ISSN 1948-5204 (online)

World Journal of *Gastrointestinal Oncology*

World J Gastrointest Oncol 2021 June 15; 13(6): 462-637

Published by Baishideng Publishing Group Inc

World Journal of Gastrointestinal Oncology

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Monthly Volume 13 Number 6 June 15, 2021

World Journal of Gastrointestinal Oncology

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INDEXING/ABSTRACTING

The *WJGO* is now indexed in Science Citation Index Expanded (also known as SciSearch®), PubMed, PubMed Central, and Scopus. The 2020 edition of Journal Citation Reports® cites the 2019 impact factor (IF) for *WJGO* as 2.898; IF without journal self cites: 2.880; 5-year IF: 3.316; Ranking: 143 among 244 journals in oncology; Quartile category: Q3; Ranking: 55 among 88 journals in gastroenterology and hepatology; and Quartile category: Q3. The *WJGO*'s CiteScore for 2019 is 2.0 and Scopus CiteScore rank 2019: Gastroenterology is 86/137.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Jia-Hui Li*; **Production Department Director:** *Xiang Li*; **Editorial Office Director:** *Ya-Juan Ma*.

NAME OF JOURNAL
World Journal of Gastrointestinal Oncology

ISSN
ISSN 1948-5204 (online)

LAUNCH DATE
February 15, 2009

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Rosa M Jimenez Rodriguez, Pashtoon Kasi, Monjur Ahmed, Florin Burada

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/1948-5204/editorialboard.htm

PUBLICATION DATE
June 15, 2021

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World J Gastrointest Oncol 2021 June 15; 13(6): 462-471

DOI: 10.4251/wjgo.v13.i6.462

ISSN 1948-5204 (online)

OPINION REVIEW

Efficacy and safety of endoscopic resection in treatment of small gastric stromal tumors: A state-of-the-art review

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Author contributions: Xu ZL conceived the idea for the manuscript; all authors reviewed the literature and drafted the manuscript.

Supported by the Natural Science Foundation of Guangdong Province of China, No. 2018A0303130278.

Conflict-of-interest statement: The author declares no conflict of interest.

Manuscript source: Invited

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Abstract

Gastrointestinal stromal tumors can occur in any part of the gastrointestinal tract, but gastric stromal tumors (GSTs) are the most common. All GSTs have the potential to become malignant, and these can be divided into four different grades by risk from low to high: Very low risk, low risk, medium risk, and high risk. Current guidelines all recommend early complete excision of GSTs larger than 2 cm in diameter. However, it is not clear whether small GSTs (sGSTs, *i.e.*, those smaller than 2 cm in diameter) should be treated as early as possible. The National Comprehensive Cancer Network recommends that endoscopic ultrasonography-guided (EUS-guided) fine-needle aspiration biopsy and imaging (computed tomography or magnetic-resonance imaging) be used to assess cancer risk for sGSTs detected by gastroscopy to determine treatment. When EUS indicates a higher risk of tumor, surgical resection is recommended. There are some questions on whether sGSTs also require early treatment. Many studies have shown that endoscopic treatment of GSTs with diameters of 2-5 cm is very effective. We here address whether endoscopic therapy is also suitable for sGSTs. In this paper, we try to explain three questions: (1) Does sGST require treatment? (2) Is digestive endoscopy a safe and effective means of treating sGST? and (3) When sGSTs are at different sites and depths, which endoscopic treatment method is more suitable?

Key Words: Gastrointestinal stromal tumors; Small gastric stromal tumors; Malignant; High risk factors; Endoscopy; Treatment

manuscript

Specialty type: Gastroenterology and hepatology

Country/Territory of origin: China

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: February 11, 2021
Peer-review started: February 11, 2021
First decision: March 29, 2021
Revised: April 4, 2021
Accepted: April 20, 2021
Article in press: April 20, 2021
Published online: June 15, 2021

P-Reviewer: Yang CW
S-Editor: Zhang H
L-Editor: Wang TQ
P-Editor: Li JH

Core Tip: Gastric stromal tumors (GSTs) are all malignant, but generally, the smaller the diameter, the more likely the tumor is inert. However, GSTs smaller than 2 cm in diameter are also at risk of growing and becoming more malignant. Endoscopic treatment of GSTs smaller than 5 cm in diameter is comparable to surgical treatment. Early endoscopic resection is safe and effective when there are high risk factors for GSTs smaller than 2 cm in diameter or the patients cannot be followed regularly, and different endoscopic treatment methods can be selected according to the tumor site and depth.

Citation: Chen ZM, Peng MS, Wang LS, Xu ZL. Efficacy and safety of endoscopic resection in treatment of small gastric stromal tumors: A state-of-the-art review. *World J Gastrointest Oncol* 2021; 13(6): 462-471
URL: https://www.wjgnet.com/1948-5204/full/v13/i6/462.htm
DOI: https://dx.doi.org/10.4251/wjgo.v13.i6.462

INTRODUCTION

Gastrointestinal stromal tumors (GISTs) originate from the Cajal stromal cells of the gastrointestinal tract[1]. These are the most common tumors arising from the mesenchymal tissue of the digestive tract and can occur in any part of the tract. Studies have shown that the most common GISTs are gastric stromal tumors (GSTs; 55.6%), followed by GISTs originating in the small intestine (31.8%), colon (6%), in other sites or with multiple occurrences (5.5%), and esophagus (only 0.7%)[2]. In China and some other developed Asian countries, the incidence of GIST is 16-22 per million[3,4]. With the advancement and popularization of high-resolution endoscopy, the detection rate of small GST (sGSTs) with diameters of < 2 cm and without obvious symptoms has been significantly improved[5].

Gastroscopy can find submucosal tumors (SMTs) of the stomach. Common gastric SMTs include GSTs, leiomyomas, lipomas, neuroendocrine tumors, granulosa cell tumors, and (relatively rarely) Schwann cell tumors[6]. However, gastroscopy cannot determine whether the lesion might be a GST, nor distinguish it from an extracavitary compressing lesion. At present, it is recognized that endoscopic ultrasonography (EUS) is the most accurate imaging method for evaluating gastric SMTs. As it can distinguish various types of SMTs of the stomach, it plays a very important role in determining tumor location and choosing treatment method[7-9]. Studies have found that EUS is better than computed tomography (CT) or magnetic-resonance imaging (MRI) for SMTs < 2 cm[10,11]. The general manifestation of GSTs under EUS is as follows: The tumor originates from the muscularis propria, except for a small part that originates from the muscularis mucosa. Small tumors often have uniform hypoechoic structures with clear boundaries, while large tumors can show irregular boundaries as well as uniform or uneven internal echo[12,13]. However, gastroscopic and EUS images of sGSTs and leiomyomas are very similar, and pathological immunohistochemical examinations are required to distinguish the two[14].

GISTs tend to be potentially malignant, but there is no absolute distinction between benign and malignant GISTs. According to degree of risk from low to high, GISTs are divided into four grades: Extremely low risk, low risk, intermediate risk, and high risk [15]. The progression of GSTs is currently uncertain; 10%-30% of cases are highly malignant[16]. Studies have found that the larger the diameter of the GST and the greater the mitotic count, the higher the metastasis rate[17]. For GSTs with a diameter > 2 cm, guidelines suggest that the tumors be completely removed early on, resection margin histology should be negative, and tumor rupture should be avoided during surgery. However, how to treat sGST remains controversial at home and abroad[18-20].

DOES SGST NEED TREATMENT?

Studies have shown[21,22] that sGSTs generally have very low proliferation activity; the tumor cells have inert biological characteristics, especially in micro-GSTs (diameter < 1 cm), which have even lower proliferation activity. A small-sample study showed

that the metastatic rate of sGSTs is negligible regardless of mitotic count[18]. However, some studies have shown that sGSTs can also pose a high risk of malignant transformation[23-26], and GIST cells can continue to proliferate, increasing the diameter of the lesion. One study showed that after an average follow-up period of 17.3 mo for sGSTs, the diameter of the lesion increased in 13.0% of patients[27]. A large-sample study of surveillance, epidemiology and end results data found that about 11.4% of sGISTs were accompanied by local progression or even distant metastasis when first diagnosed[28]. Some studies have also found that sGSTs were diagnosed as highly malignant tumors or even with distant metastasis after resection [29-31].

The United States National Comprehensive Cancer Network issued the latest soft-tissue sarcoma guidelines on May 28, 2020[32]. For sGST found by gastroscopy, EUS-guided fine-needle aspiration biopsy (FNAB) and imaging examination (CT or MRI) are recommended to assess the risk posed by the tumor and then determine treatment options. EUS manifestations suggesting a higher risk[33] are irregular edges, cystic change, ulcer formation, hyperechoic foci, and heterogeneity. The National Comprehensive Cancer Network guidelines recommend that patients with such EUS manifestations be treated by surgical resection; if there are no such manifestations, regular EUS or imaging follow-up can be considered, but no specific follow-up surveillance is suggested[32]. Lachter *et al*[34] retrospectively analyzed 70 cases of GST monitored by EUS and found that sGSTs with diameters of > 17 mm grew easily. Fang *et al*[35] conducted EUS follow-up with a median time of 24.0 mo and found that sGSTs with diameters > 14 mm were prone to tumor enlargement accompanied by clinical symptoms. Gao *et al*[26] conducted a retrospective analysis of 69 cases of sGST and found that tumors < 9.5 mm in diameter could be evaluated every 2-3 years, but those ≥ 9.5 mm in diameter should be surveyed every 6-12 mo.

Studies have shown that due to sample size limitations, the diagnosis rate of EUS-FNAB for sGSTs is 71%[36]. Therefore, only pathological evaluation of postoperative specimens can determine the malignant potential of sGSTs. Gastroscopy, EUS, and other examinations required for sGST review are all invasive procedures, and long-term, high-frequency follow-up also imposes heavy economic and psychological burdens on patients. Patients who know there is a lesion with malignant potential in their bodies and who do not undergo treatment are prone to anxiety, irritability, and other negative emotions, which seriously affect their quality of life. By communicating with sGST patients, researchers have found that most of such patient are strongly willing to undergo surgical or endoscopic treatment[37].

IS DIGESTIVE ENDOSCOPY SAFE AND EFFECTIVE FOR TREATING SGST? HOW TO CHOOSE DIFFERENT ENDOSCOPIC TREATMENT METHODS?

GST is almost completely tolerant to traditional radiotherapy and chemotherapy. The main avenue of metastasis is the blood; lymph node metastasis is rare. Therefore, lymph node dissection is generally unnecessary. Surgical treatment should completely remove the tumor and preserve gastric function as much as possible. Extensive gastrectomy cannot improve survival rate; the biological characteristics of GST greatly play to the advantages of minimally invasive endoscopic surgery[38,39].

Digestive endoscopic technology has rapidly developed and been widely popularized. Gastroscopy has significant efficacy in the treatment of GSTs. Digestive endoscopy for the treatment of GSTs with a diameter < 5 cm and no metastasis has the same efficacy as traditional surgery and laparoscopic surgery, with less trauma, shorter operation time, fewer complications, lower treatment costs, and faster recovery [40]. A recent meta-analysis of 12 studies including a total of 1292 patients with sGSTs [41] compared efficacy and safety between endoscopic resection and laparoscopic resection. The results showed that endoscopic operation time was shorter than that of laparoscopic resection; there were no significant differences between the two in intraoperative bleeding, postoperative hospital stay, postoperative exhaust time, or postoperative complication rate; and patients treated by endoscopy were able to resume eating earlier. The expert consensus issued by China in 2018 recommended the following[6]: (1) Lesions with no metastasis or with extremely low risk of metastasis; (2) Possibility of complete resection by endoscopic techniques; and (3) Low residual and recurrence risks are suitable for endoscopic resection. During endoscopic resection, the principle of tumor-free treatment should be followed: The tumor should be completely removed, and the tumor capsule should be intact during resection.

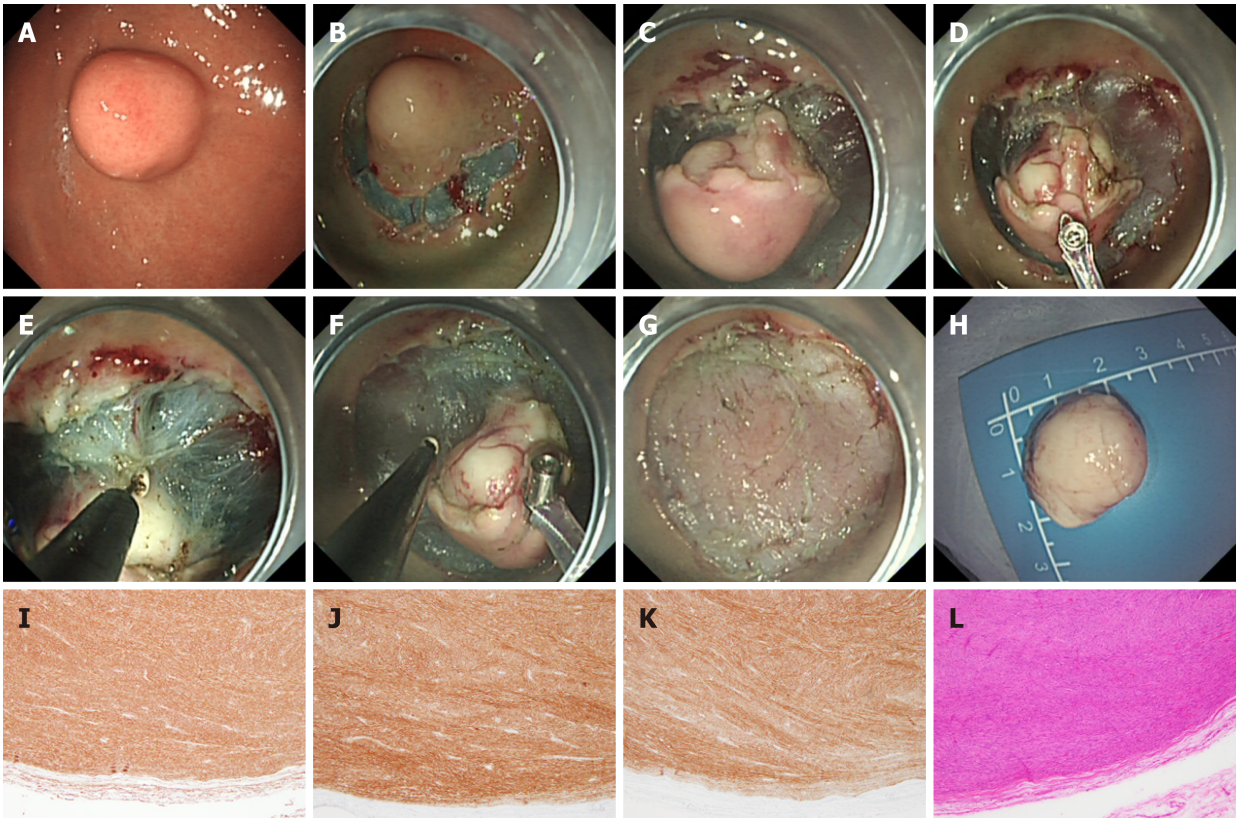

Figure 1 Endoscopic submucosal dissection treatment of gastric stromal tumor. A: Gastric stromal tumor (GST) in the fundus of the stomach; B: Submucosal injection around the GST was performed with an injection needle, and then a submucosal incision was made; C: The tumor (white) can be seen after peripheral submucosal separation; D: Traction of the tumor by the clip-and-snare method to expose its root; E and F: An IT knife was used to separate the root of the tumor; G: Wound surface after tumor resection; H: Complete resection of the tumor; I-K: CD34 (I), CD117 (J), and DOG-1 (K) were all expressed; L: Mitotic figure count ≤ 5 per 50 high-power fields.

At present, commonly used methods for gastroscopic treatment of sGST include endoscopic submucosal dissection (ESD), endoscopic submucosal excavation (ESE), endoscopic full-thickness resection (EFTR), and submucosal-tunneling endoscopic resection (STER). ESD (Figure 1) is often used to treat GSTs derived from the superficial layer of the muscle propria. A retrospective analysis of 168 patients[42] showed an overall resection rate of ESD in GST of 100%; only two (1.2%) patients had delayed bleeding, and no local recurrence or distant metastasis of the tumor was observed during a follow-up period of 6-67 mo (median, 25 mo). A study comparing ESD and laparoscopic treatment of sGSTs[43] found that ESD could significantly reduce operation time, blood loss, and patient hospital stay and that the two groups did not significantly differ in recurrence rate or survival time after tumor resection. It is reported in the literature[44,45] that the main complications of ESD treatment of GSTs are perforation (0%-8.2%) and bleeding (0%-15.6%). In most patients, bleeding and perforation complications can be controlled through endoscopic treatment. ESE (Figure 2) is a variation of ESD; the main difference is that ESE can excavate sGSTs from the deep layer of the muscularis propria. Jeong *et al*[46] reported for the first time that ESE used to treat GSTs derived from the muscularis propria has a high complete resection rate and an acceptable complication rate. The complete resection rate of ESE is reported to be 90%-100%; the main complication is perforation, with an incidence rate of 0%-20%, and most cases can be treated under endoscopy[47-50]. Studies have also pointed out that ESE resection of tumors originating from the submucosal muscularis propria would result in incomplete resection of the tumor capsule, leading to residual tumor cells, and that excessive excavation can lead to perforation[5,51]. Studies have also revealed that the complete resection rate in endoscopic treatment of sGSTs (concomitant stromal tumors) is low, especially of micro-GSTs (< 1 cm). Compared with bigger tumors, it is more difficult to remove normal tissues surrounding the capsules of small tumors and to dissect out the tumors themselves [46]. ESD and ESE resection of fundic sGST entails use of the U-type reverse endoscope, which is difficult to operate and can damage tumor capsules during removal of tumors, affecting resection integrity. Even worse, abundant blood vessels

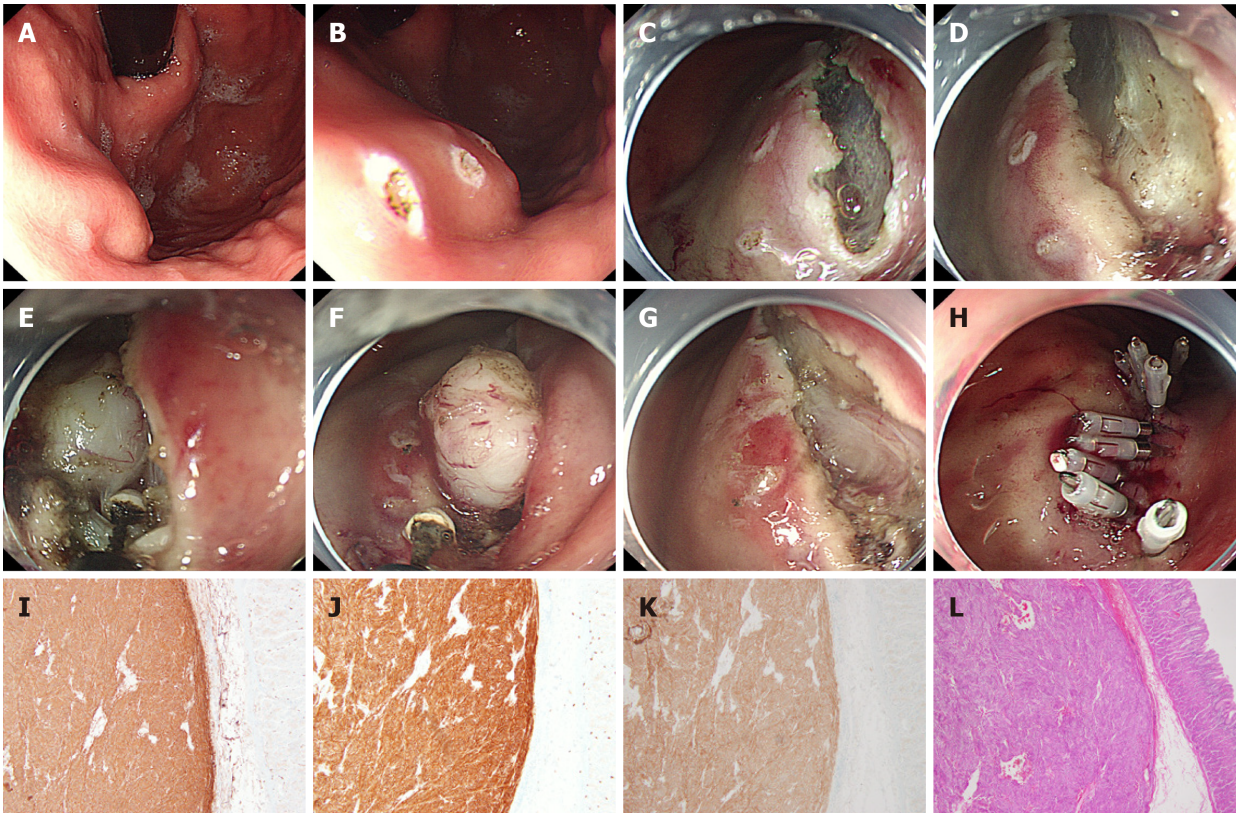

Figure 2 Endoscopic submucosal excavation treatment of gastric stromal tumor. A: Gastric stromal tumor (GST) at the junction of the gastric body and fundus; B: "Linear" electrocoagulation labeling of the GST mucosa; C and D: The tumor (white) was observed after linear incision of the GST surface mucosa and submucosa; E and F: An IT knife was used to separate the root of the tumor; G: Wound surface after tumor resection; H: Titanium clip sealing of the wound; I-K: CD34 (I), CD117 (J), and DOG-1 (K) were all expressed; L: Mitotic figure count ≤ 5/50 high-power fields.

around the tumors must be cut off, which can easily lead to intraoperative bleeding, thereby impairing physicians' vision and operational accuracy, prolonging the operation, and increasing the risk of perforation[52]. ESE is suggested to be suitable for the treatment of sGSTs derived from the muscularis propria and growing into the gastric cavity.

EFTR (Figure 3) can be classified as a special form of ESD/ESE with active rather than passive perforation; the gastric wall is closed after it undergoes a full-thickness resection. Therefore, perforation in EFTR is not considered a complication. EFTR can be used to treat sGSTs with intracavitary and extracavitary growth. Wang *et al*[53] were the first to perform EFTR in order to treat GST, removing 66 such tumors with diameters of < 3.5 cm. The complete resection rate was 100%. There were five cases of intraoperative bleeding, in all of which bleeding was successfully stopped and the perforation completely closed under endoscopy. Studies have reported that the complete resection rate in EFTR treatment can be as high as 87.5%-100.0%, and the complication rate is low. There are a few reports of abdominal infection after EFTR, which improved after treatment[54,55]. Another study[56] showed that EFTR is equivalent to laparoscopic surgery in the safety and efficacy of resectioning smaller-diameter GSTs, but EFTR can significantly reduce operation time, intraoperative blood loss, and patient hospital stay. It has been reported that the incidence of electrocoagulation syndrome is 3.1% after EFTR; although in such cases the syndrome is less severe than bleeding and perforation, it has a high incidence in the stomach and its symptoms can be similar to those of ordinary postoperative perforation[37]. However, active EFTR perforation to completely remove the tumor is relatively traumatic, and the endoscopist must have a high level of skill to close the perforation fistula; therefore, the procedure is recommended to be completed by a physician experienced in endoscopic treatment. When perforation occurs during endoscopic treatment, the use of metal clips alone or combined with nylon string (the purse string suture) has a good preventive effect against complications[57].

STER (Figure 4) is suitable for the cardia where a tunnel is easy to build, or sGSTs at the fundus of the stomach near the cardia. When sGST is not close to the cardia, it is difficult to establish a tunnel, and therefore the STER technique is not applicable. STER

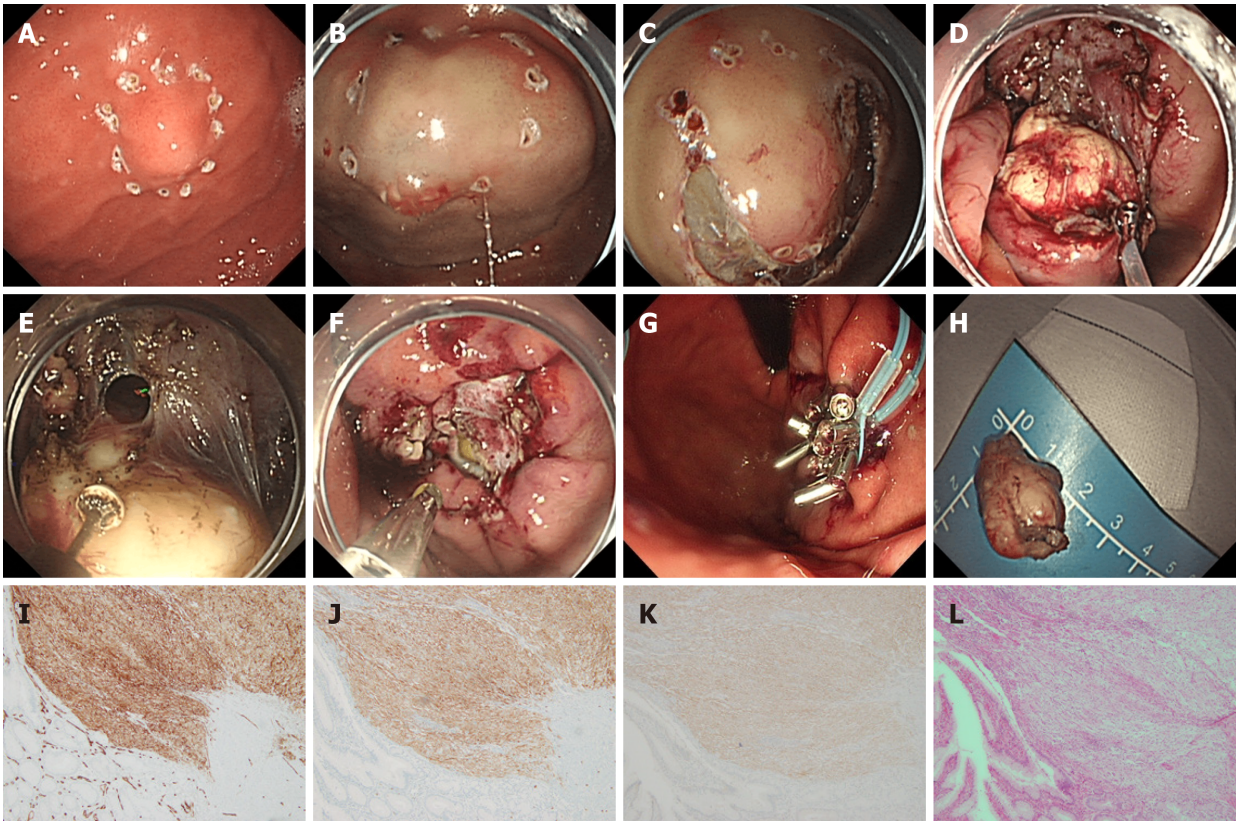

Figure 3 Endoscopic full-thickness resection treatment of gastric stromal tumor. A: Gastric stromal tumor (GST) of the gastric body near the fundus; B: Submucosal injection around the GST was performed by injection needle; C: Circumferential incision of the submucosa around the tumor; D: Traction of the exposed tumor with the clip-and-snare method; E: An IT knife was used to separate the root of the tumor, and the local full-thickness gastric wall was cut open; F: After full-layer resection, the wound was treated with hot biopsy forceps for hot coagulation and hemostasis; G: After tumor resection, the wound was sealed with a titanium clip and a nylon ring for a purse pocket suture; H: Complete resection of the tumor body for examination; I-K: CD34 (I), CD117 (J), and DOG-1 (K) were all expressed; L: Mitotic figure count ≤ 5/50 high-power fields.

has many advantages, including maintenance of mucosal integrity, small wounds, fast healing, clear operating vision, and reduced risk of pleural and abdominal infections [58]. A clinical study involving 290 cases showed that the overall incidence of complications was high [23.4% (68/290)], but only a small percentage thereof (10.0%) required therapeutic intervention[59]. A study involving 430 cases[60] showed that the complete resection rate of STER was 98.1%, the rate of postoperative gas-related complications was 21.5%, that of inflammation-related complications was 8.4%, and that of delayed bleeding was 2.2%. There were no cases of death or recurrence of tumors related to STER.

CONCLUSION

At present, there is no consensus on whether sGSTs need treatment. However, if EUS examination determines that the sGST has high-risk manifestations, resection is recommended. Even EUS-FNAB examination cannot completely determine the risk grade of sGST, which tends to be potentially malignant. Although the tumor cells have inert biological characteristics, patients who live with tumors and need long-term endoscopic follow-up have heavy psychological and economic burdens to bear. The efficacy of digestive endoscopy in the treatment of sGSTs is equivalent to that of surgery, with no effect on gastric function, less trauma, lower treatment cost, and shorter hospital stay. For sGST patients with high-risk manifestations or those who cannot tolerate endoscopic follow-up but who actively demand treatment, endoscopic sGST resection by physicians experienced in endoscopic treatment is effective and safe.

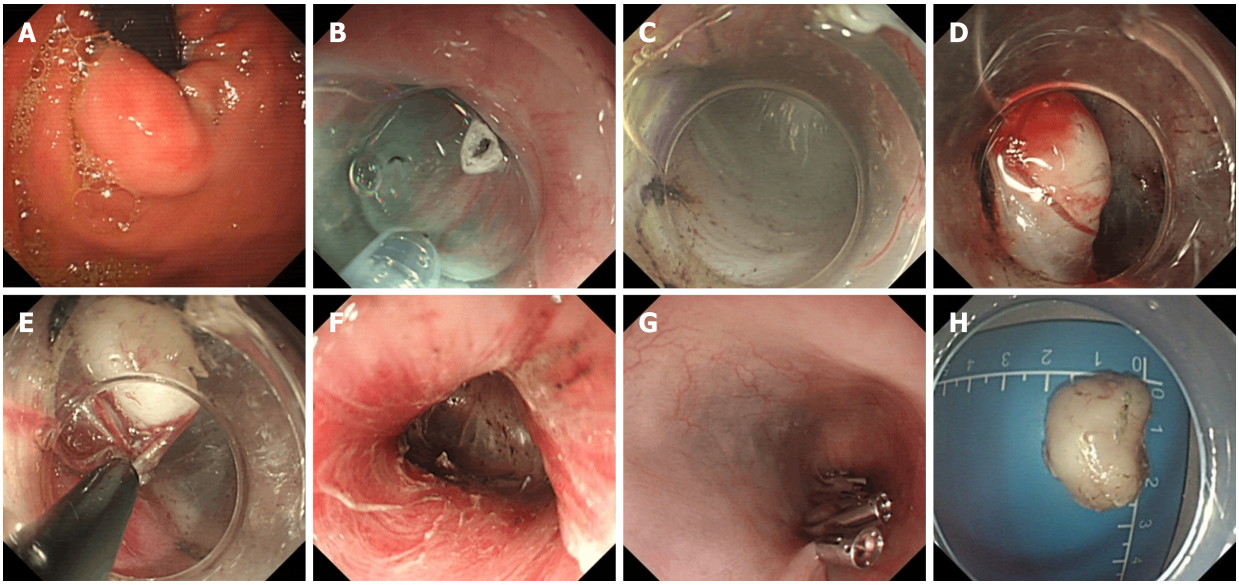

Figure 4 Submucosal-tunneling endoscopic resection treatment of gastric stromal tumor. A: Gastric stromal tumor (GST) at the esophagogastric junction; B: Submucosal injection was initiated by needle in the esophagus about 5 cm away from the GST; C and D: The esophageal mucosa was cut open to establish a submucosal tunnel to the tumor; E: An IT knife was used to separate the root of the tumor; F: Heat coagulation and hemostatic therapy on wound surface after tumor resection; G: The opening of the esophageal tunnel was sealed with titanium clamps; H: Complete resection of the tumor.

REFERENCES

1 **Kwon JG**, Hwang SJ, Hennig GW, Bayguinov Y, McCann C, Chen H, Rossi F, Besmer P, Sanders KM, Ward SM. Changes in the structure and function of ICC networks in ICC hyperplasia and gastrointestinal stromal tumors. *Gastroenterology* 2009; **136**: 630-639 [PMID: 19032955 DOI: 10.1053/j.gastro.2008.10.031]

2 **Søreide K**, Sandvik OM, Søreide JA, Giljaca V, Jureckova A, Bulusu VR. Global epidemiology of gastrointestinal stromal tumours (GIST): A systematic review of population-based cohort studies. *Cancer Epidemiol* 2016; **40**: 39-46 [PMID: 26618334 DOI: 10.1016/j.canep.2015.10.031]

3 **Cho MY**, Sohn JH, Kim JM, Kim KM, Park YS, Kim WH, Jung JS, Jung ES, Jin SY, Kang DY, Park JB, Park HS, Choi YD, Sung SH, Kim YB, Kim H, Bae YK, Kang M, Chang HJ, Chae YS, Lee HE, Park DY, Lee YS, Kang YK, Kim HK, Chang HK, Hong SW, Choi YH, Shin O, Gu M, Kim YW, Kim GI, Chang SJ. Current trends in the epidemiological and pathological characteristics of gastrointestinal stromal tumors in Korea, 2003-2004. *J Korean Med Sci* 2010; **25**: 853-862 [PMID: 20514305 DOI: 10.3346/jkms.2010.25.6.853]

4 **Chan KH**, Chan CW, Chow WH, Kwan WK, Kong CK, Mak KF, Leung MY, Lau LK. Gastrointestinal stromal tumors in a cohort of Chinese patients in Hong Kong. *World J Gastroenterol* 2006; **12**: 2223-2228 [PMID: 16610025 DOI: 10.3748/wjg.v12.i14.2223]

5 **Nishida T**, Goto O, Raut CP, Yahagi N. Diagnostic and treatment strategy for small gastrointestinal stromal tumors. *Cancer* 2016; **122**: 3110-3118 [PMID: 27478963 DOI: 10.1002/cncr.30239]

6 **Gastrointestinal Surgery Group**; Chinese Medical Association Gastroenterology Endoscopy Branch, Chinese Medical Association Endoscopy Specialized Committee, Chinese Medical Association Gastrointestinal Endoscopy Branch, Chinese Medical Association Surgery Branch. Chinese expert consensus on endoscopic diagnosis and treatment of digestive tract submucosal oncology (2018 edition). *Zhonghua Xiaohua Neijing Zazhi* 2018; **35**: 536-546

7 **Landi B**, Palazzo L. The role of endosonography in submucosal tumours. *Best Pract Res Clin Gastroenterol* 2009; **23**: 679-701 [PMID: 19744633 DOI: 10.1016/j.bpg.2009.05.009]

8 **Polkowski M**, Butruk E. Submucosal lesions. *Gastrointest Endosc Clin N Am* 2005; **15**: 33-54, viii [PMID: 15555950 DOI: 10.1016/j.giec.2004.07.005]

9 **Hwang JH**, Saunders MD, Rulyak SJ, Shaw S, Nietsch H, Kimmey MB. A prospective study comparing endoscopy and EUS in the evaluation of GI subepithelial masses. *Gastrointest Endosc* 2005; **62**: 202-208 [PMID: 16046979 DOI: 10.1016/s0016-5107(05)01567-1]

10 **Okten RS**, Kacar S, Kucukay F, Sasmaz N, Cumhur T. Gastric subepithelial masses: evaluation of multidetector CT (multiplanar reconstruction and virtual gastroscopy) versus endoscopic ultrasonography. *Abdom Imaging* 2012; **37**: 519-530 [PMID: 21822967 DOI: 10.1007/s00261-011-9791-0]

11 **Brand B**, Oesterhelweg L, Binmoeller KF, Sriram PV, Bohnacker S, Seewald S, De Weerth A, Soehendra N. Impact of endoscopic ultrasound for evaluation of submucosal lesions in gastrointestinal tract. *Dig Liver Dis* 2002; **34**: 290-297 [PMID: 12038814 DOI: 10.1016/s1590-8658(02)80150-5]

12 **Chen TH**, Hsu CM, Chu YY, Wu CH, Chen TC, Hsu JT, Yeh TS, Lin CJ, Chiu CT. Association of

endoscopic ultrasonographic parameters and gastrointestinal stromal tumors (GISTs): can endoscopic ultrasonography be used to screen gastric GISTs for potential malignancy? *Scand J Gastroenterol* 2016; **51**: 374-377 [PMID: 26489709 DOI: 10.3109/00365521.2015.1095350]

13 **Jeon SW**, Park YD, Chung YJ, Cho CM, Tak WY, Kweon YO, Kim SK, Choi YH. Gastrointestinal stromal tumors of the stomach: endosonographic differentiation in relation to histological risk. *J Gastroenterol Hepatol* 2007; **22**: 2069-2075 [PMID: 18031362 DOI: 10.1111/j.1440-1746.2006.04767.x]

14 **Humphris JL**, Jones DB. Subepithelial mass lesions in the upper gastrointestinal tract. *J Gastroenterol Hepatol* 2008; **23**: 556-566 [PMID: 18086121 DOI: 10.1111/j.1440-1746.2007.05232.x]

15 **Joensuu H**. Risk stratification of patients diagnosed with gastrointestinal stromal tumor. *Hum Pathol* 2008; **39**: 1411-1419 [PMID: 18774375 DOI: 10.1016/j.humpath.2008.06.025]

16 **Chun SY**, Kim KO, Park DS, Lee IJ, Park JW, Moon SH, Baek IH, Kim JH, Park CK, Kwon MJ. Endoscopic submucosal dissection as a treatment for gastric subepithelial tumors that originate from the muscularis propria layer: a preliminary analysis of appropriate indications. *Surg Endosc* 2013; **27**: 3271-3279 [PMID: 23519491 DOI: 10.1007/s00464-013-2904-9]

17 **Miettinen M**, Lasota J. Gastrointestinal stromal tumors: pathology and prognosis at different sites. *Semin Diagn Pathol* 2006; **23**: 70-83 [PMID: 17193820 DOI: 10.1053/j.semdp.2006.09.001]

18 **von Mehren M**, Randall RL, Benjamin RS, Boles S, Bui MM, Ganjoo KN, George S, Gonzalez RJ, Heslin MJ, Kane JM, Keedy V, Kim E, Koon H, Mayerson J, McCarter M, McGarry SV, Meyer C, Morris ZS, O'Donnell RJ, Pappo AS, Paz IB, Petersen IA, Pfeifer JD, Riedel RF, Ruo B, Schuetze S, Tap WD, Wayne JD, Bergman MA, Scavone JL. Soft Tissue Sarcoma, Version 2.2018, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 2018; **16**: 536-563 [PMID: 29752328 DOI: 10.6004/jnccn.2018.0025]

19 **Li J**, Ye Y, Wang J, Zhang B, Qin S, Shi Y, He Y, Liang X, Liu X, Zhou Y, Wu X, Zhang X, Wang M, Gao Z, Lin T, Cao H, Shen L; Chinese Society Of Clinical Oncology Csco Expert Committee On Gastrointestinal Stromal Tumor. Chinese consensus guidelines for diagnosis and management of gastrointestinal stromal tumor. *Chin J Cancer Res* 2017; **29**: 281-293 [PMID: 28947860 DOI: 10.21147/j.issn.1000-9604.2017.04.01]

20 **Casali PG**, Abecassis N, Aro HT, Bauer S, Biagini R, Bielack S, Bonvalot S, Boukovinas I, Bovee JVMG, Brodowicz T, Broto JM, Buonadonna A, De Álava E, Dei Tos AP, Del Muro XG, Dileo P, Eriksson M, Fedenko A, Ferraresi V, Ferrari A, Ferrari S, Frezza AM, Gasperoni S, Gelderblom H, Gil T, Grignani G, Gronchi A, Haas RL, Hassan B, Hohenberger P, Issels R, Joensuu H, Jones RL, Judson I, Jutte P, Kaal S, Kasper B, Kopeckova K, Krákorová DA, Le Cesne A, Lugowska I, Merimsky O, Montemurro M, Pantaleo MA, Piana R, Picci P, Piperno-Neumann S, Pousa AL, Reichardt P, Robinson MH, Rutkowski P, Safwat AA, Schöffski P, Sleijfer S, Stacchiotti S, Sundby Hall K, Unk M, Van Coevorden F, van der Graaf WTA, Whelan J, Wardelmann E, Zaikova O, Blay JY; ESMO Guidelines Committee and EURACAN. Gastrointestinal stromal tumours: ESMO-EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2018; **29**: iv68-iv78 [PMID: 29846513 DOI: 10.1093/annonc/mdy095]

21 **Rossi S**, Gasparotto D, Toffolatti L, Pastrello C, Gallina G, Marzotto A, Sartor C, Barbareschi M, Cantaloni C, Messerini L, Bearzi I, Arrigoni G, Mazzoleni G, Fletcher JA, Casali PG, Talamini R, Maestro R, Dei Tos AP. Molecular and clinicopathologic characterization of gastrointestinal stromal tumors (GISTs) of small size. *Am J Surg Pathol* 2010; **34**: 1480-1491 [PMID: 20861712 DOI: 10.1097/PAS.0b013e3181ef7431]

22 **Agaimy A**, Wünsch PH, Hofstaedter F, Blaszyk H, Rümmele P, Gaumann A, Dietmaier W, Hartmann A. Minute gastric sclerosing stromal tumors (GIST tumorlets) are common in adults and frequently show c-KIT mutations. *Am J Surg Pathol* 2007; **31**: 113-120 [PMID: 17197927 DOI: 10.1097/01.pas.0000213307.05811.f0]

23 **Fletcher CD**, Berman JJ, Corless C, Gorstein F, Lasota J, Longley BJ, Miettinen M, O'Leary TJ, Remotti H, Rubin BP, Shmookler B, Sobin LH, Weiss SW. Diagnosis of gastrointestinal stromal tumors: a consensus approach. *Int J Surg Pathol* 2002; **10**: 81-89 [PMID: 12075401 DOI: 10.1177/106689690201000201]

24 **Gill KR**, Camellini L, Conigliaro R, Sassatelli R, Azzolini F, Messerotti A, Woodward TA, Wallace MB, Jamil LH, Raimondo M. The natural history of upper gastrointestinal subepithelial tumors: a multicenter endoscopic ultrasound survey. *J Clin Gastroenterol* 2009; **43**: 723-726 [PMID: 19238092 DOI: 10.1097/MCG.0b013e31818a8457]

25 **Kim MY**, Jung HY, Choi KD, Song HJ, Lee JH, Kim DH, Choi KS, Lee GH, Kim JH. Natural history of asymptomatic small gastric subepithelial tumors. *J Clin Gastroenterol* 2011; **45**: 330-336 [PMID: 21278578 DOI: 10.1097/MCG.0b013e318206474e]

26 **Gao Z**, Wang C, Xue Q, Wang J, Shen Z, Jiang K, Shen K, Liang B, Yang X, Xie Q, Wang S, Ye Y. The cut-off value of tumor size and appropriate timing of follow-up for management of minimal EUS-suspected gastric gastrointestinal stromal tumors. *BMC Gastroenterol* 2017; **17**: 8 [PMID: 28077094 DOI: 10.1186/s12876-016-0567-4]

27 **Lok KH**, Lai L, Yiu HL, Szeto ML, Leung SK. Endosonographic surveillance of small gastrointestinal tumors originating from muscularis propria. *J Gastrointestin Liver Dis* 2009; **18**: 177-180 [PMID: 19565047]

28 **Coe TM**, Fero KE, Fanta PT, Mallory RJ, Tang CM, Murphy JD, Sicklick JK. Population-Based Epidemiology and Mortality of Small Malignant Gastrointestinal Stromal Tumors in the USA. *J*

Gastrointest Surg 2016; **20**: 1132-1140 [PMID: 27025710 DOI: 10.1007/s11605-016-3134-y]
29 **Bara T**, Bancu S, Bara T Jr, Mureşan M, Bancu L, Azamfirei L, Podeanu D, Mureşan S. [Gastric stromal tumor with liver and subcutaneus metastasis. Case report]. *Chirurgia (Bucur)* 2009; **104**: 621-624 [PMID: 19943565]
30 **Suzuki K**, Yasuda T, Nagao K, Hori T, Watanabe K, Kanamori M, Kimura T. Metastasis of gastrointestinal stromal tumor to skeletal muscle: a case report. *J Med Case Rep* 2014; **8**: 256 [PMID: 25037940 DOI: 10.1186/1752-1947-8-256]
31 **Sahin E**, Yetişyiğit T, Oznur M, Elboğa U. Gastric gastrointestinal stromal tumor with bone metastases - case report and review of the literature. *Klin Onkol* 2014; **27**: 56-59 [PMID: 24635439]
32 **National Comprehensive Cancer Network**. NCCN Clinical Practice Guidelines in Oncology: Soft Tissue Sarcoma, Version 2. 2020. [cited 28 May 2020]. Available from: https://www.nccn.org/professionals/physician_gls/pdf/sarcoma_blocks.pdf
33 **ASGE Standards of Practice Committee**, Evans JA, Chandrasekhara V, Chathadi KV, Decker GA, Early DS, Fisher DA, Foley K, Hwang JH, Jue TL, Lightdale JR, Pasha SF, Sharaf R, Shergill AK, Cash BD, DeWitt JM. The role of endoscopy in the management of premalignant and malignant conditions of the stomach. *Gastrointest Endosc* 2015; **82**: 1-8 [PMID: 25935705 DOI: 10.1016/j.gie.2015.03.1967]
34 **Lachter J**, Bishara N, Rahimi E, Shiller M, Cohen H, Reshef R. EUS clarifies the natural history and ideal management of GISTs. *Hepatogastroenterology* 2008; **55**: 1653-1656 [PMID: 19102362]
35 **Fang YJ**, Cheng TY, Sun MS, Yang CS, Chen JH, Liao WC, Wang HP. Suggested cutoff tumor size for management of small EUS-suspected gastric gastrointestinal stromal tumors. *J Formos Med Assoc* 2012; **111**: 88-93 [PMID: 22370287 DOI: 10.1016/j.jfma.2011.01.002]
36 **Akahoshi K**, Sumida Y, Matsui N, Oya M, Akinaga R, Kubokawa M, Motomura Y, Honda K, Watanabe M, Nagaie T. Preoperative diagnosis of gastrointestinal stromal tumor by endoscopic ultrasound-guided fine needle aspiration. *World J Gastroenterol* 2007; **13**: 2077-2082 [PMID: 17465451 DOI: 10.3748/wjg.v13.i14.2077]
37 **Li B**, Qi ZP, Zhou PH, Yao LQ, Xu MD, Ren Z, Shi Q, Chen T, Chai SL, Zhong YS. The value of endoscopic full-thickness resection for small and micro-gastrointestinal stromal tumors in the fundus of the stomach. *Zhongguo Shiyong Waike Zazhi* 2017; **37**: 1281-1285 [DOI: 10.19538/j.cjps.issn1005-2208.2017.11.23]
38 **Hiki N**, Nunobe S, Ohashi M. [Laparoscopy and endoscopy cooperative surgery (LECS) for gastric submucosal tumor]. *Nihon Geka Gakkai Zasshi* 2014; **115**: 102-104 [PMID: 24749332]
39 **Matsuhashi N**, Osada S, Yamaguchi K, Okumura N, Tanaka Y, Imai H, Sasaki Y, Nonaka K, Takahashi T, Futamura M, Yoshida K. Long-term outcomes of treatment of gastric gastrointestinal stromal tumor by laparoscopic surgery: review of the literature and our experience. *Hepatogastroenterology* 2013; **60**: 2011-2015 [PMID: 24719942]
40 **Zhang Q**, Gao LQ, Han ZL, Li XF, Wang LH, Liu SD. Effectiveness and safety of endoscopic resection for gastric GISTs: a systematic review. *Minim Invasive Ther Allied Technol* 2018; **27**: 127-137 [PMID: 28681655 DOI: 10.1080/13645706.2017.1347097]
41 **Wang C**, Gao Z, Shen K, Cao J, Shen Z, Jiang K, Wang S, Ye Y. Safety and efficiency of endoscopic resection versus laparoscopic resection in gastric gastrointestinal stromal tumours: A systematic review and meta-analysis. *Eur J Surg Oncol* 2020; **46**: 667-674 [PMID: 31864827 DOI: 10.1016/j.ejso.2019.10.030]
42 **An W**, Sun PB, Gao J, Jiang F, Liu F, Chen J, Wang D, Li ZS, Shi XG. Endoscopic submucosal dissection for gastric gastrointestinal stromal tumors: a retrospective cohort study. *Surg Endosc* 2017; **31**: 4522-4531 [PMID: 28374257 DOI: 10.1007/s00464-017-5511-3]
43 **Meng Y**, Li W, Han L, Zhang Q, Gong W, Cai J, Li A, Yan Q, Lai Q, Yu J, Bai L, Liu S, Li Y. Long-term outcomes of endoscopic submucosal dissection versus laparoscopic resection for gastric stromal tumors less than 2 cm. *J Gastroenterol Hepatol* 2017; **32**: 1693-1697 [PMID: 28220962 DOI: 10.1111/jgh.13768]
44 **Oda I**, Suzuki H, Nonaka S, Yoshinaga S. Complications of gastric endoscopic submucosal dissection. *Dig Endosc* 2013; **25** Suppl 1: 71-78 [PMID: 23368986 DOI: 10.1111/j.1443-1661.2012.01376.x]
45 **Saito I**, Tsuji Y, Sakaguchi Y, Niimi K, Ono S, Kodashima S, Yamamichi N, Fujishiro M, Koike K. Complications related to gastric endoscopic submucosal dissection and their managements. *Clin Endosc* 2014; **47**: 398-403 [PMID: 25324997 DOI: 10.5946/ce.2014.47.5.398]
46 **Jeong ID**, Jung SW, Bang SJ, Shin JW, Park NH, Kim DH. Endoscopic enucleation for gastric subepithelial tumors originating in the muscularis propria layer. *Surg Endosc* 2011; **25**: 468-474 [PMID: 20589510 DOI: 10.1007/s00464-010-1195-7]
47 **Kim HH**, Kim GH, Kim JH, Choi MG, Song GA, Kim SE. The efficacy of endoscopic submucosal dissection of type I gastric carcinoid tumors compared with conventional endoscopic mucosal resection. *Gastroenterol Res Pract* 2014; **2014**: 253860 [PMID: 24693280 DOI: 10.1155/2014/253860]
48 **Chen T**, Yao LQ, Xu MD, Zhang YQ, Chen WF, Shi Q, Cai SL, Chen YY, Xie YH, Ji Y, Chen SY, Zhou PH, Zhong YS. Efficacy and Safety of Endoscopic Submucosal Dissection for Colorectal Carcinoids. *Clin Gastroenterol Hepatol* 2016; **14**: 575-581 [PMID: 26256463 DOI: 10.1016/j.cgh.2015.07.048]
49 **Qi ZP**, Shi Q, Liu JZ, Yao LQ, Xu MD, Cai SL, Li B, Take I, Zhang YQ, Chen WF, Zhong YS, Zhou PH. Efficacy and safety of endoscopic submucosal dissection for submucosal tumors of the colon and

rectum. *Gastrointest Endosc* 2018; **87**: 540-548. e1 [PMID: 28987548 DOI: 10.1016/j.gie.2017.09.027]

50 **Wang S**, Shen L. Efficacy of Endoscopic Submucosal Excavation for Gastrointestinal Stromal Tumors in the Cardia. *Surg Laparosc Endosc Percutan Tech* 2016; **26**: 493-496 [PMID: 27846180 DOI: 10.1097/SLE.0000000000000330]

51 **Park CH**, Kim EH, Jung DH, Chung H, Park JC, Shin SK, Lee YC, Kim H, Lee SK. Impact of periodic endoscopy on incidentally diagnosed gastric gastrointestinal stromal tumors: findings in surgically resected and confirmed lesions. *Ann Surg Oncol* 2015; **22**: 2933-2939 [PMID: 25808096 DOI: 10.1245/s10434-015-4517-0]

52 **Yu FB**, Xiong HY. Value inquiry of combination of endoscopic resection and endoscopic band ligation closure in treatment of gastric submucosal tumors originated from the muscular is propria layer. *Zhongguo Neijing Zazhi* 2012; **18**: 121-124

53 **Wang L**, Ren W, Fan CQ, Li YH, Zhang X, Yu J, Zhao GC, Zhao XY. Full-thickness endoscopic resection of nonintracavitary gastric stromal tumors: a novel approach. *Surg Endosc* 2011; **25**: 641-647 [PMID: 20589511 DOI: 10.1007/s00464-010-1189-5]

54 **Zhou PH**, Yao LQ, Qin XY, Cai MY, Xu MD, Zhong YS, Chen WF, Zhang YQ, Qin WZ, Hu JW, Liu JZ. Endoscopic full-thickness resection without laparoscopic assistance for gastric submucosal tumors originated from the muscularis propria. *Surg Endosc* 2011; **25**: 2926-2931 [PMID: 21424195 DOI: 10.1007/s00464-011-1644-y]

55 **Wang H**, Feng X, Ye S, Wang J, Liang J, Mai S, Lai M, Feng H, Wang G, Zhou Y. A comparison of the efficacy and safety of endoscopic full-thickness resection and laparoscopic-assisted surgery for small gastrointestinal stromal tumors. *Surg Endosc* 2016; **30**: 3357-3361 [PMID: 26497947 DOI: 10.1007/s00464-015-4612-0]

56 **Schmidt A**, Damm M, Caca K. Endoscopic full-thickness resection using a novel over-the-scope device. *Gastroenterology* 2014; **147**: 740-742. e2 [PMID: 25083605 DOI: 10.1053/j.gastro.2014.07.045]

57 **Shi Q**, Chen T, Zhong YS, Zhou PH, Ren Z, Xu MD, Yao LQ. Complete closure of large gastric defects after endoscopic full-thickness resection, using endoloop and metallic clip interrupted suture. *Endoscopy* 2013; **45**: 329-334 [PMID: 23468195 DOI: 10.1055/s-0032-1326214]

58 **Lu J**, Lu X, Jiao T, Zheng M. Endoscopic management of upper gastrointestinal submucosal tumors arising from muscularis propria. *J Clin Gastroenterol* 2014; **48**: 667-673 [PMID: 25093319 DOI: 10.1097/MCG.0000000000000135]

59 **Chen T**, Zhang C, Yao LQ, Zhou PH, Zhong YS, Zhang YQ, Chen WF, Li QL, Cai MY, Chu Y, Xu MD. Management of the complications of submucosal tunneling endoscopic resection for upper gastrointestinal submucosal tumors. *Endoscopy* 2016; **48**: 149-155 [PMID: 26517846 DOI: 10.1055/s-0034-1393244]

60 **Song S**, Wang X, Zhang S, Li Y, Zhang X, Chu X. Efficacy and complications of submucosal tunneling endoscopic resection for upper gastrointestinal submucosal tumors and exploration for influencing factors. *Z Gastroenterol* 2018; **56**: 365-373 [PMID: 29346827 DOI: 10.1055/s-0043-123765]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2021 June 15; 13(6): 472-494

DOI: 10.4251/wjgo.v13.i6.472

ISSN 1948-5204 (online)

REVIEW

Current controversies and advances in the management of pancreatic adenocarcinoma

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Author contributions: Zeeshan MS was responsible for collecting reference articles, manuscript writing and figure/table generation; Ramzan Z provided expertise in the field of gastrointestinal endoscopy, manuscript writing and editing, and designing the aims of the manuscript.

Conflict-of-interest statement: Authors declare no conflict of interest for this article.

Manuscript source: Invited manuscript

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Abstract

Pancreatic adenocarcinoma is a lethal disease with a mortality rate that has not significantly improved over decades. This is likely due to several challenges unique to pancreatic cancer. Most patients with pancreatic cancer are diagnosed at a late stage of disease due to the lack of specific symptoms prompting an early investigation. A small subset of patients who are diagnosed at an early stage have a better chance at survival with curative surgical resection, but most patients still succumb to the disease in a few years. The dismal overall prognosis is due to suspected micro-metastasis at an early stage. Due to this reason, there is a recent interest in treating all patients with pancreatic cancers with systemic therapy upfront (including the ones that are surgically resectable). This approach is still not the standard of care due to the lack of robust prospective data available. Recent advancements in treatment regimens of chemotherapy, radiation and immunotherapy have improved the overall short-term survival but the long-term survival still remains poor. Novel approaches in diagnosis and treatment have shown promise in clinical studies but long-term clinical data is lacking. The following manuscript presents an overview of the epidemiology, diagnosis, staging, recent advances, novel approaches and controversies in the management of pancreatic adenocarcinoma.

Key Words: Pancreatic adenocarcinoma; Advances in management; Imaging; Novel approaches; Chemotherapy; Controversies

Core Tip: Early diagnosis and treatment of pancreatic adenocarcinoma has remained a challenge over the last several decades. Despite best efforts, the long-term survival rate has not significantly improved. The following manuscript highlights the current

Specialty type: Oncology

Country/Territory of origin: United States

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: January 8, 2021
Peer-review started: January 8, 2021
First decision: February 24, 2021
Revised: March 22, 2021
Accepted: May 25, 2021
Article in press: May 25, 2021
Published online: June 15, 2021

P-Reviewer: Mu PY
S-Editor: Fan JR
L-Editor: A
P-Editor: Li JH

advances and controversies in the management of pancreatic cancer. The standard systemic therapies have been presented in a format that is easy to read and follow. Novel approaches in diagnosis and management have been discussed in light of evidence-based medicine.

Citation: Zeeshan MS, Ramzan Z. Current controversies and advances in the management of pancreatic adenocarcinoma. *World J Gastrointest Oncol* 2021; 13(6): 472-494
URL: https://www.wjgnet.com/1948-5204/full/v13/i6/472.htm
DOI: https://dx.doi.org/10.4251/wjgo.v13.i6.472

INTRODUCTION

Pancreatic cancer ranks as the fourth leading cause of cancer related death in the United States and seventh leading cause of cancer deaths worldwide[1]. Approximately 57600 patients are diagnosed with pancreatic cancer annually[2], with a vast majority of these patients dying within the first year of diagnosis. The incidence of pancreatic cancer in the United States is 1.3 times higher in males than females[3]. There is a slightly increased risk in blacks than in whites[4]. Several other risk factors for pancreatic cancer have been identified[5], such as cigarette smoking[6,7], physical inactivity and obesity[8], high intake of saturated fat and/or processed or smoked meats[9], family history and genetic predisposition syndromes[10-12], nonhereditary chronic pancreatitis[13,14], and presence of pancreatic cysts such as intraductal papillary mucinous neoplasm of pancreas[15]. Due to high mortality of pancreatic cancer, many studies have looked at the prognostic indicators and predictors of mortality[16-19]. Several new modalities have been introduced in diagnosis and management of pancreatic cancer over the past few decades[20] but overall prognosis still remains poor[2].

PATHOLOGY

The most common type of pancreatic cancer is pancreatic adenocarcinoma, representing approximately 85% of all pancreatic neoplasms. Unfortunately, this accounts for the most aggressive type of pancreatic cancer with the poorest prognosis overall (Figure 1). Neoplasms arising from the endocrine pancreas (such as pancreatic neuroendocrine tumors) account for approximately 5% of all pancreatic tumors. Other rare tumors include acinar carcinoma, cystic neoplasms (mucinous cystadenoma, intraductal papillary mucinous tumors, solid pseudopapillary tumors, serous cystadenoma), metastatic tumors, *etc.*

CLINICAL PRESENTATION

The presentation of pancreatic cancer varies by the location of the tumor. Tumors located in the head of pancreas (approximately 60%-70%) usually present with painless jaundice[21] due to obstruction of the intra-pancreatic portion of distal common bile duct. Other symptoms include steatorrhea and weight loss. On the other hand, tumors in the body of pancreas (20%-25%) present somewhat late in the disease course with severe abdominal/back pain, anorexia and weight loss.

Rarely, a pancreatic mass is found as an incidental finding on computed tomography (CT) scan performed for another reason. Overall incidence of an incidental pancreatic mass over an eight year period in one study was reported as 7%, with one half of these were adenocarcinoma[22].

DIAGNOSTIC EVALUATION

Symptoms alone are not sensitive to diagnose pancreatic cancer as many other

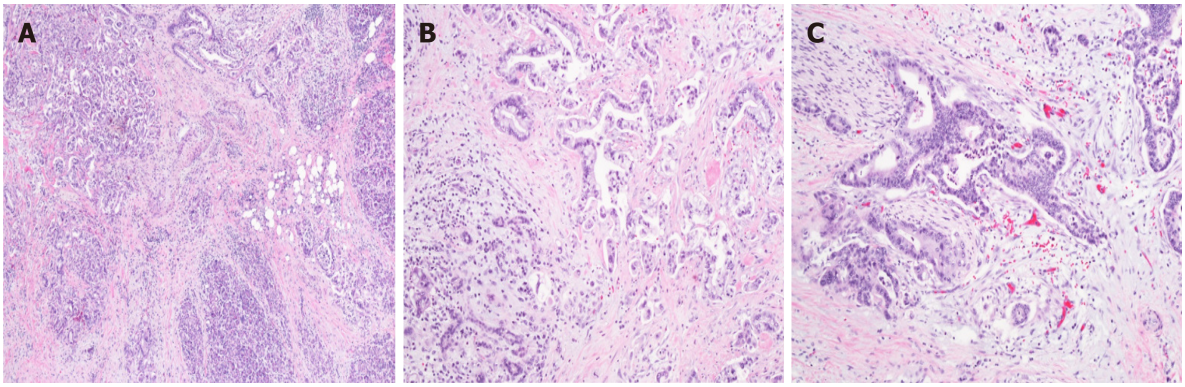

Figure 1 Hematoxylin and eosin stains[21]. A: Hematoxylin and eosin stains of normal and adjacent ductal adenocarcinoma 40 ×; B: demonstrates invasive adenocarcinoma (100 ×); C: Perineural invasion is demonstrated. Citation: Porta M, Fabregat X, Malats N, Guarner L, Carrato A, de Miguel A, Ruiz L, Jariod M, Costafreda S, Coll S, Alguacil J, Corominas JM, Solà R, Salas A, Real FX. Exocrine pancreatic cancer: symptoms at presentation and their relation to tumour site and stage. *Clin Transl Oncol* 2005; 7: 189-197. Copyright© The Authors 2005. Published by Springer Nature.

diseases can present with similar symptoms[23]. Initial workup starts with simple blood tests and cross-sectional imaging, followed by additional testing based upon clinical presentation.

Blood tests

For patients suspected to have a mass in the head of pancreas causing biliary obstruction, initial blood work should include liver function tests (such as serum aminotransferases, alkaline phosphatase, and bilirubin). Evidence of cholestasis (elevation of alkaline phosphatase and bilirubin) could suggest obstruction of distal common bile duct in the right clinical scenario. Serum amylase and lipase can be checked to rule out acute pancreatitis in patients with severe epigastric pain but is not useful to diagnose pancreatic cancer.

Abdominal ultrasound

The most important test in the diagnosis of pancreatic cancer is diagnostic imaging. Abdominal ultrasound is inexpensive, readily available and useful in certain clinical situations. It helps determine the presence of bile duct dilation, large masses in the head of pancreas (> 3 cm), any liver metastasis, ascites, *etc.* However, it has several limitations. It is not useful in the evaluation of the entire pancreas, as the retroperitoneal location of pancreas and overlying gas in the stomach and small intestine can obscure visualization of the entire pancreas.

Abdominal CT scan

The most useful test in diagnosing a pancreatic mass is abdominal CT scan. A special dedicated pancreas protocol CT scan [triple phase, helical, contrast enhanced, multidetector row CT with three dimensional reconstruction; multidetector computed tomography (MDCT)] has a sensitivity of 89%-97%[24-26]. The classic appearance of an exocrine pancreatic cancer is a poorly defined hypoattenuating mass within the pancreas, although isoattenuation can be seen in smaller tumors[27]. Other abnormalities may include abrupt cut off of pancreatic duct with proximal (upstream) pancreatic duct dilation, pancreatic atrophy, *etc.* Masses in the head of pancreas and ampulla can cause dilation of the bile duct and pancreatic duct (double duct sign)[28]. A good quality (pancreas protocol) CT also helps in the staging of tumors, which can range from delineating vascular anatomy in early-stage disease to evaluating distant metastasis in stage IV disease[29-31].

Magnetic resonance cholangiopancreatography

Magnetic resonance cholangiopancreatography (MRCP) creates a three-dimensional image of the pancreaticobiliary tree, liver and adjacent vascular structures. It is especially useful in outlining the pancreatic duct and biliary duct, obviating the need for having to inject dye during endoscopic retrograde cholangiopancreatography (ERCP) to obtain that information[32] (Figure 2). Moreover, subtle strictures, partly cystic masses and intrahepatic masses can be delineated with addition of contrast enhanced (gadolinium) injection during magnetic resonance imaging (MRI) of the abdomen. MRCP provides a road map in difficult situations such as patients with

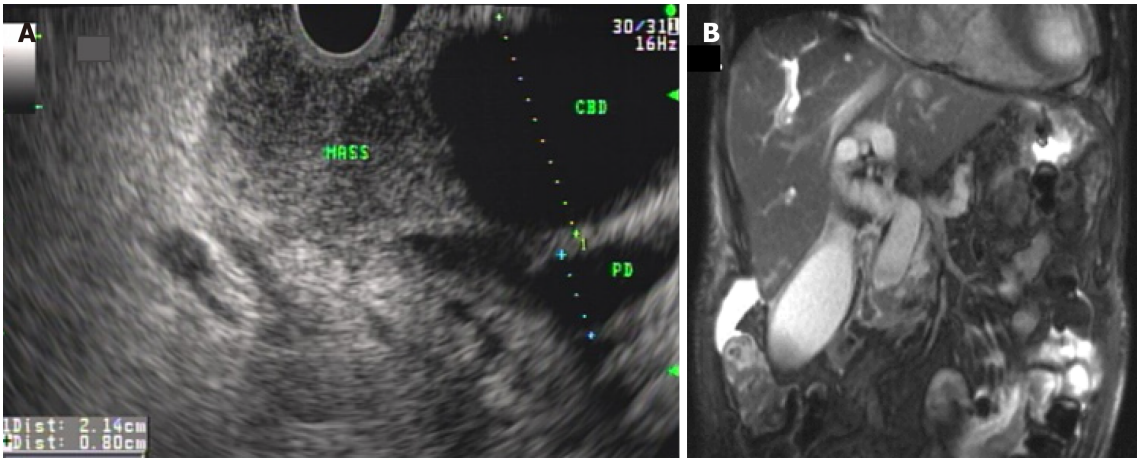

Figure 2 Malignant biliary obstruction from mass in the head of pancreas causing common bile duct and pancreatic duct dilation (double-duct sign) on endoscopic ultrasound and magnetic resonance cholangiopancreatography[21]. A: Endoscopic ultrasound; B: Magnetic resonance cholangiopancreatography, note the distended gallbladder, seen in patients with malignant biliary obstruction (Courvoisier's sign). Citation: Porta M, Fabregat X, Malats N, Guarner L, Carrato A, de Miguel A, Ruiz L, Jariod M, Costafreda S, Coll S, Alguacil J, Corominas JM, Solà R, Salas A, Real FX. Exocrine pancreatic cancer: symptoms at presentation and their relation to tumour site and stage. *Clin Transl Oncol* 2005; 7: 189-197. Copyright© The Authors 2005. Published by Springer Nature.

altered surgical anatomy (*e.g.*, Bilroth II, Roux-en-Y gastric bypass, *etc.*), gastric or duodenal stenosis, bile duct obstruction in setting of chronic pancreatitis, *etc.*[33]. Despite the value of MRI/MRCP in certain clinical situations, MRI does not offer significant advantage over MDCT in routine workup of pancreatic cancer[34-36], except probable increased sensitivity for detecting small liver metastasis[37-40].

Tumor markers

The role of tumor markers in diagnosing pancreatic cancer is controversial. The most useful and widely used tumor marker is cancer associated antigen 19-9 (CA 19-9). The reported sensitivity ranges from 70%-92% and specificity ranges from 68%-92%[41]. There are several caveats of using CA 19-9 in diagnosis of pancreatic cancer. The sensitivity is lower for smaller tumors[41,42]. In patients with a Lewis negative phenotype (approximately 5%-10% of the population), CA 19-9 is not a useful tumor marker[43,44]. The specificity is low as it is frequently elevated in patients with other cancers and various benign pancreaticobiliary tumors[44-46]. Due to low positive predictive value of CA 19-9, it is not used as a screening test for pancreatic cancer[47]. Nevertheless, there are two distinct advantages of using CA 19-9 in patients with pancreatic cancer. Firstly, it has some value as a prognostic marker, *i.e.*, a markedly elevated CA 19-9 Likely signifies occult metastasis and hence, poor overall prognosis [48-51]. Secondly, it is useful in monitoring disease activity during treatment. For example, elevation in CA 19-9 Levels after curative surgical resection may indicate early cancer recurrence even before appearance of radiographic abnormality on surveillance imaging[52-54].

Endoscopic ultrasound

The diagnosis of pancreatic cancer is made on histological confirmation of biopsy specimens. The best modality to obtain tissue diagnosis is Endoscopic ultrasound guided fine needle aspiration (EUS-FNA) of the pancreatic mass (Figure 2). The entire pancreas can be evaluated through the stomach and duodenum with the help of EUS. A special needle can be advanced through the wall of the upper gastrointestinal tract into the pancreatic mass without risking spread of cancer cells into the peritoneum as is seen with US or CT guided aspiration. The sensitivity and specificity of EUS-FNA has been reported at 89%-92% and 96% respectively[55,56]. Several advantages of EUS-FNA over US or CT guided approach (other than superior accuracy) include less risk of needle tract seeding[57], less risk of peritoneal seeding[58], ability to perform local staging, and cost[59]. Limitations of EUS are that it is operator dependent and it is suboptimal for evaluation of distant metastasis.

As an imaging tool, EUS is very sensitive and is commonly used in screening patients-with familial pancreatic cancer or other hereditary syndromes[60]. EUS not only helps biopsy the tumor but also provides simultaneous access to sampling of regional nodes, -ascites, liver lesions and malignant cyst fluid. Moreover, it helps

assess resectability of tumor duringdiagnostic evaluation (Figure 3). Additionally, recent studies have explored the utility of EUS in many other sophisticated ways, such as injection of cytotoxic agents, application of radiofrequency ablation to ablate neoplastic lesions, introducing instruments directly into the lesions for diagnostic purposes, *etc.*

Despite overwhelming evidence to support the utility of EUS in evaluation of pancreatic cancer, there are certain limitations and challenges in its use. In certain situations, masses in setting of focal chronic pancreatitis (with focus of ductal adenocarcinoma) and/or autoimmune pancreatitis can be indistinguishable from pancreatic cancer and hence, pose a clinical challenge in diagnosis and management of these lesions. In these difficult situations, a multimodality approach involving clinical history (*i.e.*, lack of alarm symptoms), radiological interpretation and short term follow up may be necessary. Additionally, certain technical limitations of EUS include difficulty in accessing tumors in the uncinate process of pancreas due to acute angulation in the second portion of duodenum, as well as the inherent limitations in obtaining rich aspirate with small FNA needles. The introduction of new and better needles such as fine needle biopsy needles with different designs and compositions have largely solved these problems. Despite these rare challenges and limitations, in the hands of experts, EUS imaging and sampling is very accurate and considered the gold standard in detecting and diagnosing pancreatic cancer.

Centers without personnel experienced with EUS-FNA rely on percutaneous biopsy of pancreatic masses to establish a diagnosis. Potential disadvantages of CT guided percutaneous biopsy approach include malignant seeding of the needle tract, though this theory has not been proven convincingly.

ERCP

ERCP is a useful tool in evaluating the duodenum, ampulla, biliary and pancreatic system. In addition to direct visualization, ERCP can help obtain tissue samples for diagnosis, such as brush samples of indeterminate strictures for cytology, as well as intraductal biopsies. As ERCP has potential risks such as bleeding, perforation and pancreatitis, it is generally not considered the initial test for the diagnosis of suspected pancreatic cancer. EUS is still considered the gold standard in obtaining samples for tissue diagnosis. However, ERCP has great clinical utility in relieving malignant biliary obstruction by stent placement (Figure 4).

Positron emission tomography scan

The role of positron emission tomography (PET) scan in routine staging of pancreatic cancer is controversial. Studies have shown data supporting the utility of PET scan in staging of pancreatic cancer[61-63], whereas other studies have shown conflicting results[64,65]. Use of 18F-flurodeoxyglucose (FDG) PET combined with CT (PET/CT) and MRI (PET/MRI) has generated interest in diagnosis, staging (lymph node involvement and metastasis)[66], assessment of pathological grade[67], assessment of treatment response, planning of radiation treatment, *etc.*[68-70]. There are certain advantages of PET/MRI over PET/CT such as lower radiation dose and superior soft tissue contrast[68]. However, the subgroup of patients with pancreatic cancer who will benefit from PET/CT or PET/MRI is not clearly understood. Hence, PET scan is not used routinely but only in certain select situations as illustrated in National Comprehensive Cancer Network and European Society for Medical Oncology guidelines[71].

Staging laparoscopy

The role of staging laparoscopy has evolved over time. The utility of staging laparoscopy relies on the pretext that small occult metastatic lesions can be missed by the available diagnostic imaging modalities and can be picked up by diagnostic laparoscopy. Hence, in certain clinical situations where the pre-test clinical probability of occult metastatic disease is high, staging laparoscopy can detect small sub-cm metastatic lesions on the peritoneum and surface of the liver and upstage the disease from resectable to stage IV metastatic disease. This also helps in re-directing the focus to palliative chemotherapy rather than neoadjuvant treatment in preparation for eventual needless surgical resection. Ideal candidates who may benefit from diagnostic laparoscopy include large tumors (> 3 cm), tumors in the body and tail of pancreas, elevated CA 19-9 > 1000, locally advanced but resectable disease, imaging suspicious for occult metastatic disease, *etc.*[72-74].

Routine use of laparoscopic ultrasound during staging laparoscopy has the potential of finding small metastatic lesions that can be missed by routine cross-sectional imaging or visual inspection during laparoscopy. When used in conjunction with

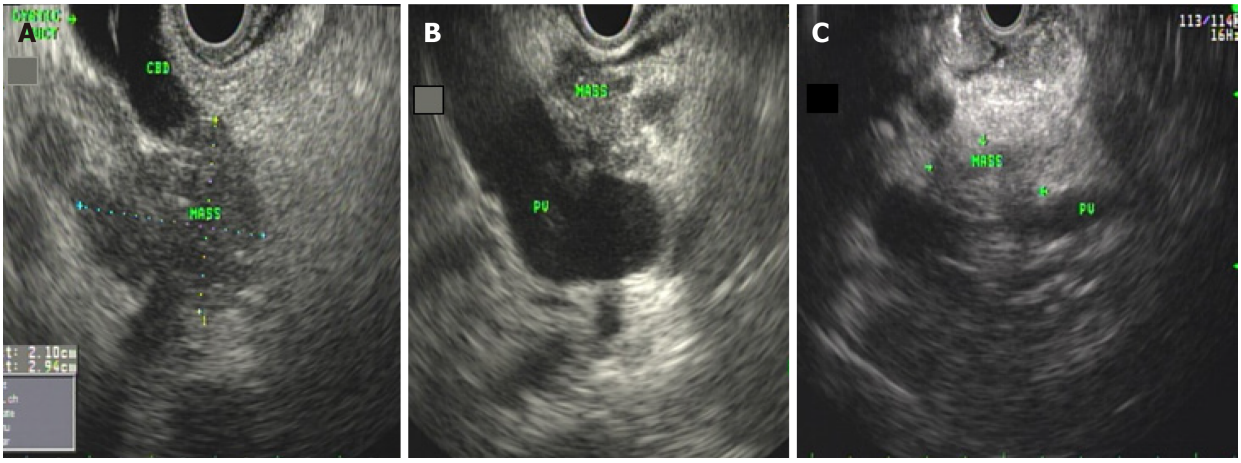

Figure 3 Endoscopic ultrasound images[21]. A: Endoscopic ultrasound (EUS) images of pancreatic adenocarcinoma invading the distal common bile duct; B: EUS images of pancreatic adenocarcinoma invading the portal vein; C: EUS images of pancreatic adenocarcinoma invading the portal vein confluence. Citation: Porta M, Fabregat X, Malats N, Guarner L, Carrato A, de Miguel A, Ruiz L, Jariod M, Costafreda S, Coll S, Alguacil J, Corominas JM, Solà R, Salas A, Real FX. Exocrine pancreatic cancer: symptoms at presentation and their relation to tumour site and stage. *Clin Transl Oncol* 2005; 7: 189-197. Copyright© The Authors 2005. Published by Springer Nature.

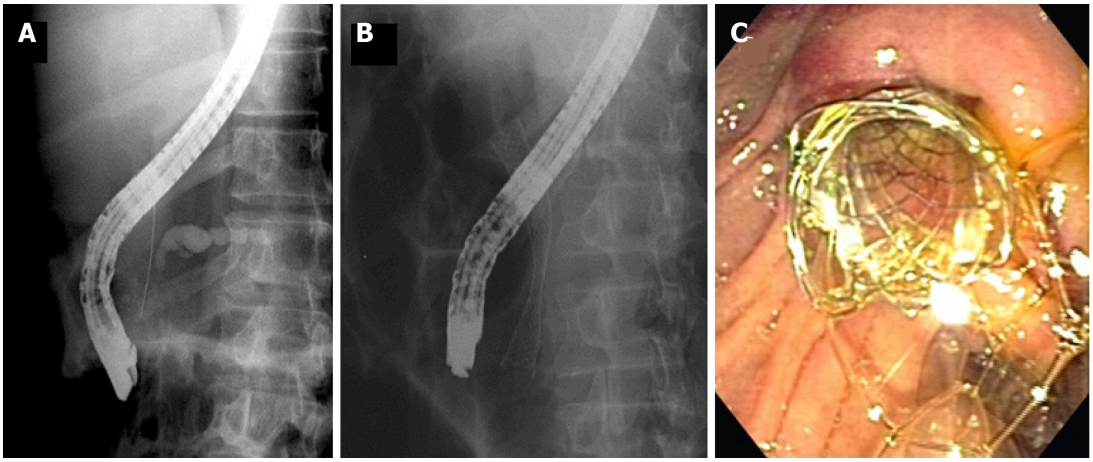

Figure 4 Malignant pancreatic stricture causing upstream pancreatic duct dilation[21]. A: Note that the wire was advanced into the bile duct during endoscopic retrograde cholangiopancreatography to place a biliary stent for palliation of obstructive jaundice; B and C: Placement of a metallic biliary stent for palliation of obstructive jaundice in a patient with unresectable pancreatic cancer [fluoroscopic picture (B); endoscopic picture (C)]. Citation: Porta M, Fabregat X, Malats N, Guarner L, Carrato A, de Miguel A, Ruiz L, Jariod M, Costafreda S, Coll S, Alguacil J, Corominas JM, Solà R, Salas A, Real FX. Exocrine pancreatic cancer: symptoms at presentation and their relation to tumour site and stage. *Clin Transl Oncol* 2005; 7: 189-197. Copyright© The Authors 2005. Published by Springer Nature.

laparoscopy, laparoscopic ultrasound can help in evaluation of primary tumors, peripancreatic vascular anatomy, detect small occult metastatic lesions and hence, change the surgical approach and prevent unnecessary radical surgery[75-79].

NOVEL DIAGNOSTIC IMAGING MODALITIES

Several studies in the last decade have sparked an interest in novel mucosal imaging, but none has yet been accepted as a routine investigation in the evaluation of suspected pancreatic mass. Narrow band imaging technology uses light of specific blue and green wavelengths to augment certain mucosal features while visualizing the wall of pancreatic duct (with a small catheter inserted into the pancreatic duct) ('pancreatoscopy')[80].

Optical endomicroscopy permits imaging of the lining of pancreatic duct and wall of pancreatic cyst with the help of a small diameter probe introduced into the pancreatic duct at the time of EUS or ERCP. Such sophisticated imaging has a potential to increase diagnostic yield of sampling by targeted biopsies in the high yield area.

Two imaging technologies used in this manner include confocal laser endomicroscopy [81,82] and high resolution microendoscopy[83]. Other imaging modalities such as optical coherence tomography have even lower clinical applicability as it employs infrared light to scan a few millimeters beneath the lining of the duct making it a time consuming and a low yield test[84-86].

Intraductal ultrasound (IDUS), a mini-ultrasound probe, can be used to evaluate indeterminate strictures. It is introduced within the pancreatic duct which makes it more invasive. In some studies it has been found to be useful in evaluation of early pancreatic cancers and determine margins of malignant cystic lesions such as intraductal papillary mucinous neoplasms before surgical resection[87]. IDUS is not commonly used in the United States due to limited clinical application and risk of pancreatitis associated with the procedure[88]. On the other hand, contrast enhanced EUS, which utilizes intravenous contrast to enhance a pancreatic lesion has been received with more interest in recent times[89]. In a meta-analysis, the pooled sensitivity of contrast-enhanced EUS for the differential diagnosis of pancreatic adenocarcinomas was 94% (95%CI: 0.91-0.95), and the specificity was 89% (95%CI: 0.85-0.92)[90].

Another modality using EUS, EUS elastography, helps distinguish between benign focal mass in chronic pancreatitis from pancreatic cancer by performing quantitative analysis of tissue stiffness. In one study, the sensitivity and specificity for detecting pancreatic malignancies were 100% and 92.9% respectively[91]. Three-dimensional reconstruction and spectrum analysis using EUS has shown -good results, and has the potential to be used more often in the future[92].

As pancreatic cancer has the potential to cause micro metastasis even in early stage of disease, research has been carried out to determine the molecular profiling of these tumors. Imaging agents such as peptides that bind to specific factors on the surface of pancreatic tumors have been developed and include: cathepsin E, integrin $\alpha_v\beta_6$, plectin 1, claudin-4 and oncolytic adenovarirus mutant[92-97] Similarly, engineered biological agents such as oncolytic adenovirus have shown efficacy and tumor selectivity in preclinical pancreatic cancer models[98,99]. More robust clinical studies are needed before it can be used in routine evaluation of early pancreatic cancer.

A different approach focused on investigating normal pancreatic parenchyma has been developed. Unlike pancreatic tumor, normal pancreatic tissue expresses receptor for bombesin. Hence, a bombesin peptide-coupled nanoparticle (BN-CLIO[Cy5.5]) can be used to image normal pancreas and hence, differentiate it from pancreatic tumors [100]. Similarly, in other studies, microbubbles (small gas-filled microspheres) have been used to image the peri-tumoral vasculature with the help of ultrasound. This technology can be potentially used to deliver anti-cancer therapies in future studies [101].

Do we need tissue diagnosis before initiating treatment?

A controversial subject is the need for pre-operative biopsy in patients with classic clinical and radiographic presentation of pancreatic adenocarcinoma. The advantage of performing biopsy is to confirm the diagnosis and minimize the risk of needless surgery for unsuspected benign disease. Disadvantages include the changes of false negative biopsy and risk of delaying definite and curative surgical resection in early pancreatic cancer. Another potential downside is the risk of rare iatrogenic complications, such as post-procedure pancreatitis or theoretical dissemination of tumor cells along the needle tract (and beyond) during CT guided biopsy. In light of these controversies, the decision to perform pre-operative biopsy rests on the discussion between the surgeon and the patient. Most centers in the US favor pre-operative biopsy as a routine. However, several experts, especially from non-US centers, favor proceeding to surgery directly (without pre-operative biopsy) in clearly resectable pancreatic head cancers[102], with an understanding that the presence of unsuspected benign diseases have been reported in 5%-11% of all resected tumors on final pathology results[103-105]. On the flip side, patients who definitely require tissue diagnosis include high risk surgical candidates, non-surgical candidates, patients due to undergo neoadjuvant or palliative chemotherapy. EUS/FNA is the ideal modality for tissue diagnosis in these patients.

Not all patients presenting with pancreatic masses have the classic presentation and supporting radiographic imaging for pancreatic cancer. Two important examples include chronic pancreatitis and autoimmune pancreatitis, where clinical presentation (lack of alarm symptoms) and imaging characteristics favor a non-malignant etiology. In these situations, a pre-operative biopsy is essential to rule out malignancy so that unnecessary surgery can be avoided.

TREATMENT

A detailed discussion on all available treatment options is beyond the scope of this article. A brief overview of the treatment options with an emphasis on controversies and recent advancements will be discussed. Patients with pancreatic cancer should ideally be evaluated and treated in a high volume center in a multidisciplinary environment. The actual treatment algorithm depends upon the stage of disease (Tables 1-4) and is generally divided into four subgroups (resectable, borderline resectable, locally advanced and unresectable, and metastatic) (Table 5).

Resectable tumors

Early curative resection of pancreatic cancer offers the best meaningful overall survival. However, only 15%-20% of pancreatic cancers are potentially resectable at presentation. Resectable tumors are the ones in which tumors have no contact with major surrounding arteries (such as celiac artery, superior mesenteric artery, or common hepatic artery) and surrounding veins (superior mesenteric vein or portal vein) (Table 5). Surgery of choice for tumors in the head of pancreas include Whipple surgery (pancreaticoduodenectomy) and distal pancreatectomy for tumors in the body and tail of pancreas. As *per* all major guidelines, these patients should undergo surgical resection if they are appropriate surgical candidates.

Pancreatic surgery carries a high morbidity and mortality but if done by experienced surgeons in high volume centers, the outcomes are superior[106,107]. Surgery also provides useful diagnostic and prognostic information[108]. Several factors such as tumor stage, status of surgical margins[17,109], lymph node status [110], tumor differentiation[111], pre and post- resection serum CA 19-9[109] and cigarette smoking[112,113] help predict overall prognosis. Five-year survival after pancreaticoduodenectomy is 10% in node positive disease[114] and 30% in node negative disease[115]. More importantly, about two thirds of patients undergoing surgical resection with curative intent will find positive lymph nodes, which correlates with a poor prognosis. Hence, this justification is used by some experts to support the use of neoadjuvant therapy upfront in all resectable tumors.

Is there a role of neoadjuvant therapy in clearly resectable tumors?

In contrast to the traditional practice of early resection in resectable tumors, the use of upfront neo-adjuvant therapy in clearly "resectable" pancreatic cancers has increased recently[116]. Some studies have supported its use[105,117-122] and others have largely debunked the idea[123,124]. The proponents of this approach highlight the fact that many such patients may already have micro-metastasis at the time of diagnosis. By providing chemo-radiation upfront, the tumors can be restaged after treatment and surgery can be offered only to the group of patients who still have localized disease. This approach will help decrease the incidence of patients presenting with grossly visible metastasis soon after surgery. Moreover, this approach helps systemic chemotherapy to be started as soon as possible, in contrast to the delay in starting chemotherapy up to 4 wk after surgery (as is routinely advised by the Oncologists). The decision to start upfront neoadjuvant therapy should ideally be made in a multidisciplinary environment in the setting of a clinical trial. There is no consensus on the best therapy for this purpose. In most centers, neoadjuvant therapy is not yet a standard treatment modality in resectable tumors outside of the context of a clinical trial[125]. For most patients with good functional status, the preferred treatment is a multiagent modified FOLFIRINOX regimen (oxaliplatin plus irinotecan with leucovorin and short term infusional flurouracil regimen), followed by chemoradio-therapy.

Is pre-operative biliary drainage necessary before surgical resection?

Another controversial subject in the management of patients with potentially resectable tumors in the head of pancreas presenting with biliary obstruction revolves around the need to perform pre-operative biliary drainage. Several studies have been done to address this question with the results revealing benefit[126], no clear benefit [127-129] or harm with this approach[130-134]. Despite overwhelming data showing potential lack of benefit or even harm in routine preoperative biliary drainage in patients with malignant biliary obstruction, many surgeons in the US routinely request biliary drainage due to perceived better post-operative outcome with this approach. The decision to choose the modality for biliary drainage (percutaneous transhepatic *vs* endoscopic) rests on the availability of expertise at the respective institution, location of the obstruction *etc.* Both techniques have advantages and disadvantages and should

Table 1 Tumor-node-metastasis staging of pancreatic adenocarcinoma [American Joint Committee on Cancer Tumor-Node-Metastasis Staging of Pancreatic Cancer (8th edition, 2017)]-T staging

T	Primary tumor
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ. This includes high-grade pancreatic intraepithelial neoplasia (PanIn-3), intraductal papillary mucinous neoplasm with high-grade dysplasia, intraductal tubulopapillary neoplasm with high-grade dysplasia, and mucinous cystic neoplasm with high-grade dysplasia
T1	Tumor ≤ 2 cm in greatest dimension
T1a	Tumor ≤ 0.5 cm in greatest dimension
T1b	Tumor > 0.5 cm and < 1 cm in greatest dimension
T1c	Tumor 1–2 cm in greatest dimension
T2	Tumor > 2 cm and ≤ 4 cm in greatest dimension
T3	Tumor > 4 cm in greatest dimension
T4	Tumor involves the celiac axis, superior mesenteric artery, and/or common hepatic artery, regardless of size

Table 2 Tumor-node-metastasis staging of pancreatic adenocarcinoma [American Joint Committee on Cancer Tumor-Node-Metastasis Staging of Pancreatic Cancer (8th edition, 2017)]-N staging

N	Regional lymph nodes
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastases
N1	Metastasis in one to three regional lymph nodes
N2	Metastasis in four or more regional lymph nodes

Table 3 Tumor-node-metastasis staging of pancreatic adenocarcinoma [American Joint Committee on Cancer Tumor-Node-Metastasis Staging of Pancreatic Cancer (8th edition, 2017)]-M staging

M	Distant metastasis
M0	No distant metastasis
M1	Distant metastasis

be used in the right clinical scenario after due consultation in a multidisciplinary environment[135]. Transhepatic biliary drainage is generally performed for more proximal intrahepatic biliary obstruction and endoscopic biliary drainage is performed for extrahepatic biliary obstruction. The type of stent used (plastic *vs* metal stent) depends upon the endoscopist and the surgeon's preference. A permanent metal stent is more commonly used as it does not need to be replaced if the tumor is deemed unresectable at the time of surgery[136].

All patients who undergo resection of tumor (without neoadjuvant therapy) should undergo repeat staging of the disease with CT scan and tumor markers before starting adjuvant chemotherapy. Adjuvant chemotherapy should be started within 2 mo of the surgery and should be continued for six months. As in neoadjuvant therapy, for patients with good functional status, the preferred treatment is a multiagent modified FOLFIRINOX regimen (oxaliplatin plus irinotecan with leucovorin and short term infusional flurouracil regimen). For patients with poor functional status, gemcitabine alone or gemcitabine plus capecitabine are reasonable options (Table 6). Addition of radiation therapy in the adjuvant setting is somewhat controversial and is usually reserved in a subgroup of patients with excellent performance status[137].

Borderline resectable and locally advanced unresectable disease

Pancreatic tumors are considered borderline resectable if there is suspected solid tumor contact with major surrounding vasculature (but less than 180 degrees of

Table 4 Tumor-node-metastasis staging of pancreatic adenocarcinoma [American Joint Committee on Cancer Tumor-Node-Metastasis Staging of Pancreatic Cancer (8th edition, 2017)]-tumor-node-metastasis staging

Stages	T	N	M
Stage 0	Tis	N0	M0
Stage IA	T1	N0	M0
Stage IB	T2	N0	M0
Stage IIA	T3	N0	M0
Stage IIB	T1, T2, T3	N1	M0
Stage III	T1, T2, T3	N2	M0
	T4	Any N	M0
Stage IV	Any T	Any N	M1

Table 5 Criteria defining resectability status of pancreatic adenocarcinoma[30]

Resectability status	Arterial	Venous
Resectable	No arterial tumor contact (CA, SMA, or CHA)	No tumor contact with the SMV or PV or ≤ 180° contact without vein contour irregularity
Borderline resectable	Pancreatic head/uncinate process: Solid tumor contact with CHA without extension to CA or hepatic artery bifurcation. Solid tumor contact with the SMA of ≤ 180°; Solid tumor contact with variant arterial anatomy (ex: Accessory right hepatic artery, replaced right hepatic artery, replaced CHA, and the origin of replaced or accessory artery). Pancreatic body/tail: Solid tumor contact with the CA of ≤ 180°; Solid tumor contact with the CA of > 180° without involvement of the aorta and with intact and uninvolved gastroduodenal artery thereby permitting a modified Appleby procedure (controversial)	Solid tumor contact with the SMV or PV of > 180°, contact of ≤ 180° with contour irregularity of the vein or thrombosis of the vein but with suitable vessel proximal and distal to the site of involvement allowing for safe and complete resection and vein reconstruction. Solid tumor contact with the IVC
Locally advanced	Head/uncinate process: Solid tumor contact with SMA > 180°; Solid tumor contact with the CA > 180°. Pancreatic body/tail: Solid tumor contact of > 180° with the SMA or CA; Solid tumor contact with the CA and aortic involvement	Unreconstructible SMV/PV due to tumor involvement or occlusion (can be due to tumor or bland thrombus)

CA: Celiac axis; SMA: Superior mesenteric artery; CHA: Common hepatic artery; SMV: Superior mesenteric vein; PV: Portal vein; IVC: Inferior vena cava.

Table 6 Treatment protocols for pancreatic adenocarcinoma in adjuvant setting

Drug	Dose and route	Administration	Toxicity
Adjuvant gemcitabine (cycle length: 4 wk)[156]			
Gemcitabine	1000 mg/m^2 IV	Weekly (× 3 wk) followed by one week of rest	Myelotoxicity; Hepatotoxicity; Pulmonary toxicity; Thrombotic microangiopathy
Adjuvant gemcitabine plus capecitabine (GemCap; cycle length: 28 d)[157]			
Gemcitabine	1000 mg/m^2 IV	Given on days 1, 8, and 15	Myelotoxicity; Nonhematologic toxicity (including hepatoxicity); Pulmonary toxicity; Thrombotic microangiopathy
Capecitabine	830 mg/m^2 *per* dose by mouth	Given on days 1 through 21	
Modified FOLFIRINOX (cycle length: 14 d)[158-162]			
Oxaliplatin	85 mg/m^2 IV	Given on day 1	Myelotoxicity; Diarrhea; Mucositis or hand-foot syndrome; Pulmonary toxicity; Neurotoxicity; Cardiotoxicity
Leucovorin	400 mg/m^2 IV	Given on day 1	
Irinotecan	150 mg/m^2 IV	Given on day 1	
Fluorouracil	2400 mg/m^2 IV	Given on day 1	

vascular involvement) on pre-operative imaging. If an obvious direct vascular invasion of > 180 degrees is noted such that a resection is not possible, then it is called locally advanced and unresectable disease (which accounts for approximately 40% of all pancreatic tumors) (Table 5).

There is no universal consensus on how to approach the treatment of these tumors. These patients are discussed in a multidisciplinary tumor board where appropriate treatment strategy is discussed in light of the patient's functional status, tumor biology (status of genetic mutations), pre-treatment imaging and many other factors.

A reasonable approach in patients with a borderline resectable disease is to attempt at downstaging with chemotherapy/chemoradiation followed by surgical exploration (if no metastatic disease is found on restaging)[137]. For patients with unresectable disease, enrollment in clinical trials using new treatment strategies should be encouraged. Prompt initiation of chemotherapy is warranted. Patients with homologous recombination repair (HRR) mutations and good performance status could benefit from aggressive medical therapy with FOLFIRINOX (short term fluorouracil, plus leucovorin, irinotecan, and oxaliplatin). A detailed discussion on the various treatment regimens and available clinical trials is beyond the scope of this article but a brief summary of the most common treatment regimens is summarized in Table 7.

Metastatic pancreatic cancer

Prognosis of metastatic pancreatic cancer is poor with an expected 5-year mortality to be greater than 97%. Hence, it is important to discuss the patient's preference and goals of care before initiation of treatment. Early involvement of the palliative care team is beneficial. Genetic testing should be performed to determine the presence of HRR deficiency. Genes associated with HRR deficiency include *BRCA1/2, PALB2, ATM, BAP1, BARD1, BLM, BRIP1, CHEK2, FAM175A, FANCA, FANCC, NBN, RAD50, RAD51, RAD51C, and RTEL1.*

For metastatic disease in the setting of known HRR mutation, a platinum based chemotherapy regimen is preferred[138]. For patients with excellent functional status (ECOG PS 0 or 1) and serum bilirubin < 1.5x upper limit of normal, an aggressive medical therapy with FOLFIRINOX or modified FOLFIRINOX should be considered. Other alternatives included FOLFOX (leucovorin plus infusional fluorouracil plus oxaliplatin, if serum bilirubin is > 1.5), and a combination of gemcitabine plus cisplatin (Table 7).

After 16 wk of chemotherapy, maintenance treatment is considered based upon the results of genetic mutation analysis. For patients with certain genetic mutations such as germline *BRCA* mutation and *PALB2* mutation, maintenance therapy with poly(ADP-ribose) polymerase inhibitor Olaparib is initiated[138,139].

If no HRR mutation is detected in patients with good functional status and low serum bilirubin (< 1.5 × ULN), an aggressive regimen such as FOLFIRINOX should be considered. Other alternatives include modified FOLFIRINOX and gemcitabine plus nonparticle albumin-bound paclitaxel (nabpaclitaxel). However, for patients with higher bilirubin (> 1.5 × ULN), a gemcitabine-based regimen can prove to be toxic and should be avoided; instead, a FOLFOX based regimen should be considered in this setting) (Table 7).

For patients with suboptimal functional status (ECOG PS of 2), monotherapy with gemcitabine or gemcitabine plus capecitabine can be considered. Gemcitabine plus nabpaclitaxel can be toxic and should be reserved for highly selected patients with high tumor burden (Table 7).

For patients with very poor functional status or severe existing co-morbidities, systemic chemotherapy should be considered cautiously. Palliative care should be involved early on with an emphasis on the control of symptoms (such as severe pain).

PALLIATION

Symptom palliation in patients with advanced pancreatic cancer is very important and is an integral part of the overall treatment plan[140]. The most common symptoms that require palliation include relief of obstructive jaundice (in tumors of the head of pancreas), duodenal obstruction (from tumor invasion) and severe debilitating pain. Other symptoms include risk of thromboembolism, anxiety/depression, anorexia and weight loss.

Palliative options in patients with malignant obstructive jaundice include biliary stenting and surgical biliary bypass. Randomized trials between the two approaches have shown no difference in survival; patients with stents have less procedure related morbidity and mortality but a higher rate of hospital readmissions from stent occlusion[141-143]. Since the advent of self-expandable metal biliary stents, however, stent occlusion has become less common compared to the traditional plastic biliary stents[143,144]. Biliary stenting can be performed endoscopically or percutaneously

Table 7 Treatment protocols for locally advanced/metastatic pancreatic adenocarcinoma

Drug	Dose and route	Administration	Toxicity
Gemcitabine monotherapy (cycle length: 8 wk for first cycle, then 4 wk)[163-166]			
Gemcitabine	1000 mg/m^2 IV	Weekly (× 7 wk) followed by one week of rest in the first cycle, then weekly (× 3 wk) followed by one week of rest in all subsequent cycles	Myelotoxicity; Hepatoxicity; Pulmonary toxicity; Thrombotic microangiopathy
Gemcitabine plus nanoparticle albumin-bound paclitaxel (nabpaclitaxel) (cycle length: 4 wk)[167,168]			
Nabpaclitaxel	125 mg/m^2 IV	Given on days 1, 8, and 15	Myelotoxicity; Sepsis; Thrombotic microangiopathy; Peripheral neuropathy; Hepatotoxicity; Pulmonary toxicity
Gemcitabine	1000 mg/m^2 IV	Given on days 1, 8, and 15	
Gemcitabine plus capecitabine (cycle length: 21 d)[157,169]			
Gemcitabine	1000 mg/m^2 IV	Given on days 1 and 8	Myelotoxicity; Nonhematologic toxicity (including hepatoxicity); Pulmonary toxicity; Thrombotic microangiopathy
Capecitabine	650 mg/m^2 *per* dose by mouth	Given on days 1 through 14	
Gemcitabine plus cisplatin (cycle length: 21 d)[170]			
Cisplatin	25 mg/m^2 IV daily	Given on days 1 and 8	Myelotoxicity; Thrombotic microangiopathy; Pulmonary toxicity; Hepatotoxicity; Neurotoxicity; Nephrotoxicity
Gemcitabine	1000 mg/m^2 IV daily	Given on days 1 and 8	
FOLFIRINOX (fluorouracil plus leucovorin, irinotecan, and oxaliplatin) (cycle length: 14 d)[160,161]			
Oxaliplatin	85 mg/m^2 IV	Given on day 1	Myelotoxicity; Diarrhea; Mucositis or hand-foot syndrome; Pulmonary toxicity; Neurotoxicity; Cardiotoxicity
Leucovorin	400 mg/m^2 IV	Given on day 1	
Irinotecan	180 mg/m^2 IV	Given on day 1	
Fluorouracil	400 mg/m^2 IV bolus	Given on day 1	
FU	2400 mg/m^2 IV	Given on day 1	
Modified FOLFIRINOX (cycle length: 14 d)[158,159,161]			
Oxaliplatin	85 mg/m^2 IV	Given on day 1	Myelotoxicity; Diarrhea; Mucositis or hand-foot syndrome; Pulmonary toxicity; Neurotoxicity; Cardiotoxicity
Leucovorin	400 mg/m^2 IV	Given on day 1	
Irinotecan	150 mg/m^2 IV	Given on day 1	
Fluorouracil	2400 mg/m^2 IV	Given on day 1	
Modified FOLFOX6 (fluorouracil plus leucovorin and oxaliplatin) (cycle length: 14 d)[160,171,172]			
Oxaliplatin	85 mg/m^2 IV	Given on day 1	Myelotoxicity; Neurotoxicity; Diarrhea; Cardiopulmonary toxicity
Leucovorin	400 mg/m^2 IV	Given on day 1	
Fluorouracil	400 mg/m^2 IV bolus	Given on day 1	
FU	2400 mg/m^2 IV	Given on day 1	
Liposomal irinotecan and fluorouracil (cycle length: 14 d)[173]			
Liposomal irinotecan	70 mg/m^2 IV	Given on day 1	Myelotoxicity; Diarrhea; Neurotoxicity; Cardiotoxicity
Leucovorin	400 mg/m^2 IV	Given on day 1	
Fluorouracil	2400 mg/m^2 IV	Given on day 1	
Pembrolizumab monotherapy for microsatellite-unstable (mismatch repair-deficient) advanced cancer (cycle length: q3 weeks or q6 weeks)[174,175]			
Pembrolizumab	200 mg IV	Given on day 1, every 3 wk	Pulmonary toxicity; Hepatotoxicity; Neurotoxicity;

OR			Dermatologic toxicityCardiotoxicity
Pembrolizumab	400 mg IV	Given on day 1, every 6 wk	

(by Interventional Radiology). Endoscopic biliary stenting is preferable as it is associated with much lower complication rates and shorter hospital stays[145-147]. A permanent expandable metal biliary stent can be placed right after obtaining samples for tissue diagnosis (during EUS-FNA), allowing for one-step, efficient and effective care to these patients[148]. As there is no surgery involved, patients can be started on chemotherapy soon afterwards (without waiting for the post-op recovery as is seen in patients undergoing surgical bypass procedure). If endoscopic management is not feasible, external biliary drainage can be attempted. Percutaneous transhepatic biliary access (by Interventional Radiology) results in the placement of a percutaneous internal-external drain which can be replaced by percutaneous metal biliary stent placement in a few weeks[146]. In rare situations, surgical biliary bypass (such as hepaticojejunostomy, choledochojejunostomy or cholecystojejunostomy) may be needed.

Locally advanced pancreatic cancer can infiltrate the wall of duodenum resulting in malignant duodenal obstruction in in approximately 15%-20% of patients[149]. This can be treated by surgical gastrojejunostomy or endoscopic enteral stent placement. Recent data on the utility of endoscopic stent placement has revealed good short-term efficacy, improved cost-effectiveness and shorter recovery time[150]. There are few studies comparing surgical bypass (gastrojejunostomy) to endoscopic stent placement in patients with malignant gastric outlet obstruction[151]. The decision on proceeding with one option vs. the other should be made in light of the patient's preference, performance status, disease stage, overall health condition and expected life expectancy. Overall, if the life expectancy is short (say 2-3 mo), an endoscopic stent is favored due to prompt relief of symptoms and short duration of recovery. If the expected life expectancy is longer, then surgical bypass is a more reasonable and durable approach due to better long-term results[151].

Cancer-related pain is a very common symptom in patients with advanced pancreatic cancer[152], resulting in decreased performance status and dismal quality of life. Opioid analgesics are most commonly used in managing severe pain associated with pancreatic cancer. Other adjunctive medications include gabapentin, pregabalin, nortriptyline, or duloxetine. In select situations, celiac plexus neurolysis (CPN) can be more effective for immediate and long-term pain relief[152,153]. CPN is preferred over radiation as the onset of action is quicker and long lasting[154,155]. In patients with pain associated with underlying depression and anxiety, antidepressant medications may be beneficial.

The risk of venous thromboembolism is 4-7 folds higher in pancreatic cancer as compared to other common adenocarcinomas. Patient education is key to recognize early signs of thromboembolism. Prophylaxis is recommended with low molecular weight heparin, low dose unfractionated heparin or fondaparinux in high-risk patients such as hospitalized patients with known pancreatic cancer. Lifelong treatment is generally required in patients who develop thromboembolism. Common recommended agents include low molecular weight heparin or a direct oral anticoagulant (*e.g.*, rivaroxaban, apixaban, edoxaban).

Poor appetite and weight loss in patients with advanced pancreatic cancer is common. Unfortunately, this correlates with disease activity and in many situations is a direct biological sequelae of tumor progression. Other factors such as severe depression and severe debilitating pain may contribute to these symptoms as well. Early referral to Nutritionist and/or dietician, dietary supplements and appetite stimulants (such as megestrol acetate) may help in these difficult situations. Patients who exhibit signs of pancreatic insufficiency (such as diarrhea and weight loss) may benefit from oral pancreatic enzyme replacement therapy.

CONCLUSION

Despite recent advances in the diagnosis and treatment of pancreatic cancer, the survival of pancreatic cancer has not significantly improved. This poor prognosis is mainly due to the aggressive tumor biology of pancreatic adenocarcinoma and its potential for micro metastasis at an early stage of the disease. Early diagnosis and curative resection, when possible, correlates with improved survival but surgery in

itself carries a definite morbidity and mortality, even in specialized centers. Neoadjuvant therapy (instead of surgery upfront) in these patients is being offered in some centers but whether this approach consistently translates to better survival is not known. On the other hand, controversies such as the need for routine pre-operative biliary drainage and histological diagnosis before surgery have been addressed by good quality studies, but the results have not translated into clinical practice universally. Nevertheless, there is an overall consensus that while we continue to find the best treatment options, patients with pancreatic cancer should be managed in light of published guidelines at high volume centers in a multi-disciplinary setting.

REFERENCES

1 **GBD 2017 Pancreatic Cancer Collaborators**. The global, regional, and national burden of pancreatic cancer and its attributable risk factors in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol* 2019; **4**: 934-947 [PMID: 31648972 DOI: 10.1016/S2468-1253(19)30347-4]

2 **Miller KD**, Fidler-Benaoudia M, Keegan TH, Hipp HS, Jemal A, Siegel RL. Cancer statistics for adolescents and young adults, 2020. *CA Cancer J Clin* 2020; **70**: 443-459 [PMID: 32940362 DOI: 10.3322/caac.21637]

3 **Zhang J**, Dhakal I, Ning B, Kesteloot H. Patterns and trends of pancreatic cancer mortality rates in Arkansas, 1969-2002: a comparison with the US population. *Eur J Cancer Prev* 2008; **17**: 18-27 [PMID: 18090906 DOI: 10.1097/CEJ.0b013e32809b4ccd]

4 **Yeo TP**, Hruban RH, Leach SD, Wilentz RE, Sohn TA, Kern SE, Iacobuzio-Donahue CA, Maitra A, Goggins M, Canto MI, Abrams RA, Laheru D, Jaffee EM, Hidalgo M, Yeo CJ. Pancreatic cancer. *Curr Probl Cancer* 2002; **26**: 176-275 [PMID: 12399802 DOI: 10.1067/mcn.2002.129579]

5 **Midha S**, Chawla S, Garg PK. Modifiable and non-modifiable risk factors for pancreatic cancer: A review. *Cancer Lett* 2016; **381**: 269-277 [PMID: 27461582 DOI: 10.1016/j.canlet.2016.07.022]

6 **Bosetti C**, Lucenteforte E, Silverman DT, Petersen G, Bracci PM, Ji BT, Negri E, Li D, Risch HA, Olson SH, Gallinger S, Miller AB, Bueno-de-Mesquita HB, Talamini R, Polesel J, Ghadirian P, Baghurst PA, Zatonski W, Fontham E, Bamlet WR, Holly EA, Bertuccio P, Gao YT, Hassan M, Yu H, Kurtz RC, Cotterchio M, Su J, Maisonneuve P, Duell EJ, Boffetta P, La Vecchia C. Cigarette smoking and pancreatic cancer: an analysis from the International Pancreatic Cancer Case-Control Consortium (Panc4). *Ann Oncol* 2012; **23**: 1880-1888 [PMID: 22104574 DOI: 10.1093/annonc/mdr541]

7 **Lynch SM**, Vrieling A, Lubin JH, Kraft P, Mendelsohn JB, Hartge P, Canzian F, Steplowski E, Arslan AA, Gross M, Helzlsouer K, Jacobs EJ, LaCroix A, Petersen G, Zheng W, Albanes D, Amundadottir L, Bingham SA, Boffetta P, Boutron-Ruault MC, Chanock SJ, Clipp S, Hoover RN, Jacobs K, Johnson KC, Kooperberg C, Luo J, Messina C, Palli D, Patel AV, Riboli E, Shu XO, Rodriguez Suarez L, Thomas G, Tjønneland A, Tobias GS, Tong E, Trichopoulos D, Virtamo J, Ye W, Yu K, Zeleniuch-Jacquette A, Bueno-de-Mesquita HB, Stolzenberg-Solomon RZ. Cigarette smoking and pancreatic cancer: a pooled analysis from the pancreatic cancer cohort consortium. *Am J Epidemiol* 2009; **170**: 403-413 [PMID: 19561064 DOI: 10.1093/aje/kwp134]

8 **Michaud DS**, Giovannucci E, Willett WC, Colditz GA, Stampfer MJ, Fuchs CS. Physical activity, obesity, height, and the risk of pancreatic cancer. *JAMA* 2001; **286**: 921-929 [PMID: 11509056 DOI: 10.1001/jama.286.8.921]

9 **Arem H**, Reedy J, Sampson J, Jiao L, Hollenbeck AR, Risch H, Mayne ST, Stolzenberg-Solomon RZ. The Healthy Eating Index 2005 and risk for pancreatic cancer in the NIH-AARP study. *J Natl Cancer Inst* 2013; **105**: 1298-1305 [PMID: 23949329 DOI: 10.1093/jnci/djt185]

10 **Benzel J**, Fendrich V. Familial Pancreatic Cancer. *Oncol Res Treat* 2018; **41**: 611-618 [PMID: 30269130 DOI: 10.1159/000493473]

11 **Olson SH**, Kurtz RC. Epidemiology of pancreatic cancer and the role of family history. *J Surg Oncol* 2013; **107**: 1-7 [PMID: 22589078 DOI: 10.1002/jso.23149]

12 **Pilarski R**. The Role of *BRCA* Testing in Hereditary Pancreatic and Prostate Cancer Families. *Am Soc Clin Oncol Educ Book* 2019; **39**: 79-86 [PMID: 31099688 DOI: 10.1200/EDBK_238977]

13 **Bang UC**, Benfield T, Hyldstrup L, Bendtsen F, Beck Jensen JE. Mortality, cancer, and comorbidities associated with chronic pancreatitis: a Danish nationwide matched-cohort study. *Gastroenterology* 2014; **146**: 989-994 [PMID: 24389306 DOI: 10.1053/j.gastro.2013.12.033]

14 **Duell EJ**, Lucenteforte E, Olson SH, Bracci PM, Li D, Risch HA, Silverman DT, Ji BT, Gallinger S, Holly EA, Fontham EH, Maisonneuve P, Bueno-de-Mesquita HB, Ghadirian P, Kurtz RC, Ludwig E, Yu H, Lowenfels AB, Seminara D, Petersen GM, La Vecchia C, Boffetta P. Pancreatitis and pancreatic cancer risk: a pooled analysis in the International Pancreatic Cancer Case-Control Consortium (PanC4). *Ann Oncol* 2012; **23**: 2964-2970 [PMID: 22767586 DOI: 10.1093/annonc/mds140]

15 **Pergolini I**, Sahora K, Ferrone CR, Morales-Oyarvide V, Wolpin BM, Mucci LA, Brugge WR, Mino-Kenudson M, Patino M, Sahani DV, Warshaw AL, Lillemoe KD, Fernández-Del Castillo C. Long-term Risk of Pancreatic Malignancy in Patients With Branch Duct Intraductal Papillary Mucinous Neoplasm in a Referral Center. *Gastroenterology* 2017; **153**: 1284-1294. e1 [PMID:

28739282 DOI: 10.1053/j.gastro.2017.07.019]
16 **Singh G**, Nassri A, Kim D, Zhu H, Ramzan Z. Lymphocyte-to-monocyte ratio can predict mortality in pancreatic adenocarcinoma. *World J Gastrointest Pharmacol Ther* 2017; **8**: 60-66 [PMID: 28217375 DOI: 10.4292/wjgpt.v8.i1.60]
17 **Chang DK**, Johns AL, Merrett ND, Gill AJ, Colvin EK, Scarlett CJ, Nguyen NQ, Leong RW, Cosman PH, Kelly MI, Sutherland RL, Henshall SM, Kench JG, Biankin AV. Margin clearance and outcome in resected pancreatic cancer. *J Clin Oncol* 2009; **27**: 2855-2862 [PMID: 19398572 DOI: 10.1200/JCO.2008.20.5104]
18 **Sohn TA**, Yeo CJ, Cameron JL, Koniaris L, Kaushal S, Abrams RA, Sauter PK, Coleman J, Hruban RH, Lillemoe KD. Resected adenocarcinoma of the pancreas-616 patients: results, outcomes, and prognostic indicators. *J Gastrointest Surg* 2000; **4**: 567-579 [PMID: 11307091 DOI: 10.1016/s1091-255x(00)80105-5]
19 **Kim D**, Zhu H, Nassri A, Mokdad A, Kukreja S, Polanco P, Huerta S, Ramzan Z. Survival analysis of veteran patients with pancreatic cancer. *J Dig Dis* 2016; **17**: 399-407 [PMID: 27235863 DOI: 10.1111/1751-2980.12361]
20 **Conroy T**, Bachet JB, Ayav A, Huguet F, Lambert A, Caramella C, Maréchal R, Van Laethem JL, Ducreux M. Current standards and new innovative approaches for treatment of pancreatic cancer. *Eur J Cancer* 2016; **57**: 10-22 [PMID: 26851397 DOI: 10.1016/j.ejca.2015.12.026]
21 **Porta M**, Fabregat X, Malats N, Guarner L, Carrato A, de Miguel A, Ruiz L, Jariod M, Costafreda S, Coll S, Alguacil J, Corominas JM, Solà R, Salas A, Real FX. Exocrine pancreatic cancer: symptoms at presentation and their relation to tumour site and stage. *Clin Transl Oncol* 2005; **7**: 189-197 [PMID: 15960930 DOI: 10.1007/BF02712816]
22 **Goodman M**, Willmann JK, Jeffrey RB. Incidentally discovered solid pancreatic masses: imaging and clinical observations. *Abdom Imaging* 2012; **37**: 91-97 [PMID: 21394600 DOI: 10.1007/s00261-011-9720-2]
23 **DiMagno EP**, Malagelada JR, Taylor WF, Go VL. A prospective comparison of current diagnostic tests for pancreatic cancer. *N Engl J Med* 1977; **297**: 737-742 [PMID: 895803 DOI: 10.1056/NEJM197710062971401]
24 **Valls C**, Andía E, Sanchez A, Fabregat J, Pozuelo O, Quintero JC, Serrano T, Garcia-Borobia F, Jorba R. Dual-phase helical CT of pancreatic adenocarcinoma: assessment of resectability before surgery. *AJR Am J Roentgenol* 2002; **178**: 821-826 [PMID: 11906855 DOI: 10.2214/ajr.178.4.1780821]
25 **Bronstein YL**, Loyer EM, Kaur H, Choi H, David C, DuBrow RA, Broemeling LD, Cleary KR, Charnsangavej C. Detection of small pancreatic tumors with multiphasic helical CT. *AJR Am J Roentgenol* 2004; **182**: 619-623 [PMID: 14975959 DOI: 10.2214/ajr.182.3.1820619]
26 **Karmazanovsky G**, Fedorov V, Kubyshkin V, Kotchatkov A. Pancreatic head cancer: accuracy of CT in determination of resectability. *Abdom Imaging* 2005; **30**: 488-500 [PMID: 15759205 DOI: 10.1007/s00261-004-0279-z]
27 **Yoon SH**, Lee JM, Cho JY, Lee KB, Kim JE, Moon SK, Kim SJ, Baek JH, Kim SH, Lee JY, Han JK, Choi BI. Small (≤ 20 mm) pancreatic adenocarcinomas: analysis of enhancement patterns and secondary signs with multiphasic multidetector CT. *Radiology* 2011; **259**: 442-452 [PMID: 21406627 DOI: 10.1148/radiol.11101133]
28 **Nino-Murcia M**, Jeffrey RB Jr, Beaulieu CF, Li KC, Rubin GD. Multidetector CT of the pancreas and bile duct system: value of curved planar reformations. *AJR Am J Roentgenol* 2001; **176**: 689-693 [PMID: 11222206 DOI: 10.2214/ajr.176.3.1760689]
29 **Ryan DP**, Hong TS, Bardeesy N. Pancreatic adenocarcinoma. *N Engl J Med* 2014; **371**: 1039-1049 [PMID: 25207767 DOI: 10.1056/NEJMra1404198]
30 **Al-Hawary MM**, Francis IR, Chari ST, Fishman EK, Hough DM, Lu DS, Macari M, Megibow AJ, Miller FH, Mortele KJ, Merchant NB, Minter RM, Tamm EP, Sahani DV, Simeone DM. Pancreatic ductal adenocarcinoma radiology reporting template: consensus statement of the society of abdominal radiology and the american pancreatic association. *Gastroenterology* 2014; **146**: 291-304.e1 [PMID: 24355035 DOI: 10.1053/j.gastro.2013.11.004]
31 **Al-Hawary MM**, Francis IR, Chari ST, Fishman EK, Hough DM, Lu DS, Macari M, Megibow AJ, Miller FH, Mortele KJ, Merchant NB, Minter RM, Tamm EP, Sahani DV, Simeone DM. Pancreatic ductal adenocarcinoma radiology reporting template: consensus statement of the Society of Abdominal Radiology and the American Pancreatic Association. *Radiology* 2014; **270**: 248-260 [PMID: 24354378 DOI: 10.1148/radiol.13131184]
32 **Adamek HE**, Albert J, Breer H, Weitz M, Schilling D, Riemann JF. Pancreatic cancer detection with magnetic resonance cholangiopancreatography and endoscopic retrograde cholangiopancreatography: a prospective controlled study. *Lancet* 2000; **356**: 190-193 [PMID: 10963196 DOI: 10.1016/S0140-6736(00)02479-X]
33 **Lopez Hänninen E**, Amthauer H, Hosten N, Ricke J, Böhmig M, Langrehr J, Hintze R, Neuhaus P, Wiedenmann B, Rosewicz S, Felix R. Prospective evaluation of pancreatic tumors: accuracy of MR imaging with MR cholangiopancreatography and MR angiography. *Radiology* 2002; **224**: 34-41 [PMID: 12091659 DOI: 10.1148/radiol.2241010798]
34 **Megibow AJ**, Zhou XH, Rotterdam H, Francis IR, Zerhouni EA, Balfe DM, Weinreb JC, Aisen A, Kuhlman J, Heiken JP. Pancreatic adenocarcinoma: CT *vs* MR imaging in the evaluation of resectability--report of the Radiology Diagnostic Oncology Group. *Radiology* 1995; **195**: 327-332 [PMID: 7724748 DOI: 10.1148/radiology.195.2.7724748]

35 **Irie H**, Honda H, Kaneko K, Kuroiwa T, Yoshimitsu K, Masuda K. Comparison of helical CT and MR imaging in detecting and staging small pancreatic adenocarcinoma. *Abdom Imaging* 1997; **22**: 429-433 [PMID: 9157866 DOI: 10.1007/s002619900226]

36 **Sheridan MB**, Ward J, Guthrie JA, Spencer JA, Craven CM, Wilson D, Guillou PJ, Robinson PJ. Dynamic contrast-enhanced MR imaging and dual-phase helical CT in the preoperative assessment of suspected pancreatic cancer: a comparative study with receiver operating characteristic analysis. *AJR Am J Roentgenol* 1999; **173**: 583-590 [PMID: 10470884 DOI: 10.2214/ajr.173.3.10470884]

37 **Holzapfel K**, Reiser-Erkan C, Fingerle AA, Erkan M, Eiber MJ, Rummeny EJ, Friess H, Kleeff J, Gaa J. Comparison of diffusion-weighted MR imaging and multidetector-row CT in the detection of liver metastases in patients operated for pancreatic cancer. *Abdom Imaging* 2011; **36**: 179-184 [PMID: 20563868 DOI: 10.1007/s00261-010-9633-5]

38 **Motosugi U**, Ichikawa T, Morisaka H, Sou H, Muhi A, Kimura K, Sano K, Araki T. Detection of pancreatic carcinoma and liver metastases with gadoxetic acid-enhanced MR imaging: comparison with contrast-enhanced multi-detector row CT. *Radiology* 2011; **260**: 446-453 [PMID: 21693662 DOI: 10.1148/radiol.11103548]

39 **Trede M**, Rumstadt B, Wendl K, Gaa J, Tesdal K, Lehmann KJ, Meier-Willersen HJ, Pescatore P, Schmoll J. Ultrafast magnetic resonance imaging improves the staging of pancreatic tumors. *Ann Surg* 1997; **226**: 393-405; discussion 405 [PMID: 9351708 DOI: 10.1097/00000658-199710000-00001]

40 **Balci NC**, Semelka RC. Radiologic diagnosis and staging of pancreatic ductal adenocarcinoma. *Eur J Radiol* 2001; **38**: 105-112 [PMID: 11335092 DOI: 10.1016/s0720-048x(01)00295-9]

41 **Ballehaninna UK**, Chamberlain RS. The clinical utility of serum CA 19-9 in the diagnosis, prognosis and management of pancreatic adenocarcinoma: An evidence based appraisal. *J Gastrointest Oncol* 2012; **3**: 105-119 [PMID: 22811878 DOI: 10.3978/j.issn.2078-6891.2011.021]

42 **Steinberg W**. The clinical utility of the CA 19-9 tumor-associated antigen. *Am J Gastroenterol* 1990; **85**: 350-355 [PMID: 2183589]

43 **Tempero MA**, Uchida E, Takasaki H, Burnett DA, Steplewski Z, Pour PM. Relationship of carbohydrate antigen 19-9 and Lewis antigens in pancreatic cancer. *Cancer Res* 1987; **47**: 5501-5503 [PMID: 3308077]

44 **Lamerz R**. Role of tumour markers, cytogenetics. *Ann Oncol* 1999; **10** Suppl 4: 145-149 [PMID: 10436809]

45 **Molina V**, Visa L, Conill C, Navarro S, Escudero JM, Auge JM, Filella X, Lopez-Boado MA, Ferrer J, Fernandez-Cruz L, Molina R. CA 19-9 in pancreatic cancer: retrospective evaluation of patients with suspicion of pancreatic cancer. *Tumour Biol* 2012; **33**: 799-807 [PMID: 22203495 DOI: 10.1007/s13277-011-0297-8]

46 **DiMagno EP**, Reber HA, Tempero MA. AGA technical review on the epidemiology, diagnosis, and treatment of pancreatic ductal adenocarcinoma. American Gastroenterological Association. *Gastroenterology* 1999; **117**: 1464-1484 [PMID: 10579989 DOI: 10.1016/s0016-5085(99)70298-2]

47 **Kim JE**, Lee KT, Lee JK, Paik SW, Rhee JC, Choi KW. Clinical usefulness of carbohydrate antigen 19-9 as a screening test for pancreatic cancer in an asymptomatic population. *J Gastroenterol Hepatol* 2004; **19**: 182-186 [PMID: 14731128 DOI: 10.1111/j.1440-1746.2004.03219.x]

48 **Maithel SK**, Maloney S, Winston C, Gönen M, D'Angelica MI, Dematteo RP, Jarnagin WR, Brennan MF, Allen PJ. Preoperative CA 19-9 and the yield of staging laparoscopy in patients with radiographically resectable pancreatic adenocarcinoma. *Ann Surg Oncol* 2008; **15**: 3512-3520 [PMID: 18781364 DOI: 10.1245/s10434-008-0134-5]

49 **Maisey NR**, Norman AR, Hill A, Massey A, Oates J, Cunningham D. CA19-9 as a prognostic factor in inoperable pancreatic cancer: the implication for clinical trials. *Br J Cancer* 2005; **93**: 740-743 [PMID: 16175188 DOI: 10.1038/sj.bjc.6602760]

50 **Kondo N**, Murakami Y, Uemura K, Hayashidani Y, Sudo T, Hashimoto Y, Nakashima A, Sakabe R, Shigemoto N, Kato Y, Ohge H, Sueda T. Prognostic impact of perioperative serum CA 19-9 Levels in patients with resectable pancreatic cancer. *Ann Surg Oncol* 2010; **17**: 2321-2329 [PMID: 20336387 DOI: 10.1245/s10434-010-1033-0]

51 **Humphris JL**, Chang DK, Johns AL, Scarlett CJ, Pajic M, Jones MD, Colvin EK, Nagrial A, Chin VT, Chantrill LA, Samra JS, Gill AJ, Kench JG, Merrett ND, Das A, Musgrove EA, Sutherland RL, Biankin AV; NSW Pancreatic Cancer Network. The prognostic and predictive value of serum CA19.9 in pancreatic cancer. *Ann Oncol* 2012; **23**: 1713-1722 [PMID: 22241899 DOI: 10.1093/annonc/mdr561]

52 **Koom WS**, Seong J, Kim YB, Pyun HO, Song SY. CA 19-9 as a predictor for response and survival in advanced pancreatic cancer patients treated with chemoradiotherapy. *Int J Radiat Oncol Biol Phys* 2009; **73**: 1148-1154 [PMID: 18760544 DOI: 10.1016/j.ijrobp.2008.06.1483]

53 **Abdel-Misih SR**, Hatzaras I, Schmidt C, Saab TB, Klemanski D, Muscarella P, Melvin WS, Ellison EC, Bloomston M. Failure of normalization of CA19-9 following resection for pancreatic cancer is tantamount to metastatic disease. *Ann Surg Oncol* 2011; **18**: 1116-1121 [PMID: 21042945 DOI: 10.1245/s10434-010-1397-1]

54 **Berger AC**, Garcia M Jr, Hoffman JP, Regine WF, Abrams RA, Safran H, Konski A, Benson AB 3rd, MacDonald J, Willett CG. Postresection CA 19-9 predicts overall survival in patients with pancreatic cancer treated with adjuvant chemoradiation: a prospective validation by RTOG 9704. *J Clin Oncol* 2008; **26**: 5918-5922 [PMID: 19029412 DOI: 10.1200/JCO.2008.18.6288]

55 **Chen J**, Yang R, Lu Y, Xia Y, Zhou H. Diagnostic accuracy of endoscopic ultrasound-guided fine-

needle aspiration for solid pancreatic lesion: a systematic review. *J Cancer Res Clin Oncol* 2012; **138**: 1433-1441 [PMID: 22752601 DOI: 10.1007/s00432-012-1268-1]

56 **Puli SR**, Bechtold ML, Buxbaum JL, Eloubeidi MA. How good is endoscopic ultrasound-guided fine-needle aspiration in diagnosing the correct etiology for a solid pancreatic mass? *Pancreas* 2013; **42**: 20-26 [PMID: 23254913 DOI: 10.1097/MPA.0b013e3182546e79]

57 **Fornari F**, Civardi G, Cavanna L, Di Stasi M, Rossi S, Sbolli G, Buscarini L. Complications of ultrasonically guided fine-needle abdominal biopsy. Results of a multicenter Italian study and review of the literature. The Cooperative Italian Study Group. *Scand J Gastroenterol* 1989; **24**: 949-955 [PMID: 2688068 DOI: 10.3109/00365528909089239]

58 **Micames C**, Jowell PS, White R, Paulson E, Nelson R, Morse M, Hurwitz H, Pappas T, Tyler D, McGrath K. Lower frequency of peritoneal carcinomatosis in patients with pancreatic cancer diagnosed by EUS-guided FNA vs. percutaneous FNA. *Gastrointest Endosc* 2003; **58**: 690-695 [PMID: 14595302 DOI: 10.1016/s0016-5107(03)02009-1]

59 **Chen VK**, Arguedas MR, Kilgore ML, Eloubeidi MA. A cost-minimization analysis of alternative strategies in diagnosing pancreatic cancer. *Am J Gastroenterol* 2004; **99**: 2223-2234 [PMID: 15555006 DOI: 10.1111/j.1572-0241.2004.40042.x]

60 **Canto MI**, Hruban RH, Fishman EK, Kamel IR, Schulick R, Zhang Z, Topazian M, Takahashi N, Fletcher J, Petersen G, Klein AP, Axilbund J, Griffin C, Syngal S, Saltzman JR, Mortele KJ, Lee J, Tamm E, Vikram R, Bhosale P, Margolis D, Farrell J, Goggins M; American Cancer of the Pancreas Screening (CAPS) Consortium. Frequent detection of pancreatic lesions in asymptomatic high-risk individuals. *Gastroenterology* 2012; **142**: 796-804; quiz e14 [PMID: 22245846 DOI: 10.1053/j.gastro.2012.01.005]

61 **Farma JM**, Santillan AA, Melis M, Walters J, Belinc D, Chen DT, Eikman EA, Malafa M. PET/CT fusion scan enhances CT staging in patients with pancreatic neoplasms. *Ann Surg Oncol* 2008; **15**: 2465-2471 [PMID: 18551347 DOI: 10.1245/s10434-008-9992-0]

62 **Kauhanen SP**, Komar G, Seppänen MP, Dean KI, Minn HR, Kajander SA, Rinta-Kiikka I, Alanen K, Borra RJ, Puolakkainen PA, Nuutila P, Ovaska JT. A prospective diagnostic accuracy study of 18F-fluorodeoxyglucose positron emission tomography/computed tomography, multidetector row computed tomography, and magnetic resonance imaging in primary diagnosis and staging of pancreatic cancer. *Ann Surg* 2009; **250**: 957-963 [PMID: 19687736 DOI: 10.1097/SLA.0b013e3181b2fafa]

63 **Nishiyama Y**, Yamamoto Y, Yokoe K, Monden T, Sasakawa Y, Tsutsui K, Satoh K, Ohkawa M. Contribution of whole body FDG-PET to the detection of distant metastasis in pancreatic cancer. *Ann Nucl Med* 2005; **19**: 491-497 [PMID: 16248386 DOI: 10.1007/BF02985577]

64 **Izuishi K**, Yamamoto Y, Sano T, Takebayashi R, Masaki T, Suzuki Y. Impact of 18-fluorodeoxyglucose positron emission tomography on the management of pancreatic cancer. *J Gastrointest Surg* 2010; **14**: 1151-1158 [PMID: 20443074 DOI: 10.1007/s11605-010-1207-x]

65 **Diederichs CG**, Staib L, Vogel J, Glasbrenner B, Glatting G, Brambs HJ, Beger HG, Reske SN. Values and limitations of 18F-fluorodeoxyglucose-positron-emission tomography with preoperative evaluation of patients with pancreatic masses. *Pancreas* 2000; **20**: 109-116 [PMID: 10707924 DOI: 10.1097/00006676-200003000-00001]

66 **Wartski M**, Sauvanet A. 18F-FDG PET/CT in pancreatic adenocarcinoma: A role at initial imaging staging? *Diagn Interv Imaging* 2019; **100**: 735-741 [PMID: 31402332 DOI: 10.1016/j.diii.2019.07.006]

67 **Xing H**, Hao Z, Zhu W, Sun D, Ding J, Zhang H, Liu Y, Huo L. Preoperative prediction of pathological grade in pancreatic ductal adenocarcinoma based on ^{18}F-FDG PET/CT radiomics. *EJNMMI Res* 2021; **11**: 19 [PMID: 33630176 DOI: 10.1186/s13550-021-00760-3]

68 **Yeh R**, Dercle L, Garg I, Wang ZJ, Hough DM, Goenka AH. The Role of 18F-FDG PET/CT and PET/MRI in Pancreatic Ductal Adenocarcinoma. *Abdom Radiol (NY)* 2018; **43**: 415-434 [PMID: 29143875 DOI: 10.1007/s00261-017-1374-2]

69 **Arnone A**, Laudicella R, Caobelli F, Guglielmo P, Spallino M, Abenavoli E, Martini AL, Filice R, Comis AD, Cuzzocrea M, Linguanti F, Evangelista L, Alongi P. Clinical Impact of ^{18}F-FDG PET/CT in the Diagnostic Workup of Pancreatic Ductal Adenocarcinoma: A Systematic Review. *Diagnostics (Basel)* 2020; **10** [PMID: 33287195 DOI: 10.3390/diagnostics10121042]

70 **Lee JW**, O JH, Choi M, Choi JY. Impact of F-18 Fluorodeoxyglucose PET/CT and PET/MRI on Initial Staging and Changes in Management of Pancreatic Ductal Adenocarcinoma: A Systemic Review and Meta-Analysis. *Diagnostics (Basel)* 2020; **10** [PMID: 33202682 DOI: 10.3390/diagnostics10110952]

71 **Seufferlein T**, Bachet JB, Van Cutsem E, Rougier P; ESMO Guidelines Working Group. Pancreatic adenocarcinoma: ESMO-ESDO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2012; **23** Suppl 7: vii33-vii40 [PMID: 22997452 DOI: 10.1093/annonc/mds224]

72 **Liu RC**, Traverso LW. Diagnostic laparoscopy improves staging of pancreatic cancer deemed locally unresectable by computed tomography. *Surg Endosc* 2005; **19**: 638-642 [PMID: 15776215 DOI: 10.1007/s00464-004-8165-x]

73 **Mayo SC**, Austin DF, Sheppard BC, Mori M, Shipley DK, Billingsley KG. Evolving preoperative evaluation of patients with pancreatic cancer: does laparoscopy have a role in the current era? *J Am Coll Surg* 2009; **208**: 87-95 [PMID: 19228509 DOI: 10.1016/j.jamcollsurg.2008.10.014]

74 **Allen VB**, Gurusamy KS, Takwoingi Y, Kalia A, Davidson BR. Diagnostic accuracy of laparoscopy following computed tomography (CT) scanning for assessing the resectability with curative intent in

pancreatic and periampullary cancer. *Cochrane Database Syst Rev* 2016; **7**: CD009323 [PMID: 27383694 DOI: 10.1002/14651858.CD009323.pub3]

75 **Piccolboni P**, Settembre A, Angelini P, Esposito F, Palladino S, Corcione F. Laparoscopic ultrasound: a surgical "must" for second line intra-operative evaluation of pancreatic cancer resectability. *G Chir* 2015; **36**: 5-8 [PMID: 25827662]

76 **de Werra C**, Quarto G, Aloia S, Perrotta S, Del Giudice R, Di Filippo G, Furino E, Amato B, Benassai G. The use of intraoperative ultrasound for diagnosis and stadiation in pancreatic head neoformations. *Int J Surg* 2015; **21** Suppl 1: S55-S58 [PMID: 26118609 DOI: 10.1016/j.ijsu.2015.04.091]

77 **Cirimbei S**, Puşcu C, Lucenco L, Brătucu E. The role of intraoperative ultrasound in establishing the surgical strategy regarding hepato-bilio-pancreatic pathology. *Chirurgia (Bucur)* 2013; **108**: 643-651 [PMID: 24157106]

78 **Doran HE**, Bosonnet L, Connor S, Jones L, Garvey C, Hughes M, Campbell F, Hartley M, Ghaneh P, Neoptolemos JP, Sutton R. Laparoscopy and laparoscopic ultrasound in the evaluation of pancreatic and periampullary tumours. *Dig Surg* 2004; **21**: 305-313 [PMID: 15365229 DOI: 10.1159/000080885]

79 **Minnard EA**, Conlon KC, Hoos A, Dougherty EC, Hann LE, Brennan MF. Laparoscopic ultrasound enhances standard laparoscopy in the staging of pancreatic cancer. *Ann Surg* 1998; **228**: 182-187 [PMID: 9712562 DOI: 10.1097/00000658-199808000-00006]

80 **Yelamali A**, Mansard MJ, Dama R, Rebela P, Rao GV, Reddy DN. Intraoperative pancreatoscopy with narrow band imaging: a novel method for assessment of resection margins in case of intraductal papillary mucinous neoplasm. *Surg Endosc* 2012; **26**: 3682-3685 [PMID: 22678173 DOI: 10.1007/s00464-012-2365-6]

81 **Giovannini M**, Bories E, Monges G, Pesenti C, Caillol F, Delpero JR. Results of a phase I-II study on intraductal confocal microscopy (IDCM) in patients with common bile duct (CBD) stenosis. *Surg Endosc* 2011; **25**: 2247-2253 [PMID: 21424206 DOI: 10.1007/s00464-010-1542-8]

82 **Konda VJ**, Aslanian HR, Wallace MB, Siddiqui UD, Hart J, Waxman I. First assessment of needle-based confocal laser endomicroscopy during EUS-FNA procedures of the pancreas (with videos). *Gastrointest Endosc* 2011; **74**: 1049-1060 [PMID: 21924718 DOI: 10.1016/j.gie.2011.07.018]

83 **Regunathan R**, Woo J, Pierce MC, Polydorides AD, Raoufi M, Roayaie S, Schwartz M, Labow D, Shin D, Suzuki R, Bhutani MS, Coghlan LG, Richards-Kortum R, Anandasabapathy S, Kim MK. Feasibility and preliminary accuracy of high-resolution imaging of the liver and pancreas using FNA compatible microendoscopy (with video). *Gastrointest Endosc* 2012; **76**: 293-300 [PMID: 22817784 DOI: 10.1016/j.gie.2012.04.445]

84 **Testoni PA**, Mariani A, Mangiavillano B, Arcidiacono PG, Di Pietro S, Masci E. Intraductal optical coherence tomography for investigating main pancreatic duct strictures. *Am J Gastroenterol* 2007; **102**: 269-274 [PMID: 17100970 DOI: 10.1111/j.1572-0241.2006.00940.x]

85 **Hwang JH**, Cobb MJ, Kimmey MB, Li X. Optical coherence tomography imaging of the pancreas: a needle-based approach. *Clin Gastroenterol Hepatol* 2005; **3**: S49-S52 [PMID: 16012997 DOI: 10.1016/s1542-3565(05)00259-4]

86 **Testoni PA**, Mangiavillano B. Optical coherence tomography in detection of dysplasia and cancer of the gastrointestinal tract and bilio-pancreatic ductal system. *World J Gastroenterol* 2008; **14**: 6444-6452 [PMID: 19030194 DOI: 10.3748/wjg.14.6444]

87 **Cheon YK**, Cho YD, Jeon SR, Moon JH, Jeong SW, Hur KY, Jin SY, Lee JS. Pancreatic resection guided by preoperative intraductal ultrasonography for intraductal papillary mucinous neoplasm. *Am J Gastroenterol* 2010; **105**: 1963-1969 [PMID: 20407429 DOI: 10.1038/ajg.2010.169]

88 **Varadarajulu S**, Eloubeidi MA, Wilcox CM. Prospective evaluation of indeterminate ERCP findings by intraductal ultrasound. *J Gastroenterol Hepatol* 2007; **22**: 2086-2092 [PMID: 18031365 DOI: 10.1111/j.1440-1746.2006.04823.x]

89 **Kitano M**, Kudo M, Yamao K, Takagi T, Sakamoto H, Komaki T, Kamata K, Imai H, Chiba Y, Okada M, Murakami T, Takeyama Y. Characterization of small solid tumors in the pancreas: the value of contrast-enhanced harmonic endoscopic ultrasonography. *Am J Gastroenterol* 2012; **107**: 303-310 [PMID: 22008892 DOI: 10.1038/ajg.2011.354]

90 **Gong TT**, Hu DM, Zhu Q. Contrast-enhanced EUS for differential diagnosis of pancreatic mass lesions: a meta-analysis. *Gastrointest Endosc* 2012; **76**: 301-309 [PMID: 22703697 DOI: 10.1016/j.gie.2012.02.051]

91 **Iglesias-Garcia J**, Larino-Noia J, Abdulkader I, Forteza J, Dominguez-Munoz JE. Quantitative endoscopic ultrasound elastography: an accurate method for the differentiation of solid pancreatic masses. *Gastroenterology* 2010; **139**: 1172-1180 [PMID: 20600020 DOI: 10.1053/j.gastro.2010.06.059]

92 **Kumon RE**, Repaka A, Atkinson M, Faulx AL, Wong RC, Isenberg GA, Hsiao YS, Gudur MS, Deng CX, Chak A. Characterization of the pancreas *in vivo* using EUS spectrum analysis with electronic array echoendoscopes. *Gastrointest Endosc* 2012; **75**: 1175-1183 [PMID: 22498178 DOI: 10.1016/j.gie.2012.01.039]

93 **Cruz-Monserrate Z**, Abd-Elgaliel WR, Grote T, Deng D, Ji B, Arumugam T, Wang H, Tung CH, Logsdon CD. Detection of pancreatic cancer tumours and precursor lesions by cathepsin E activity in mouse models. *Gut* 2012; **61**: 1315-1322 [PMID: 22068166 DOI: 10.1136/gutjnl-2011-300544]

94 **Hausner SH**, Abbey CK, Bold RJ, Gagnon MK, Marik J, Marshall JF, Stanecki CE, Sutcliffe JL. Targeted *in vivo* imaging of integrin alphavbeta6 with an improved radiotracer and its relevance in a

pancreatic tumor model. *Cancer Res* 2009; **69**: 5843-5850 [PMID: 19549907 DOI: 10.1158/0008-5472.CAN-08-4410]

95 **Neesse A**, Hahnenkamp A, Griesmann H, Buchholz M, Hahn SA, Maghnouj A, Fendrich V, Ring J, Sipos B, Tuveson DA, Bremer C, Gress TM, Michl P. Claudin-4-targeted optical imaging detects pancreatic cancer and its precursor lesions. *Gut* 2013; **62**: 1034-1043 [PMID: 22677720 DOI: 10.1136/gutjnl-2012-302577]

96 **Kojima T**, Kyuno D, Sawada N. Targeting claudin-4 in human pancreatic cancer. *Expert Opin Ther Targets* 2012; **16**: 881-887 [PMID: 22800288 DOI: 10.1517/14728222.2012.708340]

97 **Stella Man YK**, Foster J, Carapuça E, Davies JA, Parker AL, Sosabowski J, Halldén G. Systemic delivery and SPECT/CT *in vivo* imaging of ^{125}I-labelled oncolytic adenoviral mutants in models of pancreatic cancer. *Sci Rep* 2019; **9**: 12840 [PMID: 31492884 DOI: 10.1038/s41598-019-49150-9]

98 **Nattress CB**, Halldén G. Advances in oncolytic adenovirus therapy for pancreatic cancer. *Cancer Lett* 2018; **434**: 56-69 [PMID: 29981812 DOI: 10.1016/j.canlet.2018.07.006]

99 **Man YKS**, Davies JA, Coughlan L, Pantelidou C, Blázquez-Moreno A, Marshall JF, Parker AL, Halldén G. The Novel Oncolytic Adenoviral Mutant Ad5-3Δ-A20T Retargeted to αvβ6 Integrins Efficiently Eliminates Pancreatic Cancer Cells. *Mol Cancer Ther* 2018; **17**: 575-587 [PMID: 29367266 DOI: 10.1158/1535-7163.MCT-17-0671]

100 **Montet X**, Weissleder R, Josephson L. Imaging pancreatic cancer with a peptide-nanoparticle conjugate targeted to normal pancreas. *Bioconjug Chem* 2006; **17**: 905-911 [PMID: 16848396 DOI: 10.1021/bc060035+]

101 **Korpanty G**, Carbon JG, Grayburn PA, Fleming JB, Brekken RA. Monitoring response to anticancer therapy by targeting microbubbles to tumor vasculature. *Clin Cancer Res* 2007; **13**: 323-330 [PMID: 17200371 DOI: 10.1158/1078-0432.CCR-06-1313]

102 **Hartwig W**, Schneider L, Diener MK, Bergmann F, Büchler MW, Werner J. Preoperative tissue diagnosis for tumours of the pancreas. *Br J Surg* 2009; **96**: 5-20 [PMID: 19016272 DOI: 10.1002/bjs.6407]

103 **van Heerde MJ**, Biermann K, Zondervan PE, Kazemier G, van Eijck CH, Pek C, Kuipers EJ, van Buuren HR. Prevalence of autoimmune pancreatitis and other benign disorders in pancreatoduodenectomy for presumed malignancy of the pancreatic head. *Dig Dis Sci* 2012; **57**: 2458-2465 [PMID: 22588243 DOI: 10.1007/s10620-012-2191-7]

104 **de la Fuente SG**, Ceppa EP, Reddy SK, Clary BM, Tyler DS, Pappas TN. Incidence of benign disease in patients that underwent resection for presumed pancreatic cancer diagnosed by endoscopic ultrasonography (EUS) and fine-needle aspiration (FNA). *J Gastrointest Surg* 2010; **14**: 1139-1142 [PMID: 20424928 DOI: 10.1007/s11605-010-1196-9]

105 **Gerritsen A**, Molenaar IQ, Bollen TL, Nio CY, Dijkgraaf MG, van Santvoort HC, Offerhaus GJ, Brosens LA, Biermann K, Sieders E, de Jong KP, van Dam RM, van der Harst E, van Goor H, van Ramshorst B, Bonsing BA, de Hingh IH, Gerhards MF, van Eijck CH, Gouma DJ, Borel Rinkes IH, Busch OR, Besselink MG; Dutch Pancreatic Cancer Group. Preoperative characteristics of patients with presumed pancreatic cancer but ultimately benign disease: a multicenter series of 344 pancreatoduodenectomies. *Ann Surg Oncol* 2014; **21**: 3999-4006 [PMID: 24871781 DOI: 10.1245/s10434-014-3810-7]

106 **Yeo CJ**, Cameron JL, Sohn TA, Lillemoe KD, Pitt HA, Talamini MA, Hruban RH, Ord SE, Sauter PK, Coleman J, Zahurak ML, Grochow LB, Abrams RA. Six hundred fifty consecutive pancreaticoduodenectomies in the 1990s: pathology, complications, and outcomes. *Ann Surg* 1997; **226**: 248-57; discussion 257 [PMID: 9339931 DOI: 10.1097/00000658-199709000-00004]

107 **Cameron JL**, Riall TS, Coleman J, Belcher KA. One thousand consecutive pancreaticoduodenectomies. *Ann Surg* 2006; **244**: 10-15 [PMID: 16794383 DOI: 10.1097/01.sla.0000217673.04165.ea]

108 **Mayo SC**, Nathan H, Cameron JL, Olino K, Edil BH, Herman JM, Hirose K, Schulick RD, Choti MA, Wolfgang CL, Pawlik TM. Conditional survival in patients with pancreatic ductal adenocarcinoma resected with curative intent. *Cancer* 2012; **118**: 2674-2681 [PMID: 21935914 DOI: 10.1002/cncr.26553]

109 **Kinsella TJ**, Seo Y, Willis J, Stellato TA, Siegel CT, Harpp D, Willson JK, Gibbons J, Sanabria JR, Hardacre JM, Schulak JP. The impact of resection margin status and postoperative CA19-9 Levels on survival and patterns of recurrence after postoperative high-dose radiotherapy with 5-FU-based concurrent chemotherapy for resectable pancreatic cancer. *Am J Clin Oncol* 2008; **31**: 446-453 [PMID: 18838880 DOI: 10.1097/COC.0b013e318168f6c4]

110 **Helm J**, Centeno BA, Coppola D, Melis M, Lloyd M, Park JY, Chen DT, Malafa MP. Histologic characteristics enhance predictive value of American Joint Committee on Cancer staging in resectable pancreas cancer. *Cancer* 2009; **115**: 4080-4089 [PMID: 19626671 DOI: 10.1002/cncr.24503]

111 **Meyer W**, Jurowich C, Reichel M, Steinhäuser B, Wünsch PH, Gebhardt C. Pathomorphological and histological prognostic factors in curatively resected ductal adenocarcinoma of the pancreas. *Surg Today* 2000; **30**: 582-587 [PMID: 10930222 DOI: 10.1007/s005950070096]

112 **Yuan C**, Morales-Oyarvide V, Babic A, Clish CB, Kraft P, Bao Y, Qian ZR, Rubinson DA, Ng K, Giovannucci EL, Ogino S, Stampfer MJ, Gaziano JM, Sesso HD, Cochrane BB, Manson JE, Fuchs CS, Wolpin BM. Cigarette Smoking and Pancreatic Cancer Survival. *J Clin Oncol* 2017; **35**: 1822-1828 [PMID: 28358654 DOI: 10.1200/JCO.2016.71.2026]

113 **Pelucchi C**, Galeone C, Polesel J, Manzari M, Zucchetto A, Talamini R, Franceschi S, Negri E, La

Vecchia C. Smoking and body mass index and survival in pancreatic cancer patients. *Pancreas* 2014; **43**: 47-52 [PMID: 24177141 DOI: 10.1097/MPA.0b013e3182a7c74b]

114 **Kang MJ**, Jang JY, Chang YR, Kwon W, Jung W, Kim SW. Revisiting the concept of lymph node metastases of pancreatic head cancer: number of metastatic lymph nodes and lymph node ratio according to N stage. *Ann Surg Oncol* 2014; **21**: 1545-1551 [PMID: 24419758 DOI: 10.1245/s10434-013-3473-9]

115 **Allen PJ**, Kuk D, Castillo CF, Basturk O, Wolfgang CL, Cameron JL, Lillemoe KD, Ferrone CR, Morales-Oyarvide V, He J, Weiss MJ, Hruban RH, Gönen M, Klimstra DS, Mino-Kenudson M. Multi-institutional Validation Study of the American Joint Commission on Cancer (8th Edition) Changes for T and N Staging in Patients With Pancreatic Adenocarcinoma. *Ann Surg* 2017; **265**: 185-191 [PMID: 27163957 DOI: 10.1097/SLA.0000000000001763]

116 **Ellis RJ**, Ho JW, Schlick CJR, Merkow RP, Bentrem DJ, Bilimoria KY, Yang AD. National Use of Chemotherapy in Initial Management of Stage I Pancreatic Cancer and Failure to Perform Subsequent Resection. *Ann Surg Oncol* 2020; **27**: 909-918 [PMID: 31691112 DOI: 10.1245/s10434-019-08023-1]

117 **Hu Q**, Wang D, Chen Y, Li X, Cao P, Cao D. Network meta-analysis comparing neoadjuvant chemoradiation, neoadjuvant chemotherapy and upfront surgery in patients with resectable, borderline resectable, and locally advanced pancreatic ductal adenocarcinoma. *Radiat Oncol* 2019; **14**: 120 [PMID: 31291998 DOI: 10.1186/s13014-019-1330-0]

118 **Mokdad AA**, Minter RM, Zhu H, Augustine MM, Porembka MR, Wang SC, Yopp AC, Mansour JC, Choti MA, Polanco PM. Neoadjuvant Therapy Followed by Resection Versus Upfront Resection for Resectable Pancreatic Cancer: A Propensity Score Matched Analysis. *J Clin Oncol* 2017; **35**: 515-522 [PMID: 27621388 DOI: 10.1200/JCO.2016.68.5081]

119 **de Geus SW**, Eskander MF, Bliss LA, Kasumova GG, Ng SC, Callery MP, Tseng JF. Neoadjuvant therapy *vs* upfront surgery for resected pancreatic adenocarcinoma: A nationwide propensity score matched analysis. *Surgery* 2017; **161**: 592-601 [PMID: 28341441 DOI: 10.1016/j.surg.2016.08.040]

120 **Fathi A**, Christians KK, George B, Ritch PS, Erickson BA, Tolat P, Johnston FM, Evans DB, Tsai S. Neoadjuvant therapy for localized pancreatic cancer: guiding principles. *J Gastrointest Oncol* 2015; **6**: 418-429 [PMID: 26261728 DOI: 10.3978/j.issn.2078-6891.2015.053]

121 **Lee AJ**, Simoneau E, Chiang YJ, Lee JE, Kim MP, Aloia TA, Vauthey JN, Katz MH, Tzeng CD. Is early-stage pancreatic adenocarcinoma truly early: stage migration on final pathology with surgery-first *vs* neoadjuvant therapy sequencing. *HPB (Oxford)* 2019; **21**: 1203-1210 [PMID: 30799277 DOI: 10.1016/j.hpb.2019.01.011]

122 **Rangarajan K**, Pucher PH, Armstrong T, Bateman A, Hamady Z. Systemic neoadjuvant chemotherapy in modern pancreatic cancer treatment: a systematic review and meta-analysis. *Ann R Coll Surg Engl* 2019; **101**: 453-462 [PMID: 31304767 DOI: 10.1308/rcsann.2019.0060]

123 **Zhan HX**, Xu JW, Wu D, Wu ZY, Wang L, Hu SY, Zhang GY. Neoadjuvant therapy in pancreatic cancer: a systematic review and meta-analysis of prospective studies. *Cancer Med* 2017; **6**: 1201-1219 [PMID: 28544758 DOI: 10.1002/cam4.1071]

124 **Khorana AA**, Mangu PB, Berlin J, Engebretson A, Hong TS, Maitra A, Mohile SG, Mumber M, Schulick R, Shapiro M, Urba S, Zeh HJ, Katz MH. Potentially Curable Pancreatic Cancer: American Society of Clinical Oncology Clinical Practice Guideline. *J Clin Oncol* 2016; **34**: 2541-2556 [PMID: 27247221 DOI: 10.1200/JCO.2016.67.5553]

125 **Oba A**, Ho F, Bao QR, Al-Musawi MH, Schulick RD, Del Chiaro M. Neoadjuvant Treatment in Pancreatic Cancer. *Front Oncol* 2020; **10**: 245 [PMID: 32185128 DOI: 10.3389/fonc.2020.00245]

126 **Smith RC**, Pooley M, George CR, Faithful GR. Preoperative percutaneous transhepatic internal drainage in obstructive jaundice: a randomized, controlled trial examining renal function. *Surgery* 1985; **97**: 641-648 [PMID: 3890241]

127 **Eshuis WJ**, van der Gaag NA, Rauws EA, van Eijck CH, Bruno MJ, Kuipers EJ, Coene PP, Kubben FJ, Gerritsen JJ, Greve JW, Gerhards MF, de Hingh IH, Klinkenbijl JH, Nio CY, de Castro SM, Busch OR, van Gulik TM, Bossuyt PM, Gouma DJ. Therapeutic delay and survival after surgery for cancer of the pancreatic head with or without preoperative biliary drainage. *Ann Surg* 2010; **252**: 840-849 [PMID: 21037440 DOI: 10.1097/SLA.0b013e3181fd36a2]

128 **Hatfield AR**, Tobias R, Terblanche J, Girdwood AH, Fataar S, Harries-Jones R, Kernoff L, Marks IN. Preoperative external biliary drainage in obstructive jaundice. A prospective controlled clinical trial. *Lancet* 1982; **2**: 896-899 [PMID: 6126752 DOI: 10.1016/s0140-6736(82)90866-2]

129 **Mumtaz K**, Hamid S, Jafri W. Endoscopic retrograde cholangiopancreaticography with or without stenting in patients with pancreaticobiliary malignancy, prior to surgery. *Cochrane Database Syst Rev* 2007; CD006001 [PMID: 17636818 DOI: 10.1002/14651858.CD006001.pub2]

130 **van der Gaag NA**, Rauws EA, van Eijck CH, Bruno MJ, van der Harst E, Kubben FJ, Gerritsen JJ, Greve JW, Gerhards MF, de Hingh IH, Klinkenbijl JH, Nio CY, de Castro SM, Busch OR, van Gulik TM, Bossuyt PM, Gouma DJ. Preoperative biliary drainage for cancer of the head of the pancreas. *N Engl J Med* 2010; **362**: 129-137 [PMID: 20071702 DOI: 10.1056/NEJMoa0903230]

131 **Lai EC**, Mok FP, Fan ST, Lo CM, Chu KM, Liu CL, Wong J. Preoperative endoscopic drainage for malignant obstructive jaundice. *Br J Surg* 1994; **81**: 1195-1198 [PMID: 7741850 DOI: 10.1002/bjs.1800810839]

132 **Fang Y**, Gurusamy KS, Wang Q, Davidson BR, Lin H, Xie X, Wang C. Pre-operative biliary drainage for obstructive jaundice. *Cochrane Database Syst Rev* 2012; CD005444 [PMID: 22972086 DOI: 10.1002/14651858.CD005444.pub3]

133 **Fang Y**, Gurusamy KS, Wang Q, Davidson BR, Lin H, Xie X, Wang C. Meta-analysis of randomized clinical trials on safety and efficacy of biliary drainage before surgery for obstructive jaundice. *Br J Surg* 2013; **100**: 1589-1596 [PMID: 24264780 DOI: 10.1002/bjs.9260]

134 **Scheufele F**, Schorn S, Demir IE, Sargut M, Tieftrunk E, Calavrezos L, Jäger C, Friess H, Ceyhan GO. Preoperative biliary stenting *vs* operation first in jaundiced patients due to malignant lesions in the pancreatic head: A meta-analysis of current literature. *Surgery* 2017; **161**: 939-950 [PMID: 28043693 DOI: 10.1016/j.surg.2016.11.001]

135 **Rizzo A**, Ricci AD, Frega G, Palloni A, DE Lorenzo S, Abbati F, Mollica V, Tavolari S, DI Marco M, Brandi G. How to Choose Between Percutaneous Transhepatic and Endoscopic Biliary Drainage in Malignant Obstructive Jaundice: An Updated Systematic Review and Meta-analysis. *In Vivo* 2020; **34**: 1701-1714 [PMID: 32606139 DOI: 10.21873/invivo.11964]

136 **Singal AK**, Ross WA, Guturu P, Varadhachary GR, Javle M, Jaganmohan SR, Raju RP, Fleming JB, Raju GS, Kuo YF, Lee JH. Self-expanding metal stents for biliary drainage in patients with resectable pancreatic cancer: single-center experience with 79 cases. *Dig Dis Sci* 2011; **56**: 3678-3684 [PMID: 21750930 DOI: 10.1007/s10620-011-1815-7]

137 **Cellini F**, Arcelli A, Simoni N, Caravatta L, Buwenge M, Calabrese A, Brunetti O, Genovesi D, Mazzarotto R, Deodato F, Mattiucci GC, Silvestris N, Valentini V, Morganti AG. Basics and Frontiers on Pancreatic Cancer for Radiation Oncology: Target Delineation, SBRT, SIB technique, MRgRT, Particle Therapy, Immunotherapy and Clinical Guidelines. *Cancers (Basel)* 2020; **12** [PMID: 32610592 DOI: 10.3390/cancers12071729]

138 **Hammel P**, Kindler HL, Reni M, Van Cutsem E, Macarulla T, Hall MJ, Park JO, Hochhauser D, Arnold D, Oh DY, Reinacher-Schick A, Tortora G, Algül H, O'Reilly EM, McGuinness D, Cui KY, Joo S, Yoo HK, Patel N, Golan T; POLO Investigators. Health-related quality of life in patients with a germline BRCA mutation and metastatic pancreatic cancer receiving maintenance olaparib. *Ann Oncol* 2019; **30**: 1959-1968 [PMID: 31562758 DOI: 10.1093/annonc/mdz406]

139 **Golan T**, Hammel P, Reni M, Van Cutsem E, Macarulla T, Hall MJ, Park JO, Hochhauser D, Arnold D, Oh DY, Reinacher-Schick A, Tortora G, Algül H, O'Reilly EM, McGuinness D, Cui KY, Schlienger K, Locker GY, Kindler HL. Maintenance Olaparib for Germline *BRCA*-Mutated Metastatic Pancreatic Cancer. *N Engl J Med* 2019; **381**: 317-327 [PMID: 31157963 DOI: 10.1056/NEJMoa1903387]

140 **House MG**, Choti MA. Palliative therapy for pancreatic/biliary cancer. *Surg Clin North Am* 2005; **85**: 359-371 [PMID: 15833477 DOI: 10.1016/j.suc.2005.01.022]

141 **Smith AC**, Dowsett JF, Russell RC, Hatfield AR, Cotton PB. Randomised trial of endoscopic stenting *vs* surgical bypass in malignant low bileduct obstruction. *Lancet* 1994; **344**: 1655-1660 [PMID: 7996958 DOI: 10.1016/s0140-6736(94)90455-3]

142 **Andersen JR**, Sørensen SM, Kruse A, Rokkjaer M, Matzen P. Randomised trial of endoscopic endoprosthesis *vs* operative bypass in malignant obstructive jaundice. *Gut* 1989; **30**: 1132-1135 [PMID: 2475392 DOI: 10.1136/gut.30.8.1132]

143 **Moss AC**, Morris E, Leyden J, MacMathuna P. Malignant distal biliary obstruction: a systematic review and meta-analysis of endoscopic and surgical bypass results. *Cancer Treat Rev* 2007; **33**: 213-221 [PMID: 17157990 DOI: 10.1016/j.ctrv.2006.10.006]

144 **Levy MJ**, Baron TH, Gostout CJ, Petersen BT, Farnell MB. Palliation of malignant extrahepatic biliary obstruction with plastic *vs* expandable metal stents: An evidence-based approach. *Clin Gastroenterol Hepatol* 2004; **2**: 273-285 [PMID: 15067620 DOI: 10.1016/s1542-3565(04)00055-2]

145 **Moss AC**, Morris E, Mac Mathuna P. Palliative biliary stents for obstructing pancreatic carcinoma. *Cochrane Database Syst Rev* 2006; CD004200 [PMID: 16437477 DOI: 10.1002/14651858.CD004200.pub2]

146 **Speer AG**, Cotton PB, Russell RC, Mason RR, Hatfield AR, Leung JW, MacRae KD, Houghton J, Lennon CA. Randomised trial of endoscopic *vs* percutaneous stent insertion in malignant obstructive jaundice. *Lancet* 1987; **2**: 57-62 [PMID: 2439854 DOI: 10.1016/s0140-6736(87)92733-4]

147 **Inamdar S**, Slattery E, Bhalla R, Sejpal DV, Trindade AJ. Comparison of Adverse Events for Endoscopic *vs* Percutaneous Biliary Drainage in the Treatment of Malignant Biliary Tract Obstruction in an Inpatient National Cohort. *JAMA Oncol* 2016; **2**: 112-117 [PMID: 26513013 DOI: 10.1001/jamaoncol.2015.3670]

148 **Chen VK**, Arguedas MR, Baron TH. Expandable metal biliary stents before pancreaticoduodenectomy for pancreatic cancer: a Monte-Carlo decision analysis. *Clin Gastroenterol Hepatol* 2005; **3**: 1229-1237 [PMID: 16361049 DOI: 10.1016/s1542-3565(05)00886-4]

149 **Singh SM**, Longmire WP Jr, Reber HA. Surgical palliation for pancreatic cancer. The UCLA experience. *Ann Surg* 1990; **212**: 132-139 [PMID: 1695834 DOI: 10.1097/00000658-199008000-00003]

150 **Yim HB**, Jacobson BC, Saltzman JR, Johannes RS, Bounds BC, Lee JH, Shields SJ, Ruymann FW, Van Dam J, Carr-Locke DL. Clinical outcome of the use of enteral stents for palliation of patients with malignant upper GI obstruction. *Gastrointest Endosc* 2001; **53**: 329-332 [PMID: 11231392 DOI: 10.1016/s0016-5107(01)70407-5]

151 **Jeurnink SM**, Steyerberg EW, van Hooft JE, van Eijck CH, Schwartz MP, Vleggaar FP, Kuipers EJ, Siersema PD; Dutch SUSTENT Study Group. Surgical gastrojejunostomy or endoscopic stent placement for the palliation of malignant gastric outlet obstruction (SUSTENT study): a multicenter randomized trial. *Gastrointest Endosc* 2010; **71**: 490-499 [PMID: 20003966 DOI:

10.1016/j.gie.2009.09.042]
152 **Arcidiacono PG**, Calori G, Carrara S, McNicol ED, Testoni PA. Celiac plexus block for pancreatic cancer pain in adults. *Cochrane Database Syst Rev* 2011; CD007519 [PMID: 21412903 DOI: 10.1002/14651858.CD007519.pub2]
153 **Amr YM**, Makharita MY. Neurolytic sympathectomy in the management of cancer pain-time effect: a prospective, randomized multicenter study. *J Pain Symptom Manage* 2014; **48**: 944-56. e2 [PMID: 24798104 DOI: 10.1016/j.jpainsymman.2014.01.015]
154 **Ceha HM**, van Tienhoven G, Gouma DJ, Veenhof CH, Schneider CJ, Rauws EA, Phoa SS, González González D. Feasibility and efficacy of high dose conformal radiotherapy for patients with locally advanced pancreatic carcinoma. *Cancer* 2000; **89**: 2222-2229 [PMID: 11147592 DOI: 10.1002/1097-0142(20001201)89:11<2222::aid-cncr10>3.0.co;2-v]
155 **Morganti AG**, Trodella L, Valentini V, Barbi S, Macchia G, Mantini G, Turriziani A, Cellini N. Pain relief with short-term irradiation in locally advanced carcinoma of the pancreas. *J Palliat Care* 2003; **19**: 258-262 [PMID: 14959596]
156 **Oettle H**, Post S, Neuhaus P, Gellert K, Langrehr J, Ridwelski K, Schramm H, Fahlke J, Zuelke C, Burkart C, Gutberlet K, Kettner E, Schmalenberg H, Weigang-Koehler K, Bechstein WO, Niedergethmann M, Schmidt-Wolf I, Roll L, Doerken B, Riess H. Adjuvant chemotherapy with gemcitabine *vs* observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. *JAMA* 2007; **297**: 267-277 [PMID: 17227978 DOI: 10.1001/jama.297.3.267]
157 **Knox JJ**, Hedley D, Oza A, Feld R, Siu LL, Chen E, Nematollahi M, Pond GR, Zhang J, Moore MJ. Combining gemcitabine and capecitabine in patients with advanced biliary cancer: a phase II trial. *J Clin Oncol* 2005; **23**: 2332-2338 [PMID: 15800324 DOI: 10.1200/JCO.2005.51.008]
158 **Ozaka M**, Ishii H, Sato T, Ueno M, Ikeda M, Uesugi K, Sata N, Miyashita K, Mizuno N, Tsuji K, Okusaka T, Furuse J. A phase II study of modified FOLFIRINOX for chemotherapy-naïve patients with metastatic pancreatic cancer. *Cancer Chemother Pharmacol* 2018; **81**: 1017-1023 [PMID: 29633005 DOI: 10.1007/s00280-018-3577-9]
159 **Sohal DPS**, Kennedy EB, Cinar P, Conroy T, Copur MS, Crane CH, Garrido-Laguna I, Lau MW, Johnson T, Krishnamurthi S, Moravek C, O'Reilly EM, Philip PA, Pant S, Shah MA, Sahai V, Uronis HE, Zaidi N, Laheru D. Metastatic Pancreatic Cancer: ASCO Guideline Update. *J Clin Oncol* 2020; JCO2001364 [PMID: 32755482 DOI: 10.1200/JCO.20.01364]
160 **Cercek A**, Park V, Yaeger R, Reidy-Lagunes D, Kemeny NE, Stadler ZK, Segal NH, Varghese A, Saltz LB. Faster FOLFOX: Oxaliplatin Can Be Safely Infused at a Rate of 1 mg/m^2/min. *J Oncol Pract* 2016; **12**: e548-e553 [PMID: 27072569 DOI: 10.1200/JOP.2015.008417]
161 **Conroy T**, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, Adenis A, Raoul JL, Gourgou-Bourgade S, de la Fouchardière C, Bennouna J, Bachet JB, Khemissa-Akouz F, Péré-Vergé D, Delbaldo C, Assenat E, Chauffert B, Michel P, Montoto-Grillot C, Ducreux M; Groupe Tumeurs Digestives of Unicancer; PRODIGE Intergroup. FOLFIRINOX *vs* gemcitabine for metastatic pancreatic cancer. *N Engl J Med* 2011; **364**: 1817-1825 [PMID: 21561347 DOI: 10.1056/NEJMoa1011923]
162 **Conroy T**, Hammel P, Hebbar M, Ben Abdelghani M, Wei AC, Raoul JL, Choné L, Francois E, Artru P, Biagi JJ, Lecomte T, Assenat E, Faroux R, Ychou M, Volet J, Sauvanet A, Breysacher G, Di Fiore F, Cripps C, Kavan P, Texereau P, Bouhier-Leporrier K, Khemissa-Akouz F, Legoux JL, Juzyna B, Gourgou S, O'Callaghan CJ, Jouffroy-Zeller C, Rat P, Malka D, Castan F, Bachet JB; Canadian Cancer Trials Group and the Unicancer-GI–PRODIGE Group. FOLFIRINOX or Gemcitabine as Adjuvant Therapy for Pancreatic Cancer. *N Engl J Med* 2018; **379**: 2395-2406 [PMID: 30575490 DOI: 10.1056/NEJMoa1809775]
163 **Burris HA 3rd**, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, Cripps MC, Portenoy RK, Storniolo AM, Tarassoff P, Nelson R, Dorr FA, Stephens CD, Von Hoff DD. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. *J Clin Oncol* 1997; **15**: 2403-2413 [PMID: 9196156 DOI: 10.1200/JCO.1997.15.6.2403]
164 **Oettle H**, Richards D, Ramanathan RK, van Laethem JL, Peeters M, Fuchs M, Zimmermann A, John W, Von Hoff D, Arning M, Kindler HL. A phase III trial of pemetrexed plus gemcitabine *vs* gemcitabine in patients with unresectable or metastatic pancreatic cancer. *Ann Oncol* 2005; **16**: 1639-1645 [PMID: 16087696 DOI: 10.1093/annonc/mdi309]
165 **Stathopoulos GP**, Syrigos K, Aravantinos G, Polyzos A, Papakotoulas P, Fountzilas G, Potamianou A, Ziras N, Boukovinas J, Varthalitis J, Androulakis N, Kotsakis A, Samonis G, Georgoulias V. A multicenter phase III trial comparing irinotecan-gemcitabine (IG) with gemcitabine (G) monotherapy as first-line treatment in patients with locally advanced or metastatic pancreatic cancer. *Br J Cancer* 2006; **95**: 587-592 [PMID: 16909140 DOI: 10.1038/sj.bjc.6603301]
166 **Herrmann R**, Bodoky G, Ruhstaller T, Glimelius B, Bajetta E, Schüller J, Saletti P, Bauer J, Figer A, Pestalozzi B, Köhne CH, Mingrone W, Stemmer SM, Tàmas K, Kornek GV, Koeberle D, Cina S, Bernhard J, Dietrich D, Scheithauer W; Swiss Group for Clinical Cancer Research; Central European Cooperative Oncology Group. Gemcitabine plus capecitabine compared with gemcitabine alone in advanced pancreatic cancer: a randomized, multicenter, phase III trial of the Swiss Group for Clinical Cancer Research and the Central European Cooperative Oncology Group. *J Clin Oncol* 2007; **25**: 2212-2217 [PMID: 17538165 DOI: 10.1200/JCO.2006.09.0886]
167 **Von Hoff DD**, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, Seay T, Tjulandin SA, Ma

WW, Saleh MN, Harris M, Reni M, Dowden S, Laheru D, Bahary N, Ramanathan RK, Tabernero J, Hidalgo M, Goldstein D, Van Cutsem E, Wei X, Iglesias J, Renschler MF. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N Engl J Med* 2013; **369**: 1691-1703 [PMID: 24131140 DOI: 10.1056/NEJMoa1304369]

168 **Sahai V**, Catalano PJ, Zalupski MM, Lubner SJ, Menge MR, Nimeiri HS, Munshi HG, Benson AB 3rd, O'Dwyer PJ. Nab-Paclitaxel and Gemcitabine as First-line Treatment of Advanced or Metastatic Cholangiocarcinoma: A Phase 2 Clinical Trial. *JAMA Oncol* 2018; **4**: 1707-1712 [PMID: 30178032 DOI: 10.1001/jamaoncol.2018.3277]

169 **Riechelmann RP**, Townsley CA, Chin SN, Pond GR, Knox JJ. Expanded phase II trial of gemcitabine and capecitabine for advanced biliary cancer. *Cancer* 2007; **110**: 1307-1312 [PMID: 17628484 DOI: 10.1002/cncr.22902]

170 **Valle J**, Wasan H, Palmer DH, Cunningham D, Anthoney A, Maraveyas A, Madhusudan S, Iveson T, Hughes S, Pereira SP, Roughton M, Bridgewater J; ABC-02 Trial Investigators. Cisplatin plus gemcitabine *vs* gemcitabine for biliary tract cancer. *N Engl J Med* 2010; **362**: 1273-1281 [PMID: 20375404 DOI: 10.1056/NEJMoa0908721]

171 **Cheeseman SL**, Joel SP, Chester JD, Wilson G, Dent JT, Richards FJ, Seymour MT. A 'modified de Gramont' regimen of fluorouracil, alone and with oxaliplatin, for advanced colorectal cancer. *Br J Cancer* 2002; **87**: 393-399 [PMID: 12177775 DOI: 10.1038/sj.bjc.6600467]

172 **Tournigand C**, André T, Achille E, Lledo G, Flesh M, Mery-Mignard D, Quinaux E, Couteau C, Buyse M, Ganem G, Landi B, Colin P, Louvet C, de Gramont A. FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. *J Clin Oncol* 2004; **22**: 229-237 [PMID: 14657227 DOI: 10.1200/jco.2004.05.113]

173 **Wang-Gillam A**, Li CP, Bodoky G, Dean A, Shan YS, Jameson G, Macarulla T, Lee KH, Cunningham D, Blanc JF, Hubner RA, Chiu CF, Schwartsmann G, Siveke JT, Braiteh F, Moyo V, Belanger B, Dhindsa N, Bayever E, Von Hoff DD, Chen LT; NAPOLI-1 Study Group. Nanoliposomal irinotecan with fluorouracil and folinic acid in metastatic pancreatic cancer after previous gemcitabine-based therapy (NAPOLI-1): a global, randomised, open-label, phase 3 trial. *Lancet* 2016; **387**: 545-557 [PMID: 26615328 DOI: 10.1016/S0140-6736(15)00986-1]

174 **Pantuck M**, McDermott D, Drakaki A. To treat or not to treat: Patient exclusion in immune oncology clinical trials due to preexisting autoimmune disease. *Cancer* 2019; **125**: 3506-3513 [PMID: 31318445 DOI: 10.1002/cncr.32326]

175 **Brahmer JR**, Lacchetti C, Schneider BJ, Atkins MB, Brassil KJ, Caterino JM, Chau I, Ernstoff MS, Gardner JM, Ginex P, Hallmeyer S, Holter Chakrabarty J, Leighl NB, Mammen JS, McDermott DF, Naing A, Nastoupil LJ, Phillips T, Porter LD, Puzanov I, Reichner CA, Santomasso BD, Seigel C, Spira A, Suarez-Almazor ME, Wang Y, Weber JS, Wolchok JD, Thompson JA; National Comprehensive Cancer Network. Management of Immune-Related Adverse Events in Patients Treated With Immune Checkpoint Inhibitor Therapy: American Society of Clinical Oncology Clinical Practice Guideline. *J Clin Oncol* 2018; **36**: 1714-1768 [PMID: 29442540 DOI: 10.1200/JCO.2017.77.6385]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2021 June 15; 13(6): 495-508

DOI: 10.4251/wjgo.v13.i6.495

ISSN 1948-5204 (online)

REVIEW

Familial adenomatous polyposis and changes in the gut microbiota: New insights into colorectal cancer carcinogenesis

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Author contributions: All authors contributed to the writing and reading of the manuscript and gave approval of the final version. All authors have read and agreed with publication of the manuscript.

Conflict-of-interest statement: The authors have no competing interests to declare.

Manuscript source: Invited manuscript

Specialty type: Oncology

Country/Territory of origin: Italy

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Abstract

Patients with familial adenomatous polyposis (FAP), an autosomal dominant hereditary colorectal cancer syndrome, have a lifetime risk of developing cancer of nearly 100%. Recent studies have pointed out that the gut microbiota could play a crucial role in the development of colorectal adenomas and the consequent progression to colorectal cancer. Some gut bacteria, such as *Fusobacterium nucleatum*, *Escherichia coli*, *Clostridium difficile*, *Peptostreptococcus*, and enterotoxigenic *Bacteroides fragilis*, could be implicated in colorectal carcinogenesis through different mechanisms, including the maintenance of a chronic inflammatory state, production of bioactive tumorigenic metabolites, and DNA damage. Studies using the adenomatous polyposis coli$^{Min/+}$ mouse model, which resembles FAP in most respects, have shown that specific changes in the intestinal microbial community could influence a multistep progression, the intestinal "adenoma-carcinoma sequence", which involves mucosal barrier injury, low-grade inflammation, activation of the Wnt pathway. Therefore, modulation of gut microbiota might represent a novel therapeutic target for patients with FAP. Administration of probiotics, prebiotics, antibiotics, and nonsteroidal anti-inflammatory drugs could potentially prevent the progression of the adenoma-carcinoma sequence in FAP. The aim of this review was to summarize the best available knowledge on the role of gut microbiota in colorectal carcinogenesis in patients with FAP.

Key Words: Familial adenomatous polyposis; Microbiota; Colorectal cancer; Polyps; Carcinogenesis; Bacteria

Peer-review report's scientific quality classification
Grade A (Excellent): A, A
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Received: February 20, 2021
Peer-review started: February 20, 2021
First decision: March 15, 2021
Revised: March 15, 2021
Accepted: May 8, 2021
Article in press: May 8, 2021
Published online: June 15, 2021

P-Reviewer: Caba O, Vieth M
S-Editor: Gao CC
L-Editor: Filipodia
P-Editor: Yuan YY

Core Tip: A number of studies have demonstrated that gut microbiota dysbiosis could be a key factor in colorectal carcinogenesis. The adenomatous polyposis coli (*APC*)$^{Min/+}$ mouse model has been extensively used to study the underlying mechanisms of colorectal carcinogenesis in familial adenomatous polyposis. Interventions aimed at improving dysbiosis by administration of probiotics, prebiotics, or antibiotics could decrease colorectal cancer development in *APC* mutation carriers.

Citation: Biondi A, Basile F, Vacante M. Familial adenomatous polyposis and changes in the gut microbiota: New insights into colorectal cancer carcinogenesis. *World J Gastrointest Oncol* 2021; 13(6): 495-508
URL: https://www.wjgnet.com/1948-5204/full/v13/i6/495.htm
DOI: https://dx.doi.org/10.4251/wjgo.v13.i6.495

INTRODUCTION

Familial adenomatous polyposis (FAP) is an autosomal dominant hereditary colorectal cancer (CRC) syndrome characterized by the development of numerous (*i.e.* tens to thousands) colorectal adenomas[1,2]. A mutation in the adenomatous polyposis coli (*APC*) gene, found on chromosome 5q21, is responsible for FAP[3]. The incidence of FAP is around 1/8300, and the onset is commonly in the second or third decade of life. The risk of CRC is nearly 100% by the time patients with FAP reach the age of 40-50 years[4,5]. Such patients have an increased risk of desmoid tumors and gastric, duodenal, biliary duct, and thyroid cancers[6]. Extraintestinal manifestations of FAP may include osteomas, dental abnormalities such as unerupted or supernumerary teeth, congenital absence of one or more teeth, odontomas, and dentigerous cysts; and congenital hypertrophy of the retinal pigment epithelium[7,8]. Prophylactic colectomy is generally performed by age 40 in patients with FAP, and is the gold standard treatment to reduce the risk of developing CRC[9]. Nonetheless, colectomy is associated with postoperative morbidity and does not reduce the risk of developing extraintestinal manifestations of FAP[10]. Endoscopic surveillance of patients with FAP and their family members has decreased the occurrence of CRC at the time of FAP diagnosis by 55% and has also increased overall survival[4,11].

Recent studies have shown that the gut microbiota could play an important role in the development of colorectal adenomas and the consequent progression to CRC[12]. Indeed, gut bacteria such as *Fusobacterium nucleatum*, *Escherichia coli*, *Clostridium difficile*, *Peptostreptococcus*, and enterotoxigenic *Bacteroides fragilis*, could be responsible for colorectal carcinogenesis through a number of mechanisms, including the maintenance of a chronic inflammatory state, production of bioactive tumorigenic metabolites, and DNA damage[13-15]. A number of studies investigated the interaction between gut microbiota and host genetics in patients with intestinal adenomatous polyps. A study by Liang *et al*[16] showed a close relationship between the presence of *APC* mutation and modification of the gut microbiota and serum metabolites. Low levels of *Faecalibacterium prausnitzii* and an abundance of *Fusobacterium mortiferum* had the potential to predict the development of CRC from adenomatous polyps. It has been also observed that mutation of the *APC* gene could modify colonic-microbial interactions before the development of polyposis in mouse models[17]. After *F. nucleatum* infection, *APC*$^{Min/+}$ mice, carrying an inactivated allele of the *APC* gene, had an increase of small intestinal and colonic adenoma formation and an acceleration of small intestinal adenocarcinoma development[18]. Thus, it has been hypothesized that interventions aimed at improving dysbiosis in *APC* mutation carriers, including administration of probiotics, prebiotics, or antibiotics, could decrease CRC development. The aim of this review was to summarize the best available knowledge on the role of gut microbiota on colorectal carcinogenesis in patients with FAP.

GENETIC FEATURES

The classic colorectal carcinogenesis model described by Fearon and Vogelstein[19] includes development of most CRCs from a minimum of five or more genetic

alterations, while adenomas require fewer alterations. It has been hypothesized that inactivating mutations of the *APC* gene could represent the initial step of the "adenoma-carcinoma sequence" (Figure 1). The *APC* gene is a fundamental component of the β-catenin and Wnt signaling pathways, modulating cell differentiation, adhesion, migration, and apoptosis[20]. Somatic mutations of the *APC* gene occur in around 80% of sporadic CRCs, whereas germline *APC* mutations are responsible for FAP, making this a key target to study the environmental and genetic modifiers of CRC[16,17]. Loss of *APC* gene function has been shown to produce a survival advantage by mimicking hypoxic conditions and stimulate the accumulation of β-catenin and abnormal cell proliferation, associated with development of adenomatous polyposis[21-24].

Mouse models of FAP

Laboratory mouse models have proven to be valuable in the study of CRC[25]. The Min (multiple intestinal neoplasia) is the first key CRC mouse model and is induced by treatment with ethylnitrosourea[26]. Adult $APC^{Min/+}$ mice develop multiple intestinal polyps and anemia and usually die at a young age because of intestinal blockage and bleeding from the larger polyps[27]. Other mouse models have also been reported, such as conditional *APC* mutant alleles[28]. The $APC^{Min/+}$ mouse model shares numerous phenotypic and genetic similarities with FAP. However, patients with FAP develop adenomas mainly in the colon, while adenomas in $APC^{Min/+}$ mice are mainly located in the small intestine and have benign characteristics. Also, desmoid tumors and epidermoid cysts are rarely seen in mouse models compared with patients with FAP[29]. Nonetheless, the $APC^{Min/+}$ mouse represents an outstanding experimental model for investigating genetic features and therapeutic responses of CRC in humans.

Bacterial genotoxicity

Interplay between the gut microbiota and genetic characteristics could be responsible for the genetic pattern of the adenoma-carcinoma sequence. It has been hypothesized that bacterial drivers could initiate the development of precancerous lesions and the subsequent accumulation of gene mutations[30,31]. Different gut bacteria, such as *E. coli*, *Enterococcus faecalis*, *Streptococcus gallolyticus* and *B. fragilis* have been shown to promote carcinogenesis through genotoxic effects[32]. Some *E. coli* strains, mainly B2 and D, strongly express virulence genes, such as those encoding toxins and effectors that could promote carcinogenesis (*e.g.*, colibactin, cytotoxic necrotizing factors, cytolethal distending toxins, and cycle-inhibiting factor)[33,34]. Colibactin could be responsible for DNA alkylation on adenine residues, thus favoring double-strand breaks[35]. A recent study showed that expression of colibactin-producing polyketide synthase (*pks*+) in *E. coli* could was associated with the occurrence of a specific mutational signature in human gut organoids. The same mutational signature was detected in 5876 human cancer genomes in two independent study cohorts, especially in CRC[36]. Also, *pks*+ *E. coli* could be responsible for aneuploidy and abnormal cellular division, an effect promoted by the mutagen colibactin[37]. Such effects of *pks*+ *E. coli* were mainly observed in $APC^{Min/+}$ mice that lacked the autophagy gene *Atg16 L1*, and consequently were not able to recruit the DNA repair protein RAD51, thus accumulating DNA double-strand breaks and developing tumors[38]. *Enterococcus faecalis* was shown to promote DNA damage by induction of inflammation and oxidative stress resulting from the release of reactive oxygen species and reactive nitrogen species[39]. Fragilysin (also known as BST), is a toxic virulence factor released by enterotoxigenic *B. fragilis* (ETBF) that can induce DNA damage *in vivo*[40]. Colonization by sulfidogenic bacteria, such as *F. nucleatum*, has been associated with genomic or chromosomal instability and CRC development associated with the genotoxic effects of hydrogen sulfide (H_2S)[41,42]. A prior state of dysbiosis could enhance these specific bacterial genotoxic effects[31].

GUT MICROBIOTA AND CARCINOGENESIS

There is extensive evidence of an association between infectious agents and development of tumors[43]. It has also been demonstrated that specific mucosa-associated bacterial species could play a pivotal role in the pathogenesis of CRC[44-46]. Indeed, bacterial toxins and effector proteins have been shown to damage host cell DNA, and therefore affect crucial host cell signaling pathways that regulate cell differentiation, apoptosis, proliferation, and immune signaling[47-57] (Table 1).

Table 1 Studies of colorectal cancer-associated bacteria in the *APC*$^{Min/+}$ mouse model

Ref.	Bacterial strain	Mechanism of carcinogenesis
Kostic *et al*[18], 2013	*F. nucleatum*	Infiltration of CD11^{+} myeloid-derived immune cells
Tomkovich *et al*[49], 2017	*F. nucleatum* and *pks*+ *E. coli*	Mediated by inflammation, with colibactin-producing *E. coli* but not with *F. nucleatum* (FadA^{+} or Fap2^{+})
Yang *et al*[50], 2017	*F. nucleatum*	Regulation of miR-21 *via* TLR4/MYD88/NF-κB pathway
Wu *et al*[51], 2018	*F. nucleatum*	TLR4/p-PAK1/p-β-catenin S675 pathway
Chen *et al*[52], 2018	*F. nucleatum*	Induction of M2 macrophage polarization *via* TLR4. Activation of the IL-6/p-STAT3/c-MYC signaling pathway
Rubinstein *et al*[53], 2019	*F. nucleatum*	FadA adhesin upregulates Annexin A1 expression through E-cadherin
Dejea *et al*[54], 2018	Mono- or co-colonization of ETBF and *pks*+ *E. coli*	Upregulation of IL-17 and DNA damage
Chung *et al*[55], 2018	ETBF	Pathway involving activation of IL-17R, NF-κB, Stat3, and CXCL1
Goodwin *et al*[56], 2011	ETBF	Production of spermine oxidase, reactive oxygen species and DNA damage
He *et al*[57], 2019	*Campylobacter jejuni*	DNA damage due to cytolethal distending toxin
Li *et al*[15], 2019	Mixed strains from fecal samples of CRC patients after antibiotic cocktails	Wnt/β-catenin and cyclin D1 pathway

CRC: Colorectal cancer; *E. coli*: *Escherichia coli*; ETBF: Enterotoxigenic *Bacteroides fragilis*; *F. nucleatum*: *Fusobacterium nucleatum*; IL: Interleukin; NF-κB: Nuclear factor-kappa B; *pks*: Producing polyketide synthase; TLR: Toll-like receptor.

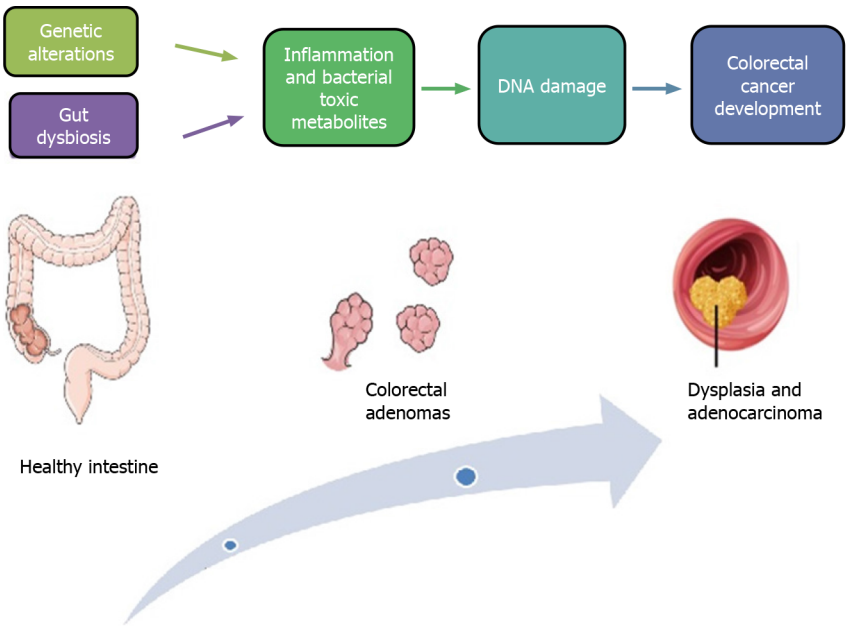

Figure 1 Pathway of the development of colorectal adenomas and the consequent progression to colorectal cancer.

Dysbiosis and bacterial toxins

Changes in the gut microbiota, can stimulate the c-Jun/JNK and STAT3 signaling pathways, thus promoting, in combination with anemia, tumor growth in *APC*$^{Min/+}$ mice[58]. A study carried out in *APC*$^{Min/+}$ mice by Son *et al*[17] reported that mutation of the *APC* gene modified colonic-microbial interactions prior to polyposis. Indeed, changes in the gut microbiota, characterized by an increased relative growth of *Bacteroidetes spp.* identified in association with intestinal tumors, has been shown to precede the development of microscopically evident intestinal tumors in 6-wk-old *APC*$^{Min/+}$ mice. A recent study by Dejea *et al*[54] detected colonic biofilms mainly composed of *E. coli* and *B. fragilis* in patients with FAP. Genes for colibactin (*clbB*) and *B. fragilis* toxin (*bft*) were highly expressed in the colonic mucosa of patients with FAP

compared with healthy subjects. Co-colonization with *E. coli* and ETBF led to an increase in interleukin-17 (IL-17) and DNA damage in colonic epithelium of tumor-prone mice, compared with mice with either bacterial strain alone. As ETBF and *pks*+ *E. coli* frequently colonize young children, it has been suggested that constant co-colonization in the colon mucosa from a young age could play a role in the pathogenesis of FAP[54]. The *B. fragilis* toxin (BFT) can bind to intestinal epithelial-cell receptors, promoting cell proliferation through cleavage of the tumor suppressor protein E-cadherin[55]. It has been shown that BFT can provoke acute and chronic colitis in C57BL/6 mice, and colon tumors in an $APC^{Min/+}$ mouse model[59-61]. Infections with enterotoxigenic strains of *B. fragilis*, compared with non-toxigenic strains, were more frequently observed in patients with CRCs. Enterotoxigenic strains were detected in only 10%-20% of healthy controls, but enterotoxigenic *B. fragilis* was found in stool samples from 40% of CRC patients[62]. A study by Tomkovich *et al*[49] carried out in germ-free, specific-pathogen-free, and gnotobiotic $APC^{Min/+}$;$IL10^{-/-}$ mice reported that colon carcinogenesis was associated with an inflammatory state. CRC did not develop in germ-free $APC^{Min/+}$;$IL\text{-}10^{-/-}$, and *pks*+ mice. *E. coli* promoted carcinogenesis in the $APC^{Min/+}$;$IL\text{-}10^{-/-}$ model in a colibactin-dependent way. An interesting study by Li *et al*[15] investigated the role of gut microbiota on adenoma progression in $APC^{Min/+}$ mice. Transplants of gut microbiota from CRC patients into $APC^{Min/+}$ mice enhanced the progression of adenoma, damaged the intestinal barrier, promoted chronic low-grade inflammation, and stimulated the Wnt signaling pathway. These results suggest that microbial targeted therapy could represent a novel FAP therapy.

Inflammation

Commensal and pathogenic bacteria were found to promote CRC development after colonizing normal colonic mucosa and promoting sustained local inflammation, and by releasing genotoxic compounds against colonic epithelial cells to induce their tumorigenic transformation[63]. Conversely, a balanced population of microbiota prevented development of CRC by producing bacterial metabolites that reduced inflammation[64]. Chronic inflammation is associated with the development of various tumors, including CRC. Inflammation of the colonic mucosa may enhance carcinogenic mutagenesis, thus favoring CRC initiation[65]. Also, a chronic inflammatory state is characterized by loss of IL-10-secreting regulatory T cells (Tregs) and stimulation of Th17cells producing IL-17A, which supports IL-17A-dependent tumor growth, and promotes colonic carcinogenesis in the $APC^{Min/+}$ mouse model, which resembles FAP in most respects[66]. An association between *F. nucleatum* infection and increased expression of the nuclear factor-kappa beta (NF-κB) pro-inflammatory profile in mouse intestinal cancers has been observed, consistent with the development of human CRC[18]. FadA, a *Fusobacterium*-specific adhesion molecule, can facilitate *F. nucleatum* adherence to host cells[67], and *F. nucleatum* colonization was found to recruit tumor-infiltrating myeloid cells and stimulate the Wnt/β-catenin pathway, leading to NF-κB activation and cancer cell proliferation[68]. Chronic inflammation in $APC^{Min/+}$;$IL\text{-}10^{-/-}$ mice was shown to modify the gut microbiota composition and selectively favor the growth of *Enterobacteriaceae*. Chronic inflammation also supported the selection of pathogenic strains of *E. coli* and was essential for the cancer-promoting effects of those bacteria[69]. Colonization of $APC^{Min/+}$ mice with ETBF led to the activation of a pro-tumorigenic multistep inflammatory cascade involving IL-17R, NF-κB, and Stat3 signaling in colonic epithelial cells. Indeed, BFT could stimulate a protumorigenic signal in colon mucosal epithelial cells that led to a Th17 response that in turn activated NFκB and myeloid cell-dependent carcinogenesis in the distal colon [55]. Grivennikov *et al*[70] reported that the loss of intestinal barrier function in $APC^{Min/+}$ mice induced by CRC-initiating genetic alterations led to adenoma invasion by microbial metabolites that stimulated inflammation and, in turn, cancer growth. It is noteworthy that even colonization of commensal bacteria can promote CRC. Indeed, infection of germ-free $APC^{Min/+}$;$IL\text{-}10^{-/-}$ mice with commensals of specific-pathogen free mice enhanced the tumor load[49]. Commensal bacteria and their constituents have been shown to stimulate Toll-like receptors on tumor-infiltrating myeloid cells and MyD88-mediated production of inflammatory cytokines, such as IL-23. Therefore, IL-23 supported CRC development by activating the release of other cytokines, such as IL-6, IL-17A, and IL-22[71].

Short-chain fatty acids and bacterial metabolites

A number of studies demonstrated that the gut microbiota was responsible for the production of various bioactive food elements and micronutrients, such as essential vitamins, and the fermentation of dietary fibers and complex carbohydrates, producing short-chain fatty acids (SCFAs), such as butyrate, acetate, and propionate

[72-74]. The role of butyrate in colorectal carcinogenesis is controversial[75]. In fact, in *APC*$^{Min/+}$; Msh2$^{-/-}$ mice that were also deficient for the DNA mismatch repair gene MutS homolog 2, Belcheva *et al*[76] found that microbial metabolism of carbohydrates into SCFAs, such as butyrate, enhanced the proliferation of tumor-initiated epithelial cells, thus promoting carcinogenesis. In their study, the growth of SCFA-producing bacteria, such as *Clostridiaceae*, *Ruminococcaceae*, and *Lachnospiraceae*, was inhibited by antibiotic therapy or a low-carbohydrate diet, and in turn the number of polyps detected in *APC*$^{Min/+}$; Msh2$^{-/-}$ mice was also reduced. On the other hand, many studies have described antineoplastic effects SCFAs, such as the suppression of inflammation, stimulation of apoptosis, and inhibition of cancer cell progression[77]. Nonetheless, further investigation is needed for clarifying the role of butyrate in CRC protection or promotion. Other bacterial metabolites, such as H_2S, secondary bile acids, and nitric oxide, have been shown to contribute to progression of adenomatous colon polyps to CRC by affecting host metabolism and immunity[78].

CURRENT CLINICAL TRIALS

A growing number of clinical trials have reported an association between gut bacteria and their metabolites and progression of CRC through various mechanisms[79,80]. However, the role of the gut microbiota in the progression and development of CRC is intricate and still not entirely understood, especially in patients with FAP. Currently, only a few clinical trials are recruiting subjects with FAP to determine whether modifying the gut microbiota might influence CRC development[81]. The Memorial Sloan Kettering Cancer Center in New York (United States), is conducting a clinical trial (Clinicaltrials.gov ID: NCT02371135) enrolling patients with Lynch syndrome or other hereditary colonic polyposis syndromes, in order to assess the role of the gut bacteria in CRC development. Investigators collect fecal samples, colon biopsies, and questionnaire responses on diet and lifestyle[82]. A phase 2, randomized, double-blind, placebo-controlled study sponsored by the Tel Aviv Sourasky Medical Center (Israel) is evaluating the efficacy of curcumin supplementation on polyp number and size in patients with FAP (Clinicaltrials.gov ID: NCT03061591)[83].

POTENTIAL THERAPEUTIC APPROACHES AND FUTURE DIRECTIONS

It has been suggested that interventions directed at improving gut dysbiosis in *APC* $^{Min/+}$ mice, for instance through probiotics, prebiotics, some antibiotics, and nonsteroidal anti-inflammatory drugs (NSAIDs), can inhibit the progression of the adenoma-carcinoma sequence, thus reducing the development of CRC[84-86].

Fap-related pouch

The ileoanal pouch is the surgical procedure of choice for patients with the classical phenotype of FAP[87]. Many studies have shown that the gut microbiota play a key role in the development of pouchitis, as supported by clinical evidence of the benefits of antibiotic therapy[88,89]. Metronidazole, ciprofloxacin, or a combination of both, is usually the initial approach, and it is often effective in chronic pouchitis[90]. A meta-analysis of 21 studies showed that antibiotics induced a significant remission rate (74%) in patients with chronic pouchitis (95% confidence interval: 56-93; $P < 0.001$), whereas the remission rate after administration of biologics was 53% (95% confidence interval: 30-76; $P < 0.001$). Conversely, steroids, bismuth, tacrolimus, and an elemental diet did not result in a significant remission, which was achieved by fecal microbiota transplantation[88]. Probiotics have been shown to be effective in the prevention of pouchitis[91]. Indeed, Shen *et al*[92] showed that administration of a probiotic treatment (*Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Bifidobacterium bifidus*) prevented pouchitis, decreased the Modified Pouch Disease Activity Index score, and reduced fecal pyruvate kinase and calprotectin in FAP patients after restorative proctocolectomy[93].

Probiotics and prebiotics

Gut microbiota composition and function are considerably modulated by diet[14]. An association between the intake of nondigestible fibers, such as prebiotics, and an abundance of beneficial bacteria in the gut, including *Bifidobacterium*, *Lactobacillus*, *Faecalibacterium*, *Ruminococcaceae*, and *Roseburia* has been widely reported. Indeed

administration of both probiotics and prebiotics has shown beneficial effects in prevention and reduction of the prevalence of adenomatous colon polyps[94,95]. A metagenomic study by Ni *et al*[96] reported a preventive effect of *Lactobacillus rhamnosus* GG (LGG) on polyp formation in *APC*$^{Min/+}$ mice. The results showed that LGG had beneficial effects and reduced polyp development in mice by preserving gut microbial functionality. A study by Urbanska *et al*[97] reported similar results using an orally delivered probiotic formulation that reduced overall intestinal inflammation and the number of polyps in the small intestine of *APC*$^{Min/+}$ mice after administration of microencapsulated live *Lactobacillus acidophilus* cells.

Antibiotics

There is evidence that antibiotic treatment can modify the gut microbiota physiological processes and functions[98]. Some studies showed that shifts in the composition of the intestinal community caused by antibiotics were associated with development of polyps and progression to CRC. Other studies reported a possible protective effect on carcinogenesis[99-101]. A nested case-control study by Dik *et al*[102] reported a significant dose-dependent association between administration of penicillin and quinolone antibiotics and increased risk of CRC development. Another nested case-control study by Boursi *et al*[103] carried out in a large population-based database in the United Kingdom, showed similar results, and concluded that past exposure to several courses of penicillin was associated with a slight increase in CRC risk. A recent study found that long-term treatment of *APC*$^{Min/+}$ mice with an antibiotic cocktail composed of vancomycin, neomycin, and streptomycin resulted in gut inflammation with polyposis and cancer progression, perhaps caused by specific changes of the gut microbiota and thinning of the protective mucus layer[104]. On the contrary, Belcheva *et al*[76] observed a decreased number of polyps in both the small and large intestine of C57BL/6 *APC*$^{Min/+}$; Msh2$^{-/-}$ mice treated with ampicillin, metronidazole, neomycin, and vancomycin. The gut microbiota in *APC*$^{Min/+}$; Msh2$^{-/-}$ mice might affect the development of CRC at an early stage, thus acting as a tumor initiator. These contrasting results suggest that the changes of gut bacteria caused by antibiotic treatment can be either detrimental or beneficial in a context-dependent way[105]. Further studies are needed to investigate the role of specific antibiotics in modulating the microbiota response and the relationship with colorectal carcinogenesis.

Diet and anti-inflammatory drugs

A number of epidemiological studies have shown an association between diet, inflammation, and cancer, including CRC[106-109]. So far, there is a lack of preventive dietary recommendations for FAP patients. A nonrandomized prospective pilot study carried out on FAP patients showed that a low-inflammatory diet based on the Mediterranean diet pattern decreased gastrointestinal markers of inflammation, such as C-reactive protein and pro-inflammatory cytokines, through a modulation of the gut microbiota composition[110]. Combination treatment with curcumin and quercetin has been reported to reduce the development of adenomas in FAP. This beneficial effect might be a result of their antioxidative, anti-inflammatory, and antiproliferative properties and the maintenance of a diverse gut microbial community[111-113]. Black raspberry powder supplementation in FAP patients significantly decreased the burden of rectal polyps and reduced staining of the mucosal proliferation marker Ki-67, compared with placebo[114]. The results could have a response to beneficial effects of the anthocyanin and fiber content of the raspberries on the diversity and composition of the gut microbiota[115,116]. Administration of berberine, an alkaloid that can be isolated from many plants including barberry (*Berberis vulgaris*), significantly reduced the development of CRC and restored the gut microbiota community in *APC*$^{Min/+}$ mice fed a high fat diet[117].

There is evidence that the combination of anti-inflammatory drugs and regular endoscopic surveillance can decrease the risk of new adenomas in the rectal stump of FAP patients[118-120]. Administration of NSAIDs and omega-3 essential fatty acids reduced recurrence[121]. Even though long-term therapy with NSAIDs has been shown to increase gastrointestinal and cardiological risk, the use of omega-3 supplements can be expensive for patients[122,123]. NSAIDs may modify the composition and diversity of gut microbiota by inhibiting or facilitating bacterial growth, inducing bacterial cell death, or affecting bacterial metabolism[123]. The bacterial composition of the gut has been shown to change with the type of NSAID administered[124]. Specific shifts in the microbiota such as an increase in *Coriobacteriaceae* or reduction in *Bifidobacteriaceae* and *Lactobacillaceae* after chronic oral treatment with celecoxib, have been associated with a decrease of polyp burden in *APC*$^{Min/+}$ mice[125]. *APC*$^{Min/+}$ mice treated with aspirin showed a decrease in CRC number and load that depended on the

presence of gut microbes. Of interest, *Lysinibacillus sphaericus* in the gut degraded aspirin, thereby reducing its chemopreventive effects in mice. Stool samples from mice treated with aspirin had increased populations of beneficial bacteria such as *Lactobacillus and Bifidobacterium*, and decreased populations of pathogenic bacteria such as *Alistipes finegoldii* and *B. fragilis*[126].

CONCLUSION

The *APC*$^{Min/+}$ mouse model has been widely used to study the underlying mechanisms of colorectal carcinogenesis in FAP. Several studies demonstrated that gut microbiota dysbiosis as a key factor in colorectal carcinogenesis. Indeed, the intestinal microbial community played an important role in the multistep process of the intestinal adenoma-carcinoma sequence, and changes in the gut microbiota were found to be responsible for mucosal barrier injury, low-grade inflammation, activation of the Wnt pathway, and subsequent progression of adenomas. Recent evidence suggests that the modulation of gut microbiota could be a novel therapeutic target in FAP patients. Administration of probiotics, prebiotics, antibiotics, and NSAIDs can prevent the progression of the adenoma-carcinoma sequence in FAP. However, further study of the role of the gut microbiota in the malignant transformation of colorectal adenoma and how microbe-targeted therapies might be useful in preventing CRC development in FAP is needed.

REFERENCES

1 **Kemp Bohan PM**, Mankaney G, Vreeland TJ, Chick RC, Hale DF, Cindass JL, Hickerson AT, Ensley DC, Sohn V, Clifton GT, Peoples GE, Burke CA. Chemoprevention in familial adenomatous polyposis: past, present and future. *Fam Cancer* 2021; **20**: 23-33 [PMID: 32507936 DOI: 10.1007/s10689-020-00189-y]

2 **Jung I**, Gurzu S, Turdean GS. Current status of familial gastrointestinal polyposis syndromes. *World J Gastrointest Oncol* 2015; **7**: 347-355 [PMID: 26600934 DOI: 10.4251/wjgo.v7.i11.347]

3 **Leoz ML**, Carballal S, Moreira L, Ocaña T, Balaguer F. The genetic basis of familial adenomatous polyposis and its implications for clinical practice and risk management. *Appl Clin Genet* 2015; **8**: 95-107 [PMID: 25931827 DOI: 10.2147/TACG.S51484]

4 **Monahan KJ**, Bradshaw N, Dolwani S, Desouza B, Dunlop MG, East JE, Ilyas M, Kaur A, Lalloo F, Latchford A, Rutter MD, Tomlinson I, Thomas HJW, Hill J; Hereditary CRC guidelines eDelphi consensus group. Guidelines for the management of hereditary colorectal cancer from the British Society of Gastroenterology (BSG)/Association of Coloproctology of Great Britain and Ireland (ACPGBI)/United Kingdom Cancer Genetics Group (UKCGG). *Gut* 2020; **69**: 411-444 [PMID: 31780574 DOI: 10.1136/gutjnl-2019-319915]

5 **GBD 2017 Colorectal Cancer Collaborators**. The global, regional, and national burden of colorectal cancer and its attributable risk factors in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol* 2019; **4**: 913-933 [PMID: 31648977 DOI: 10.1016/S2468-1253(19)30345-0]

6 **Half E**, Bercovich D, Rozen P. Familial adenomatous polyposis. *Orphanet J Rare Dis* 2009; **4**: 22 [PMID: 19822006 DOI: 10.1186/1750-1172-4-22]

7 **Wang XP**, Fan J. Molecular genetics of supernumerary tooth formation. *Genesis* 2011; **49**: 261-277 [PMID: 21309064 DOI: 10.1002/dvg.20715]

8 **Byrne RM**, Tsikitis VL. Colorectal polyposis and inherited colorectal cancer syndromes. *Ann Gastroenterol* 2018; **31**: 24-34 [PMID: 29333064 DOI: 10.20524/aog.2017.0218]

9 **Herzig D**, Hardiman K, Weiser M, You N, Paquette I, Feingold DL, Steele SR. The American Society of Colon and Rectal Surgeons Clinical Practice Guidelines for the Management of Inherited Polyposis Syndromes. *Dis Colon Rectum* 2017; **60**: 881-894 [PMID: 28796726 DOI: 10.1097/DCR.0000000000000912]

10 **Dinarvand P**, Davaro EP, Doan JV, Ising ME, Evans NR, Phillips NJ, Lai J, Guzman MA. Familial Adenomatous Polyposis Syndrome: An Update and Review of Extraintestinal Manifestations. *Arch Pathol Lab Med* 2019; **143**: 1382-1398 [PMID: 31070935 DOI: 10.5858/arpa.2018-0570-RA]

11 **Bülow S**. Results of national registration of familial adenomatous polyposis. *Gut* 2003; **52**: 742-746 [PMID: 12692062 DOI: 10.1136/gut.52.5.742]

12 **Vacante M**, Ciuni R, Basile F, Biondi A. Gut Microbiota and Colorectal Cancer Development: A Closer Look to the Adenoma-Carcinoma Sequence. *Biomedicines* 2020; **8** [PMID: 33182693 DOI: 10.3390/biomedicines8110489]

13 **Wong SH**, Yu J. Gut microbiota in colorectal cancer: mechanisms of action and clinical applications. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 690-704 [PMID: 31554963 DOI: 10.1038/s41575-019-0209-8]

14 **Pop OL**, Vodnar DC, Diaconeasa Z, Istrati M, Bințințan A, Bințințan VV, Suharoschi R,

Gabbianelli R. An Overview of Gut Microbiota and Colon Diseases with a Focus on Adenomatous Colon Polyps. *Int J Mol Sci* 2020; **21** [PMID: 33028024 DOI: 10.3390/ijms21197359]

15 **Li L**, Li X, Zhong W, Yang M, Xu M, Sun Y, Ma J, Liu T, Song X, Dong W, Liu X, Chen Y, Liu Y, Abla Z, Liu W, Wang B, Jiang K, Cao H. Gut microbiota from colorectal cancer patients enhances the progression of intestinal adenoma in Apc$^{min/+}$ mice. *EBioMedicine* 2019; **48**: 301-315 [PMID: 31594750 DOI: 10.1016/j.ebiom.2019.09.021]

16 **Liang S**, Mao Y, Liao M, Xu Y, Chen Y, Huang X, Wei C, Wu C, Wang Q, Pan X, Tang W. Gut microbiome associated with APC gene mutation in patients with intestinal adenomatous polyps. *Int J Biol Sci* 2020; **16**: 135-146 [PMID: 31892851 DOI: 10.7150/ijbs.37399]

17 **Son JS**, Khair S, Pettet DW 3rd, Ouyang N, Tian X, Zhang Y, Zhu W, Mackenzie GG, Robertson CE, Ir D, Frank DN, Rigas B, Li E. Altered Interactions between the Gut Microbiome and Colonic Mucosa Precede Polyposis in APCMin/+ Mice. *PLoS One* 2015; **10**: e0127985 [PMID: 26121046 DOI: 10.1371/journal.pone.0127985]

18 **Kostic AD**, Chun E, Robertson L, Glickman JN, Gallini CA, Michaud M, Clancy TE, Chung DC, Lochhead P, Hold GL, El-Omar EM, Brenner D, Fuchs CS, Meyerson M, Garrett WS. Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe* 2013; **14**: 207-215 [PMID: 23954159 DOI: 10.1016/j.chom.2013.07.007]

19 **Fearon ER**, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990; **61**: 759-767 [PMID: 2188735 DOI: 10.1016/0092-8674(90)90186-i]

20 **Pai SG**, Carneiro BA, Mota JM, Costa R, Leite CA, Barroso-Sousa R, Kaplan JB, Chae YK, Giles FJ. Wnt/beta-catenin pathway: modulating anticancer immune response. *J Hematol Oncol* 2017; **10**: 101 [PMID: 28476164 DOI: 10.1186/s13045-017-0471-6]

21 **Valli A**, Rodriguez M, Moutsianas L, Fischer R, Fedele V, Huang HL, Van Stiphout R, Jones D, Mccarthy M, Vinaxia M, Igarashi K, Sato M, Soga T, Buffa F, Mccullagh J, Yanes O, Harris A, Kessler B. Hypoxia induces a lipogenic cancer cell phenotype *via* HIF1α-dependent and -independent pathways. *Oncotarget* 2015; **6**: 1920-1941 [PMID: 25605240 DOI: 10.18632/oncotarget.3058]

22 **Newton IP**, Kenneth NS, Appleton PL, Näthke I, Rocha S. Adenomatous polyposis coli and hypoxia-inducible factor-1{alpha} have an antagonistic connection. *Mol Biol Cell* 2010; **21**: 3630-3638 [PMID: 20844082 DOI: 10.1091/mbc.E10-04-0312]

23 **Liu W**, Zhang R, Shu R, Yu J, Li H, Long H, Jin S, Li S, Hu Q, Yao F, Zhou C, Huang Q, Hu X, Chen M, Hu W, Wang Q, Fang S, Wu Q. Study of the Relationship between Microbiome and Colorectal Cancer Susceptibility Using 16SrRNA Sequencing. *Biomed Res Int* 2020; **2020**: 7828392 [PMID: 32083132 DOI: 10.1155/2020/7828392]

24 **Valli A**, Morotti M, Zois CE, Albers PK, Soga T, Feldinger K, Fischer R, Frejno M, McIntyre A, Bridges E, Haider S, Buffa FM, Baban D, Rodriguez M, Yanes O, Whittington HJ, Lake HA, Zervou S, Lygate CA, Kessler BM, Harris AL. Adaptation to HIF1α Deletion in Hypoxic Cancer Cells by Upregulation of GLUT14 and Creatine Metabolism. *Mol Cancer Res* 2019; **17**: 1531-1544 [PMID: 30885992 DOI: 10.1158/1541-7786.MCR-18-0315]

25 **Bürtin F**, Mullins CS, Linnebacher M. Mouse models of colorectal cancer: Past, present and future perspectives. *World J Gastroenterol* 2020; **26**: 1394-1426 [PMID: 32308343 DOI: 10.3748/wjg.v26.i13.1394]

26 **McIntyre RE**, Buczacki SJ, Arends MJ, Adams DJ. Mouse models of colorectal cancer as preclinical models. *Bioessays* 2015; **37**: 909-920 [PMID: 26115037 DOI: 10.1002/bies.201500032]

27 **Irving AA**, Yoshimi K, Hart ML, Parker T, Clipson L, Ford MR, Kuramoto T, Dove WF, Amos-Landgraf JM. The utility of Apc-mutant rats in modeling human colon cancer. *Dis Model Mech* 2014; **7**: 1215-1225 [PMID: 25288683 DOI: 10.1242/dmm.016980]

28 **Zeineldin M**, Neufeld KL. More than two decades of Apc modeling in rodents. *Biochim Biophys Acta* 2013; **1836**: 80-89 [PMID: 23333833 DOI: 10.1016/j.bbcan.2013.01.001]

29 **Young M**, Ordonez L, Clarke AR. What are the best routes to effectively model human colorectal cancer? *Mol Oncol* 2013; **7**: 178-189 [PMID: 23465602 DOI: 10.1016/j.molonc.2013.02.006]

30 **Sobhani I**, Amiot A, Le Baleur Y, Levy M, Auriault ML, Van Nhieu JT, Delchier JC. Microbial dysbiosis and colon carcinogenesis: could colon cancer be considered a bacteria-related disease? *Therap Adv Gastroenterol* 2013; **6**: 215-229 [PMID: 23634186 DOI: 10.1177/1756283X12473674]

31 **Sheflin AM**, Whitney AK, Weir TL. Cancer-promoting effects of microbial dysbiosis. *Curr Oncol Rep* 2014; **16**: 406 [PMID: 25123079 DOI: 10.1007/s11912-014-0406-0]

32 **Alhinai EA**, Walton GE, Commane DM. The Role of the Gut Microbiota in Colorectal Cancer Causation. *Int J Mol Sci* 2019; **20** [PMID: 31653078 DOI: 10.3390/ijms20215295]

33 **Khan AA**, Khan Z, Malik A, Kalam MA, Cash P, Ashraf MT, Alshamsan A. Colorectal cancer-inflammatory bowel disease nexus and felony of Escherichia coli. *Life Sci* 2017; **180**: 60-67 [PMID: 28506682 DOI: 10.1016/j.lfs.2017.05.016]

34 **Bleich RM**, Arthur JC. Revealing a microbial carcinogen. *Science* 2019; **363**: 689-690 [PMID: 30765550 DOI: 10.1126/science.aaw5475]

35 **Wilson MR**, Jiang Y, Villalta PW, Stornetta A, Boudreau PD, Carrá A, Brennan CA, Chun E, Ngo L, Samson LD, Engelward BP, Garrett WS, Balbo S, Balskus EP. The human gut bacterial genotoxin colibactin alkylates DNA. *Science* 2019; **363** [PMID: 30765538 DOI: 10.1126/science.aar7785]

36 **Pleguezuelos-Manzano C**, Puschhof J, Rosendahl Huber A, van Hoeck A, Wood HM, Nomburg J, Gurjao C, Manders F, Dalmasso G, Stege PB, Paganelli FL, Geurts MH, Beumer J, Mizutani T,

Miao Y, van der Linden R, van der Elst S; Genomics England Research Consortium, Garcia KC, Top J, Willems RJL, Giannakis M, Bonnet R, Quirke P, Meyerson M, Cuppen E, van Boxtel R, Clevers H. Mutational signature in colorectal cancer caused by genotoxic pks^{+} E. coli. *Nature* 2020; **580**: 269-273 [PMID: 32106218 DOI: 10.1038/s41586-020-2080-8]

37 **Cougnoux A**, Delmas J, Gibold L, Faïs T, Romagnoli C, Robin F, Cuevas-Ramos G, Oswald E, Darfeuille-Michaud A, Prati F, Dalmasso G, Bonnet R. Small-molecule inhibitors prevent the genotoxic and protumoural effects induced by colibactin-producing bacteria. *Gut* 2016; **65**: 278-285 [PMID: 25588406 DOI: 10.1136/gutjnl-2014-307241]

38 **Lucas C**, Salesse L, Hoang MHT, Bonnet M, Sauvanet P, Larabi A, Godfraind C, Gagnière J, Pezet D, Rosenstiel P, Barnich N, Bonnet R, Dalmasso G, Nguyen HTT. Autophagy of Intestinal Epithelial Cells Inhibits Colorectal Carcinogenesis Induced by Colibactin-Producing Escherichia coli in Apc$^{Min/+}$ Mice. *Gastroenterology* 2020; **158**: 1373-1388 [PMID: 31917256 DOI: 10.1053/j.gastro.2019.12.026]

39 **Irrazabal T**, Thakur BK, Kang M, Malaise Y, Streutker C, Wong EOY, Copeland J, Gryfe R, Guttman DS, Navarre WW, Martin A. Limiting oxidative DNA damage reduces microbe-induced colitis-associated colorectal cancer. *Nat Commun* 2020; **11**: 1802 [PMID: 32286276 DOI: 10.1038/s41467-020-15549-6]

40 **Lv Y**, Ye T, Wang HP, Zhao JY, Chen WJ, Wang X, Shen CX, Wu YB, Cai YK. Suppression of colorectal tumorigenesis by recombinant *Bacteroides fragilis* enterotoxin-2 *in vivo*. *World J Gastroenterol* 2017; **23**: 603-613 [PMID: 28216966 DOI: 10.3748/wjg.v23.i4.603]

41 **Attene-Ramos MS**, Wagner ED, Gaskins HR, Plewa MJ. Hydrogen sulfide induces direct radical-associated DNA damage. *Mol Cancer Res* 2007; **5**: 455-459 [PMID: 17475672 DOI: 10.1158/1541-7786.MCR-06-0439]

42 **Dahmus JD**, Kotler DL, Kastenberg DM, Kistler CA. The gut microbiome and colorectal cancer: a review of bacterial pathogenesis. *J Gastrointest Oncol* 2018; **9**: 769-777 [PMID: 30151274 DOI: 10.21037/jgo.2018.04.07]

43 **van Elsland D**, Neefjes J. Bacterial infections and cancer. *EMBO Rep* 2018; **19** [PMID: 30348892 DOI: 10.15252/embr.201846632]

44 **Bhatt AP**, Redinbo MR, Bultman SJ. The role of the microbiome in cancer development and therapy. *CA Cancer J Clin* 2017; **67**: 326-344 [PMID: 28481406 DOI: 10.3322/caac.21398]

45 **Richard ML**, Liguori G, Lamas B, Brandi G, da Costa G, Hoffmann TW, Pierluigi Di Simone M, Calabrese C, Poggioli G, Langella P, Campieri M, Sokol H. Mucosa-associated microbiota dysbiosis in colitis associated cancer. *Gut Microbes* 2018; **9**: 131-142 [PMID: 28914591 DOI: 10.1080/19490976.2017.1379637]

46 **Yu LC**, Wei SC, Ni YH. Impact of microbiota in colorectal carcinogenesis: lessons from experimental models. *Intest Res* 2018; **16**: 346-357 [PMID: 30090033 DOI: 10.5217/ir.2018.16.3.346]

47 **Alto NM**, Orth K. Subversion of cell signaling by pathogens. *Cold Spring Harb Perspect Biol* 2012; **4**: a006114 [PMID: 22952390 DOI: 10.1101/cshperspect.a006114]

48 **Lahiani A**, Yavin E, Lazarovici P. The Molecular Basis of Toxins' Interactions with Intracellular Signaling *via* Discrete Portals. *Toxins (Basel)* 2017; **9** [PMID: 28300784 DOI: 10.3390/toxins9030107]

49 **Tomkovich S**, Yang Y, Winglee K, Gauthier J, Mühlbauer M, Sun X, Mohamadzadeh M, Liu X, Martin P, Wang GP, Oswald E, Fodor AA, Jobin C. Locoregional Effects of Microbiota in a Preclinical Model of Colon Carcinogenesis. *Cancer Res* 2017; **77**: 2620-2632 [PMID: 28416491 DOI: 10.1158/0008-5472.CAN-16-3472]

50 **Yang Y**, Weng W, Peng J, Hong L, Yang L, Toiyama Y, Gao R, Liu M, Yin M, Pan C, Li H, Guo B, Zhu Q, Wei Q, Moyer MP, Wang P, Cai S, Goel A, Qin H, Ma Y. Fusobacterium nucleatum Increases Proliferation of Colorectal Cancer Cells and Tumor Development in Mice by Activating Toll-Like Receptor 4 Signaling to Nuclear Factor-κB, and Up-regulating Expression of MicroRNA-21. *Gastroenterology* 2017; **152**: 851-866.e24 [PMID: 27876571 DOI: 10.1053/j.gastro.2016.11.018]

51 **Wu Y**, Wu J, Chen T, Li Q, Peng W, Li H, Tang X, Fu X. Fusobacterium nucleatum Potentiates Intestinal Tumorigenesis in Mice *via* a Toll-Like Receptor 4/p21-Activated Kinase 1 Cascade. *Dig Dis Sci* 2018; **63**: 1210-1218 [PMID: 29508166 DOI: 10.1007/s10620-018-4999-2]

52 **Chen T**, Li Q, Wu J, Wu Y, Peng W, Li H, Wang J, Tang X, Peng Y, Fu X. Fusobacterium nucleatum promotes M2 polarization of macrophages in the microenvironment of colorectal tumours *via* a TLR4-dependent mechanism. *Cancer Immunol Immunother* 2018; **67**: 1635-1646 [PMID: 30121899 DOI: 10.1007/s00262-018-2233-x]

53 **Rubinstein MR**, Baik JE, Lagana SM, Han RP, Raab WJ, Sahoo D, Dalerba P, Wang TC, Han YW. *Fusobacterium nucleatum* promotes colorectal cancer by inducing Wnt/β-catenin modulator Annexin A1. *EMBO Rep* 2019; **20** [PMID: 30833345 DOI: 10.15252/embr.201847638]

54 **Dejea CM**, Fathi P, Craig JM, Boleij A, Taddese R, Geis AL, Wu X, DeStefano Shields CE, Hechenbleikner EM, Huso DL, Anders RA, Giardiello FM, Wick EC, Wang H, Wu S, Pardoll DM, Housseau F, Sears CL. Patients with familial adenomatous polyposis harbor colonic biofilms containing tumorigenic bacteria. *Science* 2018; **359**: 592-597 [PMID: 29420293 DOI: 10.1126/science.aah3648]

55 **Chung L**, Thiele Orberg E, Geis AL, Chan JL, Fu K, DeStefano Shields CE, Dejea CM, Fathi P, Chen J, Finard BB, Tam AJ, McAllister F, Fan H, Wu X, Ganguly S, Lebid A, Metz P, Van

Meerbeke SW, Huso DL, Wick EC, Pardoll DM, Wan F, Wu S, Sears CL, Housseau F. Bacteroides fragilis Toxin Coordinates a Pro-carcinogenic Inflammatory Cascade *via* Targeting of Colonic Epithelial Cells. *Cell Host Microbe* 2018; **23**: 203-214.e5 [PMID: 29398651 DOI: 10.1016/j.chom.2018.01.007]

56 **Goodwin AC**, Destefano Shields CE, Wu S, Huso DL, Wu X, Murray-Stewart TR, Hacker-Prietz A, Rabizadeh S, Woster PM, Sears CL, Casero RA Jr. Polyamine catabolism contributes to enterotoxigenic Bacteroides fragilis-induced colon tumorigenesis. *Proc Natl Acad Sci USA* 2011; **108**: 15354-15359 [PMID: 21876161 DOI: 10.1073/pnas.1010203108]

57 **He Z**, Gharaibeh RZ, Newsome RC, Pope JL, Dougherty MW, Tomkovich S, Pons B, Mirey G, Vignard J, Hendrixson DR, Jobin C. *Campylobacter jejuni* promotes colorectal tumorigenesis through the action of cytolethal distending toxin. *Gut* 2019; **68**: 289-300 [PMID: 30377189 DOI: 10.1136/gutjnl-2018-317200]

58 **Li Y**, Kundu P, Seow SW, de Matos CT, Aronsson L, Chin KC, Kärre K, Pettersson S, Greicius G. Gut microbiota accelerate tumor growth *via* c-jun and STAT3 phosphorylation in APCMin/+ mice. *Carcinogenesis* 2012; **33**: 1231-1238 [PMID: 22461519 DOI: 10.1093/carcin/bgs137]

59 **Nalbantoglu I**, Blanc V, Davidson NO. Characterization of Colorectal Cancer Development in Apc (min/+) Mice. *Methods Mol Biol* 2016; **1422**: 309-327 [PMID: 27246043 DOI: 10.1007/978-1-4939-3603-8_27]

60 **Wu S**, Rhee KJ, Albesiano E, Rabizadeh S, Wu X, Yen HR, Huso DL, Brancati FL, Wick E, McAllister F, Housseau F, Pardoll DM, Sears CL. A human colonic commensal promotes colon tumorigenesis *via* activation of T helper type 17 T cell responses. *Nat Med* 2009; **15**: 1016-1022 [PMID: 19701202 DOI: 10.1038/nm.2015]

61 **Rhee KJ**, Wu S, Wu X, Huso DL, Karim B, Franco AA, Rabizadeh S, Golub JE, Mathews LE, Shin J, Sartor RB, Golenbock D, Hamad AR, Gan CM, Housseau F, Sears CL. Induction of persistent colitis by a human commensal, enterotoxigenic Bacteroides fragilis, in wild-type C57BL/6 mice. *Infect Immun* 2009; **77**: 1708-1718 [PMID: 19188353 DOI: 10.1128/IAI.00814-08]

62 **Toprak NU**, Yagci A, Gulluoglu BM, Akin ML, Demirkalem P, Celenk T, Soyletir G. A possible role of Bacteroides fragilis enterotoxin in the aetiology of colorectal cancer. *Clin Microbiol Infect* 2006; **12**: 782-786 [PMID: 16842574 DOI: 10.1111/j.1469-0691.2006.01494.x]

63 **Dai Z**, Zhang J, Wu Q, Chen J, Liu J, Wang L, Chen C, Xu J, Zhang H, Shi C, Li Z, Fang H, Lin C, Tang D, Wang D. The role of microbiota in the development of colorectal cancer. *Int J Cancer* 2019; **145**: 2032-2041 [PMID: 30474116 DOI: 10.1002/ijc.32017]

64 **Belkaid Y**, Hand TW. Role of the microbiota in immunity and inflammation. *Cell* 2014; **157**: 121-141 [PMID: 24679531 DOI: 10.1016/j.cell.2014.03.011]

65 **Chen J**, Pitmon E, Wang K. Microbiome, inflammation and colorectal cancer. *Semin Immunol* 2017; **32**: 43-53 [PMID: 28982615 DOI: 10.1016/j.smim.2017.09.006]

66 **McClellan JL**, Davis JM, Steiner JL, Day SD, Steck SE, Carmichael MD, Murphy EA. Intestinal inflammatory cytokine response in relation to tumorigenesis in the Apc(Min/+) mouse. *Cytokine* 2012; **57**: 113-119 [PMID: 22056354 DOI: 10.1016/j.cyto.2011.09.027]

67 **Guo P**, Tian Z, Kong X, Yang L, Shan X, Dong B, Ding X, Jing X, Jiang C, Jiang N, Yu Y. FadA promotes DNA damage and progression of Fusobacterium nucleatum-induced colorectal cancer through up-regulation of chk2. *J Exp Clin Cancer Res* 2020; **39**: 202 [PMID: 32993749 DOI: 10.1186/s13046-020-01677-w]

68 **Rubinstein MR**, Wang X, Liu W, Hao Y, Cai G, Han YW. Fusobacterium nucleatum promotes colorectal carcinogenesis by modulating E-cadherin/β-catenin signaling *via* its FadA adhesin. *Cell Host Microbe* 2013; **14**: 195-206 [PMID: 23954158 DOI: 10.1016/j.chom.2013.07.012]

69 **Arthur JC**, Perez-Chanona E, Mühlbauer M, Tomkovich S, Uronis JM, Fan TJ, Campbell BJ, Abujamel T, Dogan B, Rogers AB, Rhodes JM, Stintzi A, Simpson KW, Hansen JJ, Keku TO, Fodor AA, Jobin C. Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science* 2012; **338**: 120-123 [PMID: 22903521 DOI: 10.1126/science.1224820]

70 **Grivennikov SI**, Wang K, Mucida D, Stewart CA, Schnabl B, Jauch D, Taniguchi K, Yu GY, Osterreicher CH, Hung KE, Datz C, Feng Y, Fearon ER, Oukka M, Tessarollo L, Coppola V, Yarovinsky F, Cheroutre H, Eckmann L, Trinchieri G, Karin M. Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. *Nature* 2012; **491**: 254-258 [PMID: 23034650 DOI: 10.1038/nature11465]

71 **Mager LF**, Wasmer MH, Rau TT, Krebs P. Cytokine-Induced Modulation of Colorectal Cancer. *Front Oncol* 2016; **6**: 96 [PMID: 27148488 DOI: 10.3389/fonc.2016.00096]

72 **Rowland I**, Gibson G, Heinken A, Scott K, Swann J, Thiele I, Tuohy K. Gut microbiota functions: metabolism of nutrients and other food components. *Eur J Nutr* 2018; **57**: 1-24 [PMID: 28393285 DOI: 10.1007/s00394-017-1445-8]

73 **Conlon MA**, Bird AR. The impact of diet and lifestyle on gut microbiota and human health. *Nutrients* 2014; **7**: 17-44 [PMID: 25545101 DOI: 10.3390/nu7010017]

74 **Holscher HD**. Dietary fiber and prebiotics and the gastrointestinal microbiota. *Gut Microbes* 2017; **8**: 172-184 [PMID: 28165863 DOI: 10.1080/19490976.2017.1290756]

75 **Bultman SJ**, Jobin C. Microbial-derived butyrate: an oncometabolite or tumor-suppressive metabolite? *Cell Host Microbe* 2014; **16**: 143-145 [PMID: 25121740 DOI: 10.1016/j.chom.2014.07.011]

76 **Belcheva A**, Irrazabal T, Robertson SJ, Streutker C, Maughan H, Rubino S, Moriyama EH, Copeland JK, Surendra A, Kumar S, Green B, Geddes K, Pezo RC, Navarre WW, Milosevic M,

Wilson BC, Girardin SE, Wolever TMS, Edelmann W, Guttman DS, Philpott DJ, Martin A. Gut microbial metabolism drives transformation of MSH2-deficient colon epithelial cells. *Cell* 2014; **158**: 288-299 [PMID: 25036629 DOI: 10.1016/j.cell.2014.04.051]

77 **Gill PA**, van Zelm MC, Muir JG, Gibson PR. Review article: short chain fatty acids as potential therapeutic agents in human gastrointestinal and inflammatory disorders. *Aliment Pharmacol Ther* 2018; **48**: 15-34 [PMID: 29722430 DOI: 10.1111/apt.14689]

78 **O'Keefe SJ**. Diet, microorganisms and their metabolites, and colon cancer. *Nat Rev Gastroenterol Hepatol* 2016; **13**: 691-706 [PMID: 27848961 DOI: 10.1038/nrgastro.2016.165]

79 **Zitvogel L**, Daillère R, Roberti MP, Routy B, Kroemer G. Anticancer effects of the microbiome and its products. *Nat Rev Microbiol* 2017; **15**: 465-478 [PMID: 28529325 DOI: 10.1038/nrmicro.2017.44]

80 **Louis P**, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. *Nat Rev Microbiol* 2014; **12**: 661-672 [PMID: 25198138 DOI: 10.1038/nrmicro3344]

81 **Leavitt J**, Saleh N. The Microbiome and Colorectal Cancer: Current Clinical Trials. *Oncology (Williston Park)* 2019; **33**: 78 [PMID: 30784035]

82 **Stadler Z**. Memorial Sloan Kettering Cancer Center. Metagenomic Evaluation of the Gut Microbiome in Patients With Lynch Syndrome and Other Hereditary Colonic Polyposis Syndromes. [accessed 2021 Feb 5]. In: ClinicalTrials.gov [Internet]. New York (NY): U.S. National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/NCT02371135 ClinicalTrials.gov Identifier: NCT02371135

83 **Kariv R**. Turmeric Supplementation on Polyp Number and Size in Patients With Familial Adenomatous Polyposis. [accessed 2021 Feb 5]. In: ClinicalTrials.gov [Internet]. Tel Aviv: U.S. National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/NCT03061591 ClinicalTrials.gov Identifier: NCT03061591

84 **Fong W**, Li Q, Yu J. Gut microbiota modulation: a novel strategy for prevention and treatment of colorectal cancer. *Oncogene* 2020; **39**: 4925-4943 [PMID: 32514151 DOI: 10.1038/s41388-020-1341-1]

85 **Dutta D**, Lim SH. Bidirectional interaction between intestinal microbiome and cancer: opportunities for therapeutic interventions. *Biomark Res* 2020; **8**: 31 [PMID: 32817793 DOI: 10.1186/s40364-020-00211-6]

86 **Perillo F**, Amoroso C, Strati F, Giuffrè MR, Díaz-Basabe A, Lattanzi G, Facciotti F. Gut Microbiota Manipulation as a Tool for Colorectal Cancer Management: Recent Advances in Its Use for Therapeutic Purposes. *Int J Mol Sci* 2020; **21** [PMID: 32751239 DOI: 10.3390/ijms21155389]

87 **Möslein G**. Surgical considerations in FAP-related pouch surgery: Could we do better? *Fam Cancer* 2016; **15**: 457-466 [PMID: 27194409 DOI: 10.1007/s10689-016-9904-6]

88 **Segal JP**, Ding NS, Worley G, Mclaughlin S, Preston S, Faiz OD, Clark SK, Hart AL. Systematic review with meta-analysis: the management of chronic refractory pouchitis with an evidence-based treatment algorithm. *Aliment Pharmacol Ther* 2017; **45**: 581-592 [PMID: 28008631 DOI: 10.1111/apt.13905]

89 **Batista D**, Raffals L. Role of intestinal bacteria in the pathogenesis of pouchitis. *Inflamm Bowel Dis* 2014; **20**: 1481-1486 [PMID: 25046009 DOI: 10.1097/MIB.0000000000000055]

90 **Gionchetti P**, Calafiore A, Riso D, Liguori G, Calabrese C, Vitali G, Laureti S, Poggioli G, Campieri M, Rizzello F. The role of antibiotics and probiotics in pouchitis. *Ann Gastroenterol* 2012; **25**: 100-105 [PMID: 24714229]

91 **Kousgaard SJ**, Michaelsen TY, Nielsen HL, Kirk KF, Albertsen M, Thorlacius-Ussing O. The Microbiota Profile in Inflamed and Non-Inflamed Ileal Pouch-Anal Anastomosis. *Microorganisms* 2020; **8** [PMID: 33092101 DOI: 10.3390/microorganisms8101611]

92 **Shen B**, Achkar JP, Connor JT, Ormsby AH, Remzi FH, Bevins CL, Brzezinski A, Bambrick ML, Fazio VW, Lashner BA. Modified pouchitis disease activity index: a simplified approach to the diagnosis of pouchitis. *Dis Colon Rectum* 2003; **46**: 748-753 [PMID: 12794576 DOI: 10.1007/s10350-004-6652-8]

93 **Tomasz B**, Zoran S, Jarosław W, Ryszard M, Marcin G, Robert B, Piotr K, Lukasz K, Jacek P, Piotr G, Przemysław P, Michał D. Long-term use of probiotics Lactobacillus and Bifidobacterium has a prophylactic effect on the occurrence and severity of pouchitis: a randomized prospective study. *Biomed Res Int* 2014; **2014**: 208064 [PMID: 24579075 DOI: 10.1155/2014/208064]

94 **Drago L**. Probiotics and Colon Cancer. *Microorganisms* 2019; **7** [PMID: 30823471 DOI: 10.3390/microorganisms7030066]

95 **Liong MT**. Roles of probiotics and prebiotics in colon cancer prevention: Postulated mechanisms and in-vivo evidence. *Int J Mol Sci* 2008; **9**: 854-863 [PMID: 19325789 DOI: 10.3390/ijms9050854]

96 **Ni Y**, Wong VH, Tai WC, Li J, Wong WY, Lee MM, Fong FL, El-Nezami H, Panagiotou G. A metagenomic study of the preventive effect of Lactobacillus rhamnosus GG on intestinal polyp formation in $Apc^{Min/+}$ mice. *J Appl Microbiol* 2017; **122**: 770-784 [PMID: 28004480 DOI: 10.1111/jam.13386]

97 **Urbanska AM**, Bhathena J, Cherif S, Prakash S. Orally delivered microencapsulated probiotic formulation favorably impacts polyp formation in APC (Min/+) model of intestinal carcinogenesis. *Artif Cells Nanomed Biotechnol* 2016; **44**: 1-11 [PMID: 25060720 DOI: 10.3109/21691401.2014.898647]

98 **Kennedy EA**, King KY, Baldridge MT. Mouse Microbiota Models: Comparing Germ-Free Mice and Antibiotics Treatment as Tools for Modifying Gut Bacteria. *Front Physiol* 2018; **9**: 1534

[PMID: 30429801 DOI: 10.3389/fphys.2018.01534]

99 **Hale VL**, Chen J, Johnson S, Harrington SC, Yab TC, Smyrk TC, Nelson H, Boardman LA, Druliner BR, Levin TR, Rex DK, Ahnen DJ, Lance P, Ahlquist DA, Chia N. Shifts in the Fecal Microbiota Associated with Adenomatous Polyps. *Cancer Epidemiol Biomarkers Prev* 2017; **26**: 85-94 [PMID: 27672054 DOI: 10.1158/1055-9965.EPI-16-0337]

100 **Xu L**, Surathu A, Raplee I, Chockalingam A, Stewart S, Walker L, Sacks L, Patel V, Li Z, Rouse R. The effect of antibiotics on the gut microbiome: a metagenomics analysis of microbial shift and gut antibiotic resistance in antibiotic treated mice. *BMC Genomics* 2020; **21**: 263 [PMID: 32228448 DOI: 10.1186/s12864-020-6665-2]

101 **Sánchez-Alcoholado L**, Ramos-Molina B, Otero A, Laborda-Illanes A, Ordóñez R, Medina JA, Gómez-Millán J, Queipo-Ortuño MI. The Role of the Gut Microbiome in Colorectal Cancer Development and Therapy Response. *Cancers (Basel)* 2020; **12** [PMID: 32486066 DOI: 10.3390/cancers12061406]

102 **Dik VK**, van Oijen MG, Smeets HM, Siersema PD. Frequent Use of Antibiotics Is Associated with Colorectal Cancer Risk: Results of a Nested Case-Control Study. *Dig Dis Sci* 2016; **61**: 255-264 [PMID: 26289256 DOI: 10.1007/s10620-015-3828-0]

103 **Boursi B**, Haynes K, Mamtani R, Yang YX. Impact of antibiotic exposure on the risk of colorectal cancer. *Pharmacoepidemiol Drug Saf* 2015; **24**: 534-542 [PMID: 25808540 DOI: 10.1002/pds.3765]

104 **Kaur K**, Saxena A, Debnath I, O'Brien JL, Ajami NJ, Auchtung TA, Petrosino JF, Sougiannis AJ, Depaep S, Chumanevich A, Gummadidala PM, Omebeyinje MH, Banerjee S, Chatzistamou I, Chakraborty P, Fayad R, Berger FG, Carson JA, Chanda A. Antibiotic-mediated bacteriome depletion in $Apc^{Min/+}$ mice is associated with reduction in mucus-producing goblet cells and increased colorectal cancer progression. *Cancer Med* 2018; **7**: 2003-2012 [PMID: 29624892 DOI: 10.1002/cam4.1460]

105 **Leystra AA**, Clapper ML. Gut Microbiota Influences Experimental Outcomes in Mouse Models of Colorectal Cancer. *Genes (Basel)* 2019; **10** [PMID: 31703321 DOI: 10.3390/genes10110900]

106 **Ruiz-Canela M**, Bes-Rastrollo M, Martínez-González MA. The Role of Dietary Inflammatory Index in Cardiovascular Disease, Metabolic Syndrome and Mortality. *Int J Mol Sci* 2016; **17** [PMID: 27527152 DOI: 10.3390/ijms17081265]

107 **Adjibade M**, Andreeva VA, Lemogne C, Touvier M, Shivappa N, Hébert JR, Wirth MD, Hercberg S, Galan P, Julia C, Assmann KE, Kesse-Guyot E. The Inflammatory Potential of the Diet Is Associated with Depressive Symptoms in Different Subgroups of the General Population. *J Nutr* 2017; **147**: 879-887 [PMID: 28356432 DOI: 10.3945/jn.116.245167]

108 **Ryu I**, Kwon M, Sohn C, Shivappa N, Hébert JR, Na W, Kim MK. The Association between Dietary Inflammatory Index (DII) and Cancer Risk in Korea: A Prospective Cohort Study within the KoGES-HEXA Study. *Nutrients* 2019; **11** [PMID: 31652856 DOI: 10.3390/nu11112560]

109 **Bodén S**, Myte R, Wennberg M, Harlid S, Johansson I, Shivappa N, Hébert JR, Van Guelpen B, Nilsson LM. The inflammatory potential of diet in determining cancer risk; A prospective investigation of two dietary pattern scores. *PLoS One* 2019; **14**: e0214551 [PMID: 30978193 DOI: 10.1371/journal.pone.0214551]

110 **Pasanisi P**, Gariboldi M, Verderio P, Signoroni S, Mancini A, Rivoltini L, Milione M, Masci E, Ciniselli CM, Bruno E, Macciotta A, Belfiore A, Ricci MT, Gargano G, Morelli D, Apolone G, Vitellaro M. A Pilot Low-Inflammatory Dietary Intervention to Reduce Inflammation and Improve Quality of Life in Patients With Familial Adenomatous Polyposis: Protocol Description and Preliminary Results. *Integr Cancer Ther* 2019; **18**: 1534735419846400 [PMID: 31055940 DOI: 10.1177/1534735419846400]

111 **Cruz-Correa M**, Shoskes DA, Sanchez P, Zhao R, Hylind LM, Wexner SD, Giardiello FM. Combination treatment with curcumin and quercetin of adenomas in familial adenomatous polyposis. *Clin Gastroenterol Hepatol* 2006; **4**: 1035-1038 [PMID: 16757216 DOI: 10.1016/j.cgh.2006.03.020]

112 **Cruz-Correa M**, Hylind LM, Marrero JH, Zahurak ML, Murray-Stewart T, Casero RA Jr, Montgomery EA, Iacobuzio-Donahue C, Brosens LA, Offerhaus GJ, Umar A, Rodriguez LM, Giardiello FM. Efficacy and Safety of Curcumin in Treatment of Intestinal Adenomas in Patients With Familial Adenomatous Polyposis. *Gastroenterology* 2018; **155**: 668-673 [PMID: 29802852 DOI: 10.1053/j.gastro.2018.05.031]

113 **McFadden RM**, Larmonier CB, Shehab KW, Midura-Kiela M, Ramalingam R, Harrison CA, Besselsen DG, Chase JH, Caporaso JG, Jobin C, Ghishan FK, Kiela PR. The Role of Curcumin in Modulating Colonic Microbiota During Colitis and Colon Cancer Prevention. *Inflamm Bowel Dis* 2015; **21**: 2483-2494 [PMID: 26218141 DOI: 10.1097/MIB.0000000000000522]

114 **Wang LS**, Burke CA, Hasson H, Kuo CT, Molmenti CL, Seguin C, Liu P, Huang TH, Frankel WL, Stoner GD. A phase Ib study of the effects of black raspberries on rectal polyps in patients with familial adenomatous polyposis. *Cancer Prev Res (Phila)* 2014; **7**: 666-674 [PMID: 24764585 DOI: 10.1158/1940-6207.CAPR-14-0052]

115 **Pan P**, Lam V, Salzman N, Huang YW, Yu J, Zhang J, Wang LS. Black Raspberries and Their Anthocyanin and Fiber Fractions Alter the Composition and Diversity of Gut Microbiota in F-344 Rats. *Nutr Cancer* 2017; **69**: 943-951 [PMID: 28718724 DOI: 10.1080/01635581.2017.1340491]

116 **Kresty LA**, Fromkes JJ, Frankel WL, Hammond CD, Seeram NP, Baird M, Stoner GD. A phase I pilot study evaluating the beneficial effects of black raspberries in patients with Barrett's esophagus. *Oncotarget* 2018; **9**: 35356-35372 [PMID: 30450163 DOI: 10.18632/oncotarget.10457]

117 **Wang H**, Guan L, Li J, Lai M, Wen X. The Effects of Berberine on the Gut Microbiota in Apc $^{min/+}$ Mice Fed with a High Fat Diet. *Molecules* 2018; **23** [PMID: 30205580 DOI: 10.3390/molecules23092298]

118 **Kim B**, Giardiello FM. Chemoprevention in familial adenomatous polyposis. *Best Pract Res Clin Gastroenterol* 2011; **25**: 607-622 [PMID: 22122775 DOI: 10.1016/j.bpg.2011.08.002]

119 **Tajika M**, Niwa Y, Bhatia V, Tanaka T, Ishihara M, Yamao K. Risk of ileal pouch neoplasms in patients with familial adenomatous polyposis. *World J Gastroenterol* 2013; **19**: 6774-6783 [PMID: 24187452 DOI: 10.3748/wjg.v19.i40.6774]

120 **Vasen HF**, Möslein G, Alonso A, Aretz S, Bernstein I, Bertario L, Blanco I, Bülow S, Burn J, Capella G, Colas C, Engel C, Frayling I, Friedl W, Hes FJ, Hodgson S, Järvinen H, Mecklin JP, Møller P, Myrhøi T, Nagengast FM, Parc Y, Phillips R, Clark SK, de Leon MP, Renkonen-Sinisalo L, Sampson JR, Stormorken A, Tejpar S, Thomas HJ, Wijnen J. Guidelines for the clinical management of familial adenomatous polyposis (FAP). *Gut* 2008; **57**: 704-713 [PMID: 18194984 DOI: 10.1136/gut.2007.136127]

121 **West NJ**, Clark SK, Phillips RK, Hutchinson JM, Leicester RJ, Belluzzi A, Hull MA. Eicosapentaenoic acid reduces rectal polyp number and size in familial adenomatous polyposis. *Gut* 2010; **59**: 918-925 [PMID: 20348368 DOI: 10.1136/gut.2009.200642]

122 **Song M**, Lee IM, Manson JE, Buring JE, Dushkes R, Gordon D, Walter J, Wu K, Chan AT, Ogino S, Fuchs CS, Meyerhardt JA, Giovannucci EL; VITAL Research Group. Effect of Supplementation With Marine ω-3 Fatty Acid on Risk of Colorectal Adenomas and Serrated Polyps in the US General Population: A Prespecified Ancillary Study of a Randomized Clinical Trial. *JAMA Oncol* 2020; **6**: 108-115 [PMID: 31750855 DOI: 10.1001/jamaoncol.2019.4587]

123 **Maseda D**, Ricciotti E. NSAID-Gut Microbiota Interactions. *Front Pharmacol* 2020; **11**: 1153 [PMID: 32848762 DOI: 10.3389/fphar.2020.01153]

124 **Rogers MAM**, Aronoff DM. The influence of non-steroidal anti-inflammatory drugs on the gut microbiome. *Clin Microbiol Infect* 2016; **22**: 178.e1-178.e9 [PMID: 26482265 DOI: 10.1016/j.cmi.2015.10.003]

125 **Montrose DC**, Zhou XK, McNally EM, Sue E, Yantiss RK, Gross SS, Leve ND, Karoly ED, Suen CS, Ling L, Benezra R, Pamer EG, Dannenberg AJ. Celecoxib Alters the Intestinal Microbiota and Metabolome in Association with Reducing Polyp Burden. *Cancer Prev Res (Phila)* 2016; **9**: 721-731 [PMID: 27432344 DOI: 10.1158/1940-6207.CAPR-16-0095]

126 **Zhao R**, Coker OO, Wu J, Zhou Y, Zhao L, Nakatsu G, Bian X, Wei H, Chan AWH, Sung JJY, Chan FKL, El-Omar E, Yu J. Aspirin Reduces Colorectal Tumor Development in Mice and Gut Microbes Reduce its Bioavailability and Chemopreventive Effects. *Gastroenterology* 2020; **159**: 969-983.e4 [PMID: 32387495 DOI: 10.1053/j.gastro.2020.05.004]

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World J Gastrointest Oncol 2021 June 15; 13(6): 509-535

DOI: 10.4251/wjgo.v13.i6.509

ISSN 1948-5204 (online)

REVIEW

Application of the woodchuck animal model for the treatment of hepatitis B virus-induced liver cancer

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Author contributions: Suresh M and Menne S wrote the manuscript. All authors have read and approve the final manuscript.

Conflict-of-interest statement: Manasa Suresh declares no conflict of interest for this article. Stephan Menne serves occasionally as a paid scientific consultant to Northeastern Wildlife, Inc. (Harris, ID), the only commercial source for woodchucks within the United States.

Manuscript source: Invited manuscript

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Abstract

This review describes woodchucks chronically infected with the woodchuck hepatitis virus (WHV) as an animal model for hepatocarcinogenesis and treatment of primary liver cancer or hepatocellular carcinoma (HCC) induced by the hepatitis B virus (HBV). Since laboratory animal models susceptible to HBV infection are limited, woodchucks experimentally infected with WHV, a hepatitis virus closely related to HBV, are increasingly used to enhance our understanding of virus-host interactions, immune response, and liver disease progression. A correlation of severe liver pathogenesis with high-level viral replication and deficient antiviral immunity has been established, which are present during chronic infection after WHV inoculation of neonatal woodchucks for modeling vertical HBV transmission in humans. HCC in chronic carrier woodchucks develops 17 to 36 mo after neonatal WHV infection and involves liver tumors that are comparable in size, morphology, and molecular gene signature to those of HBV-infected patients. Accordingly, woodchucks with WHV-induced liver tumors have been used for the improvement of imaging and ablation techniques of human HCC. In addition, drug efficacy studies in woodchucks with chronic WHV infection have revealed that prolonged treatment with nucleos(t)ide analogs, alone or in combination with other compounds, minimizes the risk of liver disease progression to HCC. More recently, woodchucks have been utilized in the delineation of mechanisms involved in innate and adaptive immune responses against WHV during acute, self-limited and chronic infections. Therapeutic interventions based on modulating the deficient host antiviral immunity have been explored in woodchucks for inducing functional cure in HBV-infected patients and for reducing or even delaying associated liver disease sequelae, including the onset of HCC. Therefore, woodchucks with chronic WHV infection constitute a well-characterized, fully immunocompetent animal model for HBV-induced liver cancer and for preclinical evaluation of the safety and efficacy of new modalities, which are based on chemo, gene, and immune therapy, for the prevention and treatment of HCC in patients for which current treatment options are dismal.

Specialty type: Gastroenterology and hepatology

Country/Territory of origin: United States

Peer-review report's scientific quality classification
Grade A (Excellent): A
Grade B (Very good): 0
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: February 21, 2021
Peer-review started: February 21, 2021
First decision: April 19, 2021
Revised: May 2, 2021
Accepted: May 15, 2021
Article in press: May 15, 2021
Published online: June 15, 2021

P-Reviewer: Kai K
S-Editor: Gong ZM
L-Editor: A
P-Editor: Li JH

Key Words: Woodchuck; Hepatitis B virus; Chronic infection; Liver disease; Hepatocellular carcinoma; Cancer treatment

Core Tip: Hepatitis B virus-induced liver tumors are hard to treat with currently available interventions and the prognosis of hepatocellular carcinoma (HCC) in patients remains still poor. Immunocompetent woodchucks are a useful animal model for human HCC, because multiple tumors at different stages develop spontaneously and secondary to viral infection. This similarity to human hepatocarcinogenesis and the animal's vascular architecture allowing catheterization with human-sized products have increased the preclinical use of this model to improve existing imaging (ultrasound, magnetic resonance imaging, and positron-emission tomography) and ablation techniques (embolization and radiotherapy) and to evaluate interventions (chemo, gene, and immune therapy) intended to treat human HCC.

Citation: Suresh M, Menne S. Application of the woodchuck animal model for the treatment of hepatitis B virus-induced liver cancer. *World J Gastrointest Oncol* 2021; 13(6): 509-535
URL: https://www.wjgnet.com/1948-5204/full/v13/i6/509.htm
DOI: https://dx.doi.org/10.4251/wjgo.v13.i6.509

INTRODUCTION

Infection of adult humans with the hepatitis B virus (HBV) usually leads to self-limited liver disease (*i.e.*, acute hepatitis B) and viral resolution, as the virus is controlled *via* a strong antiviral immune response[1,2]. Progression to chronic HBV infection is observed infrequently and occurs only in 5% of infected, healthy adults[3]. However, HBV infection acquired at birth by mother-to-child transfer or during early childhood in unvaccinated infants persists in 95% of individuals[3]. Persistent HBV infection then leads to chronic liver disease (*i.e.*, chronic hepatitis B) that is associated with a diminished or impaired immune response unable to control the virus[1,2]. The immunodeficiencies developed overtime during the persistence of HBV infection are further responsible for the progression of liver disease to liver cirrhosis and hepatocellular carcinoma (HCC) later in life[1,4]. Estimates indicate that approximately 257 million people worldwide are chronic carriers of HBV[5]. Without antiviral treatment and/or liver transplantation, these individuals will die, because end-stage HCC has a low five-year survival rate of about 10%[6]. The therapeutic interventions available for the treatment of chronic HBV infection and associated liver disease sequelae are suboptimal, as they rarely induce viral clearance or significantly lower the risk of HCC development and either require lifelong administration or are associated sometimes with severe adverse effects[4,7-10]. HCC has a high mortality rate because it is frequently asymptomatic and medical attention is often sought when removal by surgery (*i.e.*, hepatectomy) is limited or impossible[11,12]. The poor prognosis of HCC at an advanced stage is mainly due to its unresponsiveness to chemotherapy [11,13-16]. Although tyrosine kinase inhibitors such as sorafenib have demonstrated survival benefits among patients with advanced liver cancer, the prognosis of patients with HCC remains dismal, with tumor recurrence rates of 50% after three years[17]. Thus, chronic HBV infection is a major source of human HCC, which is the fifth most common cancer in the world and the third leading cause of cancer deaths[11,18-20]. Compared to uninfected individuals, the lifetime risk of developing HCC is significantly increased by 15- to 20-fold in patients positive for the HBV surface antigen (HBsAg), and can reach 100-fold in individuals with high levels of HBV replication and serum positivity for HBV e antigen (HBeAg)[20]. The HCC lifetime risk remains increased even after spontaneous clearance of the infection[21]. Therefore, the large reservoir of chronic HBV carriers could benefit immensely from the development of more effective and safer antiviral and anticancer therapies that cure the infection, eliminate the risk of liver disease progression, and/or eradicate established HCC.

Woodchuck hepatitis virus (WHV) infects naturally the Eastern woodchuck (*Marmota monax*) that habitats large areas within North America, including most eastern and midwestern states in the United States, southeastern Alaska, and southern Canada[22]. WHV was initially discovered in 1977 at the Philadelphia Zoo in a colony of woodchucks where several animals died due to chronic hepatitis B and HCC[23,24]. Subsequent studies revealed that WHV is closely related to HBV in regard to the nucleic acid sequence and organization of the genome, virion morphology, and mechanisms of infection and replication[23,25-28]. Consequently, WHV and HBV were classified as members of the genus *Orthohepadnavirus* within the *Hepadnaviridae* family [29]. Comparable to HBV infection in humans, WHV in woodchucks also causes age-dependent acute, self-limited or chronic outcomes of infection[23,30-33].

Early progress in the development of the woodchuck as an animal model for HBV infection involved basic studies on virological response and liver tumor development that are associated with experimental WHV infection of neonatal and adult woodchucks. Thereafter, neonatal WHV inoculation progressing to chronic viral infection during adulthood has been initially applied for the evaluation of conventional vaccines and nucleos(t)ide analogs for safety and efficacy against HBV[30,34-37]. More recently, the neonatal inoculation model of chronic WHV infection has been increasingly used for the development of immunomodulators, including those stimulating pathogen recognition receptors (PRRs) or blocking immune checkpoint markers[34,38,39]. While some of these studies provided evidence for the prevention and treatment of liver disease progression[37,38], evaluation of interventions directly targeting liver tumors in woodchucks for the treatment of HCC is limited. Since immunopathogenesis and liver disease progression to HCC induced by WHV parallels HBV infection in humans more so than in any other animal model currently available for HBV research[30,34,38-40], woodchucks with established liver tumors have been further applied in the improvement of imaging and ablation techniques and in the evaluation of new therapeutic approaches for the treatment of human HCC. The purpose of this review is to highlight the woodchuck as an animal model for hepatitis virus-induced carcinogenesis and treatment of HCC in patients with chronic HBV infection.

WHV infection and liver disease progression

Inoculation of adult woodchucks with WHV almost always results in the acute, self-limited (*i.e.*, resolved) outcome of infection[33,41-44]. Although virtually 100% of hepatocytes in the liver become infected with WHV[45], antiviral control is achieved by strong innate and adaptive immune responses. In the liver, innate immune response is activated within hours after experimental infection and partially inhibits WHV replication[46], although the infection expands further and reaches a peak thereafter. After a lack phase of immune response induction probably due to the "stealth-like behavior" of hepatitis viruses[38,47], a second, more marked, suppression of WHV replication is observed that is mediated by a non-cytolytic mechanism of viral clearance involving type I and II interferons (IFNs)[48]. IFN-α and IFN-β are most likely produced by activated PRRs after sensing of viral DNA and RNA in the liver, while IFN-γ is mainly secreted by natural killer (NK) cells. These antiviral cytokines inhibit the transcription of viral pre-genomic RNA from the episomal, covalently-closed circular (ccc) DNA genome in the nucleus of infected hepatocytes, block its packaging into nucleocapsids, prevent viral replication through upregulation of a ribonuclease, and/or impede synthesis of viral relaxed-circular (rc) DNA within these core particles during maturation, as shown for HBV in cell culture[49-53]and animal models[54-56]. However, these antiviral cytokines do not affect the levels of WHV e and surface antigens (WHeAg and WHsAg) in the periphery of woodchucks[48,57]. This is followed by a cytolytic mechanism of viral clearance leading to a nearly complete loss of both serum viremia and antigenemia, as well as of intrahepatic WHV cccDNA[48,57]. This mechanism involves killing of infected hepatocytes *via* mainly cytolytic T lymphocytes (CTLs), apoptosis, and regeneration of hepatocytes, resulting in transient, moderate to marked hepatic inflammation and liver injury[48,58-60]. In addition, virus-neutralizing, protective antibodies against WHsAg, as well as antibodies against WHV core antigen (WHcAg) and WHeAg, are elicited by B-cells[48,61]. The concerted actions of the immune system then lead to an almost complete shutdown of viral replication in the liver and clearance of the virus from the periphery, although residual amounts of replication-competent WHV and viral cccDNA often remain detectable in serum and in liver, spleen, and blood cells after resolution[45,61-64]. Truncated and thus replication-incompetent WHV DNA is found integrated into the chromosomal DNA of hepatocytes[65-67]. Such viral DNA is typically rearranged and targets different sites within the cellular DNA, suggesting

that these integration events may contribute to hepatocarcinogenesis. The presence of unintegrated and integrated virus appears to correlate with an overall lifetime risk of HCC development in 5%-20% of woodchucks after resolution of acute WHV infection [64,68].

This is in contrast to the inoculation of neonatal woodchucks with WHV (Figure 1), which leads to the chronic outcome of infection in approximately 60%-75% of animals later in life, and thus models the effect of age on the outcome of HBV infection in humans[31,33,41]. Persistent WHV infection in these animals involves an ongoing viral replication in liver, minimal to moderate hepatic inflammation and liver injury, and high levels of viral DNA and antigens in the periphery. Compared to the virion levels in patients with chronic HBV infection that are in the range of 10^9-10^{10} particles per mL [28], WHV virions often reach 10- to 100-fold greater concentrations in woodchucks with established chronic infection (*i.e.*, 10^{10}-10^{11} particles/mL), while subviral particles containing WHsAg are produced in large excess. Like in human HBV infection[69], a WHV core-related antigen (WHcrAg), including the classical WHcAg and WHeAg, and additionally, the WHV precore-related antigen (WPreC), is produced during infection in woodchucks, with elevated levels present in chronic WHV carriers[57]. The high loads of circulating WHeAg and WHsAg produced during chronic WHV infection in woodchucks are thought to be responsible for the immunological tolerance to the virus at the T- and B-cell level[30,34,39,40], and are further associated with the liver disease progression to chronic hepatitis B and liver cancer[31,70,71]. HCC develops in all animals over a median period of 2 to 2½ years after neonatal inoculation, and the median life expectancy is approximately 6 mo that is similar to the situation in patients with HCC[37,68,72]. More specifically, HCC develops in 50% of woodchucks after 29 mo of chronic WHV infection, in 95% of animals after 3 years, and in 100% of animals by 5 years[73,74]. Thus, chronicity as an outcome of neonatal WHV infection appears to result from a suboptimal or unsuccessful immune response relatively early during the acute phase of infection[30,75,76]. During the later stage of chronic WHV infection, and comparable to chronic HBV infection in patients [1,2,77, 78], a limited type I but a moderate type II IFN response is present in liver[76,79]. Persistent WHV infection is further characterized by the inhibition of antigen presentation to immune cells[80], increases in hepatocyte cytotoxicity *via* perforin-granzyme B and Fas ligand-Fas death pathways[81,82], induction of molecules linked to T-cell exhaustion (*i.e.*, immune checkpoint markers)[79,83], and elevated levels of suppressor of cytokine signaling (SOC3)[79]. Since neutrophils accumulate in woodchuck liver[79], these cells may be responsible for the intrahepatic recruitment of mononuclear inflammatory cells *via* neutrophil-derived metalloproteinases, as observed in a transgenic mouse model of acute hepatitis B and in patients with chronic hepatitis B[84,85]. Liver disease then appears to progress to HCC due to the reduced immune-mediated clearance of WHV-infected hepatocytes by both non-cytolytic and cytolytic mechanisms[30,76], continuing chronic microinflammation [43,86-88], and viral integration events[67,72,89-91]. However, as described in more detail below, these deficiencies in humoral and cellular immune responses present in chronic WHV carrier woodchucks can be altered by different means leading to a functional cure (defined as a loss of viral DNA and surface antigen in serum, with or without seroconversion to virus-neutralizing antibodies[10]) that delays or even prevents HCC onset.

WHV-induced hepatocarcinogenesis

Virus-induced hepatocarcinogenesis in chronic WHV carrier woodchucks is a multistage process (Figure 2). Chronic hepatitis B in these animals is characterized by the mild infiltration of mononuclear cells into portal tracts, sometimes extending beyond the limiting plate[31]. Liver cells with cytoplasmic inclusions are present, which correspond to the ground glass hepatocytes found in the liver of patients with chronic HBV infection and that contain HBV surface antigen (HBsAg)[92]. In HBV transgenic mice, these HBsAg-containing ground glass hepatocytes cluster and form nodules and are seen as preneoplastic lesions[93]. In addition, scattered parenchymal hepatocellular necrosis with neutrophils and macrophages, as well as bile duct proliferation, are usually observed in woodchucks, and in some cases early fibrosis was noted but hepatic cirrhosis and ascites is essentially absent[32,86,87,94,95]. Clinical manifestation of cirrhosis, however, is also absent in a minority of human HCCs due to chronic HBV infection and approximately 20% of HCCs involve non-cirrhotic livers [96,97]. Neoplasia in woodchuck liver then progresses from foci of altered hepatocytes (FAHs) to neoplastic nodules and HCCs[88,98-100]. These altered hepatocytes often harbor viral DNA integrations[65], as also noted in HBV-infected patients[101,102]. They further have a selective regeneration or survival advantage[65] and may be able to escape immune surveillance due to limited intracellular WHV replication and/or

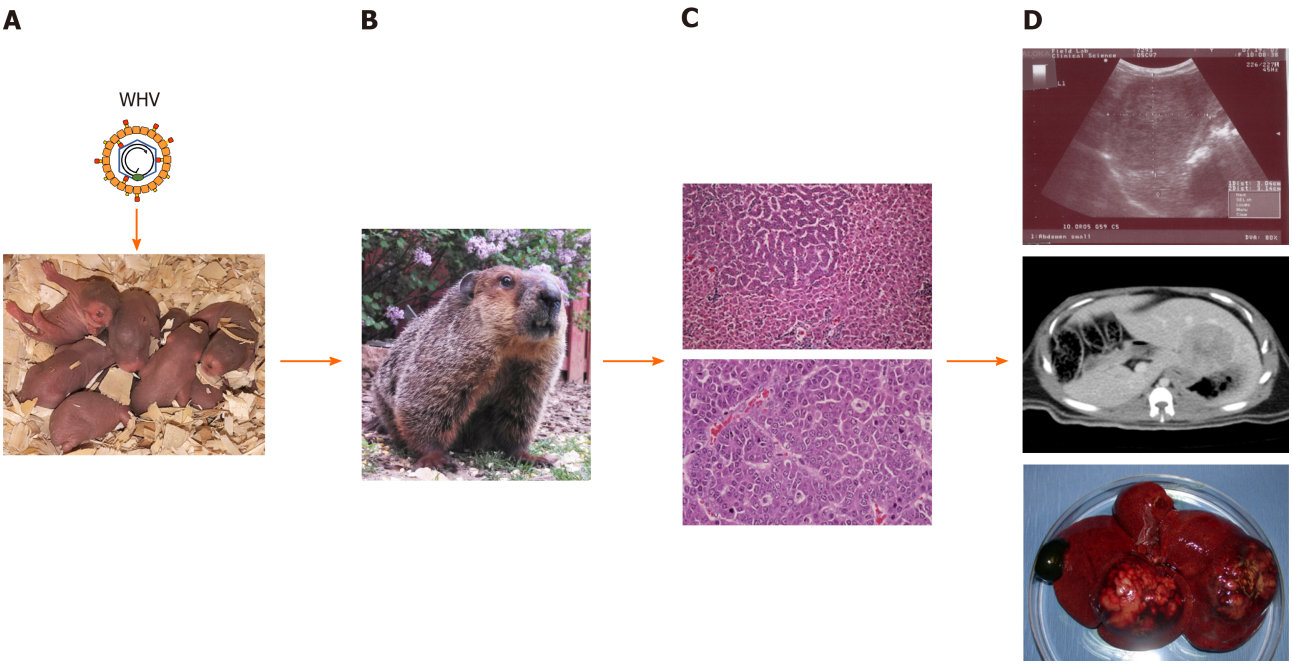

Figure 1 Schematic presentation of woodchuck hepatitis virus-induced liver disease progression and detection of tumors within woodchuck liver. A: Neonatal woodchucks are experimentally infected with woodchuck hepatitis virus (WHV) to model vertical hepatitis B virus transmission in humans; B: WHV infection progresses to chronic hepatitis B in adult woodchucks after approximately 1 year; C: Chronic WHV carrier woodchucks develop liver tumors during the next 1-1½ years. A focus of altered hepatocytes (FAH) in liver (top) and an undifferentiated liver tumor (bottom) are shown; D: Localization of liver tumors by ultrasonography (top) and computed tomography (middle). The liver of a woodchuck with two larger hepatocellular neoplasms (HCC) is shown (bottom). With permission from Elsevier, pictures shown in C were reprinted from: Tennant BC, Toshkov IA, Peek SF, Jacob JR, Menne S, Hornbuckle WE, Schinazi RD, Korba BE, Cote PJ, Gerin JL. Hepatocellular carcinoma in the woodchuck model of hepatitis B virus infection. *Gastroenterology* 2004; 127(5): S283-S293. Copyright ©American Gastroenterological Association 2004. Published by Elsevier[37]. WHV: Woodchuck hepatitis virus.

presentation of viral epitopes to immune cells. FAHs are detected as early as 6 mo after neonatal WHV inoculation, while small liver tumors occur as early as 3 mo thereafter[68]. Metastasis of HCC outside of the liver is essentially absent in woodchucks[32,87,94,103], except for rare cases of pulmonary metastasis in a few animals[86,94]. The hepatic neoplasms present in woodchucks are typically well-differentiated, trabecular HCCs, although various histologic types are found in different animals or in different tumors in the same animal[32,94,104]. A comparison of intratumoral transcriptional profiles in woodchucks and HBV-infected patients established that WHV-induced HCC shares molecular characteristics with two subtypes of human HCC[79]. One HCC signature present in woodchucks correlated well with the human HCC subclass of poor prognosis (''poor survival subclass'') that is characterized by low-level cluster of differentiation (CD) 8+ T-cell and NK-cell infiltration[105]. The second HCC signature in woodchucks was associated with the S2 subclass, a well-defined human HCC subtype[106], which is characterized by the activation of the MYC protooncogene, expression of alpha-fetoprotein (AFP) and epithelial cell adhesion molecule (EpCAM), and a relative suppression of IFN-responsive genes. The observation that HCC develops in all chronic WHV carrier woodchucks provides direct experimental evidence for the oncogenicity of WHV, and by analogy of HBV, as well as other hepatitis viruses naturally infecting several ground squirrel species[24,68]. However, infection with California ground squirrel hepatitis virus (GSHV) leads to less frequent liver cancer development and the HCC onset is much later seen than in chronic WHV infection[107]. This lower oncogenic potential of GSHV was further demonstrated in a comparative study of woodchucks infected as neonates with both WHV and GSHV, as GSHV-induced HCC developed at an later age than WHV-induced HCC in the same host[71]. Since immune cell infiltration into the liver is present during chronic WHV infection, as described above, this continuing chronic inflammatory response likely plays a role in the development of WHV-induced HCC, in addition to viral integration events and proteins, as also observed for HBV-induced HCC[108-111].

Important features of hepatitis virus-induced hepatocarcinogenesis have been investigated in woodchucks and are described here in more detail. Since replication-incompetent WHV DNA is integrated into the chromosomal DNA of woodchuck liver

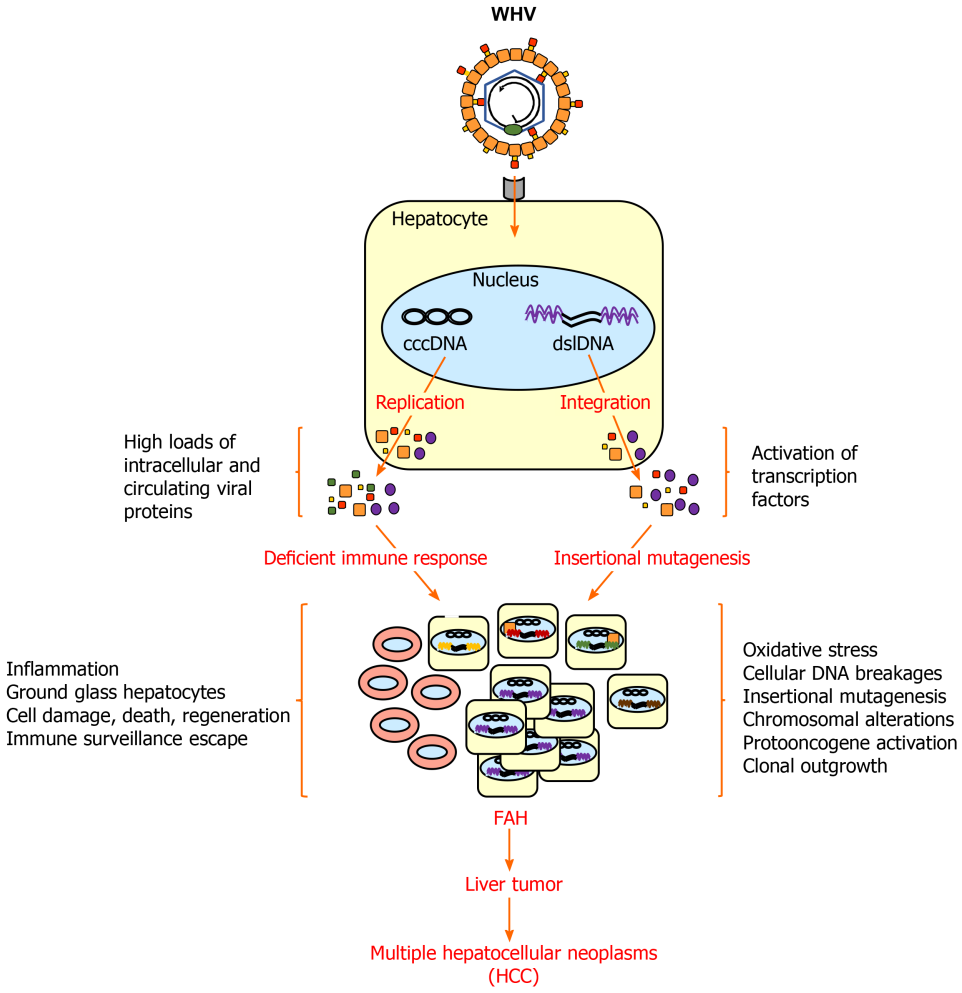

Figure 2 Woodchuck hepatitis virus-induced hepatocarcinogenesis in woodchucks. After infection of normal hepatocytes, woodchuck hepatitis virus (WHV) replicates *via* cccDNA and produces high loads of intracellular and circulating viral proteins (WHsAg, WHeAg and WPreC) that interfere with the antiviral immunity. The deficient immune response is unable to clear WHV from infected liver cells but causes inflammation. WHsAg accumulates in hepatocytes and gives rise to ground glass hepatocytes. WHV also integrates into the chromosomal DNA of hepatocytes *via* double-stranded linear (dsl) DNA leading to oxidative stress, oxidation-dependent cellular DNA breakages, insertional mutagenesis, chromosomal alterations, and protooncogene activation. WHsAg and WHV X antigen (WHxAg) are produced from viral DNA integrants. Integrant- and replication-derived viral proteins activate cellular proteins, such as transcription factors, that support the oncogenic process. The continued liver inflammation leads to cell degeneration and regeneration and facilities accumulation of genetic and epigenetic defects in hepatocytes. Individual hepatocytes with critical mutations and low WHV replication and/or antigen presentation escape immune surveillance and their clonal outgrowth results in FAHs that further develop into liver tumors and HCC. CccDNA: Covalently-closed circular DNA; Dsl DNA: Double-stranded linear DNA; FAH: Focus of altered hepatocytes; HCC: Hepatocellular carcinoma; WHV: Woodchuck hepatitis virus.

tumor cells, which is comparable to the HBV DNA integration in human HCCs[112-115], a direct molecular role of hepatitis viruses in hepatocarcinogenesis is conceivable. The main substrate for integration is viral double-stranded linear (dsl) DNA, which is sometimes produced by the viral polymerase instead of rcDNA within the nucleocapsid[114]. Integration occurs after recycling of nucleocapsids to the nucleus for replenishment of the cccDNA pool[28]. Initial integration in hepatocytes, at least *in vitro*, is mediated by virus-induced oxidative stress resulting in oxidation-dependent cellular DNA breakages[116]. The integrated viral DNA cannot serve as the source of the progeny virus, but the produced RNA transcripts for the surface and X antigens can become abundantly or even predominantly present, when compared to the viral RNAs transcribed from the cccDNA genome[117]. Thus, integration-derived RNA transcripts may serve as a considerable source for viral antigens with similar function as replication-derived viral proteins and may influence the course of chronic infection and liver disease progression by interfering with the antiviral immunity[117,118].

WHV DNA integrates into the woodchuck genome at multiple sites within hours after experimental infection[67]. Although it does not appear that there is a preferential integration site for hepatitis viruses[101,113], WHV integrates often into or near the MYC family of protooncogenes in most woodchuck HCCs[68]. Integration close to the N-MYC2 gene or in the b3n and win downstream loci then leads to activated N-

MYC genes and overexpression of their transcripts in malignant hepatocytes[89,119-122]. In coordination with N-MYC, the insulin-like growth factor-2 (IGF2) is also overexpressed in woodchuck FAHs and HCCs[123,124]. IGF2 blocks apoptosis of malignant liver cells, and thus may allow hepatocytes which otherwise might die to survive, to form FAHs, and to progress to liver tumors[123]. WHV DNA integration further causes N-MYC2 rearrangements, especially in large but less differentiated liver tumors, suggesting that these genetic alterations provide initially a proliferative stimulus or growth advantage for transformed hepatocytes[104]. However, compared with woodchucks that naturally acquired WHV infection, animals experimentally infected with WHV as neonates have more WHV DNA integrations near the N-MYC2 Locus[121]. Although the exact role of N-MYC2 rearrangements and transcripts remains to be elucidated, it was shown that transgenic mice carrying the N-MYC2 gene under the control of WHV regulatory sequences develop liver cancer, including hepatocellular adenomas and HCCs[125].

Like in human HCCs[126], woodchuck liver tumors express small non-protein-coding RNAs or microRNAs at elevated levels, such as miR-17-92 polycistron and miR-21[127]. Knockdown of these microRNAs in human- and woodchuck-derived hepatoma cell lines resulted in a 55% reduction of cell proliferation and anchorage-independent growth, as well as in a suppression of cellular antiapoptotic function. This suggests that onco-microRNAs are involved in the maintenance of malignant hepatocyte transformation during hepatitis virus-induced hepatocarcinogenesis.

Among all viral proteins, the X antigen, a multifunctional transactivator of viral and cellular genes and essential for the establishment of WHV infection in woodchucks [128], has been implicated as a cofactor in the malignant transformation of hepatocytes [129]. HBV replication and liver cell transformation by the HBV X antigen (HBxAg) are associated with the induction of the mitotic polo-like kinase 1 (PLK1) and a parallel downregulation of chromatin remodeling components, including polycomb repressive complex 2 subunit (SUZ12) and zinc finger MYM-type protein 2 (ZMYM2 or ZNF198) [130]. This inverse relationship of PLK1 and SUZ12 was also identified in woodchuck liver tumors[131]. SUZ12 targets many hepatic cancer stem cell markers and proliferation genes. Since expression of these genes is reduced in normal hepatocytes, they are also named "SUZ12 repressed" genes. During WHV-induced hepatocarcinogenesis, the SUZ12 repressed genes encoding BMP, activin membrane-bound inhibitor homolog (BAMBI), and EpCAM, as well as the proliferation gene PLK1, are selectively upregulated in woodchuck tumor cells. Furthermore, metastatic tumor antigen 1 (MTA1), a component of the nucleosome remodeling histone deacetylase complex involved in regulating transcription and chromatin remodeling, is associated with tumor invasiveness and poor prognosis in HBV-induced HCC[132]. Comparable to human HCC, the presence of MTA1 is increased in woodchuck liver tumors, its expression is regulated by the WHV X antigen (WHxAg), and the protein is essential for nuclear factor-kappa B (NF-κB) signaling and tumor progression induced by WHV [133].

Altered expression of vascular endothelial growth factor (VEGF) in the liver is used as a prognostic marker for human HCC[134] and therapeutic interventions targeting this protein or its receptors (VEGFR1/R2) can improve the clinical outcome of HCC in patients[135]. In woodchucks, WHV-induced hepatocarcinogenesis is associated with elevated VEGFR2 expression and increased ligation of VEGF to VEGFR2[136]. This VEGF-driven angiogenesis is accompanied by changes in the liver vasculature, extracellular matrix, and basement membrane, as the number of vessels positive for laminin and platelet endothelial cell adhesion molecule (PECAM1) increased while the number of collagen IV-positive blood vessels declined. This suggests that woodchucks with liver tumors can be utilized in the preclinical evaluation of VEGF-directed therapies for human HCC.

Matrix metalloproteinases (MMPs) play a central role in tumor invasion and metastasis during HBV-induced hepatocarcinogenesis[137]. For obtaining insight in the mechanisms involved in extracellular matrix remodeling in human HCCs, the expression of MMPs was investigated in woodchuck liver tumors[138]. High levels of several MMP transcripts were detected, and especially the transcript and protein levels of MMP-9 correlated with liver disease progression and tumor differentiation in woodchucks, while the protein's gelatinase activity increased during hepatocarcinogenesis. These results are comparable to findings in human liver tumors where the MMP-9 protein level was used for characterizing a more invasive and metastatic type of HCC with poor prognosis[139,140]. Since the gelatinase activities of woodchuck MMP-2 and MMP-9 could be inhibited by a commercially available drug, the use of MMP inhibitors for treatment of human HCC may be a possible treatment option and could be evaluated in woodchucks.

Hepatitis delta virus (HDV), a natural subviral agent of HBV, is known to contribute to HBV-induced hepatocarcinogenesis and to increase the overall risk of HCC in patients during concomitant infection[141-143]. Since HDV only needs the HBsAg for virion envelopment[143], persistence of HDV infection may be independent of HBV replication if integration-derived viral surface antigen can be used, as demonstrated in cell culture[118]. The contribution of HDV to HCC induction and development remains to be elucidated; however, one possible mechanism was revealed in woodchucks[144]. Intravenous inoculation of woodchucks with liver tumors using WHsAg-enveloped HDV demonstrated that malignant hepatocytes are susceptible to HDV infection. Thus, it appears likely that HDV may influence the fate of HCCs by actively replicating in tumor cells and changing the expression of host genes.

Overall, these studies demonstrated that WHV-induced hepatocarcinogenesis in woodchucks has strong similarity to HBV-induced liver carcinogenesis in humans. The features of HCC that are associated with chronic hepatitis virus infection make the woodchuck animal model unique. It further distinguishes woodchucks from other animal models, in which HCC is induced by either a chemical carcinogen, a transgene, or by transplantation of established tumor cell lines into immune-deficient or immune-compatible hosts. Additional advantages of the woodchuck model are the outbred nature of the animals and the heterogeneity of liver tumors that resemble the situation of HBV-infected patients with HCC. These studies further indicated an important role of viral DNA integration, activation of protooncogenes, microRNAs, and the viral X antigen in the malignant transformation of hepatocytes.

Development of woodchucks as an animal model for HCC

As described above, liver tumors develop in woodchucks with chronic WHV infection and HCC is fatal in 100% of cases. Tumor progression is usually monitored by serial ultrasonography (US)[86,145,146] and to a lesser degree by repeated magnetic resonance imaging (MRI)[147,148]. Changes in liver enzymes are also used for determining the degree of liver injury due to tumor development[104]. Especially, gamma-glutamyl transferase was validated as an oncogenic biomarker in woodchucks, as increases in this liver enzyme correlate with the onset of HCC[149]. In addition, elevated levels of AFP were linked with WHV-induced hepatocarcinogenesis in woodchucks[150].

Improvements in imaging techniques for human HCC

The woodchuck model of HCC has been utilized in the development of new imaging agents for enhancing the detection of hepatic neoplasms by different imaging techniques (Table 1). In the beginning, several contrast agents were evaluated for both gray scale and color Doppler US, including those that use microbubble technology, alone and in combination with hypobaric activation, a vascular imaging agent, or an agent taken up by the reticuloendothelial (RE) system. These agents facilitated tumor localization in the liver and improved measurements of tumor growth and regression in untreated *versus* treated woodchucks by increasing the sensitivity of US. Furthermore, iron oxide as a contrast agent for the detection of HCC by MRI was tested in woodchucks, either following parenteral administration for uptake by the RE system or intravenous injection as an arabinogalactan conjugate for targeting the asialoglycoprotein receptor that is highly upregulated on normal hepatocytes but not on liver tumor cells. Hepatic imaging with 99mTc-sulfur colloid also detected HCCs after uptake by the RE system and concentration in woodchuck liver. More recently, woodchucks were applied in the improvement of positron-emission tomography (PET) techniques for the early detection of human HCC by comparing radiotracers for uptake into liver tumors and surrounding hepatic tissues. HCC localization and response to radiotherapy was also assessed with MRI by applying contrast agents typically used in patients for visualizing lesions with abnormal vascularity. HCC detection and response to anticancer treatment was further tested by computed tomography (CT) with contrast agents for human use. MRI and/or CT techniques were also applied for generating a virtual three-dimensional (3D) model of the woodchuck hepatic vascular tree[151], as well as for producing virtual and printable 3D models of the woodchuck liver containing tumors that allowed accurate co-localization of imaging with histopathology[152].

Improvements in techniques for accessing human HCC and treatment by embolization

The woodchuck model was further applied in the evaluation of new techniques developed for gaining less-invasive access to liver tumors for the treatment of HCC in

Table 1 Imaging techniques and contrast agents applied for the detection of woodchuck hepatocellular carcinoma

Imaging technique	Contrast agent	Brand name	Ref.
Ultrasonography	Air-filled albumin microspheres		[195]
	Cyanacrylate polymer microparticles	SHU563A	[196]
	Dodecafluoropentane emulsion	EchoGen	[197,198]
	Galactose microparticles/palmitic acid	Levovist	[199-201]
	Perflexane-filled lipid microspheres	Imagent	[202]
	Perfluoropropane-filled albumin microspheres	Optison/FS069	[95,200,201,203,204]
Scintigraphy	99mTc-sulfur colloid		[205]
Positron-emission tomography	[1-11C]acetate		[206-208]
	[1-14C]acetate		[207]
	[N-methyl-11C]choline		[206,209,210]
	[18F]clofarabine		[211]
	[18F]fluoro-ethylcholine		[210]
	Anti-1-amino-3-[18F]fluoro-cyclobutyl-1-carboxylic acid		[212]
	2-deoxy-2-[18F]fluoro-D-glucose		[208,209,213]
	6-deoxy-6[18F]fluoro-D-glucose		[206,209,210]
	L-[S-methyl-11C]methionine		[212]
	3-deoxy-3-[18F]fluoro-thymidine		[214]
Magnetic resonance imaging	Gadolinium	Gadavist or Omniscan	[157,179,185]
	Gadopentetate dimeglumine	Magnevist	[158,189]
	Iron oxide		[215,216]
Computed tomography	Biodegradable radiopaque fiducial markers based on polymers and iodine	Ioversol, Isovue-370, Optiray300, or Optiray350	[152,153,159,186,206]
	Diatrizoic acid	Angiografin	[190]
	Meglumine iotroxate	Biliscopin	[151]
	Iohexol	Omnipaque or Omnipaque350	[157,188]

patients. For improving percutaneous liver biopsy techniques, needle-based diffuse optical spectroscopy (DOS) was tested in woodchucks[153]. This established that tissue blood and lipid content and oxygenation level declined, while tissue density increased, when the needle crossed the margin from healthy hepatic parenchyma to liver tumors, indicating that these measurements could be used in real-time as a primary discriminator of normal liver and HCC.

For the treatment of human HCC, chemoembolization and radioembolization *via* intra-arterial therapies (IAT), alone and in combination with immunotherapy, hold great promise. For the testing of IAT, rather diverse animal species, including mice, rats, rabbits, and pigs, are commonly used as preclinical models of HCC[154-156]. Translation of IAT from these animal models into patients, however, is limited due to the dissimilarity in liver disease development and the size of the vascular system that make arterial access either impossible or challenging, and often requires a surgical cut down for the use of human-size products[155]. This situation is different in woodchucks, because the size of the animals greatly facilitates IAT and other experimental approaches of intratumoral injection. Woodchucks also possess a hepatic arterial anatomy that can be accessed *via* the femoral artery and allows catheterization with clinically used microcatheters[151]. Accordingly, three studies explored IAT in woodchucks with or without liver tumors[157-159]. In these studies, arterial access *via* the femoral artery with human standard catheters allowed delivery of contrast agents for the localization of HCCs by CT or MRI. Catheterization further permitted delivery

of embolic particles routinely used in patients into liver tumors by angiography. Lobar embolization with 355–500 μm polyvinyl alcohol (PVA) particles (Boston Scientific) was successful in woodchucks without liver tumors[158]. In addition, liver tumor embolization for the targeted delivery of 100-300 μm PVA microspheres (LC- Bead; BTG, London, United Kingdom) produced a heterogeneous distribution of embolic particles in the hepatic neoplasms[157]. Moreover, chemoembolization with drug-eluting embolic 70-150 μm radiopaque PVA microspheres (LC Bead LUMI; BGT) loaded with doxorubicin resulted in a targeted drug delivery into liver tumors[159]. Doxorubicin is an anticancer drug that stops the growth of tumor cells by blocking topoisomerase II and that generates reactive oxygen species for the induction of apoptosis[160].

There is also interest in assessing the biomedical utility of nanomaterials in immunocompetent animal models for the treatment of human HCC. In particular, tumor-associated macrophages within the environment of solid tumors are a preferred target of nanoparticle-based applications, as the balance of inflammatory (tumoricidal) and immunoregulatory (tumor promoting) macrophages controls tumor development, progression, and metastasis[161]. One study evaluated the distribution and clearance of 60 nanometer gold particles into woodchuck liver and tumors after a single intravenous injection at a dose of 14 mg/kg[162]. Although these nanoparticles accumulated to some degree in the spleen after systemic administration, they were mainly found in the lysosome of immunoregulatory macrophages within the liver, as well as in liver resident macrophages. Nanoparticles were further detected in liver tumors and their accumulation in immunoregulatory macrophages was significantly greater in the periphery than in the tumor core. The study concluded that nanoparticle-based delivery of immunomodulators into tumors for treatment of HCC is feasible, especially by targeting tumor-associated macrophages and repolarizing these cells into a more inflammatory phenotype to promote anticancer immunity.

Overall, these studies established that woodchucks with liver tumors are a useful preclinical animal model for the evaluation of transarterial embolotherapies for the treatment of human HCC. They further demonstrated the feasibility of nanoparticle-based delivery of chemotherapeutics or immunomodulators into tumors and assessment of anticancer effects by CT, MRI, or PET imaging.

HCC treatment approaches in woodchucks

Woodchucks have been utilized in the evaluation of anticancer effects mediated indirectly by treatment with antiviral drugs or immunomodulators and directly by radiotherapy, tumor excision and ablation, gene therapy, or anticancer drugs (Figure 3).

Indirect treatment by antiviral drugs or immunomodulators

Woodchucks with chronic WHV infection were applied in the preclinical evaluation of antiviral drugs being developed for the treatment of HBV-infected patients [30,35,37,68,163]. Among these drugs, nucleos(t)ide analogs that suppress viral replication in the liver, and thus reduce viremia levels in the periphery, were assessed in woodchucks mainly as a single agent but also in combination (Table 2). Many of these nucleos(t)ide analogs are now approved by national regulatory agencies for administration to patients. While most woodchuck studies were focused on testing nucleos(t)ide analogs for safety and antiviral efficacy during short-term treatment, some studies were extended for the additional evaluation of effects against liver disease progression.

Lifelong, oral treatment of woodchucks with lamivudine, starting at an age of 8 mo and by applying two separate drug doses (*i.e.*, 5 mg/kg/d for approximately 10 mo and then 15 mg/kg/d for up to 50 mo in surviving animals), produced a 4-5 Log_{10} reduction in viremia and the antiviral effect was sustained for 1⅔ years while treatment continued[164]. Woodchucks experienced a significant delay in the onset of HCC and death due to severe liver cancer. In particular, lamivudine treatment delayed the development of liver tumors by 24 mo (until an animal age of 32 mo) and extended HCC-free survival by 12 mo (until an animal age of 44 mo). However, when oral lamivudine treatment was initiated in older woodchucks at an age of 13-19 mo and with relatively high doses (*i.e.*, 40 mg/kg/d for 3 mo and/or 200 mg/kg/d for up to 15 mo), the shorter treatment duration and the less pronounced antiviral effect (~2.5 Log_{10} decline in viremia) failed to delay hepatocarcinogenesis[165]. Almost all woodchucks developed liver tumors while receiving lamivudine and needed to be euthanized between 12 and 19 mo of treatment due to end-stage HCC (at an animal age of 26-38 mo). Complicating in both studies was the selection of lamivudine-resistant WHV mutants during treatment. These mutations occurred frequently in the B domain of the WHV polymerase gene[166,167] and were identical to those reported

Table 2 Nucleos(t)ide analogs evaluated in woodchucks for safety and antiviral efficacy against hepatitis B virus

Antiviral drug	Abbreviation	Brand name	Ref.
Adefovir dipivoxil	ADV	Hepsera	[217-219]
Clevudine[1]	CLV or L-FMAU	Levovir and Revovir	[172,220-222]
Emtricitabine	FTC	Coviracil	[219,221,223,224]
Entecavir[1]	ETV	Baraclude	[171,225-228]
Lamivudine[1]	LAM or 3TC	Epivir	[164,165,194,219,229]
Telbivudine	LdT	Tyzeka	[230-232]
Tenofovir alafenamide	TAF	Vemlidy	[233]
Tenofovir disoproxil fumarate	TDF	Viread	[219]
Valtorcitabine	LdC		[230-232]

[1]Long-term treatment with these drugs delayed hepatocellular carcinoma (HCC) onset and extended HCC-free survival in woodchucks. See text for more details.

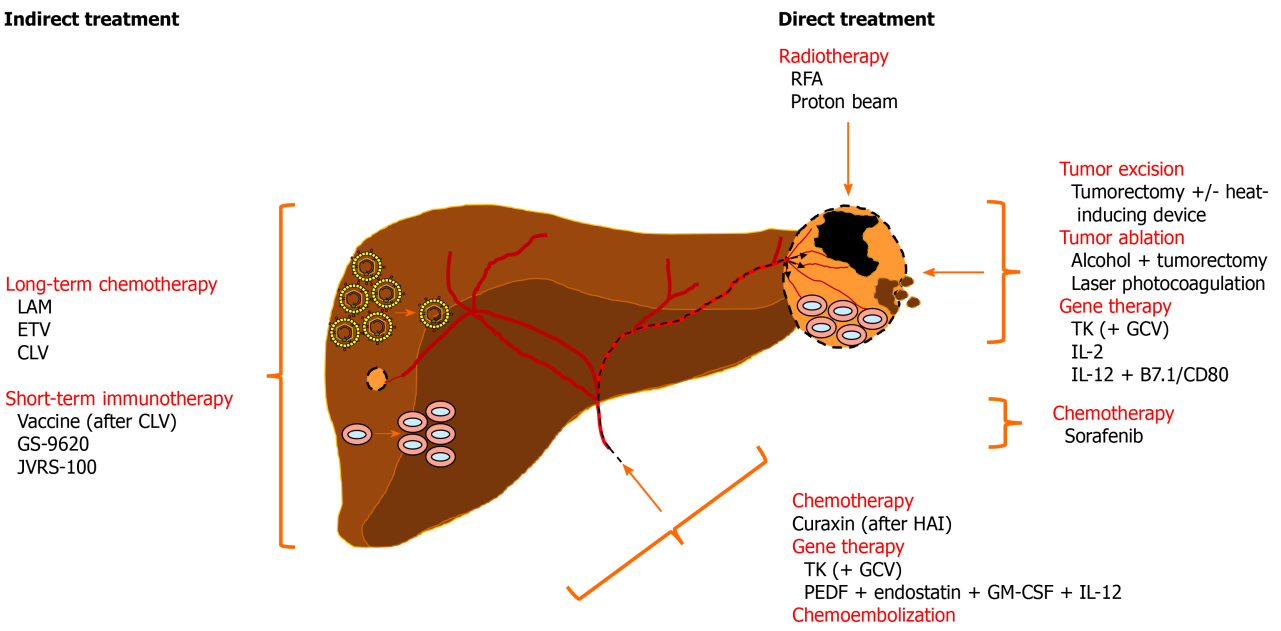

Figure 3 Overview of therapeutic interventions assessed in woodchucks with liver tumors for the treatment of human hepatocellular carcinoma. Indirect treatment of chronic WHV carrier woodchucks with nucleos(t)ide analogs or immunomodulators reduces viremia or activates antiviral and anticancer immune responses, respectively, that delay or prevent HCC onset. Direct treatment of hepatic neoplasms by radiotherapy, excision and ablation, gene therapy, or chemotherapy induce apoptosis or necrosis of tumor cells and/or activate an intratumoral, anticancer immune response that result in partial tumor remission. Chemoembolization-mediated anticancer effects have not been evaluated in woodchucks so far. See text for more details. B7.1/CD80: Costimulatory molecule; CLV: Clevudine; ETV: Entecavir; GCV: Ganciclovir; GM-CSF: Granulocyte-macrophage colony-stimulating factor; GS-9620: Toll-like receptor 7 agonist; HAI: Hepatic artery infusion; IL-12: Interleukin 12; JVRS-100: Complex of non-coding plasmid DNA and cationic liposomes; LAM: Lamivudine; PEDF: Pigment epithelium-derived factor; RFA: Radiofrequency ablation; TK: Thymidine kinase.

in lamivudine-treated patients, in addition to mutations in the C domain of the HBV polymerase gene[168-170].

Long-term, oral treatment of woodchucks with entecavir for 14 or 36 mo, starting at an animal age of 8 mo and then continuing with a lower dosing frequency from 10 mo of age onward (*i.e.*, 0.5 mg/kg/d for two months and then 0.5 mg/kg/wk for 12 or 34 mo), resulted in a 5-8 Log_{10} reduction in serum WHV DNA in 60% or 80% of animals, respectively[171]. The levels of serum WHsAg and intrahepatic WHV cccDNA declined alongside and in parallel with the marked reductions in viremia. An emergence of entecavir-resistant mutants was not observed during the study. Since woodchucks with a sustained antiviral effect stayed negative for signs of liver tumors for up to 2⅓ years after drug withdrawal, entecavir treatment prevented the

development of liver cancer in a majority of animals (*i.e.*, up to 80% HCC-free survival).

Delayed HCC onset and prolonged survival was also achieved during long-term, oral treatment of woodchucks with clevudine for 32 wk at a dose of 10 mg/kg/d, starting at an animal age of 1-2 years[37,172]. Thereafter, half of the placebo- or clevudine-treated woodchucks received intramuscularly four doses of a conventional, alum-adsorbed WHsAg vaccine that was administered monthly after drug withdrawal. Combination treatment with clevudine and vaccine reduced viremia by up to 9 Log_{10}, with undetectable serum WHV DNA in many animals. The antiviral effect was sustained for more than 1 year after treatment cessation in 75% of woodchucks and prevented HCC onset in 38% of animals. However, once HCC was established, the growth rates (*i.e.*, volume doubling times) of liver tumors were similar to those of control animals. Importantly, initiation of clevudine treatment at an animal age of 1 year, and independent of vaccination, produced a more pronounced anticancer effect than a treatment begin at an animal age of 2 years. The development of liver tumors in the younger cohort of woodchucks was further delayed and HCC-free survival increased after 3 (50% *vs* 0%) and 4 years (25% *vs* 0%). Moreover, vaccination of these animals without initial clevudine treatment improved the B- and T-cell responses to WHsAg, the protein on which the vaccine was based, but had no effect on viral replication or liver enzyme levels. In combination with clevudine, however, vaccination enhanced these B- and T-cell responses based on the higher titers of virus-neutralizing antibodies and the greater proliferation capability to stimulation with WHsAg. In addition, combination treatment broadened the antiviral immunity to include T-cell responses to other viral antigens, such as WHcAg, WHeAg, and WHxAg, while liver enzyme levels normalized.

Woodchucks were further applied in the preclinical evaluation of immunomodulating compounds being developed for the treatment of HBV-infected patients. The immunomodulators tested so far in woodchucks suppressed WHV replication in the liver and reduced viremia and antigenemia in the periphery at varying degrees. In some instances, the antiviral effect was sustained after the end of treatment, and seroconversion to antibodies against WHsAg and/or WHeAg was achieved in a subset of animals, indicating that a functional cure was induced. Immunomodulators were administered as single agents but more often in combination with nucleos(t)ide analogs and/or inhibitors of viral gene expression and immune checkpoint markers (Table 3). Comparable to the chemotherapy studies, only two immunotherapy studies were designed or extended to include the assessment of anticancer effects.

Short-term, oral administration of the small molecule GS-9620 targeting toll-like receptor (TLR) 7 induced durable antiviral efficacy in woodchucks treated with different doses and dosing frequencies[173]. In the group with the greatest antiviral effect, animals at an age of 12-14 mo received the agonist every other day for approximately 4 wk, initially at 5 mg/kg and then at 2.5 mg/kg after a treatment interruption for 9-10 d due to liver enzyme increase and thrombocytopenia that both reversed during the dose holiday. Treatment in this group induced a rapid reduction in serum WHV DNA of 6.2 Log_{10} that was accompanied by declines in intrahepatic WHV cccDNA and undetectable serum WHsAg. Suppressed WHV replication was sustained in all woodchucks during the 31-week follow-up period, and a subset of animals also seroconverted to antibodies against WHsAg during this time. At the end of the study in week 35, all animals were found to be HCC-free during postmortem examination. When combining all woodchucks enrolled in the various treatment groups of this study, and by including only animals that completed treatment and experienced sustained viral suppression, TLR7 agonism reduced the HCC incidence from 71% in placebo-treated control woodchucks to 8% in GS-9620-treated animals. The antiviral and anticancer effects were attributed to the activation of an immune response based on the induction CD8+ T-cells, NK-cells and B-cells, and the production of type I and II interferons in the liver. A follow-up study further indicated that GS-9620 not only targets TLR7 but also TLR8 when administered at high doses[174], possibly explaining the most superior antiviral effect observed so far in the woodchuck animal model with a single agent during short duration treatment.

Intravenous administration of JVRS-100, a complex of non-coding plasmid DNA and cationic liposomes, every second week for 12 wk at two separate doses to woodchucks with liver tumors at an age of 2 years resulted in antiviral and anticancer effects[175]. Since the high serum loads of viral DNA and antigens typically present during chronic WHV infection mediated immune suppression, and thus resistance to treatment, only animals with rather low levels of viremia and antigenemia were enrolled in the study. Serum WHV DNA declined by 0.9 Log_{10} during JVRS-100 treatment and during the 12-week follow-up period, especially in animals that

Table 3 Immunomodulators evaluated in woodchucks for safety and antiviral efficacy against hepatitis B virus

Immunomodulator	Compound name	Brand name	Ref.
IFN-α			[192,226,228,229]
RIG-I/NOD2 agonist	SB 9200	Inarigivir	[227]
TLR7 agonist	GS-9620[1]	Vesatolimod	[173]
	APR002		[225]
	RG7854		[38]
TLR8 agonist	GS-9688	Selgantolimod	[174]
TLR9 agonist	CpG-ODN		[234]
TLR9-dependent and -independent pathways	AIC649		[235]
	JVRS-100[2]		[175]
Viral gene expression inhibitor	RG7834		[226]
Immune checkpoint inhibitor	Anti-PD-L1		[236]

[1]Treatment delayed hepatocellular carcinoma onset in woodchucks.
[2]Treatment inhibited formation of new liver tumors in woodchucks.
See text for more details. Anti-PD-L1: Antibody against programmed death-ligand 1; CpG-ODN: CpG oligodeoxynucleotide, a short single-stranded synthetic DNA molecule containing unmethylated deoxycytosine-deoxyguanosine (CpG) motifs; IFN-α: Interferon alpha; JVRS-100: Complex of non-coding plasmid DNA and cationic liposomes; NOD2: Nucleotide-binding oligomerization domain-containing protein 2; RIG-I: Retinoic acid-inducible gene I; TLR: Toll-like receptor.

received the higher dose, but the antiviral effect was transient and less pronounced for WHsAg. Although treatment did not induce a regression of preexisting liver tumors, the higher dose prevented the formation of new tumors for 6 mo. These effects were associated with the activation of immune responses that involved CD4+ and CD8+ T-cells and T helper cell type I (Th1) cytokines, such as IFN-α, tumor necrosis factor-α (TNF-α), and interleukin (IL) 2 and 12 in liver and blood, and that apparently blocked the conversion of virus-induced chronic liver disease into HCC.

Overall, these studies demonstrated that long-term treatment with nucleos(t)ide analogs primarily delays but sometimes prevents liver tumor development in woodchucks. Since these studies established a correlation between suppressed viral replication and reduced liver disease progression, early initiation and prolonged duration of conventional antiviral treatment appear most critical for the prevention of hepatitis virus-induced HCC. Since the applied treatment regimens resulted in less cellular damage and liver injury, they most likely deferred the transformation of altered hepatocytes into liver tumors. Short-term immunomodulation, either rather broad or more targeted, mediated lasting protection against formation of new liver tumors or HCC onset. In two studies, immunomodulation was associated with improved or newly elicited humoral and cellular immune responses to viral antigens that were reduced by treatment, and thus could no longer act as endogenous tolerogens.

Direct treatment by chemotherapy, radiotherapy, or gene therapy

Since liver tumors obtain their nutrient blood supply from the hepatic artery[176], hepatic artery infusion- (HAI) supported chemotherapy has been applied for the treatment of both primary and metastatic liver cancers in patients and shown to be an effective treatment for unresectable advanced HCC[177]. Effectiveness of this intervention relates to the concentration of chemotherapeutics in HCCs by direct delivery to the tumors, with limited systemic exposure in the liver[178]. In one woodchuck study, HAI ports were placed in the gastroduodenal artery and infused with a curaxin-based experimental anticancer drug, once per week for 3 wk at a dose of 17 mg/kg[179]. Curaxin targets a histone chaperon expressed at high levels in cancer[180] and activates the p53 tumor suppressor gene, while it simultaneously suppresses inhibition of NF-κB[181]. Tumor growth in woodchucks was suppressed after repeated treatment and the anticancer effect was associated with increases in intratumoral T-cell infiltration and tumor cell apoptosis.

Woodchucks were also applied for testing the preventive effect of long-term, oral treatment with sorafenib[182]. Sorafenib, a small molecule receptor inhibitor of several surface tyrosine kinases, is a standard first-line therapy approved for the treatment of human HCC. Although this drug has both proapoptotic and antiangiogenic properties, the treatment benefit of sorafenib is modest, as only a 3-mo improvement in the overall survival is achieved and its indication is restricted to patients with well-preserved liver function[183]. The underlying mechanism of sorafenib-mediated anticancer activity has not been fully elucidated. Sorafenib was administered daily to woodchucks at two separate doses (*i.e.*, 2.5 mg/kg and 5 mg/kg) using a 5-d-on and 2-d-off schedule until tumor development was observed. Although all animals presented with liver tumors independent of the sorafenib dose applied, the lower dose was associated with smaller initial tumor volumes and delayed tumor growth that was associated with an increase in intratumoral CD3+ T-cell infiltration. An effect of sorafenib on chronic WHV infection was not noted. Interestingly, short-term, oral, daily sorafenib administration for 90 d was unable to reciprocate the anticancer effect obtained during long-term treatment. The study concluded that sorafenib has immunomodulatory activity that is dependent on the dose and treatment duration. Caution, however, is warranted when applying higher doses of sorafenib, because of its immunosuppressive function that relates to an increased activity of nuclear factor of activated T-cells 1 (NFAT1) and results in the *in vitro* inhibition of T-cell proliferation and in an increase in programmed cell death protein 1 (PD-1) expression of CD8+ T-cells, as demonstrated in woodchucks.

Woodchucks with liver tumors were further used to evaluate different ablation techniques for human HCC. One study demonstrated the feasibility of tumor excision, percutaneous alcohol ablation followed by tumorectomy, and laser photocoagulation in this animal model[86]. Extended survival for up to 16-18 mo was achieved with the first two modalities, but multiple tumor recurrence distant from the resection area occurred ultimately in all animals. A second study investigated the effect of a saline-linked dissecting sealer on the remaining tumor beds (*i.e.*, *in situ* margins) after initial removal of neoplasms by tumorectomy[184]. Surface application of this device induced a heat zone area of up to 5 mm in depth, inside which residual tumor cells, if present, were efficiently destroyed, suggesting that this approach could be beneficial in reducing marginal recurrence after tumor resection. A third study tested radiofrequency ablation (RFA) using a low energy protocol and a 1.0 cm probe that produced a consistent burn area within liver tumors, as determined by necrosis of tumor cells, but was unable to fully ablate larger lesions[185]. A final study assessed the effectiveness of passive scattering proton beams with high dose fractionation[186]. Three fractionations were applied every other day within one week to the hepatic neoplasm. A partial regression of the treated liver tumor was noticed at week 3 post-treatment, which continued until the nodule disappeared at week 9, as also confirmed during postmortem evaluation one week later. The study concluded that a delayed but complete imaging response to proton beam treatment of HCC was achieved in woodchucks without visible gastrointestinal toxicity.

Gene therapeutic strategies based on the induction of apoptosis, antiangiogenesis, or anticancer immune response were assessed in woodchucks for the treatment of human HCC. In one study, an adenoviral vector encoding for the thymidine kinase (TK) of herpes simplex virus under the control of the ubiquitous cytomegalovirus promoter for conferring sensitivity to ganciclovir (GCV) treatment was administered to liver tumors either directly or indirectly *via* the hepatic artery[187]. Transduction of tumor cells and subsequent drug administration resulted in an anticancer effect in two woodchucks that was mediated by GCV-induced apoptosis; however, a third animal died due to acute liver failure that was attributed to the transduction of adjacent, nonneoplastic hepatocytes. Although tumor regression was not achieved, necrotic areas were present in tumors one week after treatment. The study emphasized the need to make vector transduction more specific to liver tumor cells by controlling TK expression with HCC-specific promoters, such as the AFP promoter.

Two other studies tested the anticancer activity mediated by the cytokine IL-12. In the first study, murine IL-12 was expressed from a replication-competent Semliki Forest virus (SFV) vector[188]. Use of this vector has the advantage that the antitumor effect mediated by the cytokine is enhanced *via* the induction of apoptosis in tumor cells that replicate SFV. A single, intratumoral injection of the vector at increasing doses during laparotomy produced a dose-dependent tumor regression that was 80% with the highest dose. Correlating with the temporary IL-12 expression, partial tumor remission was transient and neoplasms began to regrow between 6 and 14 wk after treatment. In addition, all animals experienced a temporary reduction in serum viremia and/or antigenemia. The anticancer and antiviral effects were associated with

augmented T-cell responses to tumor and viral antigens, as well as increased expression of CD4 and CD8 markers and IFN-γ and TNF-α in peripheral blood mononuclear cells. In the second study, a single dose of an adenoviral vector encoding for murine IL-12 and the costimulatory B7.1/CD80 molecule for activating T-cells was injected into liver tumors during laparotomy or under MRI guidance[189]. Transduction of tumor cells resulted in a tumor regression of 80% on average, with one animal experiencing an almost complete tumor elimination within 7 wk. Regression was associated with the induction of an anticancer immune response, as demonstrated by a massive infiltration of CD4+ and CD8+ T-cells into tumors and an increase in intratumoral IFN-γ production. The long-term anticancer effect could not be evaluated, as almost all animals were euthanized two weeks after treatment.

A final study investigated the anticancer effect mediated by antiangiogenic proteins and cytokines in woodchucks[190]. Single dose treatment *via* the hepatic artery with an adenoviral vector encoding for human pigment epithelium-derived factor (PEDF) and endostatin in combination with an adenoviral vector for the expression of woodchuck granulocyte-macrophage colony-stimulating factor (GM-CSF) and murine IL-12 induced a tumor regression of 90%. The partial tumor remission obtained by combination treatment was superior to the 56% and 76% reduction in tumor volume that was achieved by treatment with antiangiogenic proteins or cytokines alone, respectively. An antiviral effect was not noted during the study and serum viremia and antigenemia remained unchanged in all animals. The tumor regression induced by combination treatment was attributed to several factors, including increased infiltration of CD3+ T-cells into tumors, high intratumoral levels of NK-cells, apoptosis of tumor cells, reductions in tumor vasculature (*i.e.*, reduced microvessel density), and declines in immune checkpoint markers [*i.e.*, PD-1 and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)] most likely present on regulatory or immunotolerant T-cells within tumors. Since animals were only followed for two weeks after treatment, the durability of the anticancer effect could not be evaluated.

Overall, these studies established that chemotherapy, radiotherapy, and gene therapy of liver tumors are effective means for the treatment of hepatitis virus-induced HCC in woodchucks. Since some studies established a correlation between anticancer immune response and partial tumor remission, approaches which are based on immunomodulation or checkpoint inhibition for inducing functional cure of chronic HBV infection, appear promising and should further be evaluated in woodchucks for treatment of human HCC.

CONCLUSION

WHV-infected, immunocompetent woodchucks are used to model chronic HBV infection and HCC in humans. Over the past four decades, woodchucks have been applied in the investigation of mechanisms involved in viral immunopathogenesis and hepatocarcinogenesis, in the development of new contrast agents to enhance the detection of hepatic neoplasms by various imaging techniques, in the improvement of tumor ablation strategies based on transarterial embolization and radiotherapy, and in the evaluation of therapeutic interventions directed against the severe outcome of hepatitis virus-induced liver disease. Although the latter was only assessed in a limited number of studies, in which liver tumors were targeted by indirect and direct means, the continued application of woodchucks will support not only the many efforts to cure chronic HBV infection by new antivirals and immunomodulators, but also to treat the associated disease sequelae. Future studies can take advantage of the recently identified woodchuck transcriptome[79,191,192] and genome[193] for generating all needed protein-based markers and assays, as well as of the translational value of woodchucks in predicting therapeutic efficacy against chronic HBV infection in patients[174,192,194]. Thus, chronic WHV carrier woodchucks progressing to HCC within a reasonable time will greatly aid the development and evaluation of the safety and efficacy of new anticancer prophylaxis or therapy in a relevant animal model. Increased testing of anticancer approaches in the woodchuck animal model will ultimately improve the chances for prevention and therapy of HBV-induced HCC.

ACKNOWLEDGEMENTS

In memory of Dr. Bud Tennant of Cornell University. We gratefully acknowledge Drs. John Gerin and Paul Cote of Georgetown University and Diana Berard and Dr. Rajen

Koshy of the National Institute of Allergy and Infectious Diseases for encouragement and intellectual support.

REFERENCES

1 **Chang JJ**, Lewin SR. Immunopathogenesis of hepatitis B virus infection. *Immunol Cell Biol* 2007; **85**: 16-23 [PMID: 17130898 DOI: 10.1038/sj.icb.7100009]

2 **Tan A**, Koh S, Bertoletti A. Immune Response in Hepatitis B Virus Infection. *Cold Spring Harb Perspect Med* 2015; **5**: a021428 [PMID: 26134480 DOI: 10.1101/cshperspect.a021428]

3 **Yuen MF**, Chen DS, Dusheiko GM, Janssen HLA, Lau DTY, Locarnini SA, Peters MG, Lai CL. Hepatitis B virus infection. *Nat Rev Dis Primers* 2018; **4**: 18035 [PMID: 29877316 DOI: 10.1038/nrdp.2018.35]

4 **Revill PA**, Chisari FV, Block JM, Dandri M, Gehring AJ, Guo H, Hu J, Kramvis A, Lampertico P, Janssen HLA, Levrero M, Li W, Liang TJ, Lim SG, Lu F, Penicaud MC, Tavis JE, Thimme R; Members of the ICE-HBV Working Groups; ICE-HBV Stakeholders Group Chairs; ICE-HBV Senior Advisors, Zoulim F. A global scientific strategy to cure hepatitis B. *Lancet Gastroenterol Hepatol* 2019; **4**: 545-558 [PMID: 30981686 DOI: 10.1016/S2468-1253(19)30119-0]

5 **WHO**. Hepatitis B. [accessed January 23, 2021] Available from: https://wwwwhoint/en/news-room/fact-sheets/detail/hepatitis-b

6 **Golabi P**, Fazel S, Otgonsuren M, Sayiner M, Locklear CT, Younossi ZM. Mortality assessment of patients with hepatocellular carcinoma according to underlying disease and treatment modalities. *Medicine (Baltimore)* 2017; **96**: e5904 [PMID: 28248853 DOI: 10.1097/MD.0000000000005904]

7 **Fanning GC**, Zoulim F, Hou J, Bertoletti A. Therapeutic strategies for hepatitis B virus infection: towards a cure. *Nat Rev Drug Discov* 2019; **18**: 827-844 [PMID: 31455905 DOI: 10.1038/s41573-019-0037-0]

8 **Terrault NA**, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM, Brown RS Jr, Bzowej NH, Wong JB. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. *Hepatology* 2018; **67**: 1560-1599 [PMID: 29405329 DOI: 10.1002/hep.29800]

9 **Alter H**, Block T, Brown N, Brownstein A, Brosgart C, Chang KM, Chen PJ, Chisari FV, Cohen C, El-Serag H, Feld J, Gish R, Glenn J, Greten T, Guo H, Guo JT, Hoshida Y, Hu J, Kowdley KV, Li W, Liang J, Locarnini S, Lok AS, Mason W, McMahon B, Mehta A, Perrillo R, Revill P, Rice CM, Rinaudo J, Schinazi R, Seeger C, Shetty K, Tavis J, Zoulim F. A research agenda for curing chronic hepatitis B virus infection. *Hepatology* 2018; **67**: 1127-1131 [PMID: 28877549 DOI: 10.1002/hep.29509]

10 **Lok AS**, Zoulim F, Dusheiko G, Ghany MG. Hepatitis B cure: From discovery to regulatory approval. *J Hepatol* 2017; **67**: 847-861 [PMID: 28778687 DOI: 10.1016/j.jhep.2017.05.008]

11 **Lau WY**, Lai EC. Hepatocellular carcinoma: current management and recent advances. *Hepatobiliary Pancreat Dis Int* 2008; **7**: 237-257 [PMID: 18522878]

12 **Paul SB**, Manjunatha YC, Acharya SK. Palliative treatment in advanced hepatocellular carcinoma: has it made any difference? *Trop Gastroenterol* 2009; **30**: 125-134 [PMID: 20306740]

13 **Villanueva A**, Minguez B, Forner A, Reig M, Llovet JM. Hepatocellular carcinoma: novel molecular approaches for diagnosis, prognosis, and therapy. *Annu Rev Med* 2010; **61**: 317-328 [PMID: 20059340 DOI: 10.1146/annurev.med.080608.100623]

14 **Daher S**, Massarwa M, Benson AA, Khoury T. Current and Future Treatment of Hepatocellular Carcinoma: An Updated Comprehensive Review. *J Clin Transl Hepatol* 2018; **6**: 69-78 [PMID: 29607307 DOI: 10.14218/JCTH.2017.00031]

15 **Ghavimi S**, Apfel T, Azimi H, Persaud A, Pyrsopoulos NT. Management and Treatment of Hepatocellular Carcinoma with Immunotherapy: A Review of Current and Future Options. *J Clin Transl Hepatol* 2020; **8**: 168-176 [PMID: 32832397 DOI: 10.14218/JCTH.2020.00001]

16 **Saffo S**, Taddei TH. Systemic Management for Advanced Hepatocellular Carcinoma: A Review of the Molecular Pathways of Carcinogenesis, Current and Emerging Therapies, and Novel Treatment Strategies. *Dig Dis Sci* 2019; **64**: 1016-1029 [PMID: 30887150 DOI: 10.1007/s10620-019-05582-x]

17 **Lu LC**, Cheng AL, Poon RT. Recent advances in the prevention of hepatocellular carcinoma recurrence. *Semin Liver Dis* 2014; **34**: 427-434 [PMID: 25369304 DOI: 10.1055/s-0034-1394141]

18 **Tsai WL**, Chung RT. Viral hepatocarcinogenesis. *Oncogene* 2010; **29**: 2309-2324 [PMID: 20228847 DOI: 10.1038/onc.2010.36]

19 **Ferlay J**, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359-E386 [PMID: 25220842 DOI: 10.1002/ijc.29210]

20 **El-Serag HB**. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 2012; **142**: 1264-1273. e1 [PMID: 22537432 DOI: 10.1053/j.gastro.2011.12.061]

21 **Nam SW**, Jung JJ, Bae SH, Choi JY, Yoon SK, Cho SH, Han JY, Han NI, Yang JM, Lee YS. Clinical outcomes of delayed clearance of serum HBsAG in patients with chronic HBV infection. *Korean J Intern Med* 2007; **22**: 73-76 [PMID: 17616021 DOI: 10.3904/kjim.2007.22.2.73]

22 **Bellezza CA**, Sexton S, Curtin LI, Concannon PW, Baldwin BH, Graham LA, Hornbuckle WE, Roth L, Tennant BC. The laboratory woodchuck (Marmota monax). In: Fox JG, Anderson LC, Otto GM, Pritchett-Corning KR, Whary MT (eds). American College of Laboratory Animal Medicine,

Laboratory Animal Medicine. 3rd ed. Cambridge (MA): Academic Press, 2015: 351-386 [DOI: 10.1016/B978-0-12-409527-4.00008-0]

23 **Summers J**, Smolec JM, Snyder R. A virus similar to human hepatitis B virus associated with hepatitis and hepatoma in woodchucks. *Proc Natl Acad Sci USA* 1978; **75**: 4533-4537 [PMID: 212758 DOI: 10.1073/pnas.75.9.4533]

24 **Summers J**. Three recently described animal virus models for human hepatitis B virus. *Hepatology* 1981; **1**: 179-183 [PMID: 7286898 DOI: 10.1002/hep.1840010215]

25 **Galibert F**, Chen TN, Mandart E. Nucleotide sequence of a cloned woodchuck hepatitis virus genome: comparison with the hepatitis B virus sequence. *J Virol* 1982; **41**: 51-65 [PMID: 7086958 DOI: 10.1128/JVI.41.1.51-65.1982]

26 **Girones R**, Cote PJ, Hornbuckle WE, Tennant BC, Gerin JL, Purcell RH, Miller RH. Complete nucleotide sequence of a molecular clone of woodchuck hepatitis virus that is infectious in the natural host. *Proc Natl Acad Sci USA* 1989; **86**: 1846-1849 [PMID: 2928306 DOI: 10.1073/pnas.86.6.1846]

27 **Mason WS**. Animal models and the molecular biology of hepadnavirus infection. *Cold Spring Harb Perspect Med* 2015; **5** [PMID: 25833941 DOI: 10.1101/cshperspect.a021352]

28 **Seeger C**, Mason WS. Molecular biology of hepatitis B virus infection. *Virology* 2015; **479-480**: 672-686 [PMID: 25759099 DOI: 10.1016/j.virol.2015.02.031]

29 **Gust ID**, Burrell CJ, Coulepis AG, Robinson WS, Zuckerman AJ. Taxonomic classification of human hepatitis B virus. *Intervirology* 1986; **25**: 14-29 [PMID: 3516924 DOI: 10.1159/000149651]

30 **Menne S**, Cote PJ. The woodchuck as an animal model for pathogenesis and therapy of chronic hepatitis B virus infection. *World J Gastroenterol* 2007; **13**: 104-124 [PMID: 17206759 DOI: 10.3748/wjg.v13.i1.104]

31 **Popper H**, Roth L, Purcell RH, Tennant BC, Gerin JL. Hepatocarcinogenicity of the woodchuck hepatitis virus. *Proc Natl Acad Sci USA* 1987; **84**: 866-870 [PMID: 3468514 DOI: 10.1073/pnas.84.3.866]

32 **Snyder RL**, Tyler G, Summers J. Chronic hepatitis and hepatocellular carcinoma associated with woodchuck hepatitis virus. *Am J Pathol* 1982; **107**: 422-425 [PMID: 6282133]

33 **Cote PJ**, Korba BE, Miller RH, Jacob JR, Baldwin BH, Hornbuckle WE, Purcell RH, Tennant BC, Gerin JL. Effects of age and viral determinants on chronicity as an outcome of experimental woodchuck hepatitis virus infection. *Hepatology* 2000; **31**: 190-200 [PMID: 10613745 DOI: 10.1002/hep.510310128]

34 **Roggendorf M**, Kosinska AD, Liu J, Lu M. The Woodchuck, a Nonprimate Model for Immunopathogenesis and Therapeutic Immunomodulation in Chronic Hepatitis B Virus Infection. *Cold Spring Harb Perspect Med* 2015; **5** [PMID: 26511761 DOI: 10.1101/cshperspect.a021451]

35 **Tennant BC**, Gerin JL. The woodchuck model of hepatitis B virus infection. *ILAR J* 2001; **42**: 89-102 [PMID: 11406711 DOI: 10.1093/ilar.42.2.89]

36 **Cote PJ**, Korba BE, Tennant BC, Gerin JL. Immunopathogenesis and immunomodulation of woodchuck hepatitis virus infection. In: Hollinger FB, Lemon SM, Margolis HS (eds). Viral hepatitis and liver disease. Baltimore (MD): Lippincott Williams & Wilkins, 1991: 483-486

37 **Tennant BC**, Toshkov IA, Peek SF, Jacob JR, Menne S, Hornbuckle WE, Schinazi RD, Korba BE, Cote PJ, Gerin JL. Hepatocellular carcinoma in the woodchuck model of hepatitis B virus infection. *Gastroenterology* 2004; **127**: S283-S293 [PMID: 15508096 DOI: 10.1053/j.gastro.2004.09.043]

38 **Suslov A**, Wieland S, Menne S. Modulators of innate immunity as novel therapeutics for treatment of chronic hepatitis B. *Curr Opin Virol* 2018; **30**: 9-17 [PMID: 29444493 DOI: 10.1016/j.coviro.2018.01.008]

39 **Kosinska AD**, Liu J, Lu M, Roggendorf M. Therapeutic vaccination and immunomodulation in the treatment of chronic hepatitis B: preclinical studies in the woodchuck. *Med Microbiol Immunol* 2015; **204**: 103-114 [PMID: 25535101 DOI: 10.1007/s00430-014-0379-5]

40 **Michalak TI**. Diverse Virus and Host-Dependent Mechanisms Influence the Systemic and Intrahepatic Immune Responses in the Woodchuck Model of Hepatitis B. *Front Immunol* 2020; **11**: 853 [PMID: 32536912 DOI: 10.3389/fimmu.2020.00853]

41 **Cote PJ**, Toshkov I, Bellezza C, Ascenzi M, Roneker C, Ann Graham L, Baldwin BH, Gaye K, Nakamura I, Korba BE, Tennant BC, Gerin JL. Temporal pathogenesis of experimental neonatal woodchuck hepatitis virus infection: increased initial viral load and decreased severity of acute hepatitis during the development of chronic viral infection. *Hepatology* 2000; **32**: 807-817 [PMID: 11003627 DOI: 10.1053/jhep.2000.17681]

42 **Wong DC**, Shih JW, Purcell RH, Gerin JL, London WT. Natural and experimental infection of woodchucks with woodchuck hepatitis virus, as measured by new, specific assays for woodchuck surface antigen and antibody. *J Clin Microbiol* 1982; **15**: 484-490 [PMID: 7076821 DOI: 10.1128/JCM.15.3.484-490.1982]

43 **Millman I**, Southam L, Halbherr T, Simmons H, Kang CM. Woodchuck hepatitis virus: experimental infection and natural occurrence. *Hepatology* 1984; **4**: 817-823 [PMID: 6383996 DOI: 10.1002/hep.1840040503]

44 **Tyler GV**, Snyder RL, Summers J. Experimental infection of the woodchuck (Marmota monax monax) with woodchuck hepatitis virus. *Lab Invest* 1986; **55**: 51-55 [PMID: 3724063]

45 **Kajino K**, Jilbert AR, Saputelli J, Aldrich CE, Cullen J, Mason WS. Woodchuck hepatitis virus infections: very rapid recovery after a prolonged viremia and infection of virtually every hepatocyte. *J Virol* 1994; **68**: 5792-5803 [PMID: 7914548 DOI: 10.1128/JVI.68.9.5792-5803.1994]

46 **Guy CS**, Mulrooney-Cousins PM, Churchill ND, Michalak TI. Intrahepatic expression of genes affiliated with innate and adaptive immune responses immediately after invasion and during acute infection with woodchuck hepadnavirus. *J Virol* 2008; **82**: 8579-8591 [PMID: 18596101 DOI: 10.1128/JVI.01022-08]

47 **Wieland S**, Thimme R, Purcell RH, Chisari FV. Genomic analysis of the host response to hepatitis B virus infection. *Proc Natl Acad Sci USA* 2004; **101**: 6669-6674 [PMID: 15100412 DOI: 10.1073/pnas.0401771101]

48 **Suresh M**, Czerwinski S, Murreddu MG, Kallakury BV, Ramesh A, Gudima SO, Menne S. Innate and adaptive immunity associated with resolution of acute woodchuck hepatitis virus infection in adult woodchucks. *PLoS Pathog* 2019; **15**: e1008248 [PMID: 31869393 DOI: 10.1371/journal.ppat.1008248]

49 **Lucifora J**, Xia Y, Reisinger F, Zhang K, Stadler D, Cheng X, Sprinzl MF, Koppensteiner H, Makowska Z, Volz T, Remouchamps C, Chou WM, Thasler WE, Hüser N, Durantel D, Liang TJ, Münk C, Heim MH, Browning JL, Dejardin E, Dandri M, Schindler M, Heikenwalder M, Protzer U. Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA. *Science* 2014; **343**: 1221-1228 [PMID: 24557838 DOI: 10.1126/science.1243462]

50 **Wieland SF**, Eustaquio A, Whitten-Bauer C, Boyd B, Chisari FV. Interferon prevents formation of replication-competent hepatitis B virus RNA-containing nucleocapsids. *Proc Natl Acad Sci USA* 2005; **102**: 9913-9917 [PMID: 15994231 DOI: 10.1073/pnas.0504273102]

51 **Xia Y**, Stadler D, Lucifora J, Reisinger F, Webb D, Hösel M, Michler T, Wisskirchen K, Cheng X, Zhang K, Chou WM, Wettengel JM, Malo A, Bohne F, Hoffmann D, Eyer F, Thimme R, Falk CS, Thasler WE, Heikenwalder M, Protzer U. Interferon-γ and Tumor Necrosis Factor-α Produced by T Cells Reduce the HBV Persistence Form, cccDNA, Without Cytolysis. *Gastroenterology* 2016; **150**: 194-205 [PMID: 26416327 DOI: 10.1053/j.gastro.2015.09.026]

52 **Xu C**, Guo H, Pan XB, Mao R, Yu W, Xu X, Wei L, Chang J, Block TM, Guo JT. Interferons accelerate decay of replication-competent nucleocapsids of hepatitis B virus. *J Virol* 2010; **84**: 9332-9340 [PMID: 20610715 DOI: 10.1128/JVI.00918-10]

53 **Liu Y**, Nie H, Mao R, Mitra B, Cai D, Yan R, Guo JT, Block TM, Mechti N, Guo H. Interferon-inducible ribonuclease ISG20 inhibits hepatitis B virus replication through directly binding to the epsilon stem-loop structure of viral RNA. *PLoS Pathog* 2017; **13**: e1006296 [PMID: 28399146 DOI: 10.1371/journal.ppat.1006296]

54 **Wieland SF**, Guidotti LG, Chisari FV. Intrahepatic induction of alpha/beta interferon eliminates viral RNA-containing capsids in hepatitis B virus transgenic mice. *J Virol* 2000; **74**: 4165-4173 [PMID: 10756029 DOI: 10.1128/jvi.74.9.4165-4173.2000]

55 **Wieland SF**, Spangenberg HC, Thimme R, Purcell RH, Chisari FV. Expansion and contraction of the hepatitis B virus transcriptional template in infected chimpanzees. *Proc Natl Acad Sci USA* 2004; **101**: 2129-2134 [PMID: 14764900 DOI: 10.1073/pnas.0308478100]

56 **Anderson AL**, Banks KE, Pontoglio M, Yaniv M, McLachlan A. Alpha/beta interferon differentially modulates the clearance of cytoplasmic encapsidated replication intermediates and nuclear covalently closed circular hepatitis B virus (HBV) DNA from the livers of hepatocyte nuclear factor 1alpha-null HBV transgenic mice. *J Virol* 2005; **79**: 11045-11052 [PMID: 16103155 DOI: 10.1128/JVI.79.17.11045-11052.2005]

57 **Hong X**, Luckenbaugh L, Perlman D, Revill PA, Wieland SF, Menne S, Hu J. Characterization and Application of Precore/Core-Related Antigens in Animal Models of Hepatitis B Virus Infection. *Hepatology* 2021 [PMID: 33458844 DOI: 10.1002/hep.31720]

58 **Hodgson PD**, Michalak TI. Augmented hepatic interferon gamma expression and T-cell influx characterize acute hepatitis progressing to recovery and residual lifelong virus persistence in experimental adult woodchuck hepatitis virus infection. *Hepatology* 2001; **34**: 1049-1059 [PMID: 11679978 DOI: 10.1053/jhep.2001.29004]

59 **Guo JT**, Zhou H, Liu C, Aldrich C, Saputelli J, Whitaker T, Barrasa MI, Mason WS, Seeger C. Apoptosis and regeneration of hepatocytes during recovery from transient hepadnavirus infections. *J Virol* 2000; **74**: 1495-1505 [PMID: 10627561 DOI: 10.1128/jvi.74.3.1495-1505.2000]

60 **Hodgson PD**, Grant MD, Michalak TI. Perforin and Fas/Fas ligand-mediated cytotoxicity in acute and chronic woodchuck viral hepatitis. *Clin Exp Immunol* 1999; **118**: 63-70 [PMID: 10540161 DOI: 10.1046/j.1365-2249.1999.01010.x]

61 **Korba BE**, Cote PJ, Wells FV, Baldwin B, Popper H, Purcell RH, Tennant BC, Gerin JL. Natural history of woodchuck hepatitis virus infections during the course of experimental viral infection: molecular virologic features of the liver and lymphoid tissues. *J Virol* 1989; **63**: 1360-1370 [PMID: 2915383 DOI: 10.1128/JVI.63.3.1360-1370.1989]

62 **Korba BE**, Cote PJ, Gerin JL. Mitogen-induced replication of woodchuck hepatitis virus in cultured peripheral blood lymphocytes. *Science* 1988; **241**: 1213-1216 [PMID: 3261887 DOI: 10.1126/science.3261887]

63 **Coffin CS**, Michalak TI. Persistence of infectious hepadnavirus in the offspring of woodchuck mothers recovered from viral hepatitis. *J Clin Invest* 1999; **104**: 203-212 [PMID: 10411550 DOI: 10.1172/JCI5048]

64 **Michalak TI**, Pardoe IU, Coffin CS, Churchill ND, Freake DS, Smith P, Trelegan CL. Occult lifelong persistence of infectious hepadnavirus and residual liver inflammation in woodchucks convalescent from acute viral hepatitis. *Hepatology* 1999; **29**: 928-938 [PMID: 10051500 DOI: 10.1002/hep.510290329]

65 **Mason WS**, Jilbert AR, Summers J. Clonal expansion of hepatocytes during chronic woodchuck hepatitis virus infection. *Proc Natl Acad Sci USA* 2005; **102**: 1139-1144 [PMID: 15657132 DOI: 10.1073/pnas.0409332102]

66 **Summers J**, Jilbert AR, Yang W, Aldrich CE, Saputelli J, Litwin S, Toll E, Mason WS. Hepatocyte turnover during resolution of a transient hepadnaviral infection. *Proc Natl Acad Sci USA* 2003; **100**: 11652-11659 [PMID: 14500915 DOI: 10.1073/pnas.1635109100]

67 **Chauhan R**, Churchill ND, Mulrooney-Cousins PM, Michalak TI. Initial sites of hepadnavirus integration into host genome in human hepatocytes and in the woodchuck model of hepatitis B-associated hepatocellular carcinoma. *Oncogenesis* 2017; **6**: e317 [PMID: 28414318 DOI: 10.1038/oncsis.2017.22]

68 **Tennant BC**. Animal models of hepadnavirus-associated hepatocellular carcinoma. *Clin Liver Dis* 2001; **5**: 43-68 [PMID: 11218919 DOI: 10.1016/s1089-3261(05)70153-7]

69 **Hong X**, Luckenbaugh L, Mendenhall M, Walsh R, Cabuang L, Soppe S, Revill PA, Burdette D, Feierbach B, Delaney W, Hu J. Characterization of Hepatitis B Precore/Core-Related Antigens. *J Virol* 2021; **95** [PMID: 33148795 DOI: 10.1128/JVI.01695-20]

70 **Ponzetto A**, Forzani B. Animal models of hepatocellular carcinoma: hepadnavirus-induced liver cancer in woodchucks. *Ital J Gastroenterol* 1991; **23**: 491-493 [PMID: 1751825]

71 **Seeger C**, Baldwin B, Hornbuckle WE, Yeager AE, Tennant BC, Cote P, Ferrell L, Ganem D, Varmus HE. Woodchuck hepatitis virus is a more efficient oncogenic agent than ground squirrel hepatitis virus in a common host. *J Virol* 1991; **65**: 1673-1679 [PMID: 2002538 DOI: 10.1128/JVI.65.4.1673-1679.1991]

72 **Korba BE**, Wells FV, Baldwin B, Cote PJ, Tennant BC, Popper H, Gerin JL. Hepatocellular carcinoma in woodchuck hepatitis virus-infected woodchucks: presence of viral DNA in tumor tissue from chronic carriers and animals serologically recovered from acute infections. *Hepatology* 1989; **9**: 461-470 [PMID: 2465987 DOI: 10.1002/hep.1840090321]

73 **Cote PJ**, Gerin JL. The woodchuck as a model of hepadnavirus infection, pathogenesis and therapy. *Forum Trends Exp Clin Med* 1996; **6**: 131-159

74 **Tennant BC**, Gerin JL. The woodchuck model of hepatitis B virus infection. In: Arias IM, Boyer J, Fausto N, Jakoby WB, Schachter D, Shafritz DA (eds). The liver: Biology and pathology. 3rd ed. New York (NY): Raven Press, 1994: 1455-1466

75 **Nakamura I**, Nupp JT, Cowlen M, Hall WC, Tennant BC, Casey JL, Gerin JL, Cote PJ. Pathogenesis of experimental neonatal woodchuck hepatitis virus infection: chronicity as an outcome of infection is associated with a diminished acute hepatitis that is temporally deficient for the expression of interferon gamma and tumor necrosis factor-alpha messenger RNAs. *Hepatology* 2001; **33**: 439-447 [PMID: 11172347 DOI: 10.1053/jhep.2001.21748]

76 **Wang Y**, Menne S, Jacob JR, Tennant BC, Gerin JL, Cote PJ. Role of type 1 versus type 2 immune responses in liver during the onset of chronic woodchuck hepatitis virus infection. *Hepatology* 2003; **37**: 771-780 [PMID: 12668969 DOI: 10.1053/jhep.2003.50154]

77 **Bertoletti A**, Ferrari C. Innate and adaptive immune responses in chronic hepatitis B virus infections: towards restoration of immune control of viral infection. *Gut* 2012; **61**: 1754-1764 [PMID: 22157327 DOI: 10.1136/gutjnl-2011-301073]

78 **Ferrari C**. HBV and the immune response. *Liver Int* 2015; **35** Suppl 1: 121-128 [PMID: 25529097 DOI: 10.1111/liv.12749]

79 **Fletcher SP**, Chin DJ, Ji Y, Iniguez AL, Taillon B, Swinney DC, Ravindran P, Cheng DT, Bitter H, Lopatin U, Ma H, Klumpp K, Menne S. Transcriptomic analysis of the woodchuck model of chronic hepatitis B. *Hepatology* 2012; **56**: 820-830 [PMID: 22431061 DOI: 10.1002/hep.25730]

80 **Michalak TI**, Hodgson PD, Churchill ND. Posttranscriptional inhibition of class I major histocompatibility complex presentation on hepatocytes and lymphoid cells in chronic woodchuck hepatitis virus infection. *J Virol* 2000; **74**: 4483-4494 [PMID: 10775584 DOI: 10.1128/jvi.74.10.4483-4494.2000]

81 **Guy CS**, Wang J, Michalak TI. Hepatocytes as cytotoxic effector cells can induce cell death by CD95 ligand-mediated pathway. *Hepatology* 2006; **43**: 1231-1240 [PMID: 16729304 DOI: 10.1002/hep.21201]

82 **Guy CS**, Rankin SL, Wang J, Michalak TI. Hepatocytes can induce death of contacted cells *via* perforin-dependent mechanism. *Hepatology* 2008; **47**: 1691-1701 [PMID: 18393317 DOI: 10.1002/hep.22228]

83 **Zhang E**, Zhang X, Liu J, Wang B, Tian Y, Kosinska AD, Ma Z, Xu Y, Dittmer U, Roggendorf M, Yang D, Lu M. The expression of PD-1 ligands and their involvement in regulation of T cell functions in acute and chronic woodchuck hepatitis virus infection. *PLoS One* 2011; **6**: e26196 [PMID: 22022563 DOI: 10.1371/journal.pone.0026196]

84 **Sitia G**, Isogawa M, Kakimi K, Wieland SF, Chisari FV, Guidotti LG. Depletion of neutrophils blocks the recruitment of antigen-nonspecific cells into the liver without affecting the antiviral activity of hepatitis B virus-specific cytotoxic T lymphocytes. *Proc Natl Acad Sci USA* 2002; **99**: 13717-13722 [PMID: 12368481 DOI: 10.1073/pnas.172521999]

85 **Zhang JY**, Zou ZS, Huang A, Zhang Z, Fu JL, Xu XS, Chen LM, Li BS, Wang FS. Hyper-activated pro-inflammatory CD16 monocytes correlate with the severity of liver injury and fibrosis in patients with chronic hepatitis B. *PLoS One* 2011; **6**: e17484 [PMID: 21390263 DOI: 10.1371/journal.pone.0017484]

86 **Gouillat C**, Manganas D, Zoulim F, Vitrey D, Saguier G, Guillaud M, Ain JF, Duque-Campos R,

Jamard C, Praves M, Trepo C. Woodchuck hepatitis virus-induced carcinoma as a relevant natural model for therapy of human hepatoma. *J Hepatol* 1997; **26**: 1324-1330 [PMID: 9210620 DOI: 10.1016/s0168-8278(97)80468-0]

87 **Popper H**, Shih JW, Gerin JL, Wong DC, Hoyer BH, London WT, Sly DL, Purcell RH. Woodchuck hepatitis and hepatocellular carcinoma: correlation of histologic with virologic observations. *Hepatology* 1981; **1**: 91-98 [PMID: 6269981 DOI: 10.1002/hep.1840010202]

88 **Mi LJ**, Patil J, Hornbuckle WE, Cote PJ, Gerin JL, Tennant BC, Paronetto F. DNA ploidy analysis of hepatic preneoplastic and neoplastic lesions in woodchucks experimentally infected with woodchuck hepatitis virus. *Hepatology* 1994; **20**: 21-29 [PMID: 8020890 DOI: 10.1016/0270-9139(94)90129-5]

89 **Fourel G**, Trepo C, Bougueleret L, Henglein B, Ponzetto A, Tiollais P, Buendia MA. Frequent activation of N-myc genes by hepadnavirus insertion in woodchuck liver tumours. *Nature* 1990; **347**: 294-298 [PMID: 2205804 DOI: 10.1038/347294a0]

90 **Kaneko S**, Oshima T, Kodama K, Aoyama S, Yoshikawa H, Unoura M, Fukuoka K, Matsushita F, Morimoto H, Kobayashi K. Stable integration of woodchuck hepatitis virus DNA in transplanted tumors and established tissue culture cells derived from a woodchuck primary hepatocellular carcinoma. *Cancer Res* 1986; **46**: 3608-3613 [PMID: 3011252]

91 **Fuchs K**, Heberger C, Weimer T, Roggendorf M. Characterization of woodchuck hepatitis virus DNA and RNA in the hepatocellular carcinomas of woodchucks. *Hepatology* 1989; **10**: 215-220 [PMID: 2545590 DOI: 10.1002/hep.1840100216]

92 **Popper H**, Shafritz DA, Hoofnagle JH. Relation of the hepatitis B virus carrier state to hepatocellular carcinoma. *Hepatology* 1987; **7**: 764-772 [PMID: 3038725 DOI: 10.1002/hep.1840070425]

93 **Chisari FV**, Klopchin K, Moriyama T, Pasquinelli C, Dunsford HA, Sell S, Pinkert CA, Brinster RL, Palmiter RD. Molecular pathogenesis of hepatocellular carcinoma in hepatitis B virus transgenic mice. *Cell* 1989; **59**: 1145-1156 [PMID: 2598264 DOI: 10.1016/0092-8674(89)90770-8]

94 **Roth L**, King JM, Hornbuckle WE, Harvey HJ, Tennant BC. Chronic hepatitis and hepatocellular carcinoma associated with persistent woodchuck hepatitis virus infection. *Vet Pathol* 1985; **22**: 338-343 [PMID: 2994274 DOI: 10.1177/030098588502200407]

95 **Nada T**, Moriyasu F, Kono Y, Suginoshita Y, Matsumura T, Kobayashi K, Nakamura T, Chiba T. Sonographic detection of tumor blood flow using a new contrast agent in woodchuck hepatomas. *J Ultrasound Med* 1997; **16**: 485-491 [PMID: 9315200 DOI: 10.7863/jum.1997.16.7.485]

96 **Chayanupatkul M**, Omino R, Mittal S, Kramer JR, Richardson P, Thrift AP, El-Serag HB, Kanwal F. Hepatocellular carcinoma in the absence of cirrhosis in patients with chronic hepatitis B virus infection. *J Hepatol* 2017; **66**: 355-362 [PMID: 27693539 DOI: 10.1016/j.jhep.2016.09.013]

97 **Gaddikeri S**, McNeeley MF, Wang CL, Bhargava P, Dighe MK, Yeh MM, Dubinsky TJ, Kolokythas O, Lalwani N. Hepatocellular carcinoma in the noncirrhotic liver. *AJR Am J Roentgenol* 2014; **203**: W34-W47 [PMID: 24951228 DOI: 10.2214/AJR.13.11511]

98 **Abe K**, Kurata T, Shikata T, Tennant BC. Enzyme-altered liver cell foci in woodchucks infected with woodchuck hepatitis virus. *Jpn J Cancer Res* 1988; **79**: 466-472 [PMID: 2898465 DOI: 10.1111/j.1349-7006.1988.tb01615.x]

99 **Toshkov I**, Hacker HJ, Roggendorf M, Bannasch P. Phenotypic patterns of preneoplastic and neoplastic hepatic lesions in woodchucks infected with woodchuck hepatitis virus. *J Cancer Res Clin Oncol* 1990; **116**: 581-590 [PMID: 2152341 DOI: 10.1007/BF01637078]

100 **Radaeva S**, Li Y, Hacker HJ, Burger V, Kopp-Schneider A, Bannasch P. Hepadnaviral hepatocarcinogenesis: in situ visualization of viral antigens, cytoplasmic compartmentation, enzymic patterns, and cellular proliferation in preneoplastic hepatocellular lineages in woodchucks. *J Hepatol* 2000; **33**: 580-600 [PMID: 11059863 DOI: 10.1034/j.1600-0641.2000.033004580.x]

101 **Tu T**, Mason WS, Clouston AD, Shackel NA, McCaughan GW, Yeh MM, Schiff ER, Ruszkiewicz AR, Chen JW, Harley HA, Stroeher UH, Jilbert AR. Clonal expansion of hepatocytes with a selective advantage occurs during all stages of chronic hepatitis B virus infection. *J Viral Hepat* 2015; **22**: 737-753 [PMID: 25619231 DOI: 10.1111/jvh.12380]

102 **Mason WS**, Liu C, Aldrich CE, Litwin S, Yeh MM. Clonal expansion of normal-appearing human hepatocytes during chronic hepatitis B virus infection. *J Virol* 2010; **84**: 8308-8315 [PMID: 20519397 DOI: 10.1128/JVI.00833-10]

103 **Cullen JM**, Linzey DW, Gebhard DH. Nuclear ploidy of normal and neoplastic hepatocytes from woodchuck hepatitis virus-infected and uninfected woodchucks. *Hepatology* 1994; **19**: 1072-1078 [PMID: 8175128]

104 **Jacob JR**, Sterczer A, Toshkov IA, Yeager AE, Korba BE, Cote PJ, Buendia MA, Gerin JL, Tennant BC. Integration of woodchuck hepatitis and N-myc rearrangement determine size and histologic grade of hepatic tumors. *Hepatology* 2004; **39**: 1008-1016 [PMID: 15057905 DOI: 10.1002/hep.20106]

105 **Lee JS**, Chu IS, Heo J, Calvisi DF, Sun Z, Roskams T, Durnez A, Demetris AJ, Thorgeirsson SS. Classification and prediction of survival in hepatocellular carcinoma by gene expression profiling. *Hepatology* 2004; **40**: 667-676 [PMID: 15349906 DOI: 10.1002/hep.20375]

106 **Hoshida Y**, Nijman SM, Kobayashi M, Chan JA, Brunet JP, Chiang DY, Villanueva A, Newell P, Ikeda K, Hashimoto M, Watanabe G, Gabriel S, Friedman SL, Kumada H, Llovet JM, Golub TR. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. *Cancer Res* 2009; **69**: 7385-7392 [PMID: 19723656 DOI:

10.1158/0008-5472.CAN-09-1089]
107 **Marion PL**, Van Davelaar MJ, Knight SS, Salazar FH, Garcia G, Popper H, Robinson WS. Hepatocellular carcinoma in ground squirrels persistently infected with ground squirrel hepatitis virus. *Proc Natl Acad Sci USA* 1986; **83**: 4543-4546 [PMID: 3012572 DOI: 10.1073/pnas.83.12.4543]
108 **Jayant K**, Habib N, Huang KW, Warwick J, Arasaradnam R. Recent Advances: The Imbalance of Immune Cells and Cytokines in the Pathogenesis of Hepatocellular Carcinoma. *Diagnostics (Basel)* 2020; **10** [PMID: 32466214 DOI: 10.3390/diagnostics10050338]
109 **Nakamoto Y**, Guidotti LG, Kuhlen CV, Fowler P, Chisari FV. Immune pathogenesis of hepatocellular carcinoma. *J Exp Med* 1998; **188**: 341-350 [PMID: 9670046 DOI: 10.1084/jem.188.2.341]
110 **Terradillos O**, Billet O, Renard CA, Levy R, Molina T, Briand P, Buendia MA. The hepatitis B virus X gene potentiates c-myc-induced liver oncogenesis in transgenic mice. *Oncogene* 1997; **14**: 395-404 [PMID: 9053836 DOI: 10.1038/sj.onc.1200850]
111 **Madden CR**, Finegold MJ, Slagle BL. Hepatitis B virus X protein acts as a tumor promoter in development of diethylnitrosamine-induced preneoplastic lesions. *J Virol* 2001; **75**: 3851-3858 [PMID: 11264374 DOI: 10.1128/JVI.75.8.3851-3858.2001]
112 **Feitelson MA**, Lee J. Hepatitis B virus integration, fragile sites, and hepatocarcinogenesis. *Cancer Lett* 2007; **252**: 157-170 [PMID: 17188425 DOI: 10.1016/j.canlet.2006.11.010]
113 **Fowler MJ**, Greenfield C, Chu CM, Karayiannis P, Dunk A, Lok AS, Lai CL, Yeoh EK, Monjardino JP, Wankya BM. Integration of HBV-DNA may not be a prerequisite for the maintenance of the state of malignant transformation. An analysis of 110 liver biopsies. *J Hepatol* 1986; **2**: 218-229 [PMID: 3958473 DOI: 10.1016/s0168-8278(86)80080-0]
114 **Mason WS**, Gill US, Litwin S, Zhou Y, Peri S, Pop O, Hong ML, Naik S, Quaglia A, Bertoletti A, Kennedy PT. HBV DNA Integration and Clonal Hepatocyte Expansion in Chronic Hepatitis B Patients Considered Immune Tolerant. *Gastroenterology* 2016; **151**: 986-998. e4 [PMID: 27453547 DOI: 10.1053/j.gastro.2016.07.012]
115 **Levrero M**, Zucman-Rossi J. Mechanisms of HBV-induced hepatocellular carcinoma. *J Hepatol* 2016; **64**: S84-S101 [PMID: 27084040 DOI: 10.1016/j.jhep.2016.02.021]
116 **Chauhan R**, Michalak TI. Kinetics of DNA damage repair response accompanying initial hepadnavirus-host genomic integration in woodchuck hepatitis virus infection of hepatocyte. *Cancer Genet* 2020; **244**: 1-10 [PMID: 32062411 DOI: 10.1016/j.cancergen.2020.02.001]
117 **Freitas N**, Lukash T, Gunewardena S, Chappell B, Slagle BL, Gudima SO. Relative Abundance of Integrant-Derived Viral RNAs in Infected Tissues Harvested from Chronic Hepatitis B Virus Carriers. *J Virol* 2018; **92** [PMID: 29491161 DOI: 10.1128/JVI.02221-17]
118 **Freitas N**, Cunha C, Menne S, Gudima SO. Envelope proteins derived from naturally integrated hepatitis B virus DNA support assembly and release of infectious hepatitis delta virus particles. *J Virol* 2014; **88**: 5742-5754 [PMID: 24623409 DOI: 10.1128/JVI.00430-14]
119 **Wei Y**, Fourel G, Ponzetto A, Silvestro M, Tiollais P, Buendia MA. Hepadnavirus integration: mechanisms of activation of the N-myc2 retrotransposon in woodchuck liver tumors. *J Virol* 1992; **66**: 5265-5276 [PMID: 1323693 DOI: 10.1128/JVI.66.9.5265-5276.1992]
120 **Hansen LJ**, Tennant BC, Seeger C, Ganem D. Differential activation of myc gene family members in hepatic carcinogenesis by closely related hepatitis B viruses. *Mol Cell Biol* 1993; **13**: 659-667 [PMID: 8380230 DOI: 10.1128/mcb.13.1.659]
121 **Bruni R**, Conti I, Villano U, Giuseppetti R, Palmieri G, Rapicetta M. Lack of WHV integration nearby N-myc2 and in the downstream b3n and win loci in a considerable fraction of liver tumors with activated N-myc2 from naturally infected wild woodchucks. *Virology* 2006; **345**: 258-269 [PMID: 16271377 DOI: 10.1016/j.virol.2005.09.061]
122 **Bruni R**, D'Ugo E, Villano U, Fourel G, Buendia MA, Rapicetta M. The win locus involved in activation of the distal N-myc2 gene upon WHV integration in woodchuck liver tumors harbors S/MAR elements. *Virology* 2004; **329**: 1-10 [PMID: 15476869 DOI: 10.1016/j.virol.2004.08.008]
123 **Yang D**, Faris R, Hixson D, Affigne S, Rogler CE. Insulin-like growth factor II blocks apoptosis of N-myc2-expressing woodchuck liver epithelial cells. *J Virol* 1996; **70**: 6260-6268 [PMID: 8709253 DOI: 10.1128/JVI.70.9.6260-6268.1996]
124 **Ueda K**, Ganem D. Apoptosis is induced by N-myc expression in hepatocytes, a frequent event in hepadnavirus oncogenesis, and is blocked by insulin-like growth factor II. *J Virol* 1996; **70**: 1375-1383 [PMID: 8627653 DOI: 10.1128/JVI.70.3.1375-1383.1996]
125 **Renard CA**, Fourel G, Bralet MP, Degott C, De La Coste A, Perret C, Tiollais P, Buendia MA. Hepatocellular carcinoma in WHV/N-myc2 transgenic mice: oncogenic mutations of beta-catenin and synergistic effect of p53 null alleles. *Oncogene* 2000; **19**: 2678-2686 [PMID: 10851067 DOI: 10.1038/sj.onc.1203617]
126 **Ura S**, Honda M, Yamashita T, Ueda T, Takatori H, Nishino R, Sunakozaka H, Sakai Y, Horimoto K, Kaneko S. Differential microRNA expression between hepatitis B and hepatitis C leading disease progression to hepatocellular carcinoma. *Hepatology* 2009; **49**: 1098-1112 [PMID: 19173277 DOI: 10.1002/hep.22749]
127 **Connolly E**, Melegari M, Landgraf P, Tchaikovskaya T, Tennant BC, Slagle BL, Rogler LE, Zavolan M, Tuschl T, Rogler CE. Elevated expression of the miR-17-92 polycistron and miR-21 in hepadnavirus-associated hepatocellular carcinoma contributes to the malignant phenotype. *Am J Pathol* 2008; **173**: 856-864 [PMID: 18688024 DOI: 10.2353/ajpath.2008.080096]

128 **Chen HS**, Kaneko S, Girones R, Anderson RW, Hornbuckle WE, Tennant BC, Cote PJ, Gerin JL, Purcell RH, Miller RH. The woodchuck hepatitis virus X gene is important for establishment of virus infection in woodchucks. *J Virol* 1993; **67**: 1218-1226 [PMID: 8437213 DOI: 10.1128/JVI.67.3.1218-1226.1993]

129 **Ng SA**, Lee C. Hepatitis B virus X gene and hepatocarcinogenesis. *J Gastroenterol* 2011; **46**: 974-990 [PMID: 21647825 DOI: 10.1007/s00535-011-0415-9]

130 **Wang WH**, Studach LL, Andrisani OM. Proteins ZNF198 and SUZ12 are down-regulated in hepatitis B virus (HBV) X protein-mediated hepatocyte transformation and in HBV replication. *Hepatology* 2011; **53**: 1137-1147 [PMID: 21480320 DOI: 10.1002/hep.24163]

131 **Studach LL**, Menne S, Cairo S, Buendia MA, Hullinger RL, Lefrançois L, Merle P, Andrisani OM. Subset of Suz12/PRC2 target genes is activated during hepatitis B virus replication and liver carcinogenesis associated with HBV X protein. *Hepatology* 2012; **56**: 1240-1251 [PMID: 22505317 DOI: 10.1002/hep.25781]

132 **Ryu SH**, Chung YH, Lee H, Kim JA, Shin HD, Min HJ, Seo DD, Jang MK, Yu E, Kim KW. Metastatic tumor antigen 1 is closely associated with frequent postoperative recurrence and poor survival in patients with hepatocellular carcinoma. *Hepatology* 2008; **47**: 929-936 [PMID: 18306220 DOI: 10.1002/hep.22124]

133 **Li YT**, Liu CJ, Su TH, Cheng HR, Jeng YM, Lin HL, Wang CC, Kao JH, Chen PJ, Chen DS, Wu HL. Characterization of metastatic tumor antigen 1 and its interaction with hepatitis B virus X protein in NF-κB signaling and tumor progression in a woodchuck hepatocellular carcinoma model. *Oncotarget* 2016; **7**: 47173-47185 [PMID: 27323415 DOI: 10.18632/oncotarget.9986]

134 **Tseng PL**, Tai MH, Huang CC, Wang CC, Lin JW, Hung CH, Chen CH, Wang JH, Lu SN, Lee CM, Changchien CS, Hu TH. Overexpression of VEGF is associated with positive p53 immunostaining in hepatocellular carcinoma (HCC) and adverse outcome of HCC patients. *J Surg Oncol* 2008; **98**: 349-357 [PMID: 18646041 DOI: 10.1002/jso.21109]

135 **Finn RS**, Zhu AX. Targeting angiogenesis in hepatocellular carcinoma: focus on VEGF and bevacizumab. *Expert Rev Anticancer Ther* 2009; **9**: 503-509 [PMID: 19374603 DOI: 10.1586/era.09.6]

136 **Huang H**, Salavaggione O, Rivera L, Mukherjee S, Brekken R, Tennant B, Iyer R, Adjei A. Woodchuck VEGF (wVEGF) characteristics: Model for angiogenesis and human hepatocellular carcinoma directed therapies. *Arch Biochem Biophys* 2019; **661**: 97-106 [PMID: 30439360 DOI: 10.1016/j.abb.2018.11.008]

137 **Altadill A**, Rodríguez M, González LO, Junquera S, Corte MD, González-Dieguez ML, Linares A, Barbón E, Fresno-Forcelledo M, Rodrigo L, Vizoso FJ. Liver expression of matrix metalloproteases and their inhibitors in hepatocellular carcinoma. *Dig Liver Dis* 2009; **41**: 740-748 [PMID: 19372066 DOI: 10.1016/j.dld.2009.01.016]

138 **Ochoa-Callejero L**, Toshkov I, Menne S, Martínez A. Expression of matrix metalloproteinases and their inhibitors in the woodchuck model of hepatocellular carcinoma. *J Med Virol* 2013; **85**: 1127-1138 [PMID: 23595580 DOI: 10.1002/jmv.23571]

139 **Tao K**, Qian N, Tang Y, Ti Z, Song W, Cao D, Dou K. Increased expression of a disintegrin and metalloprotease-9 in hepatocellular carcinoma: implications for tumor progression and prognosis. *Jpn J Clin Oncol* 2010; **40**: 645-651 [PMID: 20388695 DOI: 10.1093/jjco/hyq030]

140 **Qin LX**, Tang ZY. The prognostic molecular markers in hepatocellular carcinoma. *World J Gastroenterol* 2002; **8**: 385-392 [PMID: 12046056 DOI: 10.3748/wjg.v8.i3.385]

141 **Fattovich G**, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 2004; **127**: S35-S50 [PMID: 15508101 DOI: 10.1053/j.gastro.2004.09.014]

142 **Rizzetto M**. Hepatitis D Virus: Introduction and Epidemiology. *Cold Spring Harb Perspect Med* 2015; **5**: a021576 [PMID: 26134842 DOI: 10.1101/cshperspect.a021576]

143 **Taylor JM**. Virology of hepatitis D virus. *Semin Liver Dis* 2012; **32**: 195-200 [PMID: 22932967 DOI: 10.1055/s-0032-1323623]

144 **Freitas N**, Salisse J, Cunha C, Toshkov I, Menne S, Gudima SO. Hepatitis delta virus infects the cells of hepadnavirus-induced hepatocellular carcinoma in woodchucks. *Hepatology* 2012; **56**: 76-85 [PMID: 22334419 DOI: 10.1002/hep.25663]

145 **Shiga J**, Ohnishi S, Imawari M, Yamamoto K, Koshimizu K, Sasaki N. Development and growth pattern of small hepatocellular carcinomas in woodchucks--analysis of an animal model of human hepatocellular carcinoma by ultrasonography. *Jikken Dobutsu* 1991; **40**: 545-548 [PMID: 1748173 DOI: 10.1538/expanim1978.40.4_545]

146 **Lisi D**, Kondili LA, Ramieri MT, Giuseppetti R, Bruni R, Della Rocca C, De Santis A, Rapicetta M. Ultrasonography in the study of hepatocellular carcinoma in woodchucks chronically infected with WHV. *Lab Anim* 2003; **37**: 233-240 [PMID: 12869286 DOI: 10.1258/002367703766453083]

147 **McKenzie EJ**, Jackson M, Sun J, Volotovskyy V, Gruwel ML. Monitoring the development of hepatocellular carcinoma in woodchucks using 31P-MRS. *MAGMA* 2005; **18**: 201-205 [PMID: 16133593 DOI: 10.1007/s10334-005-0120-x]

148 **McKenzie EJ**, Jackson M, Turner A, Gregorash L, Harapiak L. Chronic care and monitoring of woodchucks (Marmota monax) during repeated magnetic resonance imaging of the liver. *J Am Assoc Lab Anim Sci* 2006; **45**: 26-30 [PMID: 16542039]

149 **Hornbuckle WE**, Graham ES, Roth L, Baldwin BH, Wickenden C, Tennant BC. Laboratory assessment of hepatic injury in the woodchuck (Marmota monax). *Lab Anim Sci* 1985; **35**: 376-381

[PMID: 2864472]

150 **Cote PJ**, Gerin JL, Tennant BC. Alpha-fetoprotein in the woodchuck model of hepadnavirus infection and disease: normal physiological patterns and responses to woodchuck hepatitis virus infection and hepatocellular carcinoma. *Cancer Res* 1990; **50**: 7843-7851 [PMID: 1701355]

151 **Dahmen U**, Radtke A, Schröder T, Chi H, Madrahimov N, Lu M, Schenk A, Peitgen KH, Dirsch O. Median liver lobe of woodchuck as a model to study hepatic outflow obstruction: a pilot study. *Liver Int* 2008; **28**: 1236-1244 [PMID: 18544125 DOI: 10.1111/j.1478-3231.2008.01797.x]

152 **Mikhail AS**, Mauda-Havakuk M, Partanen A, Karanian JW, Pritchard WF, Wood BJ. Liver-specific 3D sectioning molds for correlating *in vivo* CT and MRI with tumor histopathology in woodchucks (Marmota monax). *PLoS One* 2020; **15**: e0230794 [PMID: 32214365 DOI: 10.1371/journal.pone.0230794]

153 **Nachabé R**, Hendriks BH, Schierling R, Hales J, Racadio JM, Rottenberg S, Ruers TJ, Babic D. Real-Time In Vivo Characterization of Primary Liver Tumors With Diffuse Optical Spectroscopy During Percutaneous Needle Interventions: Feasibility Study in Woodchucks. *Invest Radiol* 2015; **50**: 443-448 [PMID: 25783227 DOI: 10.1097/RLI.0000000000000149]

154 **Aravalli RN**, Golzarian J, Cressman EN. Animal models of cancer in interventional radiology. *Eur Radiol* 2009; **19**: 1049-1053 [PMID: 19137307 DOI: 10.1007/s00330-008-1263-8]

155 **Mak IW**, Evaniew N, Ghert M. Lost in translation: animal models and clinical trials in cancer treatment. *Am J Transl Res* 2014; **6**: 114-118 [PMID: 24489990]

156 **Rose SC**, Halstead GD, Narsinh KH. Pressure-Directed Embolization of Hepatic Arteries in a Porcine Model Using a Temporary Occlusion Balloon Microcatheter: Proof of Concept. *Cardiovasc Intervent Radiol* 2017; **40**: 1769-1776 [PMID: 28748354 DOI: 10.1007/s00270-017-1753-7]

157 **Kim AY**, Yacoub JH, Field DH, Park BU, Kallakury B, Korolowicz KE, Menne S. Suitability of the woodchuck HCC as a preclinical model for evaluation of intra-arterial therapies. *Animal Model Exp Med* 2020; **3**: 98-102 [PMID: 32318666 DOI: 10.1002/ame2.12100]

158 **Wilkins LR**, Stone JR, Mata J, Hawrylack A, Kubicka E, Brautigan DL. The Use of the Woodchuck as an Animal Model for Evaluation of Transarterial Embolization. *J Vasc Interv Radiol* 2017; **28**: 1467-1471 [PMID: 28941521 DOI: 10.1016/j.jvir.2017.04.005]

159 **Pritchard WF**, Woods DL, Esparza-Trujillo JA, Starost MF, Mauda-Havakuk M, Mikhail AS, Bakhutashvili I, Leonard S, Jones EC, Krishnasamy V, Karanian JW, Wood BJ. Transarterial Chemoembolization in a Woodchuck Model of Hepatocellular Carcinoma. *J Vasc Interv Radiol* 2020; **31**: 812-819. e1 [PMID: 32107125 DOI: 10.1016/j.jvir.2019.08.031]

160 **Thorn CF**, Oshiro C, Marsh S, Hernandez-Boussard T, McLeod H, Klein TE, Altman RB. Doxorubicin pathways: pharmacodynamics and adverse effects. *Pharmacogenet Genomics* 2011; **21**: 440-446 [PMID: 21048526 DOI: 10.1097/FPC.0b013e32833ffb56]

161 **Lin Y**, Xu J, Lan H. Tumor-associated macrophages in tumor metastasis: biological roles and clinical therapeutic applications. *J Hematol Oncol* 2019; **12**: 76 [PMID: 31300030 DOI: 10.1186/s13045-019-0760-3]

162 **Liu LY**, Ma XZ, Ouyang B, Ings DP, Marwah S, Liu J, Chen AY, Gupta R, Manuel J, Chen XC, Gage BK, Cirlan I, Khuu N, Chung S, Camat D, Cheng M, Sekhon M, Zagorovsky K, Abdou Mohamed MA, Thoeni C, Atif J, Echeverri J, Kollmann D, Fischer S, Bader GD, Chan WCW, Michalak TI, McGilvray ID, MacParland SA. Nanoparticle Uptake in a Spontaneous and Immunocompetent Woodchuck Liver Cancer Model. *ACS Nano* 2020; **14**: 4698-4715 [PMID: 32255624 DOI: 10.1021/acsnano.0c00468]

163 **Zoulim F**. Evaluation of novel strategies to combat hepatitis B virus targetting wild-type and drug-resistant mutants in experimental models. *Antivir Chem Chemother* 2001; **12** Suppl 1: 131-142 [PMID: 11594680]

164 **Peek SF**, Toshkov IA, Erb HN, Schinazi RF, Korba BE, Cote PJ, Gerin JL, Tennant BC. 3'-Thiacytidine (3TC) delays development of hepatocellular carcinoma (HCC) in woodchucks with experimentally induced chronic woodchuck hepatitis virus (WHV) infection. Preliminary results of a lifetime study. *Hepatology* 1997; **26** Suppl: 368A

165 **Mason WS**, Cullen J, Moraleda G, Saputelli J, Aldrich CE, Miller DS, Tennant B, Frick L, Averett D, Condreay LD, Jilbert AR. Lamivudine therapy of WHV-infected woodchucks. *Virology* 1998; **245**: 18-32 [PMID: 9614864 DOI: 10.1006/viro.1998.9150]

166 **Tatti KM**, Korba BE, Stang HL, Peek S, Gerin JL, Tennant BC, Schinazi RF. Mutations in the conserved woodchuck hepatitis virus polymerase FLLA and YMDD regions conferring resistance to lamivudine. *Antiviral Res* 2002; **55**: 141-150 [PMID: 12076758 DOI: 10.1016/s0166-3542(02)00019-0]

167 **Zhou T**, Saputelli J, Aldrich CE, Deslauriers M, Condreay LD, Mason WS. Emergence of drug-resistant populations of woodchuck hepatitis virus in woodchucks treated with the antiviral nucleoside lamivudine. *Antimicrob Agents Chemother* 1999; **43**: 1947-1954 [PMID: 10428918 DOI: 10.1128/AAC.43.8.1947]

168 **Lau DT**, Khokhar MF, Doo E, Ghany MG, Herion D, Park Y, Kleiner DE, Schmid P, Condreay LD, Gauthier J, Kuhns MC, Liang TJ, Hoofnagle JH. Long-term therapy of chronic hepatitis B with lamivudine. *Hepatology* 2000; **32**: 828-834 [PMID: 11003630 DOI: 10.1053/jhep.2000.17912]

169 **Lok AS**, Hussain M, Cursano C, Margotti M, Gramenzi A, Grazi GL, Jovine E, Benardi M, Andreone P. Evolution of hepatitis B virus polymerase gene mutations in hepatitis B e antigen-negative patients receiving lamivudine therapy. *Hepatology* 2000; **32**: 1145-1153 [PMID: 11050068 DOI: 10.1053/jhep.2000.19622]

170 **Liu X**, Schinazi RF. Hepatitis B virus resistance to lamivudine and its clinical implications. *Antivir Chem Chemother* 2002; **13**: 143-155 [PMID: 12448687 DOI: 10.1177/095632020201300301]

171 **Colonno RJ**, Genovesi EV, Medina I, Lamb L, Durham SK, Huang ML, Corey L, Littlejohn M, Locarnini S, Tennant BC, Rose B, Clark JM. Long-term entecavir treatment results in sustained antiviral efficacy and prolonged life span in the woodchuck model of chronic hepatitis infection. *J Infect Dis* 2001; **184**: 1236-1245 [PMID: 11679911 DOI: 10.1086/324003]

172 **Korba BE**, Cote PJ, Menne S, Toshkov I, Baldwin BH, Wells FV, Tennant BC, Gerin JL. Clevudine therapy with vaccine inhibits progression of chronic hepatitis and delays onset of hepatocellular carcinoma in chronic woodchuck hepatitis virus infection. *Antivir Ther* 2004; **9**: 937-952 [PMID: 15651753]

173 **Menne S**, Tumas DB, Liu KH, Thampi L, AlDeghaither D, Baldwin BH, Bellezza CA, Cote PJ, Zheng J, Halcomb R, Fosdick A, Fletcher SP, Daffis S, Li L, Yue P, Wolfgang GH, Tennant BC. Sustained efficacy and seroconversion with the Toll-like receptor 7 agonist GS-9620 in the Woodchuck model of chronic hepatitis B. *J Hepatol* 2015; **62**: 1237-1245 [PMID: 25559326 DOI: 10.1016/j.jhep.2014.12.026]

174 **Daffis S**, Balsitis S, Chamberlain J, Zheng J, Santos R, Rowe W, Ramakrishnan D, Pattabiraman D, Spurlock S, Chu R, Kang D, Mish M, Ramirez R, Li L, Li B, Ma S, Hung M, Voitenleitner C, Yon C, Suresh M, Menne S, Cote P, Delaney WE 4th, Mackman R, Fletcher SP. Toll-Like Receptor 8 Agonist GS-9688 Induces Sustained Efficacy in the Woodchuck Model of Chronic Hepatitis B. *Hepatology* 2021; **73**: 53-67 [PMID: 32246499 DOI: 10.1002/hep.31255]

175 **Fairman J**, Liu KH, Menne S. Prevention of liver tumor formation in woodchucks with established hepatocellular carcinoma by treatment with cationic liposome-DNA complexes. *BMC Cancer* 2017; **17**: 172 [PMID: 28264666 DOI: 10.1186/s12885-017-3163-2]

176 **BREEDIS C**, YOUNG G. The blood supply of neoplasms in the liver. *Am J Pathol* 1954; **30**: 969-977 [PMID: 13197542]

177 **Leal JN**, Kingham TP. Hepatic artery infusion chemotherapy for liver malignancy. *Surg Oncol Clin N Am* 2015; **24**: 121-148 [PMID: 25444472 DOI: 10.1016/j.soc.2014.09.005]

178 **Kerr DJ**, Los G. Pharmacokinetic principles of locoregional chemotherapy. *Cancer Surv* 1993; **17**: 105-122 [PMID: 8137338]

179 **Kim M**, Powers CA, Curtin LI, Fisher DT, Sexton S, Gurova KV, Skitzki JJ, Iyer RV. A Translational Hepatic Artery Infusion (HAI) Model for Hepatocellular Carcinoma in Woodchucks. *J Surg Res* 2020; **251**: 126-136 [PMID: 32143057 DOI: 10.1016/j.jss.2020.02.002]

180 **Fleyshman D**, Prendergast L, Safina A, Paszkiewicz G, Commane M, Morgan K, Attwood K, Gurova K. Level of FACT defines the transcriptional landscape and aggressive phenotype of breast cancer cells. *Oncotarget* 2017; **8**: 20525-20542 [PMID: 28423528 DOI: 10.18632/oncotarget.15656]

181 **Gasparian AV**, Burkhart CA, Purmal AA, Brodsky L, Pal M, Saranadasa M, Bosykh DA, Commane M, Guryanova OA, Pal S, Safina A, Sviridov S, Koman IE, Veith J, Komar AA, Gudkov AV, Gurova KV. Curaxins: anticancer compounds that simultaneously suppress NF-κB and activate p53 by targeting FACT. *Sci Transl Med* 2011; **3**: 95ra74 [PMID: 21832239 DOI: 10.1126/scitranslmed.3002530]

182 **Iyer RV**, Maguire O, Kim M, Curtin LI, Sexton S, Fisher DT, Schihl SA, Fetterly G, Menne S, Minderman H. Dose-Dependent Sorafenib-Induced Immunosuppression Is Associated with Aberrant NFAT Activation and Expression of PD-1 in T Cells. *Cancers (Basel)* 2019; **11** [PMID: 31100868 DOI: 10.3390/cancers11050681]

183 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J; SHARP Investigators Study Group. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]

184 **Kianmanesh R**, Ogata S, Paradis V, Sauvanet A, Belghiti J. Heat-zone effect after surface application of dissecting sealer on the "in situ margin" after tumorectomy for liver tumors. *J Am Coll Surg* 2008; **206**: 1122-1128 [PMID: 18501809 DOI: 10.1016/j.jamcollsurg.2007.12.006]

185 **Burke CT**, Cullen JM, State A, Gadi S, Wilber K, Rosenthal M, Bulysheva A, Pease A, Mauro MA, Fuchs H. Development of an animal model for radiofrequency ablation of primary, virally induced hepatocellular carcinoma in the woodchuck. *J Vasc Interv Radiol* 2011; **22**: 1613-1618. e1 [PMID: 21959057 DOI: 10.1016/j.jvir.2011.08.020]

186 **Cheng CW**, Machtay M, Dorth J, Sergeeva O, Xia H, Manaspon C, Wu H, Iyer R, Sexton S, Xin W, Exner AA, Lee Z. Delayed response to proton beam treatment of hepatocellular carcinoma. *BJR Case Rep* 2020; **6**: 20180125 [PMID: 32201597 DOI: 10.1259/bjrcr.20180125]

187 **Bilbao R**, Gérolami R, Bralet MP, Qian C, Tran PL, Tennant B, Prieto J, Bréchot C. Transduction efficacy, antitumoral effect, and toxicity of adenovirus-mediated herpes simplex virus thymidine kinase/ ganciclovir therapy of hepatocellular carcinoma: the woodchuck animal model. *Cancer Gene Ther* 2000; **7**: 657-662 [PMID: 10830712 DOI: 10.1038/sj.cgt.7700175]

188 **Rodriguez-Madoz JR**, Liu KH, Quetglas JI, Ruiz-Guillen M, Otano I, Crettaz J, Butler SD, Bellezza CA, Dykes NL, Tennant BC, Prieto J, González-Aseguinolaza G, Smerdou C, Menne S. Semliki forest virus expressing interleukin-12 induces antiviral and antitumoral responses in woodchucks with chronic viral hepatitis and hepatocellular carcinoma. *J Virol* 2009; **83**: 12266-12278 [PMID: 19740992 DOI: 10.1128/JVI.01597-09]

189 **Pützer BM**, Stiewe T, Rödicker F, Schildgen O, Rühm S, Dirsch O, Fiedler M, Damen U, Tennant

B, Scherer C, Graham FL, Roggendorf M. Large nontransplanted hepatocellular carcinoma in woodchucks: treatment with adenovirus-mediated delivery of interleukin 12/B7.1 genes. *J Natl Cancer Inst* 2001; **93**: 472-479 [PMID: 11259473 DOI: 10.1093/jnci/93.6.472]

190 **Huang KW**, Wu HL, Lin HL, Liang PC, Chen PJ, Chen SH, Lee HI, Su PY, Wu WH, Lee PH, Hwang LH, Chen DS. Combining antiangiogenic therapy with immunotherapy exerts better therapeutical effects on large tumors in a woodchuck hepatoma model. *Proc Natl Acad Sci USA* 2010; **107**: 14769-14774 [PMID: 20679198 DOI: 10.1073/pnas.1009534107]

191 **Liu Y**, Wang B, Wang L, Vikash V, Wang Q, Roggendorf M, Lu M, Yang D, Liu J. Transcriptome Analysis and Comparison of Marmota monax and Marmota himalayana. *PLoS One* 2016; **11**: e0165875 [PMID: 27806133 DOI: 10.1371/journal.pone.0165875]

192 **Fletcher SP**, Chin DJ, Gruenbaum L, Bitter H, Rasmussen E, Ravindran P, Swinney DC, Birzele F, Schmucki R, Lorenz SH, Kopetzki E, Carter J, Triyatni M, Thampi LM, Yang J, AlDeghaither D, Murreddu MG, Cote P, Menne S. Intrahepatic Transcriptional Signature Associated with Response to Interferon-α Treatment in the Woodchuck Model of Chronic Hepatitis B. *PLoS Pathog* 2015; **11**: e1005103 [PMID: 26352406 DOI: 10.1371/journal.ppat.1005103]

193 **Alioto TS**, Cruz F, Gómez-Garrido J, Triyatni M, Gut M, Frias L, Esteve-Codina A, Menne S, Kiialainen A, Kumpesa N, Birzele F, Schmucki R, Gut IG, Spleiss O. The Genome Sequence of the Eastern Woodchuck (*Marmota monax*) - A Preclinical Animal Model for Chronic Hepatitis B. *G3 (Bethesda)* 2019; **9**: 3943-3952 [PMID: 31645421 DOI: 10.1534/g3.119.400413]

194 **Korba BE**, Cote P, Hornbuckle W, Tennant BC, Gerin JL. Treatment of chronic woodchuck hepatitis virus infection in the Eastern woodchuck (Marmota monax) with nucleoside analogues is predictive of therapy for chronic hepatitis B virus infection in humans. *Hepatology* 2000; **31**: 1165-1175 [PMID: 10796894 DOI: 10.1053/he.2000.5982]

195 **Goldberg BB**, Hilpert PL, Burns PN, Liu JB, Newman LM, Merton DA, Witlin LA. Hepatic tumors: signal enhancement at Doppler US after intravenous injection of a contrast agent. *Radiology* 1990; **177**: 713-717 [PMID: 2173841 DOI: 10.1148/radiology.177.3.2173841]

196 **Forsberg F**, Goldberg BB, Liu JB, Merton DA, Rawool NM, Shi WT. Tissue-specific US contrast agent for evaluation of hepatic and splenic parenchyma. *Radiology* 1999; **210**: 125-132 [PMID: 9885597 DOI: 10.1148/radiology.210.1.r99ja11125]

197 **Forsberg F**, Liu JB, Merton DA, Rawool NM, Goldberg BB. Parenchymal enhancement and tumor visualization using a new sonographic contrast agent. *J Ultrasound Med* 1995; **14**: 949-957 [PMID: 8583531 DOI: 10.7863/jum.1995.14.12.949]

198 **Forsberg F**, Roy R, Merton DA, Rawool NM, Liu JB, Huang M, Kessler D, Goldberg BB. Conventional and hypobaric activation of an ultrasound contrast agent. *Ultrasound Med Biol* 1998; **24**: 1143-1150 [PMID: 9833583 DOI: 10.1016/s0301-5629(98)00062-3]

199 **Goldberg BB**, Liu JB, Burns PN, Merton DA, Forsberg F. Galactose-based intravenous sonographic contrast agent: experimental studies. *J Ultrasound Med* 1993; **12**: 463-470 [PMID: 8411330 DOI: 10.7863/jum.1993.12.8.463]

200 **Matsumura T**, Moriyasu F, Kono Y, Chiba T. [Contrast-enhanced power Doppler imaging of the liver--preliminary animal study]. *Nihon Rinsho* 1998; **56**: 985-989 [PMID: 9577621]

201 **Nada T**, Moriyasu F, Kono Y, Matsumura T. [Sonographic depiction of woodchuck hepatomas using intravenously injected contrast agents]. *Nihon Rinsho* 1998; **56**: 980-984 [PMID: 9577620]

202 **Forsberg F**, Liu JB, Chiou HJ, Rawool NM, Parker L, Goldberg BB. Comparison of fundamental and wideband harmonic contrast imaging of liver tumors. *Ultrasonics* 2000; **38**: 110-113 [PMID: 10829639 DOI: 10.1016/s0041-624x(99)00149-3]

203 **Forsberg F**, Goldberg BB, Liu JB, Merton DA, Rawool NM. On the feasibility of real-time, *in vivo* harmonic imaging with proteinaceous microspheres. *J Ultrasound Med* 1996; **15**: 853-60; quiz 861 [PMID: 8947861 DOI: 10.7863/jum.1996.15.12.853]

204 **Kono Y**, Moriyasu F, Nada T, Suginoshita Y, Matsumura T, Kobayashi K, Nakamura T, Chiba T. Gray scale second harmonic imaging of the liver: a preliminary animal study. *Ultrasound Med Biol* 1997; **23**: 719-726 [PMID: 9253819 DOI: 10.1016/s0301-5629(97)00007-0]

205 **Kallfelz FA**, Hornbuckle WE, Harvey HJ, Wallace RJ, Potkewitz LG, Roth L, Tennant BC. Scintigraphic diagnosis of primary hepatocellular carcinoma in the woodchuck (Marmota monax). *Am J Vet Res* 1986; **47**: 573-576 [PMID: 3008602]

206 **Salem N**, Kuang Y, Wang F, Maclennan GT, Lee Z. PET imaging of hepatocellular carcinoma with 2-deoxy-2[18F]fluoro-D-glucose, 6-deoxy-6[18F] fluoro-D-glucose, [1-11C]-acetate and [N-methyl-11C]-choline. *Q J Nucl Med Mol Imaging* 2009; **53**: 144-156 [PMID: 19039303]

207 **Salem N**, Kuang Y, Corn D, Erokwu B, Kolthammer JA, Tian H, Wu C, Wang F, Wang Y, Lee Z. [(Methyl)1-(11)c]-acetate metabolism in hepatocellular carcinoma. *Mol Imaging Biol* 2011; **13**: 140-151 [PMID: 20401538 DOI: 10.1007/s11307-010-0308-y]

208 **Tenley N**, Corn DJ, Yuan L, Lee Z. The effect of fasting on PET Imaging of Hepatocellular Carcinoma. *J Cancer Ther* 2013; **4**: 561-567 [PMID: 24683497 DOI: 10.4236/jct.2013.42071]

209 **Kuang Y**, Salem N, Tian H, Kolthammer JA, Corn DJ, Wu C, Wang F, Wang Y, Lee Z. Imaging lipid synthesis in hepatocellular carcinoma with [methyl-11c]choline: correlation with *in vivo* metabolic studies. *J Nucl Med* 2011; **52**: 98-106 [PMID: 21149484 DOI: 10.2967/jnumed.110.080366]

210 **Kolthammer JA**, Corn DJ, Tenley N, Wu C, Tian H, Wang Y, Lee Z. PET imaging of hepatocellular carcinoma with 18F-fluoroethylcholine and 11C-choline. *Eur J Nucl Med Mol Imaging* 2011; **38**: 1248-1256 [PMID: 21344223 DOI: 10.1007/s00259-011-1743-y]

211 **Sergeeva O**, Kepe V, Zhang Y, Miller-Atkins GA, Keynon JD, Iyer R, Sexton S, Awadallah A, Xin W, Saunthararajah Y, Chan ER, Lee Z. [^{18}F] Clofarabine for PET Imaging of Hepatocellular Carcinoma. *Cancers (Basel)* 2019; **11** [PMID: 31703407 DOI: 10.3390/cancers11111748]

212 **Sergeeva O**, Zhang Y, Kenyon JD, Miller-Atkins GA, Wu C, Iyer R, Sexton S, Wojtylak P, Awadallah A, Xin W, Chan ER, O'Donnel JK, Lee Z. PET imaging of hepatocellular carcinoma with anti-1-amino-3-[^{18}F]fluorocyclobutanecarboxylic acid in comparison with L-[S-methyl-^{11}C]methionine. *EJNMMI Res* 2019; **9**: 47 [PMID: 31119488 DOI: 10.1186/s13550-019-0519-4]

213 **Salem N**, MacLennan GT, Kuang Y, Anderson PW, Schomisch SJ, Tochkov IA, Tennant BC, Lee Z. Quantitative evaluation of 2-deoxy-2[F-18]fluoro-D-glucose-positron emission tomography imaging on the woodchuck model of hepatocellular carcinoma with histological correlation. *Mol Imaging Biol* 2007; **9**: 135-143 [PMID: 17308952 DOI: 10.1007/s11307-007-0092-5]

214 **Sergeeva O**, Zhang Y, Kenyon J, Miller-Atkins G, Sergeev M, Verbus E, Iyer R, Sexton S, Kepe V, Avril N, Saunthararajah Y, Chan ER, Lee Z. Liver background uptake of [^{18}F]FLT in PET imaging. *Am J Nucl Med Mol Imaging* 2020; **10**: 212-225 [PMID: 33224617]

215 **Ohtomo K**, Shiga J, Sasaki Y, Itai Y. [Iron oxide-enhanced MR imaging of hepatocellular carcinoma of woodchuck]. *Nihon Igaku Hoshasen Gakkai Zasshi* 1991; **51**: 433-435 [PMID: 1648709]

216 **Reimer P**, Weissleder R, Brady TJ, Yeager AE, Baldwin BH, Tennant BC, Wittenberg J. Experimental hepatocellular carcinoma: MR receptor imaging. *Radiology* 1991; **180**: 641-645 [PMID: 1871273 DOI: 10.1148/radiology.180.3.1871273]

217 **Cullen JM**, Li DH, Brown C, Eisenberg EJ, Cundy KC, Wolfe J, Toole J, Gibbs C. Antiviral efficacy and pharmacokinetics of oral adefovir dipivoxil in chronically woodchuck hepatitis virus-infected woodchucks. *Antimicrob Agents Chemother* 2001; **45**: 2740-2745 [PMID: 11557463 DOI: 10.1128/AAC.45.10.2740-2745.2001]

218 **Jacob JR**, Korba BE, Cote PJ, Toshkov I, Delaney WE 4th, Gerin JL, Tennant BC. Suppression of lamivudine-resistant B-domain mutants by adefovir dipivoxil in the woodchuck hepatitis virus model. *Antiviral Res* 2004; **63**: 115-121 [PMID: 15302140 DOI: 10.1016/j.antiviral.2004.03.005]

219 **Menne S**, Butler SD, George AL, Tochkov IA, Zhu Y, Xiong S, Gerin JL, Cote PJ, Tennant BC. Antiviral effects of lamivudine, emtricitabine, adefovir dipivoxil, and tenofovir disoproxil fumarate administered orally alone and in combination to woodchucks with chronic woodchuck hepatitis virus infection. *Antimicrob Agents Chemother* 2008; **52**: 3617-3632 [PMID: 18676881 DOI: 10.1128/AAC.00654-08]

220 **Zhu Y**, Yamamoto T, Cullen J, Saputelli J, Aldrich CE, Miller DS, Litwin S, Furman PA, Jilbert AR, Mason WS. Kinetics of hepadnavirus loss from the liver during inhibition of viral DNA synthesis. *J Virol* 2001; **75**: 311-322 [PMID: 11119601 DOI: 10.1128/JVI.75.1.311-322.2001]

221 **Jacquard AC**, Nassal M, Pichoud C, Ren S, Schultz U, Guerret S, Chevallier M, Werle B, Peyrol S, Jamard C, Rimsky LT, Trepo C, Zoulim F. Effect of a combination of clevudine and emtricitabine with adenovirus-mediated delivery of gamma interferon in the woodchuck model of hepatitis B virus infection. *Antimicrob Agents Chemother* 2004; **48**: 2683-2692 [PMID: 15215126 DOI: 10.1128/AAC.48.7.2683-2692.2004]

222 **Peek SF**, Cote PJ, Jacob JR, Toshkov IA, Hornbuckle WE, Baldwin BH, Wells FV, Chu CK, Gerin JL, Tennant BC, Korba BE. Antiviral activity of clevudine [L-FMAU, (1-(2-fluoro-5-methyl-beta, L-arabinofuranosyl) uracil)] against woodchuck hepatitis virus replication and gene expression in chronically infected woodchucks (Marmota monax). *Hepatology* 2001; **33**: 254-266 [PMID: 11124844 DOI: 10.1053/jhep.2001.20899]

223 **Cullen JM**, Smith SL, Davis MG, Dunn SE, Botteron C, Cecchi A, Linsey D, Linzey D, Frick L, Paff MT, Goulding A, Biron K. In vivo antiviral activity and pharmacokinetics of (-)-cis-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine in woodchuck hepatitis virus-infected woodchucks. *Antimicrob Agents Chemother* 1997; **41**: 2076-2082 [PMID: 9333028 DOI: 10.1128/AAC.41.10.2076]

224 **Korba BE**, Schinazi RF, Cote P, Tennant BC, Gerin JL. Effect of oral administration of emtricitabine on woodchuck hepatitis virus replication in chronically infected woodchucks. *Antimicrob Agents Chemother* 2000; **44**: 1757-1760 [PMID: 10817750 DOI: 10.1128/aac.44.6.1757-1760.2000]

225 **Korolowizc KE**, Li B, Huang X, Yon C, Rodrigo E, Corpuz M, Plouffe DM, Kallakury BV, Suresh M, Wu TY, Miller AT, Menne S. Liver-Targeted Toll-Like Receptor 7 Agonist Combined With Entecavir Promotes a Functional Cure in the Woodchuck Model of Hepatitis B Virus. *Hepatol Commun* 2019; **3**: 1296-1310 [PMID: 31592075 DOI: 10.1002/hep4.1397]

226 **Menne S**, Wildum S, Steiner G, Suresh M, Korolowicz K, Balarezo M, Yon C, Murreddu M, Hong X, Kallakury BV, Tucker R, Yang S, Young JAT, Javanbakht H. Efficacy of an Inhibitor of Hepatitis B Virus Expression in Combination With Entecavir and Interferon-α in Woodchucks Chronically Infected With Woodchuck Hepatitis Virus. *Hepatol Commun* 2020; **4**: 916-931 [PMID: 32490326 DOI: 10.1002/hep4.1502]

227 **Suresh M**, Korolowicz KE, Balarezo M, Iyer RP, Padmanabhan S, Cleary D, Gimi R, Sheri A, Yon C, Kallakury BV, Tucker RD, Afdhal N, Menne S. Antiviral Efficacy and Host Immune Response Induction during Sequential Treatment with SB 9200 Followed by Entecavir in Woodchucks. *PLoS One* 2017; **12**: e0169631 [PMID: 28056062 DOI: 10.1371/journal.pone.0169631]

228 **Berraondo P**, Di Scala M, Korolowicz K, Thampi LM, Otano I, Suarez L, Fioravanti J, Aranda F, Ardaiz N, Yang J, Kallakury BV, Tucker RD, Vasquez M, Menne S, Prieto J, González-

Aseguinolaza G. Liver-directed gene therapy of chronic hepadnavirus infection using interferon alpha tethered to apolipoprotein A-I. *J Hepatol* 2015; **63**: 329-336 [PMID: 25772035 DOI: 10.1016/j.jhep.2015.02.048]

229 **Korba BE**, Cote P, Hornbuckle W, Schinazi R, Gangemi JD, Tennant BC, Gerin JL. Enhanced antiviral benefit of combination therapy with lamivudine and alpha interferon against WHV replication in chronic carrier woodchucks. *Antivir Ther* 2000; **5**: 95-104 [PMID: 10971862]

230 **Standring DN**, Bridges EG, Placidi L, Faraj A, Loi AG, Pierra C, Dukhan D, Gosselin G, Imbach JL, Hernandez B, Juodawlkis A, Tennant B, Korba B, Cote P, Cretton-Scott E, Schinazi RF, Myers M, Bryant ML, Sommadossi JP. Antiviral beta-L-nucleosides specific for hepatitis B virus infection. *Antivir Chem Chemother* 2001; **12** Suppl 1: 119-129 [PMID: 11594678]

231 **Bryant ML**, Bridges EG, Placidi L, Faraj A, Loi AG, Pierra C, Dukhan D, Gosselin G, Imbach JL, Hernandez B, Juodawlkis A, Tennant B, Korba B, Cote P, Marion P, Cretton-Scott E, Schinazi RF, Sommadossi JP. Antiviral L-nucleosides specific for hepatitis B virus infection. *Antimicrob Agents Chemother* 2001; **45**: 229-235 [PMID: 11120971 DOI: 10.1128/AAC.45.1.229-235.2001]

232 **Bryant ML**, Bridges EG, Placidi L, Faraj A, Loi AG, Pierra C, Dukhan D, Gosselin G, Imbach JL, Hernandez B, Juodawlkis A, Tennant B, Korba B, Cote P, Cretton-Scott E, Schinazi RF, Sommadossi JP. Anti-HBV specific beta-L-2'-deoxynucleosides. *Nucleosides Nucleotides Nucleic Acids* 2001; **20**: 597-607 [PMID: 11563077 DOI: 10.1081/NCN-100002336]

233 **Ahn B**, Lee BC, Kim HJ, Park S, Lee Y, Choi Y, Kim HE, Kim YW. hzVSF, a novel HBV therapeutic candidate, shows WHsAg loss in woodchuck hepatitis model and safety in phase I clinical study. *J Hepatol* 2020; **73** Suppl 1: S861 [DOI: 10.1016/S0168-8278(20)32164-4]

234 **Meng Z**, Zhang X, Pei R, Zhang E, Kemper T, Vollmer J, Davis HL, Glebe D, Gerlich W, Roggendorf M, Lu M. Combination therapy including CpG oligodeoxynucleotides and entecavir induces early viral response and enhanced inhibition of viral replication in a woodchuck model of chronic hepadnaviral infection. *Antiviral Res* 2016; **125**: 14-24 [PMID: 26585244 DOI: 10.1016/j.antiviral.2015.11.001]

235 **Paulsen D**, Weber O, Ruebsamen-Schaeff H, Tennant BC, Menne S. AIC649 Induces a Bi-Phasic Treatment Response in the Woodchuck Model of Chronic Hepatitis B. *PLoS One* 2015; **10**: e0144383 [PMID: 26656974 DOI: 10.1371/journal.pone.0144383]

236 **Liu J**, Zhang E, Ma Z, Wu W, Kosinska A, Zhang X, Möller I, Seiz P, Glebe D, Wang B, Yang D, Lu M, Roggendorf M. Enhancing virus-specific immunity *in vivo* by combining therapeutic vaccination and PD-L1 blockade in chronic hepadnaviral infection. *PLoS Pathog* 2014; **10**: e1003856 [PMID: 24391505 DOI: 10.1371/journal.ppat.1003856]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2021 June 15; 13(6): 536-549

DOI: 10.4251/wjgo.v13.i6.536

ISSN 1948-5204 (online)

MINIREVIEWS

Application of metabolomics in clinical and laboratory gastrointestinal oncology

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Author contributions: Gao P and Zhang Y organized the manuscript and wrote the conclusion part; Huang X and Fang XY wrote the laboratory application part; Zheng H and Cai SL wrote the introduction of metabolomics; Sun AJ and Zhao L wrote the clinical application part.

Supported by National Natural Science Foundation of China, No. 81672498; and Natural Science Foundation of Liaoning Province, China, No. 2019-ZD-1005.

Conflict-of-interest statement: The authors declare that there is no conflict of interest for this manuscript.

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Abstract

Metabolites are versatile bioactive molecules. They are not only the substrates and/or the products of enzymatic reactions but also act as the regulators in the systemic metabolism. Metabolomics is a high-throughput analytical strategy to qualify or quantify as many metabolites as possible in the metabolomes. It is an indispensable part of systems biology. The leading techniques in this field are mainly based on mass spectrometry and nuclear magnetic resonance spectroscopy. The metabolomic analysis has gained wide use in bioscience fields. In the tumor research arena, metabolomics can be employed to identify biomarkers for prediction, diagnosis, and prognosis. Chemotherapeutic effect evaluation and personalized medicine decision-making can also benefit from metabolomic analysis of patient biofluid or biopsy samples. Many cell-level studies can help in disease exploration. In this review, the basic features and principles of varied metabolomic analysis are introduced. The value of metabolomics in clinical and laboratory gastrointestinal cancer studies is discussed, especially for mass spectrometry applications. Besides, combined use of metabolomics and other tools to solve problems in cancer practice is briefly illustrated. In summary, metabolomics paves a new way to explore cancerous diseases in the light of small molecules.

Manuscript source: Invited manuscript

Specialty type: Medical laboratory technology

Country/Territory of origin: China

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: January 22, 2021
Peer-review started: January 22, 2021
First decision: February 24, 2021
Revised: March 9, 2021
Accepted: May 19, 2021
Article in press: May 19, 2021
Published online: June 15, 2021

P-Reviewer: Sridharan G
S-Editor: Zhang L
L-Editor: Wang TQ
P-Editor: Li JH

Key Words: Metabolomics; Biomarker; Mass spectrometry; Metabolite; Gastrointestinal cancer; Diagnosis

Core Tip: Genomics, transcriptomics, and proteomics aim to study the macromolecules. As a complement to systems biology, metabolomics paves a new way to explore cancerous diseases concerning temporal changes of small molecules. The metabolome is phenotype-specific. Metabolome reflects the organism's responses to environmental stimuli very directly and sensitively.

Citation: Gao P, Huang X, Fang XY, Zheng H, Cai SL, Sun AJ, Zhao L, Zhang Y. Application of metabolomics in clinical and laboratory gastrointestinal oncology. *World J Gastrointest Oncol* 2021; 13(6): 536-549
URL: https://www.wjgnet.com/1948-5204/full/v13/i6/536.htm
DOI: https://dx.doi.org/10.4251/wjgo.v13.i6.536

INTRODUCTION

Malignancies that occur in the sites from the esophagus to the rectum can be roughly classified as gastrointestinal (GI) cancers. These include the tumors rooting in the solid digestive organs and those occurring in the digestive tract. Some of them can develop from the neuroendocrine cells in the digestive system. It was estimated that about 333680 digestive cancer cases were diagnosed in the United States in 2020[1]. Many of the tumors, such as pancreatic carcinoma and hepatocellular carcinoma (HCC), have a poor prognosis even with intensive treatment. As a multifactorial process, both the individual's genetic and the relevant environmental factors contribute to oncogenesis [2]. As there is no effective therapy for cancerous diseases, early diagnosis and timely intervention play key roles in reducing mortality. Varied imaging modalities are available in cancer clinics. Because of their lower cost and easier availabilities, blood biomarkers are highly recommended by many guidelines for tumor screening, diagnosis, and therapeutic effect evaluation[3,4].

Most of the approved biomarkers for GI cancer diagnosis are proteins. With the achievement of oncogenesis research and the advances of modern analytical techniques, many other macromolecules have been explored as new types of biomarkers. For example, a panel consisting of seven plasma micro ribonucleic acids was reported to be efficient for HCC diagnosis, especially for early-stage HCC[5]. Cell-free deoxyribonucleic acid (cfDNA) was readily detected in liquid biopsy samples[6]. With some traditional protein biomarkers, cfDNA could also be used in early-stage HCC screening[7]. These newly explored biomarkers contribute to GI cancer diagnosis and management to a varied extent.

Besides macromolecules, small molecular metabolites are also indispensable for an organism. Metabolites are the direct executors of metabolism. The entity of the whole metabolites in an organism constitutes its unique metabolome. A given metabolite profile is phenotype-specific, and phenotype is substantially modulated by metabolites [8,9]. Most of the inborn metabolic diseases (IMDs) exhibit metabolite concentration abnormalities[10]. Treatment of many IMDs involves limiting intake of certain kinds of chemicals[11]. Mass spectrometry (MS) is the earliest technology that was introduced into clinical laboratories for IMD diagnosis purposes[12].

Except for acting as the substrates and the products of enzymatic reactions, metabolites can also be the biomarkers for cancer diagnosis and treatment. This review would focus on the advances in using metabolites for GI cancer study and clinical practice.

METABOLOMICS

Genomics, transcriptomics, and proteomics are the high-throughput analysis of specific molecules in biological samples. Compared with the other omics,

metabolomics is a newly coined conception. It aims at quantifying/qualifying as many metabolites as possible in a metabolome[8,13] (Figure 1). Since the advent of modern analytical technologies, high-throughput analyzing a metabolome has become possible. Nearly all the clinical specimens are compatible with metabolomic analysis [14]. Metabolomics aims at the compounds with molecular weights less than 1500 Dalton[15]. The leading techniques in this arena are MS and nuclear magnetic resonance (NMR) spectroscopy[16]. Both tactics have their inherent advantages in different analytical aspects[17]. For example, NMR is superior to MS in its analysis speed and noninvasive features[18]. MS is characterized by its high sensitivity and resolution[19]. Coupled with some separation technologies, MS or NMR can provide improved analytical abilities. This gave birth to the hyphened metabolomic analytical measures, such as liquid chromatography-MS (LC-MS), gas chromatography-MS (GC-MS), and capillary electrophoresis-MS. So far, most of the metabolomics studies were finished by employing the hyphened techniques. Many scientific groups tried to integrate NMR and MS. This approach provides distinctive advantages, especially for the analysis using isotopes[18].

Metabolites have different polarities, volatilities, and hydrophilic properties owning to their elementary compositions. These physical aspects provide analysts with the opportunity to develop varied analytical methods to meet different needs. Therefore, there have been many derivative omics conceptions from metabolomics. For example, lipidomics is the metabolomic analysis of lipids exclusively. Metabolomic analysis focusing on carbohydrates can be called glycometabolomics[20]. Nucleosides include limited members. The concentration changes of modified nucleosides are frequently encountered in different diseases. Several metabolomics groups have paid more attention to the modified nucleoside detection[21].

According to whether the potential analytes were predefined, the metabolomic analysis could be divided into targeted and untargeted analysis[22]. The former is to detect the metabolites with definite identities, and the latter is to analyze all the measurable metabolites that are compatible with the adopted methods. The targeted analysis is frequently applied to studies with definite purposes, such as for verification or accurate quantitation. The untargeted strategy is suitable for global screening or catching a glimpse of the samples. Additionally, there is an analysis called pseudotargeted metabolomics[23]. This tactic is based on the principle that certain precursor molecules can produce definite daughter ions under a specific ionized circumstance. The ion fragmentation features are compound-specific. These structurally correlated ions could be monitored in parallel by some types of MS[24]. The pseudotargeted metabolomic analysis is independent of any identity knowledge of the analytes.

For biomarker exploration, a metabolomic study should consider untargeted analysis first. This analysis helps to lock the potential valuable metabolites. Then, a targeted metabolomic analysis is carried out. It is better to employ the quantitative analytical method that is most suitable for the targeted analytes. For quantitation accuracy, any untargeted analysis method is only compatible with limited types of metabolites. The following targeted analysis with robust quantitation capacities helps to corroborate whether the untargeted analysis findings are reliable and reproducible. Ideally, the targeted analysis should use another set of samples.

A great challenge in metabolomics is metabolite identification. It is better to build a database in which all the analytical features of the metabolites are recorded. Unfortunately, it is unknown how many metabolites might exist in different biological samples. Some groups have tried to set up a database according to their routine needs. Many of the databases are free to non-commercial use[25,26]. To simplify metabolite identification, many software programs have been developed. Some of them could directly use the data collected with the analytical equipment[27]. Statistical and bioinformatic analysis is necessary for biomarker selection and annotation. Many software programs provide various online analysis tools[28].

GI CANCER PREDICTION

Any disease, including cancerous diseases, obeys their regular development progression. There must be some clues existing in the preclinical stages (Figure 2). This provides opportunities to predict diseases. In a prospective study based on LC-MS, plasma valine, leucine, and isoleucine were reported to be valuable for pancreatic ductal adenocarcinoma (PDAC) prediction especially for the onset within 2-5 years [29]. Subjects with these amino acid changes had two times higher risks than the control ones. The three branched-chain amino acids (BCAAs) belong to necessary

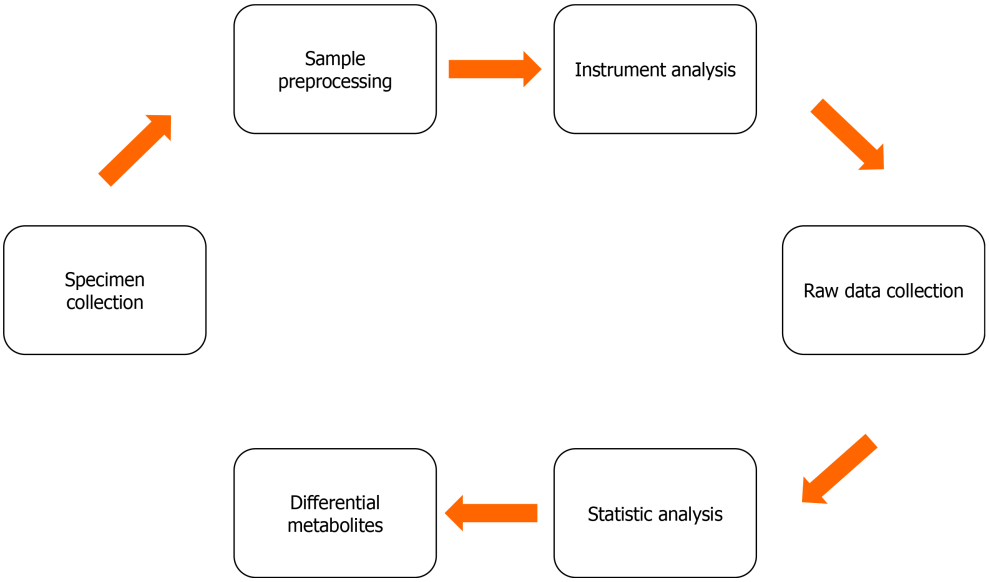

Figure 1 The basic workflow of metabolomics. Samples aiming at different purposes are first collected. The applicable specimen types include blood, biopsy, biofluid, cell, and urine samples. Some specimens must be preprocessed before they are analyzed with various equipment. The manipulations include metabolite extraction, condensation, or derivatization as possible. The metabolomics data are usually collected with the corresponding software equipped with the instruments. Some software also provides data pre-procession (*e.g.*, to remove noise signals) and statistical analysis functions. The differential metabolites are first screened out by statistical methods. These selected metabolites should be verified using another set of samples if possible. It is better to ascertain the concentration changes of each metabolite using a robust quantitation method.

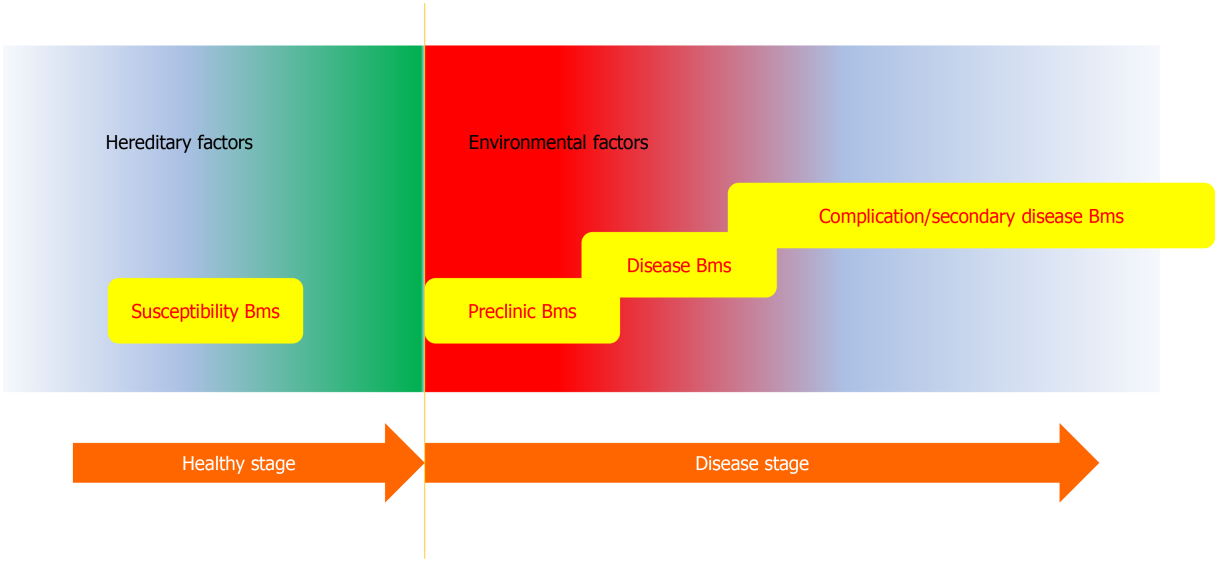

Figure 2 Schematic representation of the fluctuation of biomarker in the whole period of a disease. Disease susceptibility is usually defined by the individual's genetic background. The susceptibility biomarker (Bms) can be detected by genetic analysis most possibly. The onset of the disease would be triggered by many environmental factors. At the very beginning (preclinical stage), some prediction Bms appears. When a disease progresses to the clinical stage (with clear symptoms) the diagnosis Bms could be detected. If the disease advances further, some complications and secondary hurts would emerge. These end events give birth to the opportunities to develop the relevant Bms. Metabolomics could be applied to the whole disease period. Besides, prognosis and treatment efficacy Bms could also be explored by metabolomic analysis.

amino acids. Whereas, the authors demonstrated that the raised plasma concentrations of these BCAAs were not the results of excessive ingestion. They were linked to early-stage tissue protein breakdown driven by the *K-ras* gene. Interestingly, if the three BCAAs were combined with tyrosine and phenylalanine, they could be used to predict future diabetes onset. A 12-year follow-up study indicated that individuals with elevated blood concentrations of the five amino acids were at higher risks to develop type 2 diabetes (T2DM)[30]. T2DM and PDAC had a reciprocal relationship[31]. Thus, it is better to introduce other metabolites to improve the prediction accuracy when the metabolite panels are overlapped. To enlarge the metabolite coverage, a study

simultaneously employed LC-MS and GC-MS to analyze the blood samples. The study included 226 pairs of case and control subjects. The plasma phosphatidylcholine [PC (15:0/18:2)], coumarin, and picolinic acid levels were found to be positively related to pancreatic cancer. Six glycerophospholipids were inversely associated with pancreatic cancer incidence. After excluding the interference factors including T2DM, the PC (18:1/18:4), instead of PC (15:0/18:2), was found to be most valuable especially for predicting the onset within 5 years[32]. From the perspective of epidemiology, factors that are inversely correlated to diseases are protective. Although both studies utilized LC-MS and selected the subjects of similar backgrounds[29,32], the potential prediction markers were not identical. One reason is that tumorigenesis is a complex process. It can be triggered by different combinations of driver factors. The other reason might be that lifestyles, food appetite, and genetic backgrounds vary greatly amid different races and populations. For instance, African Americans have a higher colorectal cancer (CRC) rate than rural South Africans. Epidemic investigation proved that the former consumed more animal protein and fat in their daily life[33]. On the contrary, the latter ingested more fibers. If the food styles were exchanged between them, fecal water and urine metabolomes changed accordingly. If they ingested more protein and fat-rich food, both the Americans and the Africans were characterized with abundant fecal choline and urine trimethylamine-N-oxide[33].

Diet affects not only cancer risks but also the prognosis [34-36]. A follow-up study enrolled 463 postmenopausal CRC women. The researchers found that diet and food with anti-inflammatory potential could improve overall survival[37]. The relationship between dietary exposures and diseases was the key theme of nutritional metabolomics[36]. Unfortunately, up to now, large-scale meta-analysis data for GI cancer prediction using metabolite markers are rare. Fortunately, metabolomics analyses have identified many candidate biomarkers about specific food exposures. For example, meat and/or seafood consumption resulted in elevated plasma essential amino acids, polyunsaturated fatty acids, and D-glucose[38]. Shellfish consumption affected plasma phosphatidylethanolamine (p36: 4). Plasma 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid was related to fish intakes in the Asian population[38]. What should be mentioned is that if the fish ingestion study is carried out in European people, the candidate marker should be trimethylamine-N-oxide instead of 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid[39].

Besides tumor onset prediction, metabolites can be used to predict prognosis. Redalen *et al*[40] reported that tumor glycine was an adverse prognostic factor for locally advanced rectal cancer. Cancers with rapid growth rates were demonstrated to consume glycine excessively[41]. Too many reasons can affect the concentrations of a single amino acid. As the lessons from protein biomarker applications, a biomarker panel including several (kinds of) metabolites might be more valuable and reliable than a single metabolite.

GI CANCER DIAGNOSIS AND EARLY DIAGNOSIS

CRC poses a great challenge to public health, especially in developed countries. Early diagnosis is important to reduce mortality. To early detect CRC, plasma samples collected from stage 0/I/II patients and the controls were subjected to GC/triple-quadrupole MS (TMS) analysis[42]. A regression model consisting of eight metabolites [pyruvic acid-meto-TMS, glycolic acid-2TMS, tryptophan-3TMS (/SI), palmitoleic acid-TMS, fumaric acid-2TMS (/SI), ornithine-4TMS (/SI), lysine-4TMS, and 3-hydroxyisovaleric acid-2TMS] could realize satisfying CRC diagnosis with a sensitivity of 99.3% and specificity of 93.8%. In that study, the traditional protein markers carcinoma embryonic antigen and carbohydrate antigen19-9 showed good specificities, but their sensitivities were low (< 20%). The authors also pointed out that this model could not be applied for aggressive CRC (*e.g.*, stage III/IV). When invasive CRC metastasizes, it might affect and spread to many organs. It can be expected that the systemic metabolic changes caused by local and metastasis tumors are different.

Another notorious GI cancer is HCC. Alpha-fetoprotein (AFP) has been used for HCC surveillance and diagnosis for decades. Its limited specificity is obvious. The rapid advances of imaging modalities have excluded the utilization of AFP according to the recently approved guidelines[43]. Unfortunately, imaging examination could miss many solid neoplasms with a diameter less than 3 cm. Thus, the early diagnosis needs some alternative solutions. In this light, a large-scale metabolomic study was conducted. To pursue the robustness of the diagnosis, many diseases that might interfere with HCC were included as possible[44]. It was found that serum

phenylalanyl-tryptophan and glycocholate showed good performance in HCC diagnosis and differential diagnosis. Even for small HCC, the combined use of the two metabolites could achieve an area under the receiver-operating characteristic curve (AUC) of 0.866. According to the results, elevated glycocholate was positively correlated to HCC. Phenylalanyl-tryptophan was negatively correlated to HCC. An appropriate tumor biomarker should be in high concentrations in the blood because of its excessive release or production. Pathologically, the decreased phenylalanyl-tryptophan might be the result of tumor-related overconsumption. Technically, biomarkers with decreased concentrations causes the quantitation difficulty.

Except for the blood samples, feces sample is also a valuable specimen for metabolomics. In theory, components in the feces reflect the intestinal physiological and pathological status. A pilot metabolomic study detected 527 reproducible metabolites in the feces samples from CRC patients. Three fecal heme-related molecules, 18 peptides/amino acids, palmitoyl-sphingomyelin, mandelate, p-hydroxybenzaldehyde, acetaminophen metabolites, tocopherols, sitostanol, 3-dehydrocarnitine, pterin, conjugated-linoleate-18-2N7, N-2-furoyl-glycine, and p-aminobenzoate were found to be valuable for CRC diagnosis[45]. However, metabolites in the feces varied greatly due to diet styles and gut microflora. Many metabolites contributing to the CRC diagnosis in the above-mentioned study were bacterial metabolites or co-metabolites of human beings and the gut microbes. This resulted in the observation that not all the biomarkers were elevated in the CRC feces. For the stabilities, feces were not comparable to blood samples[45]. The markers indicating the storage stabilities of blood samples have been explored and identified[46,47]. Similar studies about feces were seldom conducted. Gut microbiota affects the intestinal microenvironment. Unhealthy microbiota contributes to many diseases including CRC. In this light, the fecal metabolomic analysis might be more valuable for prediction use[48,49].

Small-intestine neuroendocrine tumors (SINETs) are a common GI cancer stemming from the neuroendocrine cells in the small bowel. Many of these tumors have features of metastasis. By performing NMR-based metabolomic analysis, Imperiale *et al*[50] found that succinate, glutathion, taurine, myoinositol, and glycerophosphocholine were elevated in the tumor samples. The normal small intestine tissues were rich in alanine, creatine, ethanolamine, and aspartate. When the hepatic metastasis lesions were compared with the normal liver, acetate, succinate, choline, phosphocholine, taurine, lactate, and aspartate were found to be rich in the lesions. The primary SINETs were characterized with increased succinate, valine, and myoinositol when they were compared with the metastases. This study demonstrated that identical tumors found in different microenvironments could exhibit distinctive phenotypes[50].

Cholangiocarcinoma was thought to be related to bile acid metabolism[51]. Zhang *et al*[52] analyzed 329 plasma samples collected from the controls, benign biliary diseases, cholangiocarcinoma, gallbladder cancer, and HCC populations. Taurochenodeoxycholic acid and chenodeoxycholic acid played key roles in separating cholangiocarcinoma both from the healthy controls and from the HCC patients. The diagnostic performance was even superior to the commonly used carbohydrate antigen 19-9.

Recurrence is a key theme in the tumor research field. From three independent cohorts, Qiu *et al*[53] found 14 upregulated and 1 downregulated metabolite biomarkers to predict CRC relapse. The authors also pointed out the inconsistency of these metabolite changes amid different cohorts. No matter what potential uses, to validate biomarkers must need more effort.

PATHOLOGICAL DIAGNOSIS OF GI CANCER

Traditional pathological diagnosis is dependent on slice samples. Preparing a satisfying slide sample is a time-consuming and labor-intensive task. The intraoperative histological examination costs only half an hour but is expensive. Also, the diagnosis accuracy is affected by the expertise of both the technologists and the pathologists. What makes the matter worse is that the traditional pathological slides only afford limited tissues or cells. It brings about inevitable sampling bias. When it comes to metabolomics, most or all the resected tissues can be used to extract the metabolites. Additionally, the extracts can be subjected to various preprocessing such as condensation, dilution, or derivatization to meet different analytical needs.

Endoscopic examination is widely used in CRC screening. The morphological characteristics of advanced adenomas and CRC tissues are inadequate for differentiation purposes. In a study, an untargeted MS-based metabolomic technique was first

employed to analyze CRC and matched paracancerous tissues. This profiling strategy narrows the cancer-related metabolic changes to amino acid metabolism. Then, another MS-based targeted amino acid analysis was performed. The results showed that combined use of methionine, tyrosine, valine, and isoleucine was enough to distinguish CRC from advanced adenoma[54]. The notable advantages of metabolomics are its simplicity and rapidness.

As widely admitted, MS analysis is characterized by its high specificity and rich chemical information. The traditional pathological tactic has a distinguished resolution. If the advantages of both are combined, pathologists will gain more deep insight into the slice samples[55]. Fortunately, scientists have developed applicable strategies to integrate the two techniques and applied the so-called MS imaging (MSI) strategy to cancer pathological studies. Desorption electrospray ionization mass spectrometry (DESI-MS) can give chemical information from the surfaces of an intact or processed tissue specimen under ambient conditions[56]. Nagai *et al*[57] first performed an untargeted analysis of HCC and benign tissue samples by MS. They found that TG 16:0/18:1 (9Z)/20:1 (11Z) (m/z 904.83) and TG 16:0/18:1 (9Z)/18:2 (9Z, 12Z) (m/z 874.79) played roles in separating the two kinds of samples. Then, they employed MSI to explore the tissue distribution of the two TGs. Despite the overlap at the boundary regions, condensed TG 16:0/18:1 (9Z)/20:1 (11Z) distribution in the tumor regions and abundant TG 16:0/18:1 (9Z)/18:2 (9Z, 12Z) (m/z 874.79) in the nontumor regions was obvious. The results were consistent with the previous reports about the saturated and unsaturated fatty acid distribution in the tumor and nontumor tissues. These fusion images integrated traditional hematoxylin and eosin staining and MS ion imaging. The strategy provided high-quality pathological pictures at 10 μm-resolution[55]. The most valuable use of MSI might be to explore extremely small local and metastasis lesions.

MSI can not only be used to help pathological diagnosis, but it can also be used to aid tumor-related enzyme exploration. Sun *et al*[58] first employed airflow-assisted DESI-MSI to profile region-specific metabolites in esophageal squamous cell carcinoma (ESCC) and corresponding normal samples. Then, they performed metabolic pathway matching analysis based on the selected differential metabolites to lock potential tumor-associated metabolic enzymes. Subsequently, immunohistochemical staining was performed to validate the enzyme expression changes. Finally, they found that proline biosynthesis, glutamine metabolism, uridine metabolism, histidine metabolism, fatty acid biosynthesis, and polyamine biosynthesis pathways were altered in ESCC. Pyrroline-5-carboxylate reductase 2 and uridine phosphorylase 1 was upregulated in ESCC tissues. This high-coverage-based MSI analysis provided valuable information on new drug development and therapeutic target identification.

Direct, real-time, and non-invasive examination of intact tissues is highly appreciated in surgical rooms. It is affordable that partial normal tissues are damaged in some surgical operations. However, in neurosurgical resections, damaging normal brain tissues has always been avoided. Traditional DESI-MS can work under ambient conditions, but it suffers from technical incompatibilities in many facets such as the use of organic solvents, high-pressure nebulizing gas, and high voltages[59]. Zhang *et al*[59] developed a device called MasSpec Pen based on the DESI-MS. The MS was equipped with a handheld probe that could squeeze a discrete water droplet under control. The droplet was delivered on the surface of the target tissue. Metabolites in the tissue could be extracted into the droplet and transferred to the analysis system-an Orbitrap mass spectrometer. The authors employed the MasSpec Pen to analyze several kinds of benign and malignant solid tissue samples. The results demonstrated that this device could realize a diagnostic sensitivity of 96.4% and specificity of 96.2%. The overall accuracy was 96.3%. Furthermore, MasSpec Pen has ever been introduced into the porcine upper GI tracts in a study. The accuracy of distinguishing the liver from the stomach tissues *in vivo* was 98%[60]. In fact, utilizing MasSpec Pen for any cancer diagnosis was solely dependent on the availability of the corresponding tissue-specific database[59].

Like MasSpec Pen, iKnife is another rapid evaporative ionization mass spectrometry (REIMS)-based metabolomic diagnosis device. It can not only realize real-time pathological analysis but also act as an “electric lancet". iKnife does not rely on the liquid media to dissolve the metabolites. It directly analyzes the gas components released from the burned tissues. Electrosurgical devices are prevailing in the operation rooms because of their simultaneous dissection and hemostasis functions. The burned tissues would release smoke containing many oxidized metabolites. This previously discarded smoke is collected with a specifically designed device and then transferred to REIMS to be analyzed. The chemical information in the smoke can be used to identify the properties of the tissues releasing the smoke[61]. Balog *et al*[61]

analyzed 1624 cancerous, 1231 healthy, and 78 inflammatory bowel disease samples. They found a different distribution of lipid species across the specimens. Alexander *et al*[62] applied iKnife to diagnose CRC. The overall accuracy was 94.4%. Phosphatidylserines and bacterial phosphatidylglycerols were rich in the cancer samples. Ceramides were condensed in the adenomas. The normal tissues were characterized by elevated plasmalogens and triacylglycerols[62]. iKnife can be used to identify the origins of the metastatic tumors. When differentiating healthy liver parenchyma from metastasis colonic adenocarcinomas, the iKnife could give a diagnostic accuracy of 96% (73/76).

PERSONALIZED GI CANCER TREATMENT

Chemotherapy is necessary for GI cancer treatment. Chemotherapeutical drug administration brings about several side or toxic effects. Even if the physicians can correctly make their chemotherapy decisions, the one-size-fits-all approaches do not guarantee a good prognosis for all the patients. Precise prediction of the chemosensitivities would benefit both the patients and the physicians. Pharmacometabolomics is the science utilizing metabolomics to predict patient responses to drug treatments. A pilot study based on serum metabolomics indicated that elevated serum deoxyribose 1-phosphate and decreased S-lactoylglutathione correlated to chemotherapy sensitivities[63]. Capecitabine is an antimetabolic agent that could be metabolized to 5-fluorouracil-the active form for CRC treatment. Side effects of capecitabine are largely originated from its intermediate metabolite 5′-deoxy-5-fluorouridine (5′-DFUR). By performing ^{1}H NMR spectrometer-based metabolomic analysis of 52 CRC serum samples, Backshall *et al*[64] found that patients with higher LDL-like lipid particles and choline phospholipid were prone to suffering from 5′-DFUR toxicity. Also helped by NMR metabolomics, Bertini *et al*[65] analyzed 153 serum samples from metastasis CRC patients before cetuximab and irinotecan administration. They found that the patients with long and short overall survival (OS) time could be identified with an accuracy of 78.5%. The patients with OS > 24 mo and < 3 mo showed different serum metabolite profiles. They also pointed out that the potential differential metabolites contributing to separation of the two groups were also affected by some other factors such as obesity.

Postoperation chemoradiotherapies are indispensable, even if surgical resection is performed in the early stage of esophageal cancer. However, not all the cases benefit from the adjuvant strategies. A metabolomics study found that decreased serum arabitol, glycine, L-serine, and L-arginine indicated a positive response to chemoradiotherapies[66]. For predicting the chemoradiotherapy responses, the combined use of the four metabolites generated an AUC > 0.7.

Chemoresistance is frequently encountered clinically. The resistance could be acquired or innate. Many chemotherapy drugs are antimetabolites and affect cell metabolism. The built-in metabolic plasticity and the robustness of the metabolic networks render the cells with conspicuous capacities to resist perturbations from the environment. Cells can reprogram their metabolism to resist the perturbations from the chemotherapy drugs. Those cells that can not adapt to the drug stimuli will be killed. Intracellular metabolite pools are dynamic in size. The pool sizes were affected by the metabolic flux rates of the relevant metabolic pathways[67]. Cells can keep hemostasis by redirecting the metabolic fluxes of the relevant metabolic pathways. The flux rates can be calculated. The most widely used metabolic flux analysis (MFA) is ^{13}C MFA. The analysis uses the ^{13}C-labeled substrate (usually the ^{13}C-labeled glucose or amino acids) to feed the cells. After proper incubation, intracellular metabolites are quantified by metabolomic analysis. The detected metabolites are then used to calculate the metabolic fluxes through chemometrics according to the labeled element distribution in the metabolic pools[68]. Mathematically, a metabolic network is a set of stoichiometric equations. Each equation is defined by a real enzymatic reaction that can be easily retrieved from biochemical textbooks or public databases. Because the metabolic networks contain hundreds to thousands of pathways, the calculation is a tough job. Most of the tasks are finished by software models run on computers. Combined with computational and mathematical modeling tactics, MFA could shed light on cellular phenotypes from another angle[69].

Highly expressed hexokinase 2 (HK2) is frequently found in HCC cells. An MFA using (1,2-^{13}C) glucose and (U-^{13}C) glutamine as tracers exhibited that glucose uptake and lactate secretion rates dropped by 40% in Huh7 cells with HK2 silencing. Glutamine and branched-chain amino acid uptakes, secretion of alanine and

glutamate, and the tricarboxylic acid cycle-related fluxes were not affected. The HK2 silencing cells were more sensitive to one-carbon unit depletion. There was a 2-fold increase in serine uptake and glycine secretion. There was no obvious change in the intracellular glucose to serine flux. The study also found that silencing HK2 synergized sorafenib, which provided a clue to treat HCC by manipulating HK2[70].

Flux balance analysis is another type of MFA. It sets rational constraints on a metabolic network and presumes that the network is in its steady-state. Nikmanesh *et al*[71] constructed a model integrating expression data from Gene Expression Omnibus and metabolomics data. The metabolic model included 3748 reactions and 2766 metabolites. Using this model, the authors compared the metabolic flux difference of 56 normal and 67 CRC cells. Compared to the normal cells, cancer cells exhibited 503 upregulated and 560 downregulated fluxes. Reactions catalyzed by retinol dehydrogenase, bicarbonate transporter, cytosine deaminase, glutathione peroxidase, and mitochondrial adenosine diphosphate/adenosine triphosphate (ATP) transporter were the notably downregulated ones. The other pathways with decreased metabolic flux rates included pathways involving palmitoyl-CoA desaturase, glutamine synthetase, ATP synthase, and uridine triphosphate-glucose-1-phosphate uridylyltransferase. The nucleotide metabolism (catalyzed by nucleoside-diphosphate kinase) and pyruvate metabolism (catalyzed by L-lactate dehydrogenase) pathways had increased flux rates. Some reactions involved in purine catabolism, glycolysis/gluconeogenesis, and hyaluronan metabolism showed increased flux rates. In that model, the authors also included the point mutation information. This coupling strategy helped to discover the driver regulatory modules. Thus, with the help of data mining and integrating tools, metabolomics could potentially be used to uncover potential therapeutic targets and new tumor driver mechanisms. This would be good at formulating personalized therapeutic strategies.

Traditionally, the enzyme catalyzing the slowest step in a metabolic pathway is deemed as the rate-limiting enzyme. The relevant step is regarded as the rate-limiting step. At the very beginning, metabolic engineering aims at manipulating these enzymes. Unfortunately, overexpressing the relevant enzymes fails frequently. Metabolic control analysis (MCA) introduces a new conception to determine the real rate-limiting step by considering how a given enzyme exerts its influence on the fluxes and the concentrations of the involved metabolites[72]. As hemostasis is maintained by metabolism, some key metabolite changes might be lethal. The enzymes catalyzing the relevant reactions could be drug targets potentially. One of the prominent pilot studies using MCA to identify therapeutic targets was reported in the practice of treating trypanosomiasis. Scientists found that the glucose transporter, aldolase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, and glycerol-3-phosphate dehydrogenase were the Archil's heels of parasites instead of red blood cells[73,74]. Thus, relational treatment strategies could be developed by circumventing the targets that might damage the hosts.

Using MCA, Koit *et al*[75] found that HCC tissues showed suppressed respiratory chain complexes I functions. But, it was not the case for breast cancer tissues. Mitochondrial membrane permeabilities were different between the two types of tumor cells. These clues were valuable on how to select effective anti-tumor drugs. Many tumor therapies share the same drugs or drugs with similar mechanisms. Physicians could make more personalized therapeutic decisions with the MCA results.

Although the variability of a single person's metabolome is universal, every individual has his/her relatively stable metabolic phenotype. It dominates the specific responses to specific stimuli. Assfalg *et al*[76] collected 40 urine specimens from 22 healthy persons across 3 mo. According to the ^{1}H NMR urine metabolomic data, the interindividual difference was larger than the intraindividual difference. Fifteen metabolites were enough to confirm an unknown sample origin with 100% confidence. The individual-specific phenotypes contained subject-specific nutrition tolerance, drug efficacy and toxicity, disease risk, and much physical and pathological response information[76]. The authors also implied that to define an individual's phenotype needs specimens collected in a long period. This could exclude the casual influence. Thus, metabolomics could be a valuable tool for personalized medicine.

CONCLUSION

Genomics, transcriptomics, and proteomics studies have been applied in tumor fields for many decades. The findings from a single omic analysis are prone to being misinterpreted due to the tumor heterogeneities. Many analytical skills and tools could

be selected to perform metabolomic analysis. Compared to the other omics, metabolomics is still in its infancy. New methods of metabolite identification, bioinformatic analysis of the data, noise signal removal for the spectroscopic data, and analytical speed improvement are still under development. It should be noticed that all the above-mentioned GI cancer metabolite biomarkers are not "new" metabolites. All of them could be found in physiological conditions. Also, their concentration changes can be found in non-cancerous diseases. Nearly all the mentioned GI cancer metabolite biomarkers can be found in other cancers. Identical metabolite markers can be found in different GI cancers and even can be used for different purposes. Unlike the protein and the mutant gene biomarkers, metabolite biomarker concentrations are severely affected by diet styles and circadian rhythms. To use metabolite biomarkers should follow an intensive verification procession and must consider the backgrounds against which the metabolite markers are identified. Compared to the other omics, metabolomics had many advantages[77]: (1) Changes taking place at the gene or protein levels can be amplified at the metabolome level; (2) Metabolomic analysis does not need the complete gene sequence information; (3) The members of a metabolome are smaller than those of a genome or proteome; and (4) Performing a metabolomic analysis is cheaper than performing a transcriptome or a proteome analysis. Besides the above-mentioned applications, metabolomics has been used to explore gene functions[78], drug mechanisms[79], enzyme functions[80], and tumor driver metabolites (oncometabolites)[81]. Although the applications are scattered in different bioscience fields, it can be concluded that metabolomics is undoubtedly a valuable complement to the other techniques in prompting GI cancer research.

REFERENCES

1 **Siegel RL**, Miller KD, Goding Sauer A, Fedewa SA, Butterly LF, Anderson JC, Cercek A, Smith RA, Jemal A. Colorectal cancer statistics, 2020. *CA Cancer J Clin* 2020; **70**: 145-164 [PMID: 32133645 DOI: 10.3322/caac.21601]

2 **Meng C**, Bai C, Brown TD, Hood LE, Tian Q. Human Gut Microbiota and Gastrointestinal Cancer. *Genomics Proteomics Bioinformatics* 2018; **16**: 33-49 [PMID: 29474889 DOI: 10.1016/j.gpb.2017.06.002]

3 **Duffy MJ**, Sturgeon C, Lamerz R, Haglund C, Holubec VL, Klapdor R, Nicolini A, Topolcan O, Heinemann V. Tumor markers in pancreatic cancer: a European Group on Tumor Markers (EGTM) status report. *Ann Oncol* 2010; **21**: 441-447 [PMID: 19690057 DOI: 10.1093/annonc/mdp332]

4 **Duffy MJ**, Lamerz R, Haglund C, Nicolini A, Kalousová M, Holubec L, Sturgeon C. Tumor markers in colorectal cancer, gastric cancer and gastrointestinal stromal cancers: European group on tumor markers 2014 guidelines update. *Int J Cancer* 2014; **134**: 2513-2522 [PMID: 23852704 DOI: 10.1002/ijc.28384]

5 **Zhou J**, Yu L, Gao X, Hu J, Wang J, Dai Z, Wang JF, Zhang Z, Lu S, Huang X, Wang Z, Qiu S, Wang X, Yang G, Sun H, Tang Z, Wu Y, Zhu H, Fan J. Plasma microRNA panel to diagnose hepatitis B virus-related hepatocellular carcinoma. *J Clin Oncol* 2011; **29**: 4781-4788 [PMID: 22105822 DOI: 10.1200/JCO.2011.38.2697]

6 **Wu C**, Zhang J, Li H, Xu W, Zhang X. The potential of liquid biopsies in gastrointestinal cancer. *Clin Biochem* 2020; **84**: 1-12 [PMID: 32540214 DOI: 10.1016/j.clinbiochem.2020.06.007]

7 **Qu C**, Wang Y, Wang P, Chen K, Wang M, Zeng H, Lu J, Song Q, Diplas BH, Tan D, Fan C, Guo Q, Zhu Z, Yin H, Jiang L, Chen X, Zhao H, He H, Li G, Bi X, Zhao X, Chen T, Tang H, Lv C, Wang D, Chen W, Zhou J, Cai J, Wang X, Wang S, Yan H, Zeng YX, Cavenee WK, Jiao Y. Detection of early-stage hepatocellular carcinoma in asymptomatic HBsAg-seropositive individuals by liquid biopsy. *Proc Natl Acad Sci USA* 2019; **116**: 6308-6312 [PMID: 30858324 DOI: 10.1073/pnas.1819799116]

8 **Bujak R**, Struck-Lewicka W, Markuszewski MJ, Kaliszan R. Metabolomics for laboratory diagnostics. *J Pharm Biomed Anal* 2015; **113**: 108-120 [PMID: 25577715 DOI: 10.1016/j.jpba.2014.12.017]

9 **Guijas C**, Montenegro-Burke JR, Warth B, Spilker ME, Siuzdak G. Metabolomics activity screening for identifying metabolites that modulate phenotype. *Nat Biotechnol* 2018; **36**: 316-320 [PMID: 29621222 DOI: 10.1038/nbt.4101]

10 **Shibata N**, Hasegawa Y, Yamada K, Kobayashi H, Purevsuren J, Yang Y, Dung VC, Khanh NN, Verma IC, Bijarnia-Mahay S, Lee DH, Niu DM, Hoffmann GF, Shigematsu Y, Fukao T, Fukuda S, Taketani T, Yamaguchi S. Diversity in the incidence and spectrum of organic acidemias, fatty acid oxidation disorders, and amino acid disorders in Asian countries: Selective screening vs. expanded newborn screening. *Mol Genet Metab Rep* 2018; **16**: 5-10 [PMID: 29946514 DOI: 10.1016/j.ymgmr.2018.05.003]

11 **Hoskin RG**, Sasitharan T, Howard R. The use of a low phenylalanine diet with amino acid supplement in the treatment of behavioural problems in a severely mentally retarded adult female with phenylketonuria. *J Intellect Disabil Res* 1992; **36**: 183-191 [PMID: 1591502 DOI: 10.1111/j.1365-2788.1992.tb00494.x]

12 **Burlina AB**, Polo G, Salviati L, Duro G, Zizzo C, Dardis A, Bembi B, Cazzorla C, Rubert L, Zordan R, Desnick RJ, Burlina AP. Newborn screening for lysosomal storage disorders by tandem mass spectrometry in North East Italy. *J Inherit Metab Dis* 2018; **41**: 209-219 [PMID: 29143201 DOI: 10.1007/s10545-017-0098-3]

13 **Zampieri M**, Sekar K, Zamboni N, Sauer U. Frontiers of high-throughput metabolomics. *Curr Opin Chem Biol* 2017; **36**: 15-23 [PMID: 28064089 DOI: 10.1016/j.cbpa.2016.12.006]

14 **Jang C**, Chen L, Rabinowitz JD. Metabolomics and Isotope Tracing. *Cell* 2018; **173**: 822-837 [PMID: 29727671 DOI: 10.1016/j.cell.2018.03.055]

15 **Chan AW**, Mercier P, Schiller D, Bailey R, Robbins S, Eurich DT, Sawyer MB, Broadhurst D. (1)H-NMR urinary metabolomic profiling for diagnosis of gastric cancer. *Br J Cancer* 2016; **114**: 59-62 [PMID: 26645240 DOI: 10.1038/bjc.2015.414]

16 **Gao P**, Xu G. Mass-spectrometry-based microbial metabolomics: recent developments and applications. *Anal Bioanal Chem* 2015; **407**: 669-680 [PMID: 25216964 DOI: 10.1007/s00216-014-8127-7]

17 **Amberg A**, Riefke B, Schlotterbeck G, Ross A, Senn H, Dieterle F, Keck M. NMR and MS Methods for Metabolomics. *Methods Mol Biol* 2017; **1641**: 229-258 [PMID: 28748468 DOI: 10.1007/978-1-4939-7172-5_13]

18 **Markley JL**, Brüschweiler R, Edison AS, Eghbalnia HR, Powers R, Raftery D, Wishart DS. The future of NMR-based metabolomics. *Curr Opin Biotechnol* 2017; **43**: 34-40 [PMID: 27580257 DOI: 10.1016/j.copbio.2016.08.001]

19 **Cui L**, Lu H, Lee YH. Challenges and emergent solutions for LC-MS/MS based untargeted metabolomics in diseases. *Mass Spectrom Rev* 2018; **37**: 772-792 [PMID: 29486047 DOI: 10.1002/mas.21562]

20 **Fu X**, Cebo M, Ikegami T, Lämmerhofer M. Separation of carbohydrate isomers and anomers on poly-N-(1H-tetrazole-5-yl)-methacrylamide-bonded stationary phase by hydrophilic interaction chromatography as well as determination of anomer interconversion energy barriers. *J Chromatogr A* 2020; **1620**: 460981 [PMID: 32115232 DOI: 10.1016/j.chroma.2020.460981]

21 **Willmann L**, Erbes T, Krieger S, Trafkowski J, Rodamer M, Kammerer B. Metabolome analysis *via* comprehensive two-dimensional liquid chromatography: identification of modified nucleosides from RNA metabolism. *Anal Bioanal Chem* 2015; **407**: 3555-3566 [PMID: 25736241 DOI: 10.1007/s00216-015-8516-6]

22 **Roberts LD**, Souza AL, Gerszten RE, Clish CB. Targeted metabolomics. Curr Protoc Mol Biol 2012; Chapter 30: Unit 30.2.1-Unit 30.224 [PMID: 22470063 DOI: 10.1002/0471142727.mb3002s98]

23 **Zheng F**, Zhao X, Zeng Z, Wang L, Lv W, Wang Q, Xu G. Development of a plasma pseudotargeted metabolomics method based on ultra-high-performance liquid chromatography-mass spectrometry. *Nat Protoc* 2020; **15**: 2519-2537 [PMID: 32581297 DOI: 10.1038/s41596-020-0341-5]

24 **Fu X**, Anderson M, Wang Y, Zimring JC. LC-MS/MS-MRM-Based Targeted Metabolomics for Quantitative Analysis of Polyunsaturated Fatty Acids and Oxylipins. *Methods Mol Biol* 2019; **1978**: 107-120 [PMID: 31119659 DOI: 10.1007/978-1-4939-9236-2_7]

25 **Wishart DS**, Feunang YD, Marcu A, Guo AC, Liang K, Vázquez-Fresno R, Sajed T, Johnson D, Li C, Karu N, Sayeeda Z, Lo E, Assempour N, Berjanskii M, Singhal S, Arndt D, Liang Y, Badran H, Grant J, Serra-Cayuela A, Liu Y, Mandal R, Neveu V, Pon A, Knox C, Wilson M, Manach C, Scalbert A. HMDB 4.0: the human metabolome database for 2018. *Nucleic Acids Res* 2018; **46**: D608-D617 [PMID: 29140435 DOI: 10.1093/nar/gkx1089]

26 **Guijas C**, Montenegro-Burke JR, Domingo-Almenara X, Palermo A, Warth B, Hermann G, Koellensperger G, Huan T, Uritboonthai W, Aisporna AE, Wolan DW, Spilker ME, Benton HP, Siuzdak G. METLIN: A Technology Platform for Identifying Knowns and Unknowns. *Anal Chem* 2018; **90**: 3156-3164 [PMID: 29381867 DOI: 10.1021/acs.analchem.7b04424]

27 **Domingo-Almenara X**, Siuzdak G. Metabolomics Data Processing Using XCMS. *Methods Mol Biol* 2020; **2104**: 11-24 [PMID: 31953810 DOI: 10.1007/978-1-0716-0239-3_2]

28 **Chong J**, Wishart DS, Xia J. Using MetaboAnalyst 4.0 for Comprehensive and Integrative Metabolomics Data Analysis. *Curr Protoc Bioinformatics* 2019; **68**: e86 [PMID: 31756036 DOI: 10.1002/cpbi.86]

29 **Mayers JR**, Wu C, Clish CB, Kraft P, Torrence ME, Fiske BP, Yuan C, Bao Y, Townsend MK, Tworoger SS, Davidson SM, Papagiannakopoulos T, Yang A, Dayton TL, Ogino S, Stampfer MJ, Giovannucci EL, Qian ZR, Rubinson DA, Ma J, Sesso HD, Gaziano JM, Cochrane BB, Liu S, Wactawski-Wende J, Manson JE, Pollak MN, Kimmelman AC, Souza A, Pierce K, Wang TJ, Gerszten RE, Fuchs CS, Vander Heiden MG, Wolpin BM. Elevation of circulating branched-chain amino acids is an early event in human pancreatic adenocarcinoma development. *Nat Med* 2014; **20**: 1193-1198 [PMID: 25261994 DOI: 10.1038/nm.3686]

30 **Wang TJ**, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, Lewis GD, Fox CS, Jacques PF, Fernandez C, O'Donnell CJ, Carr SA, Mootha VK, Florez JC, Souza A, Melander O, Clish CB, Gerszten RE. Metabolite profiles and the risk of developing diabetes. *Nat Med* 2011; **17**: 448-453 [PMID: 21423183 DOI: 10.1038/nm.2307]

31 **Andersen DK**, Korc M, Petersen GM, Eibl G, Li D, Rickels MR, Chari ST, Abbruzzese JL. Diabetes, Pancreatogenic Diabetes, and Pancreatic Cancer. *Diabetes* 2017; **66**: 1103-1110 [PMID: 28507210 DOI: 10.2337/db16-1477]

32 **Shu X**, Zheng W, Yu D, Li HL, Lan Q, Yang G, Cai H, Ma X, Rothman N, Gao YT, Jia W, Xiang

YB, Shu XO. Prospective metabolomics study identifies potential novel blood metabolites associated with pancreatic cancer risk. *Int J Cancer* 2018; **143**: 2161-2167 [PMID: 29717485 DOI: 10.1002/ijc.31574]

33 **O'Keefe SJ**, Li JV, Lahti L, Ou J, Carbonero F, Mohammed K, Posma JM, Kinross J, Wahl E, Ruder E, Vipperla K, Naidoo V, Mtshali L, Tims S, Puylaert PG, DeLany J, Krasinskas A, Benefiel AC, Kaseb HO, Newton K, Nicholson JK, de Vos WM, Gaskins HR, Zoetendal EG. Fat, fibre and cancer risk in African Americans and rural Africans. *Nat Commun* 2015; **6**: 6342 [PMID: 25919227 DOI: 10.1038/ncomms7342]

34 **Thorburn AN**, Macia L, Mackay CR. Diet, metabolites, and "western-lifestyle" inflammatory diseases. *Immunity* 2014; **40**: 833-842 [PMID: 24950203 DOI: 10.1016/j.immuni.2014.05.014]

35 **Steck SE**, Murphy EA. Dietary patterns and cancer risk. *Nat Rev Cancer* 2020; **20**: 125-138 [PMID: 31848467 DOI: 10.1038/s41568-019-0227-4]

36 **McGee EE**, Kiblawi R, Playdon MC, Eliassen AH. Nutritional Metabolomics in Cancer Epidemiology: Current Trends, Challenges, and Future Directions. *Curr Nutr Rep* 2019; **8**: 187-201 [PMID: 31129888 DOI: 10.1007/s13668-019-00279-z]

37 **Zheng J**, Tabung FK, Zhang J, Murphy EA, Shivappa N, Ockene JK, Caan B, Kroenke CH, Hébert JR, Steck SE. Post-cancer diagnosis dietary inflammatory potential is associated with survival among women diagnosed with colorectal cancer in the Women's Health Initiative. *Eur J Nutr* 2020; **59**: 965-977 [PMID: 30955051 DOI: 10.1007/s00394-019-01956-z]

38 **Lu Y**, Zou L, Su J, Tai ES, Whitton C, Dam RMV, Ong CN. Meat and Seafood Consumption in Relation to Plasma Metabolic Profiles in a Chinese Population: A Combined Untargeted and Targeted Metabolomics Study. *Nutrients* 2017; **9** [PMID: 28665358 DOI: 10.3390/nu9070683]

39 **Cheung W**, Keski-Rahkonen P, Assi N, Ferrari P, Freisling H, Rinaldi S, Slimani N, Zamora-Ros R, Rundle M, Frost G, Gibbons H, Carr E, Brennan L, Cross AJ, Pala V, Panico S, Sacerdote C, Palli D, Tumino R, Kühn T, Kaaks R, Boeing H, Floegel A, Mancini F, Boutron-Ruault MC, Baglietto L, Trichopoulou A, Naska A, Orfanos P, Scalbert A. A metabolomic study of biomarkers of meat and fish intake. *Am J Clin Nutr* 2017; **105**: 600-608 [PMID: 28122782 DOI: 10.3945/ajcn.116.146639]

40 **Redalen KR**, Sitter B, Bathen TF, Grøholt KK, Hole KH, Dueland S, Flatmark K, Ree AH, Seierstad T. High tumor glycine concentration is an adverse prognostic factor in locally advanced rectal cancer. *Radiother Oncol* 2016; **118**: 393-398 [PMID: 26705680 DOI: 10.1016/j.radonc.2015.11.031]

41 **Jain M**, Nilsson R, Sharma S, Madhusudhan N, Kitami T, Souza AL, Kafri R, Kirschner MW, Clish CB, Mootha VK. Metabolite profiling identifies a key role for glycine in rapid cancer cell proliferation. *Science* 2012; **336**: 1040-1044 [PMID: 22628656 DOI: 10.1126/science.1218595]

42 **Nishiumi S**, Kobayashi T, Kawana S, Unno Y, Sakai T, Okamoto K, Yamada Y, Sudo K, Yamaji T, Saito Y, Kanemitsu Y, Okita NT, Saito H, Tsugane S, Azuma T, Ojima N, Yoshida M. Investigations in the possibility of early detection of colorectal cancer by gas chromatography/triple-quadrupole mass spectrometry. *Oncotarget* 2017; **8**: 17115-17126 [PMID: 28179577 DOI: 10.18632/oncotarget.15081]

43 **Song PP**, Xia JF, Inagaki Y, Hasegawa K, Sakamoto Y, Kokudo N, Tang W. Controversies regarding and perspectives on clinical utility of biomarkers in hepatocellular carcinoma. *World J Gastroenterol* 2016; **22**: 262-274 [PMID: 26755875 DOI: 10.3748/wjg.v22.i1.262]

44 **Luo P**, Yin P, Hua R, Tan Y, Li Z, Qiu G, Yin Z, Xie X, Wang X, Chen W, Zhou L, Li Y, Chen H, Gao L, Lu X, Wu T, Wang H, Niu J, Xu G. A Large-scale, multicenter serum metabolite biomarker identification study for the early detection of hepatocellular carcinoma. *Hepatology* 2018; **67**: 662-675 [PMID: 28960374 DOI: 10.1002/hep.29561]

45 **Goedert JJ**, Sampson JN, Moore SC, Xiao Q, Xiong X, Hayes RB, Ahn J, Shi J, Sinha R. Fecal metabolomics: assay performance and association with colorectal cancer. *Carcinogenesis* 2014; **35**: 2089-2096 [PMID: 25037050 DOI: 10.1093/carcin/bgu131]

46 **Yin P**, Peter A, Franken H, Zhao X, Neukamm SS, Rosenbaum L, Lucio M, Zell A, Häring HU, Xu G, Lehmann R. Preanalytical aspects and sample quality assessment in metabolomics studies of human blood. *Clin Chem* 2013; **59**: 833-845 [PMID: 23386698 DOI: 10.1373/clinchem.2012.199257]

47 **Liu X**, Hoene M, Yin P, Fritsche L, Plomgaard P, Hansen JS, Nakas CT, Niess AM, Hudemann J, Haap M, Mendy M, Weigert C, Wang X, Fritsche A, Peter A, Häring HU, Xu G, Lehmann R. Quality Control of Serum and Plasma by Quantification of (4E,14Z)-Sphingadienine-C18-1-Phosphate Uncovers Common Preanalytical Errors During Handling of Whole Blood. *Clin Chem* 2018; **64**: 810-819 [PMID: 29567661 DOI: 10.1373/clinchem.2017.277905]

48 **Jia W**, Xie G, Jia W. Bile acid-microbiota crosstalk in gastrointestinal inflammation and carcinogenesis. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 111-128 [PMID: 29018272 DOI: 10.1038/nrgastro.2017.119]

49 **Wong SH**, Yu J. Gut microbiota in colorectal cancer: mechanisms of action and clinical applications. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 690-704 [PMID: 31554963 DOI: 10.1038/s41575-019-0209-8]

50 **Imperiale A**, Poncet G, Addeo P, Ruhland E, Roche C, Battini S, Cicek AE, Chenard MP, Hervieu V, Goichot B, Bachellier P, Walter T, Namer IJ. Metabolomics of Small Intestine Neuroendocrine Tumors and Related Hepatic Metastases. *Metabolites* 2019; **9** [PMID: 31835679 DOI: 10.3390/metabo9120300]

51 **Herraez E**, Romero MR, Macias RIR, Monte MJ, Marin JJG. Clinical relevance of the relationship between changes in gut microbiota and bile acid metabolism in patients with intrahepatic

cholangiocarcinoma. *Hepatobiliary Surg Nutr* 2020; **9**: 211-214 [PMID: 32355682 DOI: 10.21037/hbsn.2019.10.11]

52 **Zhang X**, Yang Z, Shi Z, Zhu Z, Li C, Du Z, Zhang Y, Wang Z, Jiao Z, Tian X, Zhang J, Zhai W, Kan Q. Analysis of bile acid profile in plasma to differentiate cholangiocarcinoma from benign biliary diseases and healthy controls. *J Steroid Biochem Mol Biol* 2021; **205**: 105775 [PMID: 33130021 DOI: 10.1016/j.jsbmb.2020.105775]

53 **Qiu Y**, Cai G, Zhou B, Li D, Zhao A, Xie G, Li H, Cai S, Xie D, Huang C, Ge W, Zhou Z, Xu LX, Jia W, Zheng S, Yen Y. A distinct metabolic signature of human colorectal cancer with prognostic potential. *Clin Cancer Res* 2014; **20**: 2136-2146 [PMID: 24526730 DOI: 10.1158/1078-0432.CCR-13-1939]

54 **Gao P**, Zhou C, Zhao L, Zhang G, Zhang Y. Tissue amino acid profile could be used to differentiate advanced adenoma from colorectal cancer. *J Pharm Biomed Anal* 2016; **118**: 349-355 [PMID: 26595283 DOI: 10.1016/j.jpba.2015.11.007]

55 **Van de Plas R**, Yang J, Spraggins J, Caprioli RM. Image fusion of mass spectrometry and microscopy: a multimodality paradigm for molecular tissue mapping. *Nat Methods* 2015; **12**: 366-372 [PMID: 25707028 DOI: 10.1038/nmeth.3296]

56 **Wiseman JM**, Puolitaival SM, Takáts Z, Cooks RG, Caprioli RM. Mass spectrometric profiling of intact biological tissue by using desorption electrospray ionization. *Angew Chem Int Ed Engl* 2005; **44**: 7094-7097 [PMID: 16259018 DOI: 10.1002/anie.200502362]

57 **Nagai K**, Uranbileg B, Chen Z, Fujioka A, Yamazaki T, Matsumoto Y, Tsukamoto H, Ikeda H, Yatomi Y, Chiba H, Hui SP, Nakazawa T, Saito R, Koshiba S, Aoki J, Saigusa D, Tomioka Y. Identification of novel biomarkers of hepatocellular carcinoma by high-definition mass spectrometry: Ultrahigh-performance liquid chromatography quadrupole time-of-flight mass spectrometry and desorption electrospray ionization mass spectrometry imaging. *Rapid Commun Mass Spectrom* 2020; **34** Suppl 1: e8551 [PMID: 31412144 DOI: 10.1002/rcm.8551]

58 **Sun C**, Li T, Song X, Huang L, Zang Q, Xu J, Bi N, Jiao G, Hao Y, Chen Y, Zhang R, Luo Z, Li X, Wang L, Wang Z, Song Y, He J, Abliz Z. Spatially resolved metabolomics to discover tumor-associated metabolic alterations. *Proc Natl Acad Sci USA* 2019; **116**: 52-57 [PMID: 30559182 DOI: 10.1073/pnas.1808950116]

59 **Zhang J**, Rector J, Lin JQ, Young JH, Sans M, Katta N, Giese N, Yu W, Nagi C, Suliburk J, Liu J, Bensussan A, DeHoog RJ, Garza KY, Ludolph B, Sorace AG, Syed A, Zahedivash A, Milner TE, Eberlin LS. Nondestructive tissue analysis for *ex vivo* and *in vivo* cancer diagnosis using a handheld mass spectrometry system. *Sci Transl Med* 2017; **9**: eaan3968 [PMID: 28878011 DOI: 10.1126/scitranslmed.aan3968]

60 **Keating MF**, Zhang J, Feider CL, Retailleau S, Reid R, Antaris A, Hart B, Tan G, Milner TE, Miller K, Eberlin LS. Integrating the MasSpec Pen to the da Vinci Surgical System for *In Vivo* Tissue Analysis during a Robotic Assisted Porcine Surgery. *Anal Chem* 2020; **92**: 11535-11542 [PMID: 32786489 DOI: 10.1021/acs.analchem.0c02037]

61 **Balog J**, Sasi-Szabó L, Kinross J, Lewis MR, Muirhead LJ, Veselkov K, Mirnezami R, Dezső B, Damjanovich L, Darzi A, Nicholson JK, Takáts Z. Intraoperative tissue identification using rapid evaporative ionization mass spectrometry. *Sci Transl Med* 2013; **5**: 194ra93 [PMID: 23863833 DOI: 10.1126/scitranslmed.3005623]

62 **Alexander J**, Gildea L, Balog J, Speller A, McKenzie J, Muirhead L, Scott A, Kontovounisios C, Rasheed S, Teare J, Hoare J, Veselkov K, Goldin R, Tekkis P, Darzi A, Nicholson J, Kinross J, Takats Z. A novel methodology for *in vivo* endoscopic phenotyping of colorectal cancer based on real-time analysis of the mucosal lipidome: a prospective observational study of the iKnife. *Surg Endosc* 2017; **31**: 1361-1370 [PMID: 27501728 DOI: 10.1007/s00464-016-5121-5]

63 **Wang D**, Li W, Yin L, Du Y, Zhang S, Suo J. Association of serum levels of deoxyribose 1-phosphate and S-lactoylglutathione with neoadjuvant chemotherapy sensitivity in patients with gastric cancer: A metabolomics study. *Oncol Lett* 2020; **19**: 2231-2242 [PMID: 32194721 DOI: 10.3892/ol.2020.11350]

64 **Backshall A**, Sharma R, Clarke SJ, Keun HC. Pharmacometabonomic profiling as a predictor of toxicity in patients with inoperable colorectal cancer treated with capecitabine. *Clin Cancer Res* 2011; **17**: 3019-3028 [PMID: 21415219 DOI: 10.1158/1078-0432.CCR-10-2474]

65 **Bertini I**, Cacciatore S, Jensen BV, Schou JV, Johansen JS, Kruhøffer M, Luchinat C, Nielsen DL, Turano P. Metabolomic NMR fingerprinting to identify and predict survival of patients with metastatic colorectal cancer. *Cancer Res* 2012; **72**: 356-364 [PMID: 22080567 DOI: 10.1158/0008-5472.CAN-11-1543]

66 **Fujigaki S**, Nishiumi S, Kobayashi T, Suzuki M, Iemoto T, Kojima T, Ito Y, Daiko H, Kato K, Shouji H, Honda K, Azuma T, Yoshida M. Identification of serum biomarkers of chemoradiosensitivity in esophageal cancer *via* the targeted metabolomics approach. *Biomark Med* 2018; **12**: 827-840 [PMID: 30043633 DOI: 10.2217/bmm-2017-0449]

67 **Vander Heiden MG**. Targeting cancer metabolism: a therapeutic window opens. *Nat Rev Drug Discov* 2011; **10**: 671-684 [PMID: 21878982 DOI: 10.1038/nrd3504]

68 **Antoniewicz MR**. A guide to ^{13}C metabolic flux analysis for the cancer biologist. *Exp Mol Med* 2018; **50**: 1-13 [PMID: 29657327 DOI: 10.1038/s12276-018-0060-y]

69 **Lagziel S**, Lee WD, Shlomi T. Studying metabolic flux adaptations in cancer through integrated experimental-computational approaches. *BMC Biol* 2019; **17**: 51 [PMID: 31272436 DOI: 10.1186/s12915-019-0669-x]

70 **DeWaal D**, Nogueira V, Terry AR, Patra KC, Jeon SM, Guzman G, Au J, Long CP, Antoniewicz MR, Hay N. Hexokinase-2 depletion inhibits glycolysis and induces oxidative phosphorylation in hepatocellular carcinoma and sensitizes to metformin. *Nat Commun* 2018; **9**: 446 [PMID: 29386513 DOI: 10.1038/s41467-017-02733-4]

71 **Nikmanesh F**, Sarhadi S, Dadashpour M, Asghari Y, Zarghami N. Omics Integration Analysis Unravel the Landscape of Driving Mechanisms of Colorectal Cancer. *Asian Pac J Cancer Prev* 2020; **21**: 3539-3549 [PMID: 33369450 DOI: 10.31557/APJCP.2020.21.12.3539]

72 **Moreno-Sánchez R**, Saavedra E, Rodríguez-Enríquez S, Olín-Sandoval V. Metabolic control analysis: a tool for designing strategies to manipulate metabolic pathways. *J Biomed Biotechnol* 2008; **2008**: 597913 [PMID: 18629230 DOI: 10.1155/2008/597913]

73 **Bakker BM**, Westerhoff HV, Opperdoes FR, Michels PA. Metabolic control analysis of glycolysis in trypanosomes as an approach to improve selectivity and effectiveness of drugs. *Mol Biochem Parasitol* 2000; **106**: 1-10 [PMID: 10743606 DOI: 10.1016/s0166-6851(99)00197-8]

74 **Bakker BM**, Walsh MC, ter Kuile BH, Mensonides FI, Michels PA, Opperdoes FR, Westerhoff HV. Contribution of glucose transport to the control of the glycolytic flux in Trypanosoma brucei. *Proc Natl Acad Sci USA* 1999; **96**: 10098-10103 [PMID: 10468568 DOI: 10.1073/pnas.96.18.10098]

75 **Koit A**, Shevchuk I, Ounpuu L, Klepinin A, Chekulayev V, Timohhina N, Tepp K, Puurand M, Truu L, Heck K, Valvere V, Guzun R, Kaambre T. Mitochondrial Respiration in Human Colorectal and Breast Cancer Clinical Material Is Regulated Differently. *Oxid Med Cell Longev* 2017; **2017**: 1372640 [PMID: 28781720 DOI: 10.1155/2017/1372640]

76 **Assfalg M**, Bertini I, Colangiuli D, Luchinat C, Schäfer H, Schütz B, Spraul M. Evidence of different metabolic phenotypes in humans. *Proc Natl Acad Sci USA* 2008; **105**: 1420-1424 [PMID: 18230739 DOI: 10.1073/pnas.0705685105]

77 **Taylor J**, King RD, Altmann T, Fiehn O. Application of metabolomics to plant genotype discrimination using statistics and machine learning. *Bioinformatics* 2002; **18** Suppl 2: S241-S248 [PMID: 12386008 DOI: 10.1093/bioinformatics/18.suppl_2.s241]

78 **Raamsdonk LM**, Teusink B, Broadhurst D, Zhang N, Hayes A, Walsh MC, Berden JA, Brindle KM, Kell DB, Rowland JJ, Westerhoff HV, van Dam K, Oliver SG. A functional genomics strategy that uses metabolome data to reveal the phenotype of silent mutations. *Nat Biotechnol* 2001; **19**: 45-50 [PMID: 11135551 DOI: 10.1038/83496]

79 **Allen J**, Davey HM, Broadhurst D, Rowland JJ, Oliver SG, Kell DB. Discrimination of modes of action of antifungal substances by use of metabolic footprinting. *Appl Environ Microbiol* 2004; **70**: 6157-6165 [PMID: 15466562 DOI: 10.1128/AEM.70.10.6157-6165.2004]

80 **Saito N**, Robert M, Kitamura S, Baran R, Soga T, Mori H, Nishioka T, Tomita M. Metabolomics approach for enzyme discovery. *J Proteome Res* 2006; **5**: 1979-1987 [PMID: 16889420 DOI: 10.1021/pr0600576]

81 **Budczies J**, Denkert C. Tissue-Based Metabolomics to Analyze the Breast Cancer Metabolome. *Recent Results Cancer Res* 2016; **207**: 157-175 [PMID: 27557538 DOI: 10.1007/978-3-319-42118-6_7]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2021 June 15; 13(6): 550-559

DOI: 10.4251/wjgo.v13.i6.550

ISSN 1948-5204 (online)

MINIREVIEWS

Targeting of elevated cell surface phosphatidylserine with saposin C-dioleoylphosphatidylserine nanodrug as individual or combination therapy for pancreatic cancer

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Author contributions: Davis HW and Qi X conceptualized the review; Kaynak A helped generate the figures; all authors made major contributions to the review's literature search and contributed to drafting the manuscript; all authors contributed to some or all of our laboratory's original studies discussed in this review; all authors provided critical review and approved the final manuscript before submission.

Supported by Pancreatic Cancer Action Network Translational Research Grant, No. 20-65-QIXI; Give Hope Foundation, National Institutes of Health, No. R01CA158372 and No. R21NS095047; and CCTST Pilot Collaborative Studies, Bearcats Against Cancer, and Hematology-Oncology Programmatic Support from University of Cincinnati College of Medicine (to Qi X).

Conflict-of-interest statement: Qi X is listed as an inventor on the patent for SapC-DOPS technology

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Abstract

Pancreatic cancer is one of the deadliest of cancers with a five-year survival of roughly 8%. Current therapies are: surgery, radiation and chemotherapy. Surgery is curative only if the cancer is caught very early, which is rare, and the latter two modalities are only marginally effective and have significant side effects. We have developed a nanosome comprised of the lysosomal protein, saposin C (SapC) and the acidic phospholipid, dioleoylphosphatidylserine (DOPS). In the acidic tumor microenvironment, this molecule, SapC-DOPS, targets the phosphatidylserine cancer-biomarker which is predominantly elevated on the surface of cancer cells. Importantly, SapC-DOPS can selectively target pancreatic tumors and metastases. Furthermore, SapC-DOPS has exhibited an impressive safety profile with only a few minor side effects in both preclinical experiments and in phase I clinical trials. With the dismal outcomes for pancreatic cancer there is an urgent need for better treatments and SapC-DOPS is a good candidate for addition to the oncologist's toolbox.

Key Words: Pancreatic cancer; Saposin C; Dioleoylphosphatidylserine; Phosphatidylserine-targeted therapy; Chemotherapy; Radiation

that is the subject of this review. Consistent with current Cincinnati Children's Hospital Medical Center policies, the development and commercialization of this technology has been licensed to Bexion Pharmaceuticals, LLC, in which Qi X, holds a minor (< 3%) equity interest.

Manuscript source: Invited manuscript

Specialty type: Oncology

Country/Territory of origin: United States

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B, B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Received: January 29, 2021
Peer-review started: January 29, 2021
First decision: April 6, 2021
Revised: April 13, 2021
Accepted: May 10, 2021
Article in press: May 10, 2021
Published online: June 15, 2021

P-Reviewer: Tenreiro N, Wang XB, Zhou J
S-Editor: Gao CC
L-Editor: A
P-Editor: Yuan YY

Core Tip: This review presents the mechanisms and efficacy of saposin C-dioleoylphosphatidylserine (SapC-DOPS), a novel phosphatidylserine (PS) biomarker-targeted nanodrug, alone and in combination with other treatment modalities for the treatment of pancreatic ductal adenocarcinoma (PDAC) tumors. Our results indicate that SapC-DOPS preferentially targets cells with high surface PS which are primarily in the G2/M phase of the cell cycle. Other treatment modalities such as Gemcitabine, Abraxane and radiation target G1 phase cells that have low surface PS. Combination of SapC-DOPS and Gemcitabine/Abraxane or radiation significantly inhibits tumor growth of orthotopic PDAC tumors *in vivo* and increases survival compared to individual treatments.

Citation: Davis HW, Kaynak A, Vallabhapurapu SD, Qi X. Targeting of elevated cell surface phosphatidylserine with saposin C-dioleoylphosphatidylserine nanodrug as individual or combination therapy for pancreatic cancer. *World J Gastrointest Oncol* 2021; 13(6): 550-559
URL: https://www.wjgnet.com/1948-5204/full/v13/i6/550.htm
DOI: https://dx.doi.org/10.4251/wjgo.v13.i6.550

INTRODUCTION

On March 6, 2019, Alex Trebek, the beloved host of the quiz show *Jeopardy* announced that he had Stage IV pancreatic cancer. While discovering that one has cancer is unsettling, a diagnosis of pancreatic cancer is particularly terrifying due to the dismal prognosis. In the United States, pancreatic cancer is the 9th or 10th most commonly diagnosed cancer but is the fourth leading cause of cancer-associated death[1]. Pancreatic ductal adenocarcinoma (PDAC) which accounts for 85% of all forms of pancreatic cancer is also the deadliest gastrointestinal cancer[2]. Longevity from the time of diagnosis until death is the worst of any of the major cancers; the median survival for untreated advanced pancreatic cancer is about 3 1/2 mo but with surgery, radiation and chemotherapy this increases to about 8 mo. In contrast to the steady increase in survival observed for most cancer types, advances have been slow for pancreatic cancer and the 5-year survival rate is only about 8%, however for patients who are diagnosed at an advanced stage the 5-year survival rate is a discouraging 3%. While over the past 20 years there have been incremental increases in survival due to improving treatments, the incidence of pancreatic cancer is increasing by approximately 0.3%/year leading to the expectation that it will be the second leading cause of cancer death by 2030[3]. Pancreatic neuroendocrine tumors or islet cell tumors are less common and tend to have a better outcome so our emphasis in this review will be on PDAC.

The vast majority of pancreatic tumors are located in the head of the pancreas (65%) which are usually found relatively early due to symptoms of obstructive jaundice and pancreatitis. Pancreatic tumors are also located in the body (15%), in the tail (10%), or present as multifocal lesions (2%). These tumors tend to present late and are associated with a worse prognosis[4]. Unfortunately, PDAC is rarely diagnosed early and the tumor is generally between 2-4 cm when found (but can be even larger if located in the body or tail) and has already infiltrated surrounding structures (*i.e.*, peri-pancreatic adipose tissue, stomach, duodenum, portal vein). The histology of PDAC specimens is critical for assessing a PDAC case, with the three main aspects being the size of the primary tumor, the incidence and number of lymph node metastases and the presence or absence of tumor cells at the resection margins[1]. The tumor is generally a solid, firm white to pale yellow, poorly-defined mass. Regional lymph node metastases are also commonly present at diagnosis[5]. Often as little as 10% of the whole tumor volume is occupied by tumor cells, while the remainder is a network of nonmalignant cells, called the stroma, which acts as a protective barrier. In addition, the tumor usually contains a buildup of matrix proteins that cause blood vessels to collapse, preventing chemotherapeutic drugs from reaching the cancer cells in sufficient amounts.

Pancreatic cancer is staged according to the American Joint Committee on Cancer tumor-node-metastasis classification (Table 1).

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For earlier stage pancreatic cancers, surgery is the best option and is potentially curative. Unfortunately, only approximately 20% of patients are candidates for surgery and Stage III or IV cancers are unresectable. Even then in only about 15% of cases are all cancer cells removed[5]. Recently, neoadjuvant therapy (chemo- or radiation therapy prior to surgery) has been used to shrink the tumor. The most common type of surgery is the Whipple procedure, also known as pancreaticoduodenectomy, which involves removal of the head of the pancreas next to the duodenum, the duodenum, a portion of the common bile duct, gallbladder, and occasionally part of the stomach. Afterward, the remaining intestine is reconnected to itself and to the bile duct and pancreas. Although this surgery can improve 5-year survival to approximately 25% it is only available for a small percentage of patients where the tumor has not metastasized[6]. After resection, both gross and microscopic evaluation of the tumor extent is challenging in PDAC, due to dispersed growth, which is more prominent following neoadjuvant therapy, as regression and therefore tumor-induced fibrosis may be patchy. Furthermore, PDAC (even untreated) is characterized by significant inflammation and accumulation of matrix proteins in the normal surrounding stromal cells, making the extent of therapy-induced fibrosis unsatisfactory for determining efficacy [1].

Chemotherapy

Gemcitabine (GEM, Gemzar) is a first line drug for advanced pancreatic cancer. It can be used alone or combined with other drugs such as albumin-bound paclitaxel (Abraxane), capecitabine (Xeloda), or the targeted drug erlotinib (Tarceva). GEM is hydrophilic and must be transported into cells *via* molecular transporters for nucleosides. After addition of three phosphates, GEM can mimic deoxycytidine triphosphate so it is incorporated into newly synthesized DNA and creates an irreparable error that inhibits of further DNA synthesis, thereby leading to cell death [7]. However, cancer cells often become resistant to GEM after several months of treatment. Abraxane is a form of the anti-cancer drug, paclitaxel that has fewer side effects. It is an anti-microtubule agent that inhibits mitosis thus preventing cancer cells from growing and dividing, consequently killing them[8]. In a Phase III study of patients with previously untreated metastatic pancreatic cancer, there was a statistically significant median overall survival benefit of 8.5 mo *vs* 6.7 mo in the GEM/Abraxane group compared to the GEM arm[9].

Capecitabine is metabolized to 5-fluorouracil (5-FU) which inhibits the synthesis of thymidine monophosphate, the active form of thymidine required for *de novo* synthesis of DNA[10]. Erlotinib specifically targets the epidermal growth factor receptor tyrosine kinase, which is overexpressed and often mutated (to generate an overactive form) in most pancreatic cancers[11]. Interestingly, many PDAC cell lines of the classical subtype seem to be resistant to GEM therapy, but sensitive to erlotinib, while PDAC cell lines of the quasi-mesenchymal subtype seem GEM-sensitive, but erlotinib-resistant[12]. Therapeutic strategies targeting angiogenesis with bevacizumab plus GEM were evaluated in a Phase III study but showed no additional benefit[13]. A combination of chemotherapeutic drugs called FOLFIRINOX consisting of 4 drugs: 5-FU, leucovorin, irinotecan (Camptosar), and oxaliplatin (Eloxatin) improves lifespan compared to GEM alone, but it can also have more severe side effects[14,15]. For otherwise healthy patients, FOLFIRINOX is now considered a category 1 recommendation for advanced pancreatic cancer. In a recent study, adjuvant therapy with a modified FOLFIRINOX protocol led to significantly longer disease-free survival than with GEM, among patients with resected PDAC (21.6 mo *vs* 12.8 mo), but had a higher incidence of adverse events of grade 3 or 4 (75.9% *vs* 52.9%)[16]. In addition, a retrospective study, Perri *et al*[17] showed that patients with localized PDAC who received FOLFIRINOX or GEM/Abraxane as their first line of therapy, FOLFIRINOX was associated with a higher rate of RECIST partial response, allowing subsequent pancreatectomy, than GEM/Abraxane but the overall survival rates were similar (21 mo *vs* 20 mo). However, the patients treated with FOLFIRINOX were significantly younger (61 years *vs* 71 years). As indicated, none of these treatments are especially effective and Alex Trebek died on November 8, 2020, 19 mo after his announcement.

A NOVEL THERAPY

Phosphatidylserine (PS), an anionic phospholipid, is primarily located on the inner

Table 1 Staging of pancreatic cancer (from the American Joint Committee on Cancer Staging Manual, 8th edition[45])

Stage	Tumor
IA	Limited to pancreas, greatest dimension: ≤ 2 cm
IB	Limited to pancreas, greatest dimension: ≥ 2 cm
IIA	The greatest dimension is > 4 cm but there is no metastasis or lymph node involvement
IIB	The greatest dimension is ≤ 2 cm to > 4 cm but the cancer has spread to 1-3 regional lymph nodes
III	The greatest dimension is > 4 cm and there are 4 or more lymph nodes involved or the tumor has invaded the celiac axis, superior mesenteric artery, and/or common hepatic artery, regardless of tumor size or lymph nodes involved. The tumor is unresectable
IV	Metastasis to distant sites, regardless of size or number of lymph nodes involved. Metastasis of pancreatic cancer occurs mainly in the liver, peritoneum, and lungs

leaflet of the cell membrane[18-20] due to the activity of ATP-dependent phospholipid translocases (flippases)[21,22]. However, in many viable cancer cells flippase activity is depressed and PS accumulates on the outer leaflet of the membrane[22]. In normal cells the increased surface PS would represent apoptosis and the cell would be engulfed by macrophages[23,24] but cancer cells express CD47 which prevents phagocytosis by macrophages[25]. While most cancer cells express higher levels of PS on the extracellular cell membrane than normal cells, there is a wide variation of surface PS, even in cell lines from the same type of cancer[22]. We have previously demonstrated this is extant in a panel of pancreatic cancer cell lines[26].

Exploiting the increased surface PS on cancer cells, our lab has developed a therapeutic agent that consists of the membrane fusogenic protein, saposin C (SapC) which is embedded in dioleoylphosphatidylserine (DOPS) vesicles. These nanovesicles selectively target a variety of cancer cells[27-30] including pancreatic tumors, due to high affinity of SapC for cancer cell PS[26,31]. SapC is a stable 80-amino acid lysosomal protein ubiquitous in all cells, that has high affinity and exceptional specificity for PS and catabolizes glycosphingolipids in membranes[32-34].

When SapC is coupled with DOPS durable nanovesicles are formed and selectively fuse with the PS on the surface of cancer cells[27,28]. This targeting correlates with the expression of surface PS on the cells and can be blocked by specific PS-binding proteins, such as lactadherin or β2-glycoprotein[26,35]. The specificity of SapC-DOPS binding to cancer cells is further enhanced by the tumor microenvironment which is acidic due to the Warburg effect[36,37]. In cancer cells, lysosomal acid sphingomyelinase (ASMase) leaks out from lysosomes and migrates to the plasma membrane. When SapC-DOPS nanovesicles fuse with surface PS of cancer cell membranes, SapC stimulates ASMase which elevates ceramide levels and consequently activates caspases that induce apoptotic cell death[26,27,38]. In untransformed cells, asymmetric acidic phospholipid distribution results in low PS exposure on the membrane surface. This coupled with the neutral pH environment leads to weak SapC-DOPS interaction with these cells. Thus, SapC-DOPS selectively kills pancreatic tumor cells, without apparent off-target toxicity to normal cells and tissues[26-31]. Indeed, SapC-DOPS (clinical name: BXQ-350) has shown an exemplary safety profile both preclinically[26] and in Phase I clinical trials[39,40]. In mice there were no noticeable side effects and SapC-DOPS appeared to attenuate cancer-associated cachexia[31,41]. In the clinical trials, no severe adverse events were observed and most subjects showed no drug linked problems at all. Importantly, SapC-DOPS has shown strong cytotoxicity on pancreatic cancer cells regardless of their genetic modifications so it should be effective in all patients.

PS also has potential as a diagnostic biomarker, as our data indicate that pancreatic tumors have elevated surface PS compared to relatively normal pancreatic tissue from PDAC patients with no previous therapeutic exposure (Figure 1). This increased PS serves as a molecular target for SapC-DOPS and allows SapC-DOPS to invade the PDAC tumor (Figure 2A) and specifically target tumor blood vessels (Figure 2B) and PDAC cells (Figure 2C) in murine tumor models.

Chu *et al*[26] have determined that the optimal molar ratio of SapC to DOPS is 1:3-1:10 for maximal cytotoxic effects against human cancer cells and for most studies we use 1:7. This formulation of SapC-DOPS is cytotoxic to a variety of pancreatic cancer cells but harmless to normal human pancreatic ductal epithelial cells (HPDE)[26]. As anticipated, there was a correlation between surface PS and the killing effect of SapC-DOPS. Microscopic inspection of SapC-DOPS-treated cells revealed that tumor cells had morphologies consistent with apoptotic cell death, while HPDE cells appeared

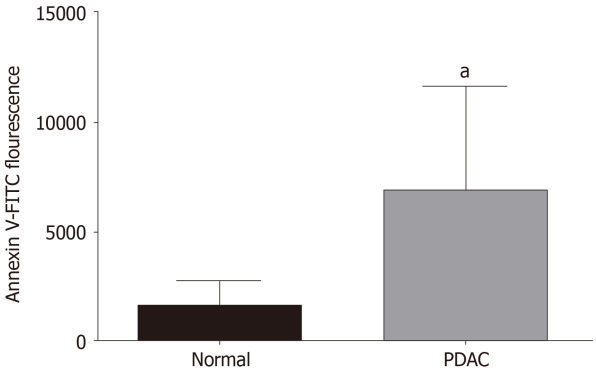

Figure 1 Surface phosphatidylserine on normal pancreata and pancreatic ductal adenocarcinoma tumors. Pancreatic tumors and neighboring more normal tissue excised from treatment-naïve pancreatic ductal adenocarcinoma (PDAC) patients were digested with collagenase IV or a Tumor Dissociation kit (Miltenyi). PDAC cells were co-stained with MUC-4-APC, a marker for epithelial cells and Annexin V-FITC, which binds cell surface phosphatidylserine (PS) and surface PS was quantified on MUC-4$^+$ cells by flow cytometry (n = 5 for each). Data are presented as the mean ± SE of the mean, and were compared between two groups using t-test. $^aP < 0.05$ *vs* normal group. PDAC: Pancreatic ductal adenocarcinoma.

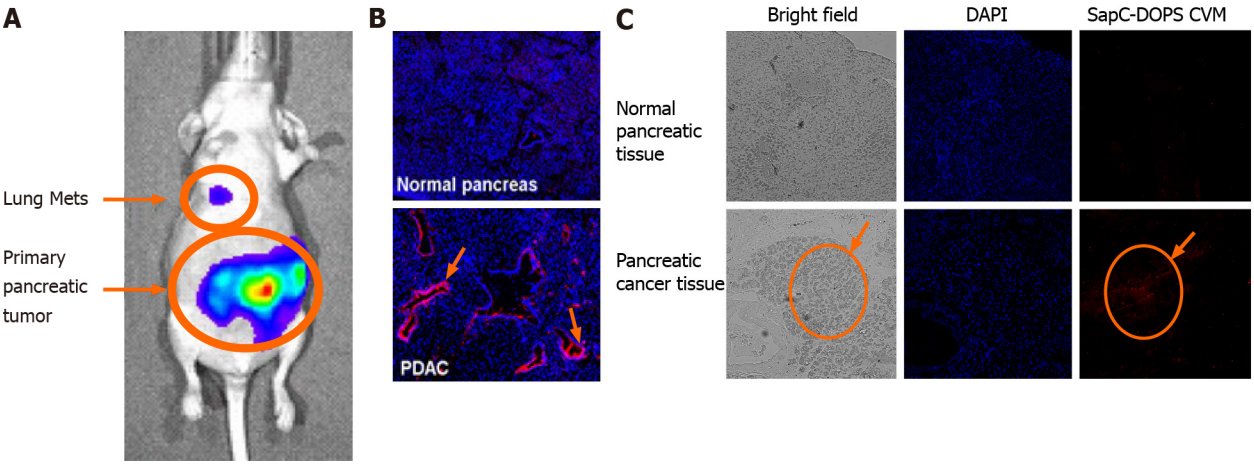

Figure 2 Saposin C-dioleoylphosphatidylserine targets pancreatic tumors. Saposin C-dioleoylphosphatidylserine (SapC-DOPS) was fluorescently labeled with CellVue Maroon (CVM, a far red fluorescent probe) and injected into mice after pancreatic tumors were established from human cancer cells (cfPac-1-Luc3) (see details in reference 26)[26]. A: SapC-DOPS-CVM localized to the primary and lung metastatic tumors detected with intravascular ultrasound live animal imaging; B: Tumors, established from human MiaPaCa-2 cells, and normal pancreata of SapC-DOPS-CVM-injected mice were isolated and prepared for fluorescent microscopy. The slides, after staining with DAPI (blue) to detect nuclei, show accumulation of SapC-DOPS-CVM in the tumors. Note preferential SapC-DOPS labeling of ductal structures (arrows) in pancreatic ductal adenocarcinoma (PDAC), and minimal binding to normal pancreas. SapC-DOPS is binding phosphatidylserine (PS) on the cancer cell surfaces as prior treatment with lactadherin, a PS binding protein, eliminates subsequent binding of SapC-DOPS; C: Frozen, unfixed sections from murine PDAC and matched normal pancreas tissues were incubated with SapC-DOPS-CVM nanovesicles for 20 min, counterstained with DAPI and mounted. The ovals localize the PDAC tumor. SapC-DOPS: Saposin C-dioleoylphosphatidylserine; CVM: CellVue Maroon; Mets: Metastatic tumors; PDAC: Pancreatic ductal adenocarcinoma.

unchanged[26].

To advance these studies, human PANC-1 or MiaPaCa-2 cells were implanted subcutaneously in nude mice then the mice were treated every 2 d to 3 d with various doses of SapC-DOPS. Our results demonstrated a dose-dependent inhibition of pancreatic tumor growth by SapC-DOPS. To investigate a more pathologically important model, mice were implanted with cfPac-1 cells orthotopically into the pancreata. In these mice, SapC-DOPS dramatically prolonged survival; tumor-bearing control mice all died within 170 d but 67% of SapC-DOPS-treated mice survived until they were euthanized at day 260 and none of the surviving mice harbored any detectable tumor. Notably, a metastatic tumor appeared in the lung of one mouse (Figure 2A) and this lesion was targeted by SapC-DOPS[26].

As mentioned, GEM provides only marginal benefit to patients so we assessed the therapeutic benefits of combining GEM with SapC-DOPS. For these studies[31] we first, treated MiaPaCa-2 cells with SapC-DOPS (48 h) and GEM (24 h) alone as well as a combination of SapC-DOPS with GEM. These data demonstrated that the combination of SapC-DOPS and GEM had a significantly greater anti-tumor effect than

either treatment alone. Interestingly, low dose GEM treatment elevates surface PS on cancer cells lines within 48 h without killing the cells, although this may be an early, aborted apoptotic response. We then implanted the pancreatic cancer cell line, p53.2.1.1, subcutaneously into c57Bl/6J mice[31]. We used suboptimal concentrations of both GEM and SapC-DOPS and only treated on days 1 and 4 post implantation to examine the combination effects. Both GEM and SapC-DOPS alone reduced tumor sizes by about 50% but the combination reached 90%. A similar experiment was conducted using subcutaneous mouse 4580P cells in c57Bl/6J mice with GEM/Abraxane and SapC-DOPS with similar results. To ascertain whether the combination could improve survival, we injected mice orthotopically with p53.2.1.1 cells and then administered saline, GEM, SapC-DOPS or the combination. All the control mice died within 29 d. The mice receiving the combination treatment lived substantially longer with one mouse being euthanized tumor-free on day 50. The mice receiving suboptimal concentrations of either GEM or SapC-DOPS alone lived for an intermediate duration. In all of these experiments SapC-DOPS was introduced shortly after the injection of the GEM or GEM/Abraxane. We had previously shown that another chemotherapeutic drug, temozolomide, also had synergistic effects with SapC-DOPS in brain cancer models[35].

Radiation, another therapy for PDAC, also increases surface PS on viable cancer cells. Pancreatic cell lines with initially low to moderate surface PS exhibited dose-dependent increases in surface PS by 12 h with a maximum increase by 24 h. In addition, subcutaneous tumors generated in nude mice from the human pancreatic cancer cell line, cfPac-1, nearly doubled their surface PS 48 h following focused exposure to 10 Gy of radiation[42]. Incidentally, we have recently demonstrated that we can incorporate the therapeutic radioisotope, ^{131}I into SapC-DOPS nanovesicles and that this radiation enhances the effects of SapC-DOPS to prolong survival in mice bearing glioblastoma multiforme, a type of brain cancer[41]. In this scenario, the radiation from ^{131}I, while directly killing the tumor cells may also increase surface PS.

It is tempting to speculate that increasing surface PS with GEM or radiation would augment the cytotoxicity of SapC-DOPS. Thus, we investigated whether sequential treatment order of SapC-DOPS and GEM altered the treatment efficacy. Treating the cells with GEM long enough to increase cell surface PS followed by SapC-DOPS was no more efficacious than SapC-DOPS followed by GEM treatment (Figure 3). These data and results from Davis *et al*[42] suggest that GEM and radiation do not sensitize cells to SapC-DOPS treatment but rather selectively kill the low surface PS cells, leaving high PS cancer cells intact which can then be targeted by SapC-DOPS.

Even within a specific pancreatic cancer cell line there is heterogeneous surface PS expression. As discussed above, SapC-DOPS targets cancer cells with higher surface PS. Indeed, when we treated a heterogeneous cell population with SapC-DOPS the high surface PS population was killed, leaving behind cells with lower surface PS[31]. Interestingly, the opposite effect was observed when pancreatic cancer cell lines were treated with GEM[31] or radiation[42]; that is GEM and radiation tend to kill low surface PS cells. Additionally, when cells were sorted into low and high surface PS fractions by flow cytometry then treated with GEM, cytotoxicity was more pronounced in the low surface PS population[31,42].

GEM preferentially kills cells in the G1 phase of the cell cycle by binding to DNA to prevent the cells from entering S phase of the cycle where DNA is duplicated[31,43]. Of note, we have demonstrated that G1 cancer cells have relatively low surface PS, and as the cells proceed through the cell cycle surface PS increases even when the expansion of the cell surface area is accounted for[31]. Interestingly, PTDSS1, the gene for the enzyme that converts phosphatidylcholine to PS, is elevated in G2/M compared to G1 phase in cfPac-1 cells but not in a non-cancerous pancreatic epithelial cell line (HPDE). PTDSS2 (the enzyme that catalyzes phosphatidylethanolamine transition to PS) is unchanged in either cell line throughout the cell cycle. On the other hand, when we sorted cells by surface PS, we found that a higher percentage of low surface PS cells were in G1 and a higher percentage of high surface PS cells were in G2/M (Qi and colleagues unpublished data). Consequently, more cells in G1 are killed by GEM than SapC-DOPS while the opposite is true for cells in G2/M[31]. When these experiments were repeated in HPDE surface PS was unaltered throughout the cell cycle.

While GEM and SapC-DOPS kill cells through different mechanisms; by preventing DNA synthesis and activating caspases, respectively, we have shown that cells in tumors are segregated into low PS, high G1 and high PS, high G2/M populations which allows the drugs to work on divergent cells and to collaborate to enhance tumor destruction (Figure 4). Thus, tumor cell surface PS may serve as a significant biomarker to assign the most effective treatment for the patient. Importantly, SapC-DOPS

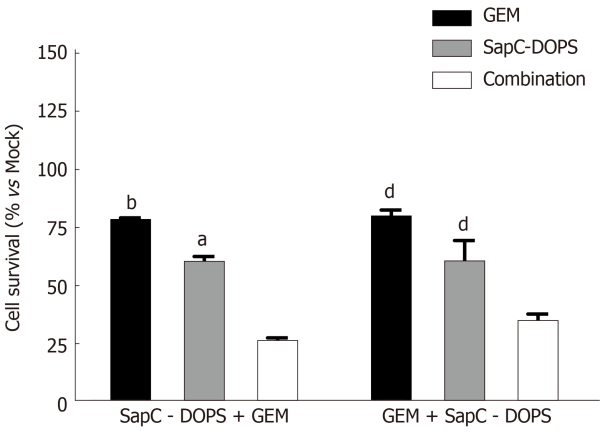

Figure 3 Sequential treatment of pancreatic ductal adenocarcinoma cells with saposin C-dioleoylphosphatidylserine nanodrug and gemcitabine. MiaPaCa-2 cells were treated with saposin C-dioleoylphosphatidylserine (SapC-DOPS) (25 μmol/L) alone, gemcitabine (GEM) (50 nmol/L) alone or in combination. Cells were seeded onto 96 well plates and the next day were exposed to drugs. In the left grouping SapC-DOPS was added for 48 h, the cells were washed twice then incubated with GEM for 24 h. In the right grouping the cells were treated with GEM for 24 h, washed twice and SapC-DOPS was added for 48 h. Untreated cells remained in the media for 72 h. After the 72 h incubation, the MTT cell viability assay was performed. Data are presented as the mean ± SE of the mean, and were compared between two groups using *t*-test. $^{a}P < 0.05$ and $^{b}P < 0.01$ *vs* combination group; $^{d}P < 0.01$ *vs* combination group. GEM: Gemcitabine; SapC-DOPS: Saposin C-dioleoylphosphatidylserine.

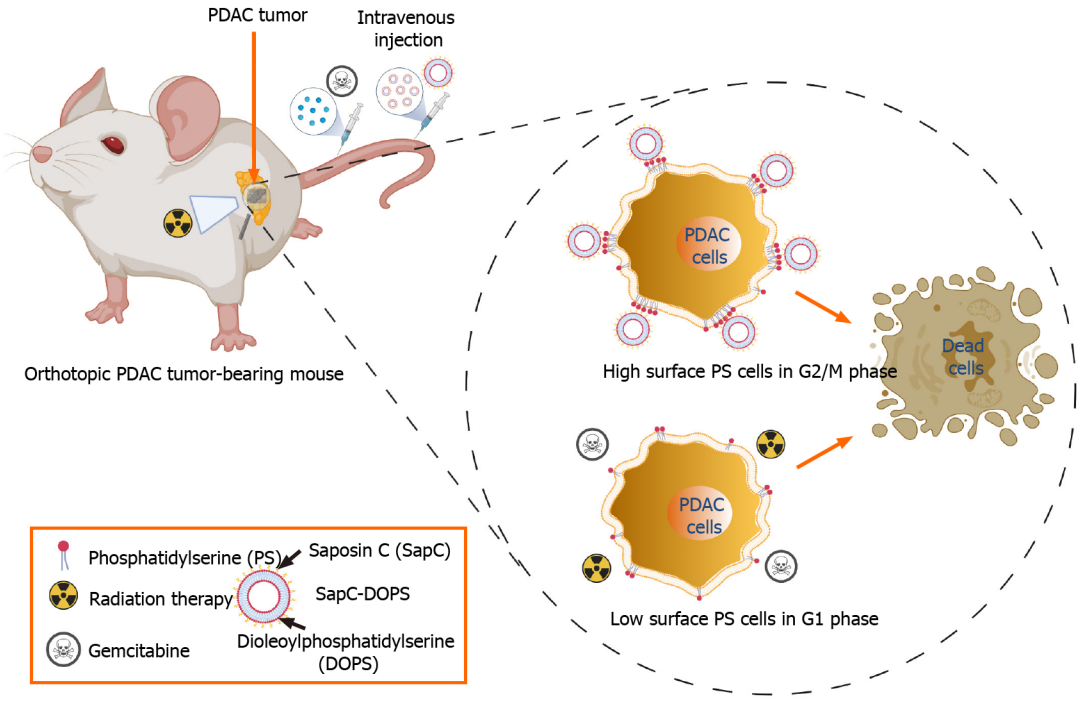

Figure 4 Variable surface phosphatidylserine on pancreatic ductal adenocarcinoma tumor cells are sensitive to phosphatidylserine-selective treatments. Chemotherapy and radiation target primarily low surface phosphatidylserine (PS) cells and may increase PS. Saposin C-dioleoylphosphatidylserine (SapC-DOPS) hones in on high surface PS cells in the acidic tumor microenvironment. Thus, a combination of chemotherapy and/or radiation therapy and SapC-DOPS has the potential to eliminate the preponderance of tumor cells. PDAC: Pancreatic ductal adenocarcinoma.

is a nanovesicle, and unlike many chemotherapeutic drugs, can penetrate the fibrosis and stroma of pancreatic tumors (see Figure 2). This makes it available for use as a carrier of therapeutic modalities such as the ^{131}I mentioned above but also as an imaging and detection agent. In fact, we have demonstrated that fluorescently labeled SapC-DOPS nanovesicles allow selective visualization of primary and metastatic pancreatic tumors *in vivo*[26]. The nanovesicles can also carry contrast agents (iron or gadolinium) for computed tomography or magnetic resonance imaging of tumors[44]. Thus, SapC-DOPS can be used for both diagnosis and treatment.

CONCLUSION

Phase I clinical trials are designed to evaluate the safety of a candidate drug. In these preliminary trials, SapC-DOPS (BXQ-350) has demonstrated an excellent safety record but impressively, has also shown remarkable efficacy in some patients that have failed all other modalities[39,40]. Our data establish that SapC-DOPS alone or in combination with GEM (GEM/Abraxane) or radiation can reduce tumor growth and enhance survival in mouse models of PDAC. We are hopeful that one day soon SapC-DOPS will be part of the cancer treatment arsenal and alleviate the dread that comes with this diagnosis.

ACKNOWLEDGEMENTS

We thank Chu Z and Blanco V for technical assistance with experiments mentioned within.

REFERENCES

1 **Haeberle L**, Esposito I. Pathology of pancreatic cancer. *Transl Gastroenterol Hepatol* 2019; **4**: 50 [PMID: 31304427 DOI: 10.21037/tgh.2019.06.02]
2 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin* 2020; **70**: 7-30 [PMID: 31912902 DOI: 10.3322/caac.21590]
3 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin* 2018; **68**: 7-30 [PMID: 29313949 DOI: 10.3322/caac.21442]
4 **Ghaneh P**, Costello E, Neoptolemos JP. Biology and management of pancreatic cancer. *Gut* 2007; **56**: 1134-1152 [PMID: 17625148 DOI: 10.1136/gut.2006.103333]
5 **Peixoto RD**, Speers C, McGahan CE, Renouf DJ, Schaeffer DF, Kennecke HF. Prognostic factors and sites of metastasis in unresectable locally advanced pancreatic cancer. *Cancer Med* 2015; **4**: 1171-1177 [PMID: 25891650 DOI: 10.1002/cam4.459]
6 **Neoptolemos JP**, Kleeff J, Michl P, Costello E, Greenhalf W, Palmer DH. Therapeutic developments in pancreatic cancer: current and future perspectives. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 333-348 [PMID: 29717230 DOI: 10.1038/s41575-018-0005-x]
7 **Ciccolini J**, Serdjebi C, Peters GJ, Giovannetti E. Pharmacokinetics and pharmacogenetics of Gemcitabine as a mainstay in adult and pediatric oncology: an EORTC-PAMM perspective. *Cancer Chemother Pharmacol* 2016; **78**: 1-12 [PMID: 27007129 DOI: 10.1007/s00280-016-3003-0]
8 **Gradishar WJ**. Albumin-bound paclitaxel: a next-generation taxane. *Expert Opin Pharmacother* 2006; **7**: 1041-1053 [PMID: 16722814 DOI: 10.1517/14656566.7.8.1041]
9 **Von Hoff DD**, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, Seay T, Tjulandin SA, Ma WW, Saleh MN, Harris M, Reni M, Dowden S, Laheru D, Bahary N, Ramanathan RK, Tabernero J, Hidalgo M, Goldstein D, Van Cutsem E, Wei X, Iglesias J, Renschler MF. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N Engl J Med* 2013; **369**: 1691-1703 [PMID: 24131140 DOI: 10.1056/NEJMoa1304369]
10 **Reigner B**, Verweij J, Dirix L, Cassidy J, Twelves C, Allman D, Weidekamm E, Roos B, Banken L, Utoh M, Osterwalder B. Effect of food on the pharmacokinetics of capecitabine and its metabolites following oral administration in cancer patients. *Clin Cancer Res* 1998; **4**: 941-948 [PMID: 9563888]
11 **Moore MJ**, Goldstein D, Hamm J, Figer A, Hecht JR, Gallinger S, Au HJ, Murawa P, Walde D, Wolff RA, Campos D, Lim R, Ding K, Clark G, Voskoglou-Nomikos T, Ptasynski M, Parulekar W; National Cancer Institute of Canada Clinical Trials Group. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol* 2007; **25**: 1960-1966 [PMID: 17452677 DOI: 10.1200/JCO.2006.07.9525]
12 **Collisson EA**, Sadanandam A, Olson P, Gibb WJ, Truitt M, Gu S, Cooc J, Weinkle J, Kim GE, Jakkula L, Feiler HS, Ko AH, Olshen AB, Danenberg KL, Tempero MA, Spellman PT, Hanahan D, Gray JW. Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy. *Nat Med* 2011; **17**: 500-503 [PMID: 21460848 DOI: 10.1038/nm.2344]
13 **Kindler HL**, Niedzwiecki D, Hollis D, Sutherland S, Schrag D, Hurwitz H, Innocenti F, Mulcahy MF, O'Reilly E, Wozniak TF, Picus J, Bhargava P, Mayer RJ, Schilsky RL, Goldberg RM. Gemcitabine plus bevacizumab compared with gemcitabine plus placebo in patients with advanced pancreatic cancer: phase III trial of the Cancer and Leukemia Group B (CALGB 80303). *J Clin Oncol* 2010; **28**: 3617-3622 [PMID: 20606091 DOI: 10.1200/JCO.2010.28.1386]
14 **Conroy T**, Gavoille C, Samalin E, Ychou M, Ducreux M. The role of the FOLFIRINOX regimen for advanced pancreatic cancer. *Curr Oncol Rep* 2013; **15**: 182-189 [PMID: 23341367 DOI: 10.1007/s11912-012-0290-4]
15 **Conroy T**, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, Adenis A, Raoul JL, Gourgou-Bourgade S, de la Fouchardière C, Bennouna J, Bachet JB, Khemissa-Akouz F, Péré-Vergé

D, Delbaldo C, Assenat E, Chauffert B, Michel P, Montoto-Grillot C, Ducreux M; Groupe Tumeurs Digestives of Unicancer; PRODIGE Intergroup. FOLFIRINOX *vs* gemcitabine for metastatic pancreatic cancer. *N Engl J Med* 2011; **364**: 1817-1825 [PMID: 21561347 DOI: 10.1056/NEJMoa1011923]

16 **Conroy T**, Hammel P, Hebbar M, Ben Abdelghani M, Wei AC, Raoul JL, Choné L, Francois E, Artru P, Biagi JJ, Lecomte T, Assenat E, Faroux R, Ychou M, Volet J, Sauvanet A, Breysacher G, Di Fiore F, Cripps C, Kavan P, Texereau P, Bouhier-Leporrier K, Khemissa-Akouz F, Legoux JL, Juzyna B, Gourgou S, O'Callaghan CJ, Jouffroy-Zeller C, Rat P, Malka D, Castan F, Bachet JB; Canadian Cancer Trials Group and the Unicancer-GI–PRODIGE Group. FOLFIRINOX or Gemcitabine as Adjuvant Therapy for Pancreatic Cancer. *N Engl J Med* 2018; **379**: 2395-2406 [PMID: 30575490 DOI: 10.1056/NEJMoa1809775]

17 **Perri G**, Prakash L, Qiao W, Varadhachary GR, Wolff R, Fogelman D, Overman M, Pant S, Javle M, Koay EJ, Herman J, Kim M, Ikoma N, Tzeng CW, Lee JE, Katz MHG. Response and Survival Associated With First-line FOLFIRINOX *vs* Gemcitabine and nab-Paclitaxel Chemotherapy for Localized Pancreatic Ductal Adenocarcinoma. *JAMA Surg* 2020; **155**: 832-839 [PMID: 32667641 DOI: 10.1001/jamasurg.2020.2286]

18 **Bevers EM**, Williamson PL. Getting to the Outer Leaflet: Physiology of Phosphatidylserine Exposure at the Plasma Membrane. *Physiol Rev* 2016; **96**: 605-645 [PMID: 26936867 DOI: 10.1152/physrev.00020.2015]

19 **Connor J**, Bucana C, Fidler IJ, Schroit AJ. Differentiation-dependent expression of phosphatidylserine in mammalian plasma membranes: quantitative assessment of outer-leaflet lipid by prothrombinase complex formation. *Proc Natl Acad Sci USA* 1989; **86**: 3184-3188 [PMID: 2717615 DOI: 10.1073/pnas.86.9.3184]

20 **Riedl S**, Rinner B, Asslaber M, Schaider H, Walzer S, Novak A, Lohner K, Zweytick D. In search of a novel target - phosphatidylserine exposed by non-apoptotic tumor cells and metastases of malignancies with poor treatment efficacy. *Biochim Biophys Acta* 2011; **1808**: 2638-2645 [PMID: 21810406 DOI: 10.1016/j.bbamem.2011.07.026]

21 **Soupene E**, Kemaladewi DU, Kuypers FA. ATP8A1 activity and phosphatidylserine transbilayer movement. *J Receptor Ligand Channel Res* 2008; **1**: 1-10 [PMID: 20224745 DOI: 10.2147/jrlcr.s3773]

22 **Vallabhapurapu SD**, Blanco VM, Sulaiman MK, Vallabhapurapu SL, Chu Z, Franco RS, Qi X. Variation in human cancer cell external phosphatidylserine is regulated by flippase activity and intracellular calcium. *Oncotarget* 2015; **6**: 34375-34388 [PMID: 26462157 DOI: 10.18632/oncotarget.6045]

23 **Huynh ML**, Fadok VA, Henson PM. Phosphatidylserine-dependent ingestion of apoptotic cells promotes TGF-beta1 secretion and the resolution of inflammation. *J Clin Invest* 2002; **109**: 41-50 [PMID: 11781349 DOI: 10.1172/JCI11638]

24 **Poon IK**, Lucas CD, Rossi AG, Ravichandran KS. Apoptotic cell clearance: basic biology and therapeutic potential. *Nat Rev Immunol* 2014; **14**: 166-180 [PMID: 24481336 DOI: 10.1038/nri3607]

25 **Jaiswal S**, Jamieson CH, Pang WW, Park CY, Chao MP, Majeti R, Traver D, van Rooijen N, Weissman IL. CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. *Cell* 2009; **138**: 271-285 [PMID: 19632178 DOI: 10.1016/j.cell.2009.05.046]

26 **Chu Z**, Abu-Baker S, Palascak MB, Ahmad SA, Franco RS, Qi X. Targeting and cytotoxicity of SapC-DOPS nanovesicles in pancreatic cancer. *PLoS One* 2013; **8**: e75507 [PMID: 24124494 DOI: 10.1371/journal.pone.0075507]

27 **Qi X**, Chu Z, Mahller YY, Stringer KF, Witte DP, Cripe TP. Cancer-selective targeting and cytotoxicity by liposomal-coupled lysosomal saposin C protein. *Clin Cancer Res* 2009; **15**: 5840-5851 [PMID: 19737950 DOI: 10.1158/1078-0432.CCR-08-3285]

28 **Abu-Baker S**, Chu Z, Stevens AM, Li J, Qi X. Cytotoxicity and Selectivity in Skin Cancer by SapC-DOPS Nanovesicles. *J Cancer Ther* 2012; **3**: 321-326 [PMID: 25485166 DOI: 10.4236/jct.2012.34041]

29 **Zhao S**, Chu Z, Blanco VM, Nie Y, Hou Y, Qi X. SapC-DOPS nanovesicles as targeted therapy for lung cancer. *Mol Cancer Ther* 2015; **14**: 491-498 [PMID: 25670331 DOI: 10.1158/1535-7163.MCT-14-0661]

30 **Blanco VM**, Curry R, Qi X. SapC-DOPS nanovesicles: a novel targeted agent for the imaging and treatment of glioblastoma. *Oncoscience* 2015; **2**: 102-110 [PMID: 25859553 DOI: 10.18632/oncoscience.122]

31 **N'Guessan KF**, Davis HW, Chu Z, Vallabhapurapu SD, Lewis CS, Franco RS, Olowokure O, Ahmad SA, Yeh JJ, Bogdanov VY, Qi X. Enhanced Efficacy of Combination of Gemcitabine and Phosphatidylserine-Targeted Nanovesicles against Pancreatic Cancer. *Mol Ther* 2020; **28**: 1876-1886 [PMID: 32516572 DOI: 10.1016/j.ymthe.2020.05.013]

32 **Qi X**, Leonova T, Grabowski GA. Functional human saposins expressed in Escherichia coli. Evidence for binding and activation properties of saposins C with acid beta-glucosidase. *J Biol Chem* 1994; **269**: 16746-16753 [PMID: 8206997 DOI: 10.1016/S0021-9258(19)89454-1]

33 **Wang Y**, Grabowski GA, Qi X. Phospholipid vesicle fusion induced by saposin C. *Arch Biochem Biophys* 2003; **415**: 43-53 [PMID: 12801511 DOI: 10.1016/s0003-9861(03)00219-4]

34 **Qi X**, Chu Z. Fusogenic domain and lysines in saposin C. *Arch Biochem Biophys* 2004; **424**: 210-218 [PMID: 15047193 DOI: 10.1016/j.abb.2004.02.023]

35 **Wojton J**, Meisen WH, Jacob NK, Thorne AH, Hardcastle J, Denton N, Chu Z, Dmitrieva N, Marsh

R, Van Meir EG, Kwon CH, Chakravarti A, Qi X, Kaur B. SapC-DOPS-induced lysosomal cell death synergizes with TMZ in glioblastoma. *Oncotarget* 2014; **5**: 9703-9709 [PMID: 25210852 DOI: 10.18632/oncotarget.2232]

36 **Hsu PP**, Sabatini DM. Cancer cell metabolism: Warburg and beyond. *Cell* 2008; **134**: 703-707 [PMID: 18775299 DOI: 10.1016/j.cell.2008.08.021]

37 **Webb BA**, Chimenti M, Jacobson MP, Barber DL. Dysregulated pH: a perfect storm for cancer progression. *Nat Rev Cancer* 2011; **11**: 671-677 [PMID: 21833026 DOI: 10.1038/nrc3110]

38 **Sulaiman MK**, Chu Z, Blanco VM, Vallabhapurapu SD, Franco RS, Qi X. SapC-DOPS nanovesicles induce Smac- and Bax-dependent apoptosis through mitochondrial activation in neuroblastomas. *Mol Cancer* 2015; **14**: 78 [PMID: 25889084 DOI: 10.1186/s12943-015-0336-y]

39 **Rixe O**, Morris JC, Puduvalli VK, Villano JL, Wise-Draper TM, Muller C, Johnson AN, Wesolowski R, Qi X. First-in-human, first-in-class phase 1a study of BXQ-350 for solid tumors and gliomas. *J Clin Oncol* 2018; **36**: 2517-17 [DOI: 10.1200/JCO.2018.36.15_suppl.2517]

40 **Abdelbaki M**, Setty B, DeWire MD, Cripe TP, Curry R. A pediatric and young adult phase I dose escalation study of BXQ-350 for solid and central nervous system tumors. *J Clin Oncol* 2020; **38**: 2541-41 [DOI: 10.1200/JCO.2020.38.15_suppl.2541]

41 **Davis HW**, Vallabhapurapu SD, Chu Z, Wyder MA, Greis KD, Fannin V, Sun Y, Desai PB, Pak KY, Gray BD, Qi X. Biotherapy of Brain Tumors with Phosphatidylserine-Targeted Radioiodinated SapC-DOPS Nanovesicles. *Cells* 2020; **9** [PMID: 32854321 DOI: 10.3390/cells9091960]

42 **Davis HW**, Vallabhapurapu SD, Chu Z, Vallabhapurapu SL, Franco RS, Mierzwa M, Kassing W, Barrett WL, Qi X. Enhanced phosphatidylserine-selective cancer therapy with irradiation and SapC-DOPS nanovesicles. *Oncotarget* 2019; **10**: 856-868 [PMID: 30783515 DOI: 10.18632/oncotarget.26615]

43 **Park SH**, Sung JH, Kim EJ, Chung N. Berberine induces apoptosis *via* ROS generation in PANC-1 and MIA-PaCa2 pancreatic cell lines. *Braz J Med Biol Res* 2015; **48**: 111-119 [PMID: 25517919 DOI: 10.1590/1414-431X20144293]

44 **Blanco VM**, Latif T, Chu Z, Qi X. Imaging and Therapy of Pancreatic Cancer with Phosphatidylserine-Targeted Nanovesicles. *Transl Oncol* 2015; **8**: 196-203 [PMID: 26055177 DOI: 10.1016/j.tranon.2015.03.011]

45 **Amin MB**, Edge S, Greene F, Byrd DR, Brookland RK, Washington MK, Gershenwald JE, Compton CC, Hess KR, Sullivan DC, Jessup JM, Brierley JD, Gaspar LE, Schilsky RL, Balch CM. AJCC Cancer Staging Manual. 8th ed. Springer International Publishing: American Joint Commission on Cancer, 2017

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2021 June 15; 13(6): 560-573

DOI: 10.4251/wjgo.v13.i6.560

ISSN 1948-5204 (online)

MINIREVIEWS

Current indications for endoscopic submucosal dissection of early gastric cancer

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Author contributions: Zheng Z, Yin J, Liu XY, and Yan XS contributed equally to this work; Zheng Z, Yin J and Yan XS carried out the studies, participated in collecting the data, and drafted the manuscript; Xu R, Liu XY, Li MY, and Cai J participated in study design; Zhang J, Yin J, Chen GY, and Zhang ZT helped to draft the manuscript; all authors read and approved the final manuscript.

Supported by Beijing Municipal Science & Technology Commission, No. D171100006517003 and No. Z181100001718223; Research Foundation of Beijing Friendship Hospital, Capital Medical University, No. Y2018-3; Beijing Municipal Administration of Hospitals Incubating Program, No. PX2020001; and Digestive Medical Coordinated Development Center of Beijing Hospital Authority, No. XXX0102.

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Abstract

The development of endoscopic treatment technology has further promoted the minimally invasive treatment of early gastric cancer (EGC). Endoscopic treatment has achieved better therapeutic effects in terms of safety and prognosis and is the preferred treatment method for patients who meet the indications for endoscopic treatment. However, the consequent problem is that some patients receiving endoscopic treatment may undergo non-curative resection, and the principle of follow-up management for non-curative resection patients deserves further attention. In addition, there are still debates on how to improve the accuracy of clinical staging, select a reasonable treatment method for patients who meet the expanded indications for endoscopic treatment, manage patients with positive endoscopic surgical margins, conduct research on function-preserving surgery, and manage the treatment of EGC under the current situation in China. Consequently, we aim to review current indications for endoscopic submucosal dissection of EGC in order to better inform treatment options.

Key Words: Early gastric cancer; Endoscopic submucosal dissection indications; Non-curative resection; Salvage surgery; Function-preserving surgery

Core Tip: Gastric cancer is a worldwide public health problem with a lower cure rate and worse prognosis. With the improvement of people's health awareness and the

Conflict-of-interest statement: All authors declare that they have no conflict of interest to disclose.

Manuscript source: Invited manuscript

Specialty type: Surgery

Country/Territory of origin: China

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: February 5, 2021
Peer-review started: February 5, 2021
First decision: March 29, 2021
Revised: March 31, 2021
Accepted: May 22, 2021
Article in press: May 22, 2021
Published online: June 15, 2021

P-Reviewer: Masaki S, Shang Y
S-Editor: Yan JP
L-Editor: Wang TQ
P-Editor: Li JH

popularization of physical examination, the detection rate of early gastric cancer is increasing each year. *Helicobacter pylori* and Epstein-Barr virus are important pathogenic factors for gastric cancer. For patients who meet with the absolute and expanded indications for endoscopic treatment, endoscopic submucosal dissection can have the same therapeutic effect as surgery while reducing surgical trauma. For non-curative resection, laparoscopic subtotal gastrectomy or function-preserving gastrectomy can be performed based on the patient's condition.

Citation: Zheng Z, Yin J, Liu XY, Yan XS, Xu R, Li MY, Cai J, Chen GY, Zhang J, Zhang ZT. Current indications for endoscopic submucosal dissection of early gastric cancer. *World J Gastrointest Oncol* 2021; 13(6): 560-573
URL: https://www.wjgnet.com/1948-5204/full/v13/i6/560.htm
DOI: https://dx.doi.org/10.4251/wjgo.v13.i6.560

INTRODUCTION

Gastric cancer is a malignant tumor originating from the gastric mucosal epithelium, and its prognosis and outcomes are closely related to tumor stage. Most patients with early gastric cancer (EGC) have no obvious clinical symptoms. If gastric cancer screening is performed properly, gastric cancer can be detected at an early stage. However, unfortunately, patients are often diagnosed at the stage of advanced gastric cancer because they have not been screened for gastric cancer. This leads to a lower radical tumor resection rate and poor prognosis, and the 5-year survival rate is less than 30%[1]. However, patients with EGC have a better prognosis, with a 5-year survival rate of more than 90%. With the gradual popularization of diagnostic techniques and endoscopic screening in China, more patients with gastric cancer can be diagnosed in the early stage and receive therapy.

At present, radical surgery is still the acknowledged treatment for EGC, and whether it is accompanied by lymph node metastasis (LNM) is an important basis for the choice of surgery. In recent years, endoscopic submucosal dissection (ESD), a minimally invasive and effective technique, has become the preferred approach for the treatment of EGC. It is advantageous because under the premise of strict control of indications, the surgical trauma is significantly less than laparoscopic or open surgery, and the long-term prognosis is not worse than surgical treatment. Furthermore, it can maximize the preservation of gastric functions and improve the life quality of patients after surgery[2]. However, difficulty in accurately assessing the histopathological conditions, such as the depth of tumor invasion, the extent of lateral invasion, and vascular invasion before treatment, as well as the deficiency of endoscopic surgical technique leads to the occurrence of non-curative resection (NCR), which is also a disadvantage of endoscopic therapy. A study found that among 194 patients with EGC who received additional surgical treatment after NCR of ESD, 10 (5.2%) had tumor recurrence and 11 (5.7%) had LNM[3]. Although patients with NCR of EGC have a higher risk of LNM and should be treated by additional surgery, follow-up results showed that most patients do not have LNM after surgery, and some patients are unable or unwilling to receive surgical treatment due to advanced age and underlying diseases[4]. Therefore, how to minimize surgical trauma and choose the optimal treatment to ensure the tumor radical resection is the focus of ongoing research. Herein, we aim to review the current indications for ESD of EGC in order to better evaluate treatment options.

EPIDEMIOLOGY OF EGC

Gastric cancer is a worldwide public health problem with a lower cure rate and worse prognosis. According to the Global Cancer statistic report of the World Health Organization and the International Agency for Research on Cancer, there were 1 million new cases of gastric cancer worldwide in 2018, ranking fifth among new patients with malignant tumors. There were 783000 deaths accounting for the third highest number of cancer-related deaths[5]. There are obvious differences in the

epidemiological characteristics of gastric cancer between East Asia and Western countries, among which Japan, South Korea, and China are the regions with a high incidence of gastric cancer, and the incidence in males is about twice that in females[5, 6]. Based on the survey results of the National Central Cancer Registry of China, there were about 410000 new cases of gastric cancer and about 290000 deaths in China in 2014, making it the second common cause of morbidity and mortality among cancer patients[7]. Although the overall incidence and mortality of gastric cancer have shown a downward trend with the gradual improvement of diagnostic methods and strategies and the deepening understanding of its molecular mechanisms, it still faces huge challenges. In South Korea and Japan, due to the mature gastroscopy screening system, the detection rate of EGC accounts for 50%-60% of the overall proportion of gastric cancer[8-10]. In China, with the improvement of people's health awareness and the popularization of physical examination, the detection rate of EGC in the overall incidence of gastric cancer is increasing each year. According to the statistics of the Chinese Association of Gastrointestinal Cancer Surgery from 2014 to 2016, EGC accounted for 19% of the total gastric cancer cases, which is still a considerable gap compared with Japan and South Korea[11] (Figure 1). Therefore, attention should be paid to the screening, early diagnosis, and treatment in order to further increase the detection rate of EGC and better improve the long-term prognosis of patients.

ETIOLOGY OF EGC

Helicobacter pylori (*H. pylori*) infection is an important pathogenic factor for gastric cancer. In addition, *H. pylori* is involved in tumor proliferation, apoptosis, and epigenetic modification of oncogenes, which ultimately leads to tumorigenesis associated with inflammatory lesions[12]. However, for patients with EGC undergoing surgery, whether *H. pylori* is routinely eradicated is still inconclusive, and whether radical *H. pylori* eradication can stop the progression from precancerous lesions to cancer is still debated. Studies have found that compared with the placebo group, the *H. pylori* eradication group showed a significantly reduced incidence of metachronous gastric cancer [hazard ratio (HR) = 0.50, 95% confidence interval (CI): 0.26-0.94, P = 0.03] after ESD in patients with EGC[13]. No serious adverse events occurred in either group, which fully affirmed the significance of eradicating *H. pylori* infection in precancerous lesions and EGC.

Epstein-Barr virus (EBV) mainly exists in gastric cancer cells and lymphoid stroma, while most normal epithelial cells do not have EBV. Currently, with the establishment of molecular classification of gastric cancer and the rise of immunotherapy, EBV-related gastric cancer has gradually attracted attention, but the mechanism of EBV in the pathogenesis of gastric cancer remains unclear[14]. In the Cancer Genome Atlas molecular classification, EBV type has higher CpG island methylation, phosphoinositide 3-kinase mutation, programmed death ligand 1/2 overexpression, silencing of cyclin-dependent kinase inhibitor 2A, and activation of immune-related signaling pathways, suggesting that EBV-associated gastric cancer may have its own independent biological and clinical characteristics[14,15]. A study found that the objective response rate of patients with EBV-positive metastatic gastric cancer was 100% after treatment with pembrolizumab, which preliminarily confirmed that EBV-positive status can be used as a potential molecular marker to predict the possibility of immunotherapy[16]. However, the limitation of this study was its retrospective design and the findings need to be further verified by prospective studies. It is believed that further studies on the molecular mechanism of EBV-related gastric cancer will provide a theoretical basis for the refinement of gastric cancer molecular classification and developing new drugs.

PRINCIPLES OF ENDOSCOPIC RESECTION

LNM is one of the important factors affecting the prognosis of patients with EGC and the choice of treatment. Therefore, endoscopic treatment is suitable for tumors with relatively limited primary lesions and an extremely low possibility of LNM[6]. At present, endoscopic resection of EGC mainly includes endoscopic mucosal resection (EMR) and ESD. ESD makes up for the problems that EMR cannot remove, such as large areas of lesions. Moreover, ESD can make more accurate judgments on the depth of tumor invasion and presence of vascular invasion. Thus, ESD has gradually replaced EMR as the preferred treatment for EGC[17]. However, there are still many

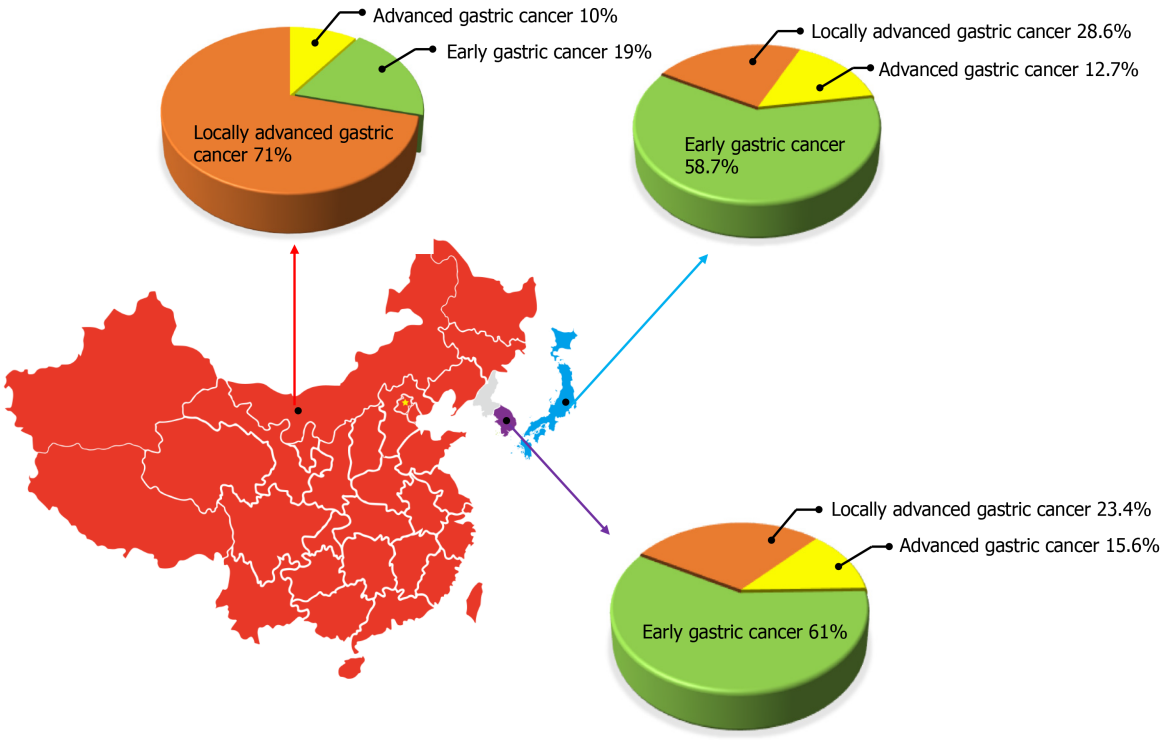

Figure 1 Epidemiology of early gastric cancer. In China, early gastric cancer (EGC) accounts for 19% of the total gastric cancer cases, while in Korea and Japan, EGC accounts for 61% and 58.7% of the total gastric cancer cases, respectively.

controversies regarding the selection of ESD indications for EGC. Consequently, how to refine the indications for ESD treatment more effectively, reduce surgical trauma, and improve safety and rationality as far as possible under the premise of curative resection has gradually become a prominent problem in the treatment of EGC.

Absolute indications

In 2016, the Japan Gastroenterological Endoscopy Society and the Japanese Gastric Cancer Association (JGCA) jointly issued the guidelines for ESD and EMR of EGC, which divided indications for endoscopic resection into absolute and expanded indications[18]. The former has good long-term prognostic evidence, while the latter lacks reliable long-term prognostic results.

Tumor lesions with a risk of LNM less than 1% for which endoscopic resection is considered to have the same effect as radical surgery are classified as absolute indications for ESD therapy[6]. Based on the research results of 5265 patients with EGC by Gotoda *et al*[19], Japanese gastric cancer treatment guidelines 2014 (version 4) clearly stated non-ulcerated and differentiated intramucosal carcinoma with a tumor diameter ≤ 2 cm as the absolute indication for endoscopic treatment of EGC[20] (Table 1). This absolute indication range is the same as that mentioned in guidelines for ESD and EMR for EGC[18]. Based on the results of the JCOG0607 trial[21], Japanese gastric cancer treatment guidelines 2018 (5th edition) adjusted tumor diameter > 2 cm, differentiated intramucosal carcinoma without ulcer lesions and tumor diameter ≤ 3 cm, and differentiated intramucosal carcinoma with ulcer lesions as the absolute indications for ESD. On the other hand, undifferentiated intramucosal carcinoma without ulcer and with a diameter ≤ 2 cm was considered an expanded indication for ESD[22] (Table 1). This version of guidelines is the same as the Korean Practice Guideline for Gastric Cancer 2018 for the absolute and expanded indications for ESD in EGC[23]. In addition, the ongoing study of JCOG1009/1010, which explores the efficacy and safety of ESD in the treatment of undifferentiated cT1a gastric cancer, has completed a 5-year follow-up. The results of the study confirmed that the patients with undifferentiated intramucosal carcinoma with tumor diameter < 2 cm, without ulcers had a satisfactory prognosis after endoscopic treatment, and no LNM occurred[24,25]. It is believed that this indication is expected to be included in the absolute indication range of endoscopic resection, which will lay a theoretical foundation for further elaboration of ESD indications.

Table 1 Absolute and expanded indications for endoscopic submucosal dissection in early gastric cancer patients in Japanese Gastric Cancer Association guideline version 4, 2014 and version 5, 2018

	T1a				T1b			
	UL (-)		UL (+)		SM1 (< 500 μm)		SM2 (> 500 μm)	
	≤ 2 cm	> 2 cm	≤ 3 cm	> 3 cm	≤ 3 cm	> 3 cm	≤ 3 cm	> 3 cm
JGCA guideline (version 4, 2014)								
Differentiated	ESD	EXPANDED(JCOG0607)	EXPANDED(JCOG0607)	SURGERY	EXPANDED	SURGERY	SURGERY	SURGERY
Undifferentiated	EXPANDED	SURGERY	SURGERY	SURGERY	SURGERY	SURGERY	SURGERY	SURGERY
JGCA guideline (version 5, 2018)								
Differentiated	ESD	ESD	ESD	SURGERY	EXPANDED	SURGERY	SURGERY	SURGERY
Undifferentiated	EXPANDED(JCOG1009/1010)	SURGERY	SURGERY	SURGERY	SURGERY	SURGERY	SURGERY	SURGERY

JGCA: Japanese Gastric Cancer Association; T1a: Mucosal carcinoma; T1b: Submucosal carcinoma; ESD: Endoscopic submucosal dissection absolute indication; EXPANDED: Endoscopic submucosal dissection expanded indication; SURGERY: Surgical indication; EGC: Early gastric cancer; UL (-): Without ulcer; UL (+): With ulcer; SM1: The submucosal invasion depth is less than 500 μm; SM2: The submucosal invasion depth is more than 500 μm.

Due to the low incidence of EGC in European and American countries, endoscopic treatment still lacks relevant evidence-based medicine. Currently, research results of endoscopic therapy for EGC are mainly based on the data from related studies in Japan and South Korea. The European Society of Gastrointestinal Endoscopy guidelines and the National Comprehensive Cancer Network (NCCN) guidelines fully adopt the absolute indications for endoscopic treatment recommended by the JGCA for endoscopic resection[26-28]. However, whether the above ESD treatment indications are suitable for the Chinese population is yet to be validated in high-quality clinical trials. Therefore, some research centers in China are carrying out exploratory research on ESD indications for EGC, hoping to establish reasonable ESD indications that meet the characteristics of the Chinese population[29]. Meanwhile, a staging diagnosis scheme for EGC suitable for China's national conditions was proposed to further achieve the purpose of precision treatment and improve the life quality and prognosis of patients.

Expanded indications

The absolute indications for ESD for the treatment of EGC have been unanimously approved, but the application of expanded indications is still controversial. Among them, assessment of the risk of LNM is the key to determining the optimal therapy for patients with expanded indications. A study from Korea found that 17.6% of the patients who were assessed to meet the expanded indications for ESD before surgery were proved to be non-compliant with the ESD indications after surgery, while only 6.7% of patients who were assessed as absolute indications before surgery did not meet the indications[30]. It indicates that improving the accuracy of preoperative diagnosis of expanded indications is a prerequisite for the rational application of ESD in the treatment of EGC. Therefore, although expanded indications can benefit some patients with EGC, its exact efficacy is still being explored. A meta-analysis showed that *en bloc* resection rates (93.6% *vs* 97%, $P < 0.0001$) and radical resection rates (82.4% *vs* 94%, $P < 0.0001$) were significantly lower in patients eligible for the expanded indications than in those with absolute indications, but there was no statistically significant difference in long-term survival ($P = 0.37$)[31]. Another retrospective study from South Korea used propensity score matching to analyze 522 patients who were eligible for expanded indications and underwent surgery or endoscopic treatment[32]. The study found that the overall and tumor-specific survival rates were not statistically different between the two groups, but the 5-year relapse-free survival rate in the surgery group was better than that in the endoscopic group (96.7% *vs* 92.7%, $P < 0.001$)[32]. Further comparison of patient recurrence patterns showed that there was no significant statistical difference in LNM rate and distant metastasis rate between the two groups, but the metachronous metastasis rate in the endoscopic group was higher than that in the surgical group[32]. This result indicates that the recurrence pattern of patients with expanded indications for endoscopic treatment is mainly local recurrence. Therefore, endoscopic treatment may be a good option for patients with expanded indications under the condition of ensuring sufficient surgical margins and

regular postoperative re-examination.

The JCOG0607 trial, which is conducted in Japan, suggested that ESD treatment is safe and effective for EGC patients with expanded indications. A total of 470 patients eligible for expanded indications for ESD were included in the study. The results showed that the *en bloc* rate of ESD was 99.1%, the curative resection rate was 67%, and the delayed bleeding and perforation rates were 8.5% and 2.6%, respectively. Of these, 86.8% of patients with NCR received surgical treatment, and the 5-year survival rate of all patients was 97%[21]. Based on the results of this study, it was confirmed that ESD was reasonable and safe in the treatment of patients with EGC who partially met the expanded indications. On this basis, the endoscopic treatment indications of the Japanese gastric cancer treatment guidelines 2018 (5th edition) have been revised to make the guidelines more in line with the needs of clinical treatment. Therefore, the current expanded indications for endoscopic treatment mainly include undifferentiated intramucosal carcinoma with tumor diameter ≤ 2 cm and without ulcer lesions and differentiated submucosal carcinoma with tumor diameter < 3 cm and invasion depth <500 μm[22]. Recently, with the publication of JCOG1009/1010 results, the application scope of existing ESD indications will be further expanded, benefiting more patients with EGC[24] (Table 1).

Although the above research results support the application of ESD expanded indications, some skeptical studies pointed out that compared with patients with absolute indications, patients with expanded indications had a higher rate of LNM, especially for undifferentiated intramucosal carcinoma with a diameter ≤ 2 cm [25/972 (2.6%), reference range = 6.79, P = 0.004] and differentiated submucosal carcinoma with a diameter < 3 cm [8/315 (2.5%), reference range = 6.30, P = 0.004][33]. Therefore, the current debate on expanded indications mainly focuses on undifferentiated carcinoma and submucosal infiltrating carcinoma. These two types of lesions seem to have a higher risk of LNM, which may not only increase the rate of NCR but also pose a challenge to preoperative evaluation of lesions. Therefore, we suggest that endoscopic therapy for patients with expanded indications should be selectively carried out by experienced centers in the context of clinical trials.

MANAGEMENT AFTER ENDOSCOPIC RESECTION

Curative resection

Curative resection refers to the complete resection of the lesions with negative margins and no vascular and lymphatic infiltration which meet the absolute and expanded indications. Complete resection is an important condition for curative resection and complete reconstruction after segmental resection of the lesions can also be considered as meeting the criteria for curative resection. According to the Japanese gastric cancer treatment guidelines 2014 (version 4)[20], complete resection, tumor diameter ≤ 2 cm, differentiated intramucosal carcinoma without ulceration, negative horizontal and vertical margins, and no lymph node or vascular infiltration are required for absolute indications. For expanded indications, one of the following four requirements is required: (1) Tumor diameter > 2 cm, differentiated intramucosal carcinoma without ulcer; (2) Tumor diameter ≤ 3 cm, differentiated intramucosal carcinoma with ulcer; (3) Tumor diameter ≤ 2 cm, undifferentiated intramucosal carcinoma without ulcer; and (4) Tumor diameter ≤ 3 cm, differentiated submucosal carcinoma with invasion depth < 500 μm. In addition, the horizontal and vertical resection margins should be negative without lymphatic and vascular infiltration (Table 2). Curative resection of EGC is the ultimate goal of endoscopic therapy and the key is that clinicians need to have a full grasp of the indications for endoscopic therapy. However, postoperative pathology confirmed that part of EGC did not reach the standard of curative resection after endoscopic treatment. Cho *et al*[34] analyzed the literature on the efficacy of ESD in the treatment of EGC in Eastern and Western countries in recent years and found that the *en bloc* rate was 92%-97% and curative resection rate was 73.6%, which indicated that NCR still had a certain proportion in postoperative pathological evaluation. Therefore, the Japanese gastric cancer treatment guidelines 2018 (5th edition) updated the expression of 'curative/non-curative resection' in the evaluation of ESD radical resection to 'endoscopic curability (eCura)'[22]. In these guidelines, curative resection, expanded curative resection, and NCR were changed to eCura A, eCura B, and eCura C, respectively (Table 2). Studies have found that patients with curative resection still have a potential recurrence risk after surgery with a local recurrence rate of 0.13%-1.3%[35], an incidence of simultaneous carcinoma and metachronous carcinoma of 4.0%-12.9% and 2.5%-5.1%, respectively[35-37], and 5-year and 10-year cumulative risk

Table 2 Curative and non-curative resection criteria for endoscopic submucosal dissection in early gastric cancer patients in Japanese Gastric Cancer Association guideline version 4, 2014 and version 5, 2018

	T1a				T1b			
	UL (-)		UL (+)		SM1 (< 500 μm)		SM2 (> 500 μm)	
	≤ 2 cm	> 2 cm	≤ 3 cm	> 3 cm	≤ 3 cm	> 3 cm	≤ 3 cm	> 3 cm
JGCA guideline (version 4, 2014)								
Differentiated	CR	CR	CR	NCR	CR	NCR	NCR	NCR
Undifferentiated	CR	NCR	NCR	NCR	NCR	NCR	NCR	NCR
JGCA guideline (version 5, 2018)								
Differentiated	eCura A	eCura A	eCura A	eCura C	eCura B	eCura C	eCura C	eCura C
Undifferentiated	eCura B	eCura C	eCura C	eCura C	eCura C	eCura C	eCura C	eCura C

JGCA: Japanese Gastric Cancer Association; T1a: Mucosal carcinoma; T1b: Submucosal carcinoma; UL (-): Without ulcer; UL (+): With ulcer; SM1: The submucosal invasion depth is less than 500 μm; SM2: The submucosal invasion depth is more than 500 μm; CR: Curative resection; NCR: Non-curative resection; eCura A and eCura B: The horizontal and vertical incisal margins were negative; eCura C: It means non-curative resection, which includes eCura C1 (non-*en bloc* resection or positive horizontal incisal margin) and eCura C2 (tumor diameter > 2 cm, undifferentiated or submucosal carcinoma).

rates as high as 9.5% and 22.7%, respectively[35]. Therefore, for patients with eCura A and eCura B, the Japanese guidelines recommend close follow-up observation to monitor the occurrence of metachronous gastric cancer and LNM[38], and regular high-quality endoscopy follow-up can detect more than 95% of metachronous carcinomas and regular abdominal computed tomography can monitor the presence or absence of LNM and distant organ metastasis.

NCR

NCR refers to the situation that does not meet the criteria for curative resection or expanded curative resection after endoscopic resection, and its incidence is approximately 14.3%-21.4%[39-42]. NCR includes eCura C1 and eCura C2, among which eCura C1 refers to non-*en bloc* resection and positive horizontal margins, while other situations belong to eCura C2[22].

For patients with eCura C1, the guidelines recommend additional ESD remedial resection, surgical treatment, and close follow-up. Follow-up is a feasible strategy for patients having only positive horizontal margins with a low rate of LNM. A study found that in 77 patients with positive horizontal margins after ESD, only 11.9% had local recurrence without distant metastasis after 60 mo of follow-up, and the 5-year overall survival rate was 94.2%[43]. Other studies have found a higher risk of recurrence in patients whose tumors were partitioned, but no tumor-related deaths during the 10-year follow-up period were observed[44]. However, due to the lack of evidence from randomized controlled studies, there is still no accepted standard treatment for NCR of eCura C1.

For eCura C2 patients with high-risk factors for LNM, the guidelines recommend additional surgical treatment. Suzuki *et al*[45] divided 1969 EGC patients with NCR into the additional surgery group and the observation group and found that the 5-year overall survival rates of the two groups were 91% and 75.5% ($P < 0.001$), and the disease-specific survival rates was 99.0% and 96.8% ($P = 0.013$), respectively. Therefore, although the current treatment of eCura C2 is still controversial, most evidence shows that additional surgery can benefit patients' survival[39,46-48]. However, salvage surgery also increases the risk of surgical complications and reduces the patient's postoperative life quality, and it is possible for these patients to obtain postoperative pathological specimens without residual cancer.

Therefore, for patients diagnosed with NCR after ESD, two factors need to be considered in the formulation of remedial strategies: (1) Positive margin or local recurrence; and (2) LNM. In the absence of LNM, complete excision can be achieved by ESD again, regardless of positive margin or local recurrence. However, how to predict the risk of LNM after NCR of ESD is the key to guiding treatment after NCR[49].

To assess the risk factors for LNM in patients with NCR, Hatta *et al*[40] proposed the eCura scoring system to make treatment decisions. Five factors including tumor size (1 point), invasion depth (1 point), lymphatic invasion (3 points), venous invasion (1 point), and vertical margin positive (1 point) were included in the eCura scoring

system. Patients with a total score of 0-1, 2-4, and 5-7 were classified as low-risk, medium-risk, and high-risk groups, with LNM rates of 2.5%, 6.7%, and 22.7%, respectively(Table 3). The eCura scoring system was used to conduct internal verification on 905 patients with EGC without additional surgical treatment. The results showed that the 5-year tumor-specific survival rates of the low-, medium-, and high-risk groups were 99.6%, 96%, and 90.2%, respectively ($P < 0.01$)[40]. In a follow-up study, compared to the patients with additional surgery, Hatta *et al*[41] demonstrated that a higher risk of tumor recurrence (HR = 3.13, $P = 0.024$) and no significant difference in specific tumor-related mortality (reference range = 2.66, $P = 0.063$) in high-risk patients as per the eCura scoring system. This indicated that the additional radical surgery after ESD is of great significance to improve the prognosis of the high-risk group, while close follow-up is also a feasible option for low-risk group. In addition, Niwa *et al*[50] retrospectively analyzed 47 patients with EGC and found that the eCura scoring system was also applicable to the selection of additional surgery after the NCR of ESD. However, in clinical practice, patients with undifferentiated EGC often choose radical gastrectomy, and there is a selection bias. Therefore, the study did not recommend the use of eCura scoring system to evaluate risk level and formulate therapy in patients with undifferentiated EGC. Consequently, how to put forward a more accurate model to predict LNM and tumor recurrence to reduce unnecessary surgical trauma is still a research hotspot in the future.

Thus, there are both correlations and differences between eCura and the eCura scoring system[22,40]. eCura is mainly used for the curative evaluation of EGC patients undergoing endoscopic resection. As per the guidelines, undifferentiated carcinoma or carcinoma with a tumor diameter > 2 cm that invades the submucosa are classified as eCura C2. However, the eCura scoring system is mainly for eCura C2 patients with EGC to predict the risk of LNM, so the histological type is not included as an evaluation index (Table 4).

MANAGEMENT AFTER ADDITIONAL SURGERY IN PATIENTS WITH ENDOSCOPIC RESECTION

Whether to add surgery after NCR of EGC should be dependent on the risk of LNM. For patients with NCR, an accurate histopathological examination should be performed on the excised specimens, risk factors for LNM should be evaluated comprehensively, and the treatment strategies should be developed based on individual conditions. At present, radical surgery is still the main treatment for EGC patients with NCR. However, conventional surgery provides survival benefits for a small number of patients while it may impose additional surgical risks on some patients who do not have LNM. Therefore, there are still controversies about the choice of additional surgery after ESD. In recent years, although laparoscopic surgery has developed rapidly, it still lacks sufficient evidence-based medicine. With the application of the first case of laparoscopic radical gastrectomy in patients with EGC in 1991, it has shown great potential in terms of safety and curative effect[51], but whether it can achieve the same curative effect as traditional open surgery is still controversial. Therefore, the JCOG0912 and KLASS01 trials compared the short-term and long-term curative effects of laparoscopic radical gastrectomy and traditional open radical gastrectomy in the treatment of EGC. The results showed that compared with traditional open surgery, laparoscopic surgery had the same safety and radical curative effects for tumors, and recurrence rate and long-term survival rate were not significantly different. However, it had the advantages of less trauma, less bleeding, lower postoperative complication rate, and faster recovery[52,53]. Meanwhile, the CLASS02 trial from China also showed that the rate of overall morbidity and mortality (rate difference = -1.1%, 95%CI: -11.8% to 9.6%) and postoperative complication occurrence were not significantly different between the laparoscopic group and open group[54]. Therefore, these studies suggested that laparoscopic radical gastrectomy is a safe and feasible way to treat EGC.

Distal and proximal gastrectomy

For additional surgery after NCR, the range of gastric resection is not clearly specified in the guidelines, but the resection range of EGC can be referred to. According to the European Society for Medical Oncology guidelines, a distal gastrectomy should be performed if the proximal margin of resection is more than 5 cm from the tumor, otherwise a total gastrectomy should be considered[55]. According to the NCCN guidelines, adequate gastrectomy for T1b-T3 stage tumors is recommended to achieve

Table 3 eCura system for predicting lymph node metastasis rate of non-curative resection in early gastric cancer

Risk factor	Score	Risk grade	Total score[1]	Lymph node metastasis rate (%)
Tumor diameter > 3 cm	1	Low	0-1	2.5
Submucosal invasion depth > 500 μm	1	Medium	2-4	6.7
Lymphatic invasion positive	3			
Vascular invasion positive	1	High	5-7	22.5
Vertical incisal margin positive	1			

[1]Total score is the sum of the risk factor scores.

Table 4 Differences and correlations between eCura system and eCura in Japanese Gastric Cancer Association guidelines version 5 in early gastric cancer

	eCura system	eCura in JGCA guidelines version 5, 2018
Evaluation index	Predicting LNM	Curative resection criteria
Scope of application	Patients with EGC who do not meet the criteria of curative resection (eCura C2)	Patients with EGC who receive endoscopic resection
Category	Low risk; medium risk; high risk	eCura A; eCura B; eCura C1; eCura C2 (using eCura system predicted LNM rate)

JGCA: Japanese Gastric Cancer Association; LNM: Lymph node metastasis; EGC: Early gastric cancer.

a negative pathologic margin. Distal gastrectomy is preferred for distal gastric tumors, while both proximal and total gastrectomy are available for proximal gastric tumors [27]. Yamasaki *et al*[56] conducted a prospective multicenter controlled study on early upper stomach cancer, which confirmed that compared with total gastrectomy, patients with proximal gastrectomy had good safety and short-term and long-term efficacy. Therefore, the Japanese guidelines recommend that a safe margin of 2 cm should be ensured for T1 patients and preoperative endoscopic positioning should be performed for tumors with unclear boundaries. Distal gastrectomy should be performed for lower stomach cancer and pylorus-preserving gastrectomy (PPG) and proximal gastrectomy should be considered for tumors in the middle of the stomach (more than 4 cm from the pylorus) and upper stomach, respectively[22].

PPG

Function-preserving gastrectomy is performed to maximize the postoperative life quality of patients by ensuring the radical resection of the tumor. With the gradual improvement in people's requirements for quality of life, the treatment of EGC has gradually shifted from radical gastrectomy to function-preserving gastrectomy which includes PPG, laparoscopic-endoscopic combined partial gastrectomy, proximal gastrectomy, segmental gastrectomy, and local gastrectomy. Although segmental gastrectomy and local gastrectomy can theoretically achieve the effects of radical oncology, there is still a lack of high-quality research evidence, so it is not often used in clinical practice. The indications for PPG, which have been studied extensively in recent years, are mainly for EGC patients with cT1N0, tumor lesions located at the greater curvature of the gastric body, and the distance from the pylorus of more than 4 cm. The advantages of surgery are mainly reflected in the reduced incidence of dumping syndrome and bile reflux due to pyloric resection, as well as better food storage[57,58] (Figure 2). Studies have confirmed that the probability of suprapyloric LNM in T1 stage EGC in the middle of the stomach is only 0.2%. Therefore, lymph node dissection in the suprapyloric region can be omitted or only partially dissected to preserve the hepatic branch, the celiac branch, and the pyloric branch of the vagus nerve and the right gastric vessel, so as to seek a balance between the radical resection of the tumor and the function preservation as far as possible, and at the same time reduce the incidence of postoperative gastric emptying and improve the postoperative life quality of patients[59,60]. We also think that anastomotic methods might be associated with gastric emptying disorder occurrence after PPG, that is, manual suture

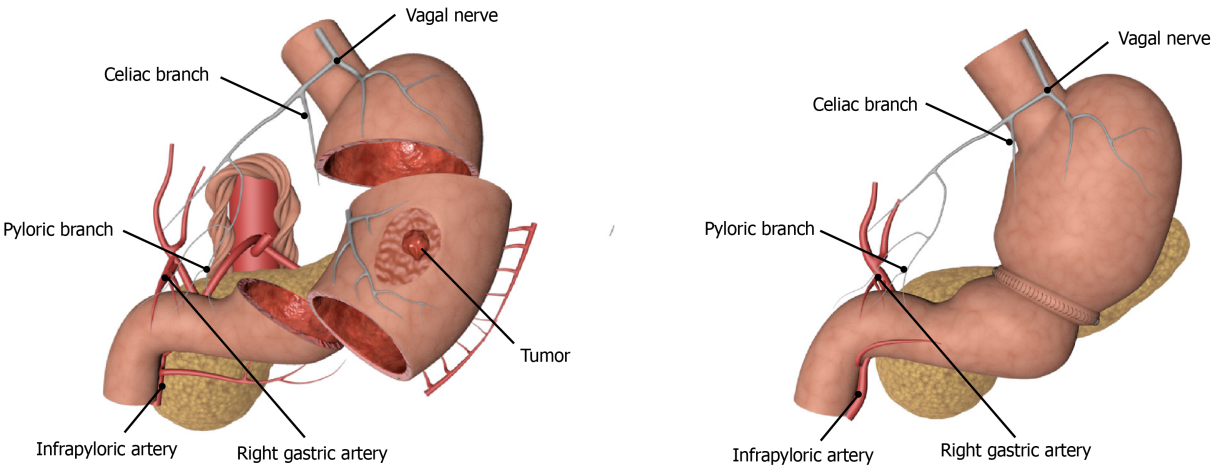

Figure 2 Indications for pylorus-preserving gastrectomy. For early gastric cancer patients with cT1N0, when the tumor lesion is located at the greater curvature of the gastric body and the distance from the pylorus is more than 4 cm, they are suitable for pylorus-preserving gastrectomy (PPG) surgery. The advantages of PPG include reduced incidence of dumping syndrome and bile reflux due to pyloric resection, as well as better food storage.

might be better than Stapler. However, we need to perform large-sample clinical trials to verify this in the future. In addition, another study conducted short- and long-term follow-ups of 2898 Japanese patients with EGC in the middle of the stomach who underwent either PPG or distal gastrectomy[61]. It was found that there were no statistically significant differences in mortality, incidence of postoperative complications, and 3-year and 5-year survival rates between the two groups[61]. Meanwhile, Tsujiura *et al*[62] evaluated the nutritional status of 465 patients undergoing PPG surgery and found that the serum total protein, albumin, and hemoglobin could be maintained at a good level, and the bodyweight ratio could be restored to 93.24% ± 7.29% one year after the surgery. Therefore, PPG can achieve the same therapeutic effects as distal gastrectomy for patients with T1N0 EGC in the middle of the stomach, but the grasp of indications, especially the accuracy of preoperative diagnosis, is an important factor affecting the therapeutic effect.

CONCLUSION

Diagnosis and treatment of EGC are the key to improving the prognosis of patients. Without affecting the radical effect of EGC, minimally invasive surgery can significantly improve the postoperative life quality of patients. For some patients with EGC, endoscopic resection is a safe and effective treatment. With the publication of JCOG1009/1010 results, the scope of indications for endoscopic therapy will be further expanded, and endoscopy will occupy an indispensable position in the treatment of EGC in the future. For patients with EGC who are not suitable for endoscopic resection or NCR, laparoscopic surgery is an appropriate treatment and may help achieve the same efficacy as traditional open surgery. Of course, though PPG preserves gastric functions and shows great potential in terms of patients' life quality and curative effects, clinicians still need to be cautious about whether it is suitable for a wide range of clinical applications. It requires strict technical standardization and large-scale, multicenter clinical trials to evaluate its safety and efficacy, hoping to provide a theoretical basis for function-preserving surgery.

REFERENCES

1 **Tanabe S**, Hirabayashi S, Oda I, Ono H, Nashimoto A, Isobe Y, Miyashiro I, Tsujitani S, Seto Y, Fukagawa T, Nunobe S, Furukawa H, Kodera Y, Kaminishi M, Katai H. Gastric cancer treated by endoscopic submucosal dissection or endoscopic mucosal resection in Japan from 2004 through 2006: JGCA nationwide registry conducted in 2013. *Gastric Cancer* 2017; **20**: 834-842 [PMID: 28205058 DOI: 10.1007/s10120-017-0699-4]

2 **Liu Q**, Ding L, Qiu X, Meng F. Updated evaluation of endoscopic submucosal dissection versus surgery for early gastric cancer: A systematic review and meta-analysis. *Int J Surg* 2020; **73**: 28-41

[PMID: 31783166 DOI: 10.1016/j.ijsu.2019.11.027]
3 **Kim ER**, Lee H, Min BH, Lee JH, Rhee PL, Kim JJ, Kim KM, Kim S. Effect of rescue surgery after non-curative endoscopic resection of early gastric cancer. *Br J Surg* 2015; **102**: 1394-1401 [PMID: 26313295 DOI: 10.1002/bjs.9873]
4 **Chang JW**, Jung DH, Park JC, Shin SK, Lee SK, Lee YC. Long-Term Outcomes and Prognostic Factors of Endoscopic Submucosal Dissection for Early Gastric Cancer in Patients Aged ≥75 Years. *Cancers (Basel)* 2020; **12** [PMID: 33142928 DOI: 10.3390/cancers12113222]
5 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]
6 **Hatta W**, Gotoda T, Koike T, Masamune A. History and future perspectives in Japanese guidelines for endoscopic resection of early gastric cancer. *Dig Endosc* 2020; **32**: 180-190 [PMID: 31529716 DOI: 10.1111/den.13531]
7 **Yang L**, Zheng R, Wang N, Yuan Y, Liu S, Li H, Zhang S, Zeng H, Chen W. Incidence and mortality of stomach cancer in China, 2014. *Chin J Cancer Res* 2018; **30**: 291-298 [PMID: 30046223 DOI: 10.21147/j.issn.1000-9604.2018.03.01]
8 **Katai H**, Ishikawa T, Akazawa K, Isobe Y, Miyashiro I, Oda I, Tsujitani S, Ono H, Tanabe S, Fukagawa T, Nunobe S, Kakeji Y, Nashimoto A; Registration Committee of the Japanese Gastric Cancer Association. Five-year survival analysis of surgically resected gastric cancer cases in Japan: a retrospective analysis of more than 100,000 patients from the nationwide registry of the Japanese Gastric Cancer Association (2001-2007). *Gastric Cancer* 2018; **21**: 144-154 [PMID: 28417260 DOI: 10.1007/s10120-017-0716-7]
9 **Hong S**, Lee YY, Lee J, Kim Y, Choi KS, Jun JK, Suh M. Trends in Cancer Screening Rates among Korean Men and Women: Results of the Korean National Cancer Screening Survey, 2004-2018. *Cancer Res Treat* 2021; **53**: 330-338 [PMID: 33091969 DOI: 10.4143/crt.2020.263]
10 **Information Committee of Korean Gastric Cancer Association**. Korean Gastric Cancer Association Nationwide Survey on Gastric Cancer in 2014. *J Gastric Cancer* 2016; **16**: 131-140 [PMID: 27752390 DOI: 10.5230/jgc.2016.16.3.131]
11 **Wang Y**, Li Z, Shan F, Miao R, Xue K, Gao C, Chen N, Gao X, Li S, Ji J. [Current status of diagnosis and treatment of early gastric cancer in China--Data from China Gastrointestinal Cancer Surgery Union]. *Zhonghua Wei Chang Wai Ke Za Zhi* 2018; **21**: 168-174 [PMID: 29492915]
12 **Wang F**, Meng W, Wang B, Qiao L. Helicobacter pylori-induced gastric inflammation and gastric cancer. *Cancer Lett* 2014; **345**: 196-202 [PMID: 23981572 DOI: 10.1016/j.canlet.2013.08.016]
13 **Choi IJ**, Kook MC, Kim YI, Cho SJ, Lee JY, Kim CG, Park B, Nam BH. Helicobacter pylori Therapy for the Prevention of Metachronous Gastric Cancer. *N Engl J Med* 2018; **378**: 1085-1095 [PMID: 29562147 DOI: 10.1056/NEJMoa1708423]
14 **Fang WL**, Chen MH, Huang KH, Lin CH, Chao Y, Lo SS, Li AF, Wu CW, Shyr YM. The Clinicopathological Features and Genetic Alterations in Epstein-Barr Virus-Associated Gastric Cancer Patients after Curative Surgery. *Cancers (Basel)* 2020; **12** [PMID: 32531970 DOI: 10.3390/cancers12061517]
15 **Cancer Genome Atlas Research Network**. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 2014; **513**: 202-209 [PMID: 25079317 DOI: 10.1038/nature13480]
16 **Kim ST**, Cristescu R, Bass AJ, Kim KM, Odegaard JI, Kim K, Liu XQ, Sher X, Jung H, Lee M, Lee S, Park SH, Park JO, Park YS, Lim HY, Lee H, Choi M, Talasaz A, Kang PS, Cheng J, Loboda A, Lee J, Kang WK. Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. *Nat Med* 2018; **24**: 1449-1458 [PMID: 30013197 DOI: 10.1038/s41591-018-0101-z]
17 **Smyth EC**, Nilsson M, Grabsch HI, van Grieken NC, Lordick F. Gastric cancer. *Lancet* 2020; **396**: 635-648 [PMID: 32861308 DOI: 10.1016/S0140-6736(20)31288-5]
18 **Ono H**, Yao K, Fujishiro M, Oda I, Nimura S, Yahagi N, Iishi H, Oka M, Ajioka Y, Ichinose M, Matsui T. Guidelines for endoscopic submucosal dissection and endoscopic mucosal resection for early gastric cancer. *Dig Endosc* 2016; **28**: 3-15 [PMID: 26234303 DOI: 10.1111/den.12518]
19 **Gotoda T**, Yanagisawa A, Sasako M, Ono H, Nakanishi Y, Shimoda T, Kato Y. Incidence of lymph node metastasis from early gastric cancer: estimation with a large number of cases at two large centers. *Gastric Cancer* 2000; **3**: 219-225 [PMID: 11984739 DOI: 10.1007/pl00011720]
20 **Japanese Gastric Cancer Association**. Japanese gastric cancer treatment guidelines 2014 (ver. 4). *Gastric Cancer* 2017; **20**: 1-19 [PMID: 27342689 DOI: 10.1007/s10120-016-0622-4]
21 **Hasuike N**, Ono H, Boku N, Mizusawa J, Takizawa K, Fukuda H, Oda I, Doyama H, Kaneko K, Hori S, Iishi H, Kurokawa Y, Muto M; Gastrointestinal Endoscopy Group of Japan Clinical Oncology Group (JCOG-GIESG). A non-randomized confirmatory trial of an expanded indication for endoscopic submucosal dissection for intestinal-type gastric cancer (cT1a): the Japan Clinical Oncology Group study (JCOG0607). *Gastric Cancer* 2018; **21**: 114-123 [PMID: 28224238 DOI: 10.1007/s10120-017-0704-y]
22 **Japanese Gastric Cancer Association**. Japanese gastric cancer treatment guidelines 2018 (5th edition). *Gastric Cancer* 2021; **24**: 1-21 [PMID: 32060757 DOI: 10.1007/s10120-020-01042-y]
23 **Guideline Committee of the Korean Gastric Cancer Association (KGCA)**; Development Working Group & Review Panel. . Korean Practice Guideline for Gastric Cancer 2018: an Evidence-based, Multi-disciplinary Approach. *J Gastric Cancer* 2019; **19**: 1-48 [PMID: 30944757 DOI: 10.5230/jgc.2019.19.e8]

24 **Takizawa K**, Ono H, Hasuike N, Takashima A, Minashi K, Boku N, Kushima R, Katayama H, Ogawa G, Fukuda H, Fujisaki J, Oda I, Yano T, Hori S, Doyama H, Hirasawa K, Yamamoto Y, Ishihara R, Tanabe S, Niwa Y, Nakagawa M, Terashima M, Muto M; Gastrointestinal Endoscopy Group (GIESG) and the Stomach Cancer Study Group (SCSG) of Japan Clinical Oncology Group. A nonrandomized, single-arm confirmatory trial of expanded endoscopic submucosal dissection indication for undifferentiated early gastric cancer: Japan Clinical Oncology Group study (JCOG1009/1010). *Gastric Cancer* 2021; **24**: 479-491 [PMID: 33161444 DOI: 10.1007/s10120-020-01134-9]

25 **Horiuchi Y**, Ida S, Yamamoto N, Nunobe S, Ishizuka N, Yoshimizu S, Ishiyama A, Yoshio T, Hirasawa T, Tsuchida T, Kumagai K, Ohashi M, Sano T, Fujisaki J. Feasibility of further expansion of the indications for endoscopic submucosal dissection in undifferentiated-type early gastric cancer. *Gastric Cancer* 2020; **23**: 285-292 [PMID: 31486980 DOI: 10.1007/s10120-019-01003-0]

26 **Pimentel-Nunes P**, Dinis-Ribeiro M, Ponchon T, Repici A, Vieth M, De Ceglie A, Amato A, Berr F, Bhandari P, Bialek A, Conio M, Haringsma J, Langner C, Meisner S, Messmann H, Morino M, Neuhaus H, Piessevaux H, Rugge M, Saunders BP, Robaszkiewicz M, Seewald S, Kashin S, Dumonceau JM, Hassan C, Deprez PH. Endoscopic submucosal dissection: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. *Endoscopy* 2015; **47**: 829-854 [PMID: 26317585 DOI: 10.1055/s-0034-1392882]

27 **Ajani JA**, D'Amico TA, Almhanna K, Bentrem DJ, Chao J, Das P, Denlinger CS, Fanta P, Farjah F, Fuchs CS, Gerdes H, Gibson M, Glasgow RE, Hayman JA, Hochwald S, Hofstetter WL, Ilson DH, Jaroszewski D, Johung KL, Keswani RN, Kleinberg LR, Korn WM, Leong S, Linn C, Lockhart AC, Ly QP, Mulcahy MF, Orringer MB, Perry KA, Poultsides GA, Scott WJ, Strong VE, Washington MK, Weksler B, Willett CG, Wright CD, Zelman D, McMillian N, Sundar H. Gastric Cancer, Version 3.2016, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 2016; **14**: 1286-1312 [PMID: 27697982 DOI: 10.6004/jnccn.2016.0137]

28 **Qiu H**, Zhou Z. [Updates and interpretation on NCCN clinical practice guidelines for gastric cancer 2017 version 5]. *Zhonghua Wei Chang Wai Ke Za Zhi* 2018; **21**: 160-164 [PMID: 29492914]

29 **Zheng Z**, Yin J, Li Z, Ye Y, Wei B, Wang X, Tian Y, Li M, Zhang Q, Zeng N, Xu R, Chen G, Zhang J, Li P, Cai J, Yao H, Zhang Z, Zhang S. Protocol for expanded indications of endoscopic submucosal dissection for early gastric cancer in China: a multicenter, ambispective, observational, open-cohort study. *BMC Cancer* 2020; **20**: 801 [PMID: 32831061 DOI: 10.1186/s12885-020-07312-3]

30 **Sohn SH**, Lee SH, Kim KO, Jang BI, Kim TN. Therapeutic outcomes of endoscopic submucosal dissection for early gastric cancer: single-center study. *Eur J Gastroenterol Hepatol* 2017; **29**: 61-67 [PMID: 27508325 DOI: 10.1097/MEG.0000000000000718]

31 **Peng LJ**, Tian SN, Lu L, Chen H, Ouyang YY, Wu YJ. Outcome of endoscopic submucosal dissection for early gastric cancer of conventional and expanded indications: systematic review and meta-analysis. *J Dig Dis* 2015; **16**: 67-74 [PMID: 25421172 DOI: 10.1111/1751-2980.12217]

32 **Lee S**, Choi KD, Han M, Na HK, Ahn JY, Jung KW, Lee JH, Kim DH, Song HJ, Lee GH, Yook JH, Kim BS, Jung HY. Long-term outcomes of endoscopic submucosal dissection versus surgery in early gastric cancer meeting expanded indication including undifferentiated-type tumors: a criteria-based analysis. *Gastric Cancer* 2018; **21**: 490-499 [PMID: 29052052 DOI: 10.1007/s10120-017-0772-z]

33 **Abdelfatah MM**, Barakat M, Lee H, Kim JJ, Uedo N, Grimm I, Othman MO. The incidence of lymph node metastasis in early gastric cancer according to the expanded criteria in comparison with the absolute criteria of the Japanese Gastric Cancer Association: a systematic review of the literature and meta-analysis. *Gastrointest Endosc* 2018; **87**: 338-347 [PMID: 28966062 DOI: 10.1016/j.gie.2017.09.025]

34 **Cho KB**, Jeon WJ, Kim JJ. Worldwide experiences of endoscopic submucosal dissection: not just Eastern acrobatics. *World J Gastroenterol* 2011; **17**: 2611-2617 [PMID: 21677828 DOI: 10.3748/wjg.v17.i21.2611]

35 **Min BH**, Kim ER, Kim KM, Park CK, Lee JH, Rhee PL, Kim JJ. Surveillance strategy based on the incidence and patterns of recurrence after curative endoscopic submucosal dissection for early gastric cancer. *Endoscopy* 2015; **47**: 784-793 [PMID: 26111362 DOI: 10.1055/s-0034-1392249]

36 **Abe S**, Oda I, Suzuki H, Nonaka S, Yoshinaga S, Nakajima T, Sekiguchi M, Mori G, Taniguchi H, Sekine S, Katai H, Saito Y. Long-term surveillance and treatment outcomes of metachronous gastric cancer occurring after curative endoscopic submucosal dissection. *Endoscopy* 2015; **47**: 1113-1118 [PMID: 26165734 DOI: 10.1055/s-0034-1392484]

37 **Jang MY**, Cho JW, Oh WG, Ko SJ, Han SH, Baek HK, Lee YJ, Kim JW, Jung GM, Cho YK. Clinicopathological characteristics of synchronous and metachronous gastric neoplasms after endoscopic submucosal dissection. *Korean J Intern Med* 2013; **28**: 687-693 [PMID: 24307844 DOI: 10.3904/kjim.2013.28.6.687]

38 **Sun K**, Chen S, Ye J, Wu H, Peng J, He Y, Xu J. Endoscopic resection versus surgery for early gastric cancer: a systematic review and meta-analysis. *Dig Endosc* 2016; **28**: 513-525 [PMID: 26701862 DOI: 10.1111/den.12596]

39 **Jeon MY**, Park JC, Hahn KY, Shin SK, Lee SK, Lee YC. Long-term outcomes after noncurative endoscopic resection of early gastric cancer: the optimal time for additional endoscopic treatment. *Gastrointest Endosc* 2018; **87**: 1003-1013. e2 [PMID: 29031882 DOI: 10.1016/j.gie.2017.10.004]

40 **Hatta W**, Gotoda T, Oyama T, Kawata N, Takahashi A, Yoshifuku Y, Hoteya S, Nakagawa M, Hirano M, Esaki M, Matsuda M, Ohnita K, Yamanouchi K, Yoshida M, Dohi O, Takada J, Tanaka K, Yamada S, Tsuji T, Ito H, Hayashi Y, Nakaya N, Nakamura T, Shimosegawa T. A Scoring System to

Stratify Curability after Endoscopic Submucosal Dissection for Early Gastric Cancer: "eCura system". *Am J Gastroenterol* 2017; **112**: 874-881 [PMID: 28397873 DOI: 10.1038/ajg.2017.95]

41 **Hatta W**, Gotoda T, Oyama T, Kawata N, Takahashi A, Yoshifuku Y, Hoteya S, Nakamura K, Hirano M, Esaki M, Matsuda M, Ohnita K, Shimoda R, Yoshida M, Dohi O, Takada J, Tanaka K, Yamada S, Tsuji T, Ito H, Hayashi Y, Nakamura T, Shimosegawa T. Is radical surgery necessary in all patients who do not meet the curative criteria for endoscopic submucosal dissection in early gastric cancer? *J Gastroenterol* 2017; **52**: 175-184 [PMID: 27098174 DOI: 10.1007/s00535-016-1210-4]

42 **Park JW**, Ahn S, Lee H, Min BH, Lee JH, Rhee PL, Kim KM, Kim JJ. Predictive factors for lymph node metastasis in early gastric cancer with lymphatic invasion after endoscopic resection. *Surg Endosc* 2017; **31**: 4419-4424 [PMID: 28378075 DOI: 10.1007/s00464-017-5490-4]

43 **Sekiguchi M**, Suzuki H, Oda I, Abe S, Nonaka S, Yoshinaga S, Taniguchi H, Sekine S, Kushima R, Saito Y. Risk of recurrent gastric cancer after endoscopic resection with a positive lateral margin. *Endoscopy* 2014; **46**: 273-278 [PMID: 24505020 DOI: 10.1055/s-0034-1364938]

44 **Horiki N**, Omata F, Uemura M, Suzuki S, Ishii N, Fukuda K, Fujita Y, Ninomiya K, Tano S, Katurahara M, Tanaka K, Gabazza EC, Takei Y. Risk for local recurrence of early gastric cancer treated with piecemeal endoscopic mucosal resection during a 10-year follow-up period. *Surg Endosc* 2012; **26**: 72-78 [PMID: 21792719 DOI: 10.1007/s00464-011-1830-y]

45 **Suzuki S**, Gotoda T, Hatta W, Oyama T, Kawata N, Takahashi A, Yoshifuku Y, Hoteya S, Nakagawa M, Hirano M, Esaki M, Matsuda M, Ohnita K, Yamanouchi K, Yoshida M, Dohi O, Takada J, Tanaka K, Yamada S, Tsuji T, Ito H, Hayashi Y, Shimosegawa T. Survival Benefit of Additional Surgery After Non-curative Endoscopic Submucosal Dissection for Early Gastric Cancer: A Propensity Score Matching Analysis. *Ann Surg Oncol* 2017; **24**: 3353-3360 [PMID: 28795364 DOI: 10.1245/s10434-017-6039-4]

46 **Suzuki H**, Oda I, Abe S, Sekiguchi M, Nonaka S, Yoshinaga S, Saito Y, Fukagawa T, Katai H. Clinical outcomes of early gastric cancer patients after noncurative endoscopic submucosal dissection in a large consecutive patient series. *Gastric Cancer* 2017; **20**: 679-689 [PMID: 27722825 DOI: 10.1007/s10120-016-0651-z]

47 **Kawata N**, Kakushima N, Takizawa K, Tanaka M, Makuuchi R, Tokunaga M, Tanizawa Y, Bando E, Kawamura T, Sugino T, Kusafuka K, Shimoda T, Nakajima T, Terashima M, Ono H. Risk factors for lymph node metastasis and long-term outcomes of patients with early gastric cancer after non-curative endoscopic submucosal dissection. *Surg Endosc* 2017; **31**: 1607-1616 [PMID: 27495338 DOI: 10.1007/s00464-016-5148-7]

48 **Kikuchi S**, Kuroda S, Nishizaki M, Kagawa T, Kanzaki H, Kawahara Y, Kagawa S, Tanaka T, Okada H, Fujiwara T. Management of early gastric cancer that meet the indication for radical lymph node dissection following endoscopic resection: a retrospective cohort analysis. *BMC Surg* 2017; **17**: 72 [PMID: 28637436 DOI: 10.1186/s12893-017-0268-0]

49 **Kang HJ**, Chung H, Kim SG, Kim J, Kim JL, Lee E, Jung HC. Synergistic Effect of Lymphatic Invasion and Venous Invasion on the Risk of Lymph Node Metastasis in Patients with Non-Curative Endoscopic Resection of Early Gastric Cancer. *J Gastrointest Surg* 2020; **24**: 1499-1509 [PMID: 31313145 DOI: 10.1007/s11605-019-04302-0]

50 **Niwa H**, Ozawa R, Kurahashi Y, Kumamoto T, Nakanishi Y, Okumura K, Matsuda I, Ishida Y, Hirota S, Shinohara H. The eCura system as a novel indicator for the necessity of salvage surgery after non-curative ESD for gastric cancer: A case-control study. *PLoS One* 2018; **13**: e0204039 [PMID: 30273388 DOI: 10.1371/journal.pone.0204039]

51 **Kitano S**, Iso Y, Moriyama M, Sugimachi K. Laparoscopy-assisted Billroth I gastrectomy. *Surg Laparosc Endosc* 1994; **4**: 146-148 [PMID: 8180768 DOI: 10.1007/BF00590967]

52 **Katai H**, Mizusawa J, Katayama H, Takagi M, Yoshikawa T, Fukagawa T, Terashima M, Misawa K, Teshima S, Koeda K, Nunobe S, Fukushima N, Yasuda T, Asao Y, Fujiwara Y, Sasako M. Short-term surgical outcomes from a phase III study of laparoscopy-assisted versus open distal gastrectomy with nodal dissection for clinical stage IA/IB gastric cancer: Japan Clinical Oncology Group Study JCOG0912. *Gastric Cancer* 2017; **20**: 699-708 [PMID: 27718137 DOI: 10.1007/s10120-016-0646-9]

53 **Kim HH**, Han SU, Kim MC, Hyung WJ, Kim W, Lee HJ, Ryu SW, Cho GS, Kim CY, Yang HK, Park DJ, Song KY, Lee SI, Ryu SY, Lee JH; Korean Laparoscopic Gastrointestinal Surgery Study (KLASS) Group. Prospective randomized controlled trial (phase III) to comparing laparoscopic distal gastrectomy with open distal gastrectomy for gastric adenocarcinoma (KLASS 01). *J Korean Surg Soc* 2013; **84**: 123-130 [PMID: 23396494 DOI: 10.4174/jkss.2013.84.2.123]

54 **Liu F**, Huang C, Xu Z, Su X, Zhao G, Ye J, Du X, Huang H, Hu J, Li G, Yu P, Li Y, Suo J, Zhao N, Zhang W, Li H, He H, Sun Y; Chinese Laparoscopic Gastrointestinal Surgery Study (CLASS) Group. Morbidity and Mortality of Laparoscopic vs Open Total Gastrectomy for Clinical Stage I Gastric Cancer: The CLASS02 Multicenter Randomized Clinical Trial. *JAMA Oncol* 2020; **6**: 1590-1597 [PMID: 32815991 DOI: 10.1001/jamaoncol.2020.3152]

55 **Smyth EC**, Verheij M, Allum W, Cunningham D, Cervantes A, Arnold D; ESMO Guidelines Committee. Gastric cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2016; **27**: v38-v49 [PMID: 27664260 DOI: 10.1093/annonc/mdw350]

56 **Yamasaki M**, Takiguchi S, Omori T, Hirao M, Imamura H, Fujitani K, Tamura S, Akamaru Y, Kishi K, Fujita J, Hirao T, Demura K, Matsuyama J, Takeno A, Ebisui C, Takachi K, Takayama O, Fukunaga H, Okada K, Adachi S, Fukuda S, Matsuura N, Saito T, Takahashi T, Kurokawa Y, Yano M, Eguchi H, Doki Y. Multicenter prospective trial of total gastrectomy versus proximal gastrectomy for upper third cT1 gastric cancer. *Gastric Cancer* 2021; **24**: 535-543 [PMID: 33118118 DOI:

10.1007/s10120-020-01129-6]
57 **Oh SY**, Lee HJ, Yang HK. Pylorus-Preserving Gastrectomy for Gastric Cancer. *J Gastric Cancer* 2016; **16**: 63-71 [PMID: 27433390 DOI: 10.5230/jgc.2016.16.2.63]
58 **Eom BW**, Park B, Yoon HM, Ryu KW, Kim YW. Laparoscopy-assisted pylorus-preserving gastrectomy for early gastric cancer: A retrospective study of long-term functional outcomes and quality of life. *World J Gastroenterol* 2019; **25**: 5494-5504 [PMID: 31576095 DOI: 10.3748/wjg.v25.i36.5494]
59 **Huang C**, Yu F, Zhao G, Xia X. Postoperative quality of life after laparoscopy-assisted pylorus-preserving gastrectomy compared with laparoscopy-assisted distal gastrectomy for early gastric cancer. *J Gastroenterol Hepatol* 2020; **35**: 1712-1719 [PMID: 31945189 DOI: 10.1111/jgh.14985]
60 **Isozaki H**, Okajima K, Momura E, Ichinona T, Fujii K, Izumi N, Takeda Y. Postoperative evaluation of pylorus-preserving gastrectomy for early gastric cancer. *Br J Surg* 1996; **83**: 266-269 [PMID: 8689185 DOI: 10.1046/j.1365-2168.1996.02093.x]
61 **Aizawa M**, Honda M, Hiki N, Kinoshita T, Yabusaki H, Nunobe S, Shibasaki H, Matsuki A, Watanabe M, Abe T. Oncological outcomes of function-preserving gastrectomy for early gastric cancer: a multicenter propensity score matched cohort analysis comparing pylorus-preserving gastrectomy versus conventional distal gastrectomy. *Gastric Cancer* 2017; **20**: 709-717 [PMID: 27672061 DOI: 10.1007/s10120-016-0644-y]
62 **Tsujiura M**, Hiki N, Ohashi M, Nunobe S, Kumagai K, Ida S, Hayami M, Sano T, Yamaguchi T. Excellent Long-Term Prognosis and Favorable Postoperative Nutritional Status After Laparoscopic Pylorus-Preserving Gastrectomy. *Ann Surg Oncol* 2017; **24**: 2233-2240 [PMID: 28280944 DOI: 10.1245/s10434-017-5828-0]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2021 June 15; 13(6): 574-588

DOI: 10.4251/wjgo.v13.i6.574

ISSN 1948-5204 (online)

MINIREVIEWS

Poly adenosine diphosphate-ribosylation, a promising target for colorectal cancer treatment

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Author contributions: Jeong KY collected references and designed the contents; Jeong KY and Park MH wrote the manuscript; all authors have read and approved the final manuscript.

Conflict-of-interest statement: We have no conflict of interest to declare.

Manuscript source: Invited manuscript

Specialty type: Oncology

Country/Territory of origin: South Korea

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Abstract

The development of colorectal cancer (CRC) can result from changes in a variety of cellular systems within the tumor microenvironment. Particularly, it is primarily associated with genomic instability that is the gradual accumulation of genetic and epigenetic changes consisting of a characteristic set of mutations crucial for pathways in CRC progression. Based on this background, the potential to focus on poly [adenosine diphosphate (ADP)-ribose] polymerase (PARP)-1 and poly-ADP ribosylation (PARylation) as the main causes of malignant formation of CRC may be considered. One of the important functions of PARP-1 and PARylation is its deoxyribonucleic acid (DNA) repair function, which plays a pivotal role in the DNA damage response and prevention of DNA damage maintaining the redox homeostasis involved in the regulation of oxidation and superoxide. PARP-1 and PARylation can also alter epigenetic markers and chromatin structure involved in transcriptional regulation for the oncogenes or tumor suppressor genes by remodeling histone and chromatin enzymes. Given the high importance of these processes in CRC, it can be considered that PARP-1 and PARylation are at the forefront of the pathological changes required for CRC progression. Therefore, this review addresses the current molecular biological features for understanding the multifactorial function of PARP-1 and PARylation in CRC related to the aforementioned roles; furthermore, it presents a summary of recent approaches with PARP-1 inhibition in non-clinical and clinical studies targeting CRC. This understanding could help embrace the importance of targeting PARP-1 and PARylation in the treatment of CRC, which may present the potential to identify various research topics that can be challenged both non-clinically and clinically.

Key Words: Colorectal cancer; Poly adenosine diphosphate-ribose polymerase-1; Poly adenosine diphosphate-ribose; Poly-adenosine diphosphate ribosylation

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: February 25, 2021
Peer-review started: February 25, 2021
First decision: April 19, 2021
Revised: April 22, 2021
Accepted: May 8, 2021
Article in press: May 8, 2021
Published online: June 15, 2021

P-Reviewer: Zhu Y
S-Editor: Zhang L
L-Editor: A
P-Editor: Li JH

Core Tip: The main focus is on highlighting the pivotal role of poly adenosine diphosphate-ribose polymerase-1 (PARP-1) and poly-adenosine diphosphate ribosylation (PARylation) in regulating deoxyribonucleic acid damage response, redox homeostasis, chromosomal instability, and transcriptional activity under the common denominator of overcoming the genomic instability in colorectal cancer (CRC). The importance of targeting PARP-1 and PARylation in the treatment of CRC will be emphasized because the level of understanding of pathological changes leading to malignant transformation of CRC by PARP-1 and PARylation may increase.

Citation: Jeong KY, Park M. Poly adenosine diphosphate-ribosylation, a promising target for colorectal cancer treatment. *World J Gastrointest Oncol* 2021; 13(6): 574-588
URL: https://www.wjgnet.com/1948-5204/full/v13/i6/574.htm
DOI: https://dx.doi.org/10.4251/wjgo.v13.i6.574

INTRODUCTION

Colorectal cancer (CRC) is a type of cancer that begins with a malignant transformation in the colon or rectum and usually begins as benign clumps of cells called polyps from the lining of the large intestine[1]. CRC proceeds gradually through three connected stages. The first is the initiation of altering the molecular signals of normal cells that are still classified as precancerous. The next step is the promotion in which an increase in abnormal signaling is induced. The final step is progression wherein the phenotypes change, transformed cells are discovered, and a CRC can be diagnosed[2]. Then, changes in various cellular systems within the tumor microenvironment can make it possible to lead a favorable direction for adaptation even with excessive cancer cell growth[1,2]. Recent studies reporting on the details of this malignant transformation have revealed that the following molecular biological and genetic changes play an important role in the development and progression of CRC[3]. The formation of CRC is predominantly associated with genomic instability caused by the gradual accumulation of genetic and epigenetic changes leading to a transformation of normal colon epithelium into colon adenocarcinoma[3,4]. The phenomena representing genomic instability, such as chromosomal and microsatellite instability, have been studied, and they are reportedly associated with defects in mitosis, telomere stability, and the deoxyribonucleic acid (DNA) damage response. Thus, allowing for the accumulation of a characteristic set of mutations crucial for activating critical pathways in CRC development[4,5]. Reactive oxygen species (ROS) are continuously produced in aerobic organisms both endogenously and through involvement in various physiological and pathological processes in the cancer cells[6]. The oxidative stress caused by ROS may play an important role in regulating genetic alterations, and mutations in genetic material can contribute to CRC cell growth, survival, and metastasis[7]. Advances in molecular biology over the past few years have increased our knowledge of the oncogenic mechanisms involved in CRC development, and oncogenes have been shown to have a major role in cancer cell proliferation, angiogenesis, and metastasis[8]. These oncogenic activities can also form an interconnected network that includes the phosphorylation of proteins related to carcinogenic transcription factors, thus leading to malignant transformation of CRC[4,5,9]. Mutations in the tumor suppressor gene, which counteracts this action, also markedly contribute to the sustained survival of CRC[4].

In recent studies, checkpoint inhibitors were expected to be potential treatments for CRC patients with high genomic instability[10]. However, the proportion of patients to whom they could be applied was low, thus showing poor overall prognosis and limited treatment options; this is particularly exemplified by deficient mismatch repair associated with rat sarcoma viral oncogene homolog (RAS) mutations[11]. Thus, finding alternative and effective treatments for patients with CRC is an urgent unmet clinical need, and the roles of various proteins that occupy a key position in genomic alterations to DNA damage responses are emerging as a new target for CRC treatment. Recent genome-wide studies have identified distinct subpopulations of CRC that possess unstable genomic properties due to mutations in their DNA repair genes[4]. Although the major mechanistic role of these mutations on DNA damage response genes in CRC has not yet been elucidated and data on clinical effects are reportedly

insufficient, widespread recognition of the clinical need for targeting DNA damage responses offered a great opportunity to arouse interest in poly [adenosine diphosphate (ADP)-ribose] polymerase (PARP)-1[12]. PARP-1 is a nuclear enzyme of cellular homeostasis that modifies nuclear proteins by poly ADP-ribosylation (PARylation), and one of its important functions is to induce a response to DNA damage[12,13]. The pivotal role of PARP-1 in regulating the DNA repair process has led to clinical investigations that potentially target this important enzyme in ovarian and pancreatic cancer patients with breast cancer susceptibility gene (BRCA) mutations[14]. However, finding the optimal biomarkers that can be used to explore their applicability for CRC remains a challenging task. In addition to the representative functions of PARP-1 involved in overcoming genetic instability, cancer-specific phenomena elicited for CRC survival can be interestingly explained by the molecular biological processes in which PARP-1 participates[14,15]. Because PARP-1 and PARylation are known to have a wide range of essential functions for cellular homeostasis, the following roles are also attracting attention toward the tumor microenvironment[13,15]. PARP-1 can regulate mitochondrial activity by occupying a prominent position characterized by the regulation of mitochondrial peroxide and oxidation[16]. Further, PARP-1 may directly participate as a transcription regulator being a member of the transcription family, and PARylation can regulate gene expression or protein activation by remodeling histone and chromatin enzymes through direct and indirect pathways (Figure 1)[17,18]. In other words, the widespread functions essential for cellular homeostasis in the tumor microenvironment that PARP-1 and PARylation can lead to malignant transformation of CRC, and these are more likely to adapt better even under inferior conditions unfavorable for survival[13-18].

Therefore, this review summarizes the current knowledge on the molecular biological and biochemical process of PARylation to understand the multifactorial functions of PARP-1 that enable the proliferation and survival of CRC. Further, cases from clinical studies involving patients with CRC targeting PARP-1 were listed, and treatment outcomes are also discussed.

PARP-1 AND PARYLATION

PARP-1 is a member of the recently well-studied PARP family and forms a domain containing approximately 106 molecules[19,20]. PARP-1 catalyzes the polymerization of ADP-ribose from the donor nicotinamide adenine dinucleotide (NAD+) on the target protein to form a linear or branched poly ADP-ribose (PAR) polymer through a biochemical action called PARylation[21]. PARP-1 forms a structure in which the N-terminal double zinc finger DNA binding domain, nuclear localization signal, central auto-transformation domain, and C-terminal catalytic domain are well conserved[22]. The functional aspect is characterized by having a composition advantageous for interaction with other molecules, particularly with DNA structures. The N-terminal DNA-binding domain has three zinc fingers and a specific sequence for localization in the nucleus, and two homologous zinc finger proteins are characterized by the zinc finger motif[23]. Auto-modifying domains include the BRCA1 C-terminal motif and are involved in the interaction with intracellular proteins or nuclear proteins or both. The C-terminal catalytic domain comprises six β-strands and one α-helical motif that functionally binds to NAD+[19,23]. The PARP signature (NAD+ binding site) motif is composed of an acceptor for adenosine and donor of nicotinamide wherein ADP ribose from NAD+ are transferred to target proteins to PAR synthesis (Figure 2)[23,24].

PARP-1 is a princeps enzyme that can mediate PAR synthesis and attach it to acceptor proteins[21]. Various molecular and biological functions essential for cancer cell survival are associated with PARylation, and more than 90% of PARylation depends on the regulatory function of PARP-1[13]. PARylation proceeds according to an integrated and dynamic biochemical process, and the hypothesis that the synthetic method is determined by two potential pathways has recently been established[13,22]. PARP-1 catalyzes the transfer of ADP-ribose units from NAD+ to form a branched-chain, PAR, which is negatively charged to specific amino acid residues, such as aspartate, arginine, serine, lysine, and glutamate, on PARP-1 itself and other acceptor proteins[13,22]. The PAR synthesis is based on the attaching of ADP-ribose to the 2'-OH end of the growing chain at the terminus adjacent to the PARylation target, depending on the reaction mechanism of PARP-1[13,21,22]. It may appear as if it is self-applicable only to the auto-modification of PARP-1; however, at certain stages of the extension reaction, reactive intermediates during PARylation may also be transferred to other acceptor molecules in their vicinity[13,21,22]. Besides, PAR may be

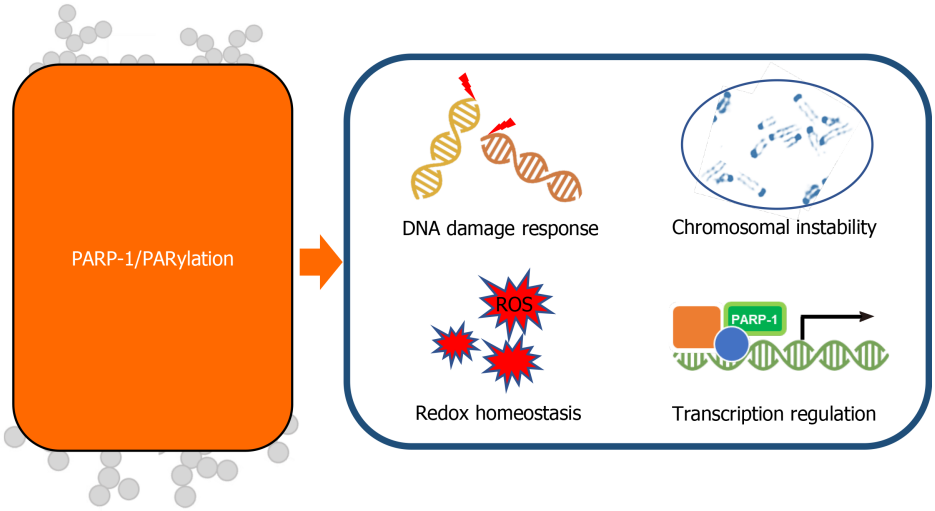

Figure 1 Multifactorial role of poly adenosine diphosphate-ribose polymerase-1 and poly-adenosine diphosphate ribosylation in cancer development. Poly adenosine diphosphate-ribose polymerase-1 (PARP-1) can occupy a position as an important regulator of deoxyribonucleic acid damage response, redox homeostasis, chromosomal instability, and transcription, which are required for the dysfunctional regulation for a crucial role in tumorigenesis. Therefore, a crucial process for malignant transformation of colorectal cancer can be attributed to the involvement of PARP-1 and Poly-adenosine diphosphate ribosylation. PARP-1: Poly adenosine diphosphate-ribose polymerase-1.

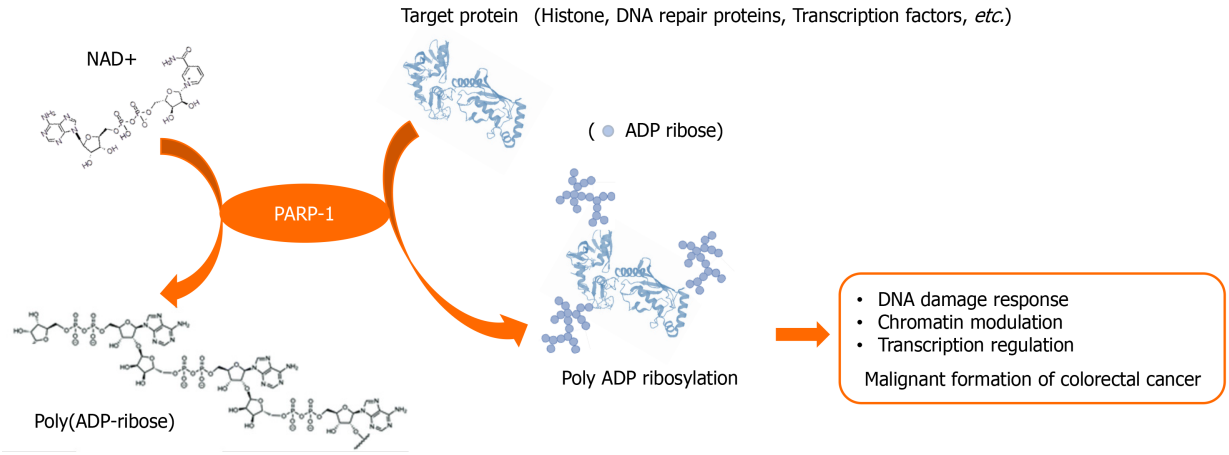

Figure 2 Poly adenosine diphosphate-ribosylation in cancer. Poly adenosine diphosphate-ribose polymerase-1 (PARP-1) branched poly adenosine diphosphate (ADP)-ribose polymers following the cleavage of nicotinamide adenine dinucleotide+ to ADP-ribose. PARP-1 enables interactions by catalyzing the covalent attachment of poly ADP-ribose polymers on acceptor proteins, such as histones, deoxyribonucleic acid repair proteins, transcription factors, and chromatin modulators. This enzymology reaction is known as poly ADP-ribosylation on target proteins, and this process may be important for the malignant transformation of colorectal cancer. ADP: Adenosine diphosphate; PARP-1: Poly adenosine diphosphate-ribose polymerase-1.

synthesized by sequentially adding the following ADP-ribose residues to the 2'-OH end of the ADP-ribose moiety[21]. Particularly because the substrate properties for PARP-1 are reduced in the extension reaction, the NAD+ analog is an ideal modification to modify most PAR acceptor sites by short ADP-ribose oligomers[21,25]. The ability of PARP-1 to link long, negatively charged PAR polymers to a variety of acceptor proteins by PARylation suggests that their role as a modulator in favor of survival signals in cancer cells may accompany potential molecular biological and biochemical changes[20,26]. The PAR binding on acceptor proteins can form deterministic structures through intramolecular interactions; these structures may have non-covalent, attractive interactions with other molecules[21]. Thus, PARylation can modulate protein activity by functioning as a site-specific covalent modification, protein binding matrix, or steric block[26,27]. Recent studies have increased our understanding of the role of PARylation in various molecular and cellular processes, including DNA damage response, chromatin modification, and transcription regulation[15,20]. It has also been demonstrated that the molecular and cellular aspects of PARylation can play a potential role in many pathophysiological outcomes,

including carcinogenesis and overcoming genomic instability[25]. In particular, carcinogenesis is a multi-step process involving abnormalities such as genomic maintenance, cell cycle regulation, proliferation, and differentiation and is closely related to initiation, promotion, and progression of cancer followed by all subsequent processes leading to advanced stages involving metastasis[2]. Since PARP-1 and PARylation have been investigated as promising regulators of all these processes, it may be considered a major target for the inhibition of malignant transformation[16-20]. Therefore, it is essential to understand the multifactorial role of PARP-1 wand PARylation in the broader framework of CRC development.

MULTIFACTORIAL ROLE OF PARP-1 AND PARYLATION IN MALIGNANT TRANSFORMATION OF CRC

DNA damage response and defense mechanisms

DNA damage refers to a single or double-strand break resulting from physical or chemical changes to DNA that can affect the interpretation and transmission of genetic information[28]. This leads to an unbeneficial environment for normal cell survival. However, DNA damage is well recognized as a critical factor in cancer development and progression[29]. A reason for endogenous DNA damage in CRC is the induction of replication stress by oncogenes[30,31]. Mutations or overexpression of proto-oncogenes can transform them into oncogenes that induce sustained cell growth and carcinogenesis[32]. The oncogenic cell cycle is usually associated with the induction of replication stress, which is also defined as irregular replication fork progression and DNA synthesis[31]. Another cause of endogenous DNA damage in CRC is an increase in ROS[7]. ROS is derived from the incomplete reduction of oxygen, a by-product of energy metabolism. And it can affect cellular function by reacting with biomolecules, including nucleic acids and proteins[6]. Consequently, damage to the nucleotide sequence causes aneuploidy by inducing DNA strand breaks and genomic instability, which is a critical contributor to induce colon carcinogenesis following gene mutations [33]. The aforementioned process supports the hypothesis that it may be related to the development and progression of CRC; however, unregulated replication stress and/or increased oxidative stress focused on sustained ROS production can have a devastating effect on the survival of cancer cells[7,31,33]. Therefore, it is necessary to activate the protective mechanism constantly.

CRC can exert a function characterized by initiating various reactions to protect the genome in response to DNA damage and ensuring cancer cell survival[4,33]. The DNA damage response pathway in CRC is characterized by a complex network of multiple effectors that promote DNA replication and cell proliferation, and genomic alterations to the DNA damage response pathway may appear[4,12]. Under such a tumor microenvironment, the roles of PARP-1 and PARylation on DNA damage responses can be considered as important factors in overcoming genomic instability[14,15]. PARP-1 functionally interacts with the DNA single-strand break (SSB) repair factor named X-ray repair cross-complementing protein 1 (XRCC1) which plays an important role in the SSB repair signaling pathway, thus facilitating the recruitment and assembly of the SSB repair machinery[17,34]. Recent studies have shown that PARylation is induced directly on the BRCA1 C-terminal domain of XRCC1 and mediates the early recruitment of XRCC1 targeting DNA lesions[35]. To deal with a double-strand break, CRC can employ DNA repair mechanisms including non-homologous end joining (NHEJ) and homologous recombination (HR)[36,37]. However, cancers that arise from BRCA1 germline mutations are deficient in HR DNA repair and are vulnerable to DNA damage[38]. If DNA lesions are detected in BRCA1-mutated cancers, PARP-1 and PARylation may play a pioneering role in constructing a platform for recruiting NHEJ repair factors, such as DNA-dependent protein kinases [37]. Further, CRC cells can activate the function of an antioxidant program to protect the cells from irreversible oxidative damage by excessive ROS accumulation [39]. The antioxidant program can be driven by defense through enzymatic antioxidants, including the detoxification of secondary metabolites and the direct removal of the electrophiles themselves[40]. Of course, PARP-1 and PARylation remove the negative aspects of oxidative stress and exert their key roles in areas of positive utilization related to cancer cell growth or oncogene expression[15,41]. Antioxidant enzymes are dependent on the activation of the transcriptional action of nuclear factor erythroid-related factor 2 (NRF2), a basic leucine zipper protein, and NRF2 is involved in maintaining intracellular homeostasis in response to physiological changes between intracellular redox actions[42]. The dissociation of NRF2 and Kelch-like ECH-related

protein 1 is promoted as the production of intracellular ROS increases to levels that threaten the survival of CRC cells[43]. It can enhance a wide range of downstream cellular defense processes regulated by NRF2, such as glutamate-cysteine ligase and glutathione S-transferase[42]. Recent studies have revealed molecular cooperation between NRF2 and PARP-1 in the transcription of antioxidant genes[41]. Evidence that PARylation is directly involved in this cooperative process is not yet available; however, the relevance of PARylation in the mechanism of action of Sirtuin 6 related to the transcriptional activity of NRF2 is well demonstrated[41,44]. In particular, PARP-1 can act by directly binding to the antioxidant response element or the promoter of a small Maf heterodimer; therefore, PARylation can be anticipated to play a direct or indirect role in NRF2 activity[41]. Furthermore, counteracting mechanism with PARP-1 and PARylation is denoted by its interaction with the protein kinase B (AKT) pathway. Phosphatidylinositol 3 phosphorylates AKT to induce an active form and acts as a redox sensor in cancer cells[7]. Active AKT contributes to hydrogen peroxide accumulation by stimulating oxidative metabolism and inhibition of class O of forkhead box-dependent catalase; however, PARP-1 and PARylation can inhibit the mammalian target of rapamycin complex 1 signaling pathway, thus resulting in downregulation of AKT activity[7,45]. At this point, it can be emphasized that PARP-1 and PARylation can directly participate in DNA repair and can maintain redox homeostasis to prevent DNA damage by regulating the oxidation state caused by the rapid growth of CRC.

Chromosomal instability

Chromosomal instability is defined as a defect that involves the loss or rearrangement of chromosomes during cell division and has been well demonstrated as the cause of genetic mutations leading to the stressful tumor microenvironment that supports the rapid growth of cancer[46]. It is a common feature that accompanies most solid tumors and can be classified as numerical or structural chromosomal instability[46,47]. Various molecular characterizations of genomic changes make it possible to elucidate the role of chromosomal instability in cancer; furthermore, these could provide important information related to the mechanisms of tumorigenesis and genetic anomalies[46,47]. Since chromosomal instability is associated with cancer progression, increased invasiveness, poor prognosis, and resistance to anticancer mechanisms, some investigations could work on elucidating therapeutic benefits by targeting chromosomal instability in cancers[46,48]. For one, the pathway that regulates chromosome segregation during mitosis and the one involved in the response mechanism to taxane were found to be similar in CRC characterized by chromosomal instability[47]. This is a promising discovery that metastatic CRC is made inherently resistant to anticancer mechanisms by a taxane, and thereafter, various studies have supported that PARP-1 and PARylation play key roles in such resistance[14,16]. An important implication in recent studies is that the role of PARP-1 and PARylation in chromosomal instability can be emphasized in the chromatic structure change and regulation of epigenetic genes and mitosis[19,25].

Regulation of chromatin structure by PARP-1 may involve direct binding to histones as well as non-histone proteins or chromatin-related proteins or the alteration of nucleosomal structure through PARylation[19,27]. It has been demonstrated that environmental stimulation for the development of cancer can induce PARP-1- and PARylation-dependent nucleosome loosening, leading to histone removal and opening of chromatin structures[49,50]. Activation of PARP-1 promotes chromatin decondensation in response to signaling pathways for cancer cell growth and differentiation[49]. Chromatin decondensation could be induced by competitive displacement of histone H1 in the nucleosomes by PARP-1 and ADP-ribosylation on histone H1[51]. The induction of negatively charged PARylation on histone proteins can reportedly lead to repulsion with DNA, thus leading to chromatin decondensation[52]. Then, PARP-1 activity on chromatin can target a wide range of domains, and at the nucleosomal level, it recognizes specific structural features and binds directly to the nucleosomes[19,53]. The histone cores of the nucleosomes, such as H2A, H2B, H3, and H4, and the linker histone H1 are well-known direct targets of PARP-1, and such a function of action can be considered as a proof to induce localized decondensation of chromatin[25,51]. Recent studies indicated that PARP-1 binds to mononucleosomes and interacts with trinucleosomes, which is consistent with its role as a chromatin architectural protein[18,54]. Thereby, the reduction in affinity for surrounding proteins caused by PARP-1 and PARylation may help protect the linker DNA from nuclease digestion; in this context, its role in the facilitation of the reassembly of free histones into nucleosomes may suggest that PARP-1 and PARylation also act as a chaperone for histone protection under chromosomal instability[18,51,54]. Studies on CRC have

demonstrated a role of PARylation in the regulation of chromatin relaxation by histone proteins H1, H2A, and H2B[14,54].

PARylation of histones leading to open chromatin morphology is well known as another function that enables epigenetic regulation[19,55]. Histones can undergo covalent modifications from conserved lysine or arginine residues by enzymes called histone acetyltransferases or methyltransferases, which are related to the regulation of oncogene expression[56]. A link between PARylation and acetylation may exist *via* the positive transcriptional control of histone acetyltransferases by PARP-1, such as that of E1A binding protein P300 and cyclic adenosine monophosphate response element-binding protein (CBP), together with the recently identified covalent PARylation on P300 and CBP[57]. PARylation also has an important role in the maintenance of histone H3 at lysine 4 as it impinges on its demethylation process through the covalent modification of the demethylase lysine demethylase 5B[18]. Undergoing such epigenetic variations with PARylation is a key event necessary for activation of nuclear factor-kappa B-dependent genes in CRC and recruiting of key proteins involved in the DNA damage response[15,58]. Further, approximately hundreds to thousands of genes are considered to be abnormally methylated in the CRC genome, and this epigenetic change may be an important part of the pathogenesis of CRC[59]. When abnormally methylated genes are detected in normal mucous membranes, they are classified into a group with a high risk of developing CRC because abnormal methylation is equally detected in adenocarcinomas as well as in adenomas[4,58,59]. Thus, methylation is considered to play an important role in the progression of CRC[4,58]. Some cases of abnormally methylated genes in CRC include integrin subunit alpha 4, O^6-methylguanine DNA methyltransferase (MGMT), sodium-coupled monocarboxylate transporter 1, human mutL homolog 1 (MLH1), and amyloid-beta precursor protein-binding family A member 1. In particular, it has been suggested that abnormal methylation of DNA repair genes, such as MGMT and MLH1, in colorectal adenoma may promote progression to adenocarcinoma[60]. There have been some reports on the regulation of MGMT or MLH1 functions by covalent or non-covalent PARylation in ovarian cancer or glioblastoma; however, only the indirect effects of PARP-1 and PARylation were investigated in CRC, and there is still no study demonstrating a direct correlation between such genes and PARylation[12,58,61,62].

CRC is genetically classified into microsatellite instability and chromosomal instability, and chromosomal instability accounts for about 85% of sporadic CRCs[63]. Since the main feature of chromosomal instability is aneuploidy, it was predicted that it could be caused by structural changes in chromosomes and abnormal mitosis[64]. A variety of genetic changes that contribute to chromosomal instability remain to be elucidated, but the main cause of the high aneuploidy because of an increase in the total chromosome number is reportedly a trait that can be shared with the occurrence of mitotic defects[65]. Potential defects in various genes that participate in many mitotic processes for CRC development can lead to uneven separation of chromosomes and have been investigated to their involvement in the aneuploidy and carcinogenesis of CRC[47]. These include chromosomal condensation, centrosome replication, microtubule dynamics, and checkpoints for proper progression of the cell cycle[46,47]. For example, centromere protein A is a centromere-specific histone-H3-like variant essential for centromere structure and function, which play a critical role in the assembly of protein complexes that perform the function of identical chromosomal separation in the CRC[66]. In addition, aurora kinases can be overexpressed in CRC, resulting in a transgenic activity[67]. Checkpoint gene budding uninhibited by benomyl (BUB)s are mutated in CRC, and exogenous expression of mutant BUBs confers abnormal spindle checkpoints[68]. The checkpoint with forkhead-associated and ring finger domains (CHFR) is a mitotic checkpoint and tumor-suppressor gene, its loss contributes to carcinogenesis of CRC[69]. Although there are still no reports demonstrating genetic benefits for cancer survival by the regulation of CRC-specific mitotic defects by PARP-1 and PARylation, the existing theory offers a chance to focus on the possibility that the function of PARP-1 and PARylation is related to the regulation of mitotic checkpoint genes, which are involved in the mitotic defect of CRC. PARP-1 is accumulated in the centrosome chromatin until metaphase during mitosis and dissociates from anaphase after interacting with centromere proteins A and B and BUB mitotic checkpoint proteins[15,68,70]. It has also been found to interact with aurora kinases to inhibit DNA damage-induced activity and reduce histone H3 serine 10 phosphorylation[71]. Furthermore, another mitotic checkpoint, known as the antephase checkpoint, precedes the spindle assembly checkpoint and occurs in the initial prophase[72]. The antephase checkpoint responds to microtubule toxicity or DNA damage and causes chromosomal decondensation and delayed mitosis[72]. CHFR has a role in the ubiquitination of polo-like kinase 1 as an E3 ubiquitin ligase,

and it can be stabilized by PARylation. The key function of CHFR is to ensure intact antephase checkpoints, and it has been demonstrated that PARylation increases interaction with CHFR to control prophase checkpoints in stressful environments during mitosis[70,73,74]. It is likely for listed genes to be potential candidates to be targeted for demonstrating the association of mitosis defects with PARP-1 and PARylation in identifying the malignancy of CRC [60,63-66,68,69,71-74].

Modulation of tumor suppressor gene and oncogene expression

The sequential acquisition of genetic and epigenetic changes in CRC has been well defined recently through widespread genetic studies[4,31,33,47]. These studies presented clear evidence that the initiation and progression of CRC depend on the mutation of tumor suppressor genes or abnormal expression of oncogenes in stages followed by invasive and metastatic CRC[4,31,33,47]. Somatic mutations in the adenomatous polyposis coli (*APC*) gene are observed in slightly over 80% of all sporadic CRC. Similarly, mutations in the DNA mismatch repair genes, such as mutS homolog 2, mutL homolog 1, and PMS2, are found in the majority of the remaining 20% of sporadic CRC[47,75]. Many kinds of genes have been recently identified, and they have a causal relationship with the formation of CRC in the later stages of neoplastic transformation. Representative examples include Kirsten ras (KRAS) oncogenic activation and mutant inactivation of several tumor suppressor genes, including deleted pancreatic cancer locus 4 and p53[76,77]. Among the changes in various genes in CRC, studies on *APC*, *p53*, and *KRAS* were the recently focused. *APC* is a key component of the β-catenin disruption complex involved in the degradation and inhibition of the Wnt/β-catenin signaling pathway; therefore, a mutation in *APC* induces the stabilization and accumulation of β-catenin in the tumor microenvironment, thus this mutation is in charge of the earliest process in the development of CRC[78,79]. p53 is a tumor suppressor gene that encodes a transcription factor that regulates the transcription of countless genes involved in various processes, such as DNA repair, cell cycle arrest, death, and metabolism[80]. The p53 mutation is associated with the progression of sporadic CRC and leads to adenoma-to-carcinoma transition as the loss of function contributes to the propagation of damaged DNA to daughter cells[4,77]. KRAS is a membrane-bound protein with intrinsic guanosine triphosphatase (GTPase) activity and belongs to a family of RAS genes involved in signaling pathways that regulate cell proliferation, differentiation, and survival[76]. KRAS mutations impair the intrinsic GTPase activity of KRAS, causing the accumulation of KRAS protein in the state of GTPase binding activity, resulting in constitutive activation of downstream proliferative signaling pathways[76]. Following this context, since the current understanding of PARP1-induced PARylation can be emphasized owing to its potential involvement in transcriptional regulation by interaction with PARylated proteins, it is necessary to give an eye to the function of PARP-1 and PARylation concerning the gene regulation of *APC*, *p53*, and *KRAS* in CRC.

Defects of the function of the *APC* tumor suppressor gene are associated with familial and sporadic CRC, resulting in the accumulation of β-catenin and activation of T-cell factor 4 and lymphoid enhancer factor[81,82]. PARP-1 interacts with the T-cell factor 4 in CRC to act as a bridge for the complex interaction of T-cell factor 4 with β-catenin[83]. Through this function, PARP-1 increases the transcriptional activation of T-cell factor 4 and lymphoid enhancer factor with β-catenin[50,83]. mRNA and protein expression level of PARP-1 is reportedly elevated in the clinical biopsy of familial adenomatous polyposis and sporadic CRC, suggesting that they may be a possible cause of PARP-1 regulatory transcriptional activation in CRC[84]. It has also been demonstrated that PARP-1-mediated transcription up-regulation with T-cell factor 4 and lymphoid enhancer factor may be increased in sporadic CRC compared to normal tissues[50,82,83]. A direct correlation of PARylation with T-cell factor 4 or lymphatic system enhancer has not yet been established; however, it is possible to deduce that transcriptional regulation of PARP-1 is carried out in conjunction with PARylation based on the evidence for PAR accumulation in the nucleus of CRC cells[27]. That is, PARP-1 can positively regulate the transcriptional activity of T-cell factor 4 and lymphoid enhancer factor in CRC, and it can be inferred that *APC* may be more active in CRC when PARP-1 and PARylation are actively involved[27,81-83]. PARP-1 also has a unique function that allows direct regulation of sequence-specific transcription factors, and it can form a complex that allows down-regulation of all transcription processes involving p53[50,85]. The formation of a transcription inhibitory complex is made possible by direct covalent binding of PAR to p53 to induce p53 stabilization[14,50,85]. PARylation of p53 first leads to recruitment of histone deacetylases; this transcriptional inhibitory complex can upregulate cancer-related genes and phenotypes by raising the level of expression of hypoxia-inducing factor-1α and vascular

endothelial growth factor, which is related to malignant transformation of CRC[14,85]. It has been suggested that PARP-1 interacts with the G4 motif region of the KRAS promoter under the tumor microenvironment subjected to oxidative stress, such as increased ROS levels[86-88]. As aforementioned, oxidative stress caused by ROS can play an important role in the regulation of genetic changes and can be considered a common feature in most solid cancers, particularly contributing to the growth, survival, and metastasis of CRC[6,7,88]. Under such a condition, it has been proved that PARP-1 is recruited to the KRAS promoter G4 structure after which it undergoes auto-PARylation[88]. The results revealed the mobilization of the transcription factors, heterogeneous nuclear ribonucleoprotein A1 (HNRNPA1), and the protooncogene (MYC)-associated zinc finger protein, as well as the formation of a transcription pre-initiation complex[88,89]. It may be characterized by favoring recruitment to the promoter of cationic transcription factors required for KRAS transcription, such as HNRNPA1 and MYC-associated zinc finger protein, because of the strong anionic properties of PAR[88].

NON-CLINICAL AND CLINICAL STUDIES ON CRC TREATMENT

The first-generation PARP1 inhibitor, a nicotinamide analog, was found to have a cytotoxic effect on tumor cells when combined with genotoxic stress agents; however, it was not applicable for *in vivo* experiments because they had to be used in millimolar concentrations for *in vitro* studies[90]. More effective second-generation PARP-1 inhibitors were developed based on quinazoline analogs, some of which have become the basis for further development of more effective PARP-1 inhibitors, and they applied to the investigations targeting most cancer types[90]. However, non-clinical studies on inhibiting PARP-1 in CRC are still in their infancy, and regarding anticancer effects targeting CRC through a method of inhibiting PARP-1, results of only small-scale studies conducted mainly using a few small molecules, such as PJ34, NU1085, and AG14361, are available[91-94]. The phenanthridine PJ34 treatment reportedly decreased the viability of CRC cells by G2/M cell cycle arrest and subsequent clustering of additional centroids[91]. Previous studies have shown that NU1085, a family of benzimidazole PARP-1 inhibitors, exhibited potent anticancer effects in a panel of CRC cell lines at low concentrations regardless of the status of p53[92]. AG14361, binding to the catalytic domain of PARP-1, inhibited the growth of CRC even at extremely low concentrations, and its combination with irinotecan impeded the growth of human CRC in the xenograft model by 2-to 3-fold without body-weight loss[93,94]. Recently, five PARP inhibitors, olaparib, niraparib, veliparib, rucaparib, and talazoparib, are drawing attention[95-100]. Olaparib is an oral PARP inhibitor first approved for the treatment of advanced ovarian cancer; however, today, it is also being applied to patients with other cancer types with *BRCA* mutations[96]. Niraparib is an oral medicine for the highly selective inhibitor of PARP-1 and-2 used for the treatment of women with advanced ovarian cancer regardless of *BRCA* mutation or HR deficiency status[100]. Veliparib, an oral inhibitor of PARP-1 and-2, is also being studied for its applicability to treating many types of cancer with *BRCA* mutations, as well as advanced ovarian cancer[97]. Rucaparib is an oral, small-molecule inhibitor of PARP-1, -2, and -3[98]. Talazoparib is an orally bioavailable PARP inhibitor with the potential antineoplastic activity that targets cancer with BRCA mutations or with deficiencies in DNA damage repair[99]. The five mentioned PARP-1 inhibitors reportedly have anticancer effects, primarily under the characteristics of the tumor microenvironment associated with genetic changes. CRC cells with short hairpin RNA depletion of ataxia telangiectasia mutated protein kinase are sensitive to olaparib, and the depletion of p53 enhances this sensitivity[101]. The combination of niraparip or rucaparip with the topoisomerase I inhibitor irinotecan obtained results showing enhanced anticancer efficacy targeting CRC cells regardless of microsatellite status [102,103]. Veliparib could be more sensitive to CRC cells undergoing mutations in mismatch repair or mutS Homolog 3 genes, and talazoparib could increase antitumor effects through the formation of DNA a double-strand break in CRC cell lines and xenograft animal models with wild-type *BRCA* genes[104,105].

According to the outcomes in previous clinical trials, the lack of anticancer activity in PARP-1 inhibitors mainly targeting CRC has led to little interest in further clinical development. This is because a clinical trial for talazoparib involving patients with breast cancer including CRC with HR pathway gene mutation is ongoing; however, no tumor response targeting CRC has been noted so far[106]. The exact indications were defined as non-breast tumors, such as the pancreas, uterine, testicular, parotid

Table 1 The ongoing clinical trials of poly adenosine diphosphate-ribose polymerase-1 inhibitor for the treatment of colorectal cancer

Drug	Trial ID	Target Indication	Outcome measurement	Arm
Olaparib	NCT04456699	Unresectable or metastatic CRC patients who have not progressed following first-line therapy of FOLFOX with bevacizumab	Primary: PFS (up to 6 yr); Secondary: OS, ORR, DOR, AE (up to 6 yr)	(1) Olaparib; (2) Olaparib + Bevacizumab; (3) 5-FU + Bevacizumab; Triple-arms
Olaparib	NCT04166435	O6-MGMT hypermethylated CRC patients	Primary: ORR (up to 2 yr); Secondary: AE, PFS, OS (up to 2 yr)	Temozolomide + Olaparib; Single-arm
Niraparib	NCT03983993	Patients with metastatic CRC	Primary: Clinical benefit rate (CR + PR + SD, up to 5 yr); Secondary: ORR, DOR, PFS, OS (up to 5 yr)	Niraparib + Panitumumab; Single-arm
Rucaparib	NCT03337087	Treating patients with metastatic CRC up to third-line of prior therapy	Primary: MTD, DLT, ORR (SD, CR, PR; up to 3 yr); Secondary: DCR, PFS, OS, AE (up to 3 yr)	liposomal irinotecan + 5-FU + rucaparib; Single-arm

FOLFOX: Leucovorin + 5-FU + Oxaliplatin; PFS: Progression-free survival; OS: Overall survival; ORR: Objective response rate; DOR: Duration of response; AE: Adverse events; CR: Complete response; PR: Partial response; SD: Stable disease; DLT: Dose-limiting toxicity; MTD: Maximum tolerated dose; DCR: Disease control rate; MGMT: Methylguanine deoxyribonucleic acid methyltransferase.

salivary, and CRC[106]. And, a placebo-controlled phase 2 study evaluated the efficacy and tolerability of veliparib in combination with 5-fluorouracil, leucovorin, and irinotecan (FOLFIRI) compared to placebo plus FOLFIRI in patients with refractory and metastatic CRC[107]. Although there were no unexpected safety issues, it solely showed similar efficacy between the two groups[107]. However, the feasibility of its applicability to CRC treatment is being investigated in recent clinical trials in combination with existing anticancer drugs. The ongoing cases of clinical trials involving combination with PARP-1 inhibitors are as follows (Table 1): (1) In patients with histologically confirmed metastatic or unresectable CRC who have not recovered following first-line therapy of 5-fluorouracil, leucovorin, and oxaliplatin with bevacizumab, the efficacy and safety of olaparib monotherapy or in combination with bevacizumab has been evaluated in comparison with bevacizumab with 5-fluorouracil [108]; (2) The efficacy of temozolomide in combination with olaparib has been evaluated in patients with MGMT promoter hypermethylated advanced CRC[109]; (3) The adverse effects and activity of the combination of niraparib with an epidermal growth factor receptor inhibitor, panitumumab, has been evaluated in previously treated patients with RAS wild-type, microsatellite stable, and microsatellite instable metastatic CRC[110]; and (4) Phase I/II study for the investigation of side effects and best dose of liposomal irinotecan and rucaparib when given together with 5-fluorouracil and to see how well they work in treating patients with metastatic CRC [111]. The bright side is that the cases of clinical trials using PARP-1 inhibitors for CRC treatment is still only at the beginning stage, which can be counted with a finger, and it is anticipated that clinical trials that take into account the aforementioned various functions of PARP-1 and PARylation in CRC have not been initiated in earnest. This is because obvious challenges still exist to clear up scattered tasks, such as finding optimal biomarkers to screen applicable and appropriate patients with CRC.

CONCLUSION

The pathogenic roles of PARP-1-driven PARylation contributing to CRC are being actively considered in various processes required for CRC development, such as DNA damage response, transcriptional regulation, and overcoming chromosomal instability. In particular, further understanding the genetic characteristics of CRC related to the aforementioned functions for achieving significant clinical benefits by targeting PARP-1 is necessary. Therefore, it is essential to continue the discovery of optimal biomarkers that can be appropriately applied to the treatment of CRC and the pathogenetic investigations to overcome the predicted toxicity or resistance. If a clearer scientific background is supported, it is strongly inferred that the feasibility of clinical trials targeting PARP and PARylation for CRC treatment could increase.

REFERENCES

1 **Kuipers EJ**, Grady WM, Lieberman D, Seufferlein T, Sung JJ, Boelens PG, van de Velde CJ, Watanabe T. Colorectal cancer. *Nat Rev Dis Primers* 2015; **1**: 15065 [PMID: 27189416 DOI: 10.1038/nrdp.2015.65]

2 **Centelles JJ**. General aspects of colorectal cancer. *ISRN Oncol* 2012; **2012**: 139268 [PMID: 23209942 DOI: 10.5402/2012/139268]

3 **Liu J**, Cho YB, Hong HK, Wu S, Ebert PJ, Bray SM, Wong SS, Ting JC, Calley JN, Whittington CF, Bhagwat SV, Reinhard C, Wild R, Nam DH, Aggarwal A, Lee WY, Peng SB. Molecular dissection of CRC primary tumors and their matched liver metastases reveals critical role of immune microenvironment, EMT and angiogenesis in cancer metastasis. *Sci Rep* 2020; **10**: 10725 [PMID: 32612211 DOI: 10.1038/s41598-020-67842-5]

4 **Testa U**, Pelosi E, Castelli G. Colorectal cancer: genetic abnormalities, tumor progression, tumor heterogeneity, clonal evolution and tumor-initiating cells. *Med Sci (Basel)* 2018; **6** [PMID: 29652830 DOI: 10.3390/medsci6020031]

5 **Issa JP**. Colon cancer: it's CIN or CIMP. *Clin Cancer Res* 2008; **14**: 5939-5940 [PMID: 18829469 DOI: 10.1158/1078-0432.CCR-08-1596]

6 **Bardaweel SK**, Gul M, Alzweiri M, Ishaqat A, ALSalamat HA, Bashatwah RM. Reactive Oxygen Species: the Dual Role in Physiological and Pathological Conditions of the Human Body. *Eurasian J Med* 2018; **50**: 193-201 [PMID: 30515042 DOI: 10.5152/eurasianjmed.2018.17397]

7 **Lin S**, Li Y, Zamyatnin AA Jr, Werner J, Bazhin AV. Reactive oxygen species and colorectal cancer. *J Cell Physiol* 2018; **233**: 5119-5132 [PMID: 29215746 DOI: 10.1002/jcp.26356]

8 **Koveitypour Z**, Panahi F, Vakilian M, Peymani M, Seyed Forootan F, Nasr Esfahani MH, Ghaedi K. Signaling pathways involved in colorectal cancer progression. *Cell Biosci* 2019; **9**: 97 [PMID: 31827763 DOI: 10.1186/s13578-019-0361-4]

9 **García-Cárdenas JM**, Guerrero S, López-Cortés A, Armendáriz-Castillo I, Guevara-Ramírez P, Pérez-Villa A, Yumiceba V, Zambrano AK, Leone PE, Paz-Y-Miño C. Post-transcriptional Regulation of Colorectal Cancer: A Focus on RNA-Binding Proteins. *Front Mol Biosci* 2019; **6**: 65 [PMID: 31440515 DOI: 10.3389/fmolb.2019.00065]

10 **Morse MA**, Hochster H, Benson A. Perspectives on Treatment of Metastatic Colorectal Cancer with Immune Checkpoint Inhibitor Therapy. *Oncologist* 2020; **25**: 33-45 [PMID: 31383813 DOI: 10.1634/theoncologist.2019-0176]

11 **Marmorino F**, Boccaccino A, Germani MM, Falcone A, Cremolini C. Immune Checkpoint Inhibitors in pMMR Metastatic Colorectal Cancer: A Tough Challenge. *Cancers (Basel)* 2020; **12** [PMID: 32824490 DOI: 10.3390/cancers12082317]

12 **Mauri G**, Arena S, Siena S, Bardelli A, Sartore-Bianchi A. The DNA damage response pathway as a land of therapeutic opportunities for colorectal cancer. *Ann Oncol* 2020; **31**: 1135-1147 [PMID: 32512040 DOI: 10.1016/j.annonc.2020.05.027]

13 **Kamaletdinova T**, Fanaei-Kahrani Z, Wang ZQ. The Enigmatic Function of PARP1: From PARylation Activity to PAR Readers. *Cells* 2019; **8**: 1625 [PMID: 31842403 DOI: 10.3390/cells8121625]

14 **Weaver AN**, Yang ES. Beyond DNA Repair: Additional Functions of PARP-1 in Cancer. *Front Oncol* 2013; **3**: 290 [PMID: 24350055 DOI: 10.3389/fonc.2013.00290]

15 **Martí JM**, Fernández-Cortés M, Serrano-Sáenz S, Zamudio-Martinez E, Delgado-Bellido D, Garcia-Diaz A, Oliver FJ. The Multifactorial Role of PARP-1 in Tumor Microenvironment. *Cancers (Basel)* 2020; **12**: 739 [PMID: 32245040 DOI: 10.3390/cancers12030739]

16 **Gallyas F Jr**, Sumegi B. Mitochondrial Protection by PARP Inhibition. *Int J Mol Sci* 2020; **21**: 2767 [PMID: 32316192 DOI: 10.3390/ijms21082767]

17 **Ko HL**, Ren EC. Functional Aspects of PARP1 in DNA Repair and Transcription. *Biomolecules* 2012; **2**: 524-548 [PMID: 24970148 DOI: 10.3390/biom2040524]

18 **Ciccarone F**, Zampieri M, Caiafa P. PARP1 orchestrates epigenetic events setting up chromatin domains. *Semin Cell Dev Biol* 2017; **63**: 123-134 [PMID: 27908606 DOI: 10.1016/j.semcdb.2016.11.010]

19 **Gupte R**, Liu Z, Kraus WL. PARPs and ADP-ribosylation: recent advances linking molecular functions to biological outcomes. *Genes Dev* 2017; **31**: 101-126 [PMID: 28202539 DOI: 10.1101/gad.291518.116]

20 **Gibson BA**, Kraus WL. New insights into the molecular and cellular functions of poly(ADP-ribose) and PARPs. *Nat Rev Mol Cell Biol* 2012; **13**: 411-424 [PMID: 22713970 DOI: 10.1038/nrm3376]

21 **Alemasova EE**, Lavrik OI. Poly(ADP-ribosyl)ation by PARP1: reaction mechanism and regulatory proteins. *Nucleic Acids Res* 2019; **47**: 3811-3827 [PMID: 30799503 DOI: 10.1093/nar/gkz120]

22 **Bürkle A**. Poly(ADP-ribose). The most elaborate metabolite of NAD+. *FEBS J* 2005; **272**: 4576-4589 [PMID: 16156780 DOI: 10.1111/j.1742-4658.2005.04864.x]

23 **Langelier MF**, Planck JL, Roy S, Pascal JM. Crystal structures of poly(ADP-ribose) polymerase-1 (PARP-1) zinc fingers bound to DNA: structural and functional insights into DNA-dependent PARP-1 activity. *J Biol Chem* 2011; **286**: 10690-10701 [PMID: 21233213 DOI: 10.1074/jbc.M110.202507]

24 **Mansoorabadi SO**, Wu M, Tao Z, Gao P, Pingali SV, Guo L, Liu HW. Conformational activation of poly(ADP-ribose) polymerase-1 upon DNA binding revealed by small-angle X-ray scattering. *Biochemistry* 2014; **53**: 1779-1788 [PMID: 24588584 DOI: 10.1021/bi401439n]

25 **Ryu KW**, Kim DS, Kraus WL. New facets in the regulation of gene expression by ADP-ribosylation and poly(ADP-ribose) polymerases. *Chem Rev* 2015; **115**: 2453-2481 [PMID: 25575290 DOI: 10.1021/cr5004248]

26 **Langelier MF**, Eisemann T, Riccio AA, Pascal JM. PARP family enzymes: regulation and catalysis of the poly(ADP-ribose) posttranslational modification. *Curr Opin Struct Biol* 2018; **53**: 187-198 [PMID: 30481609 DOI: 10.1016/j.sbi.2018.11.002]

27 **Krishnakumar R**, Kraus WL. The PARP side of the nucleus: molecular actions, physiological outcomes, and clinical targets. *Mol Cell* 2010; **39**: 8-24 [PMID: 20603072 DOI: 10.1016/j.molcel.2010.06.017]

28 **Cannan WJ**, Pederson DS. Mechanisms and Consequences of Double-Strand DNA Break Formation in Chromatin. *J Cell Physiol* 2016; **231**: 3-14 [PMID: 26040249 DOI: 10.1002/jcp.25048]

29 **Alhmoud JF**, Woolley JF, Al Moustafa AE, Malki MI. DNA Damage/Repair Management in Cancers. *Cancers (Basel)* 2020; **12**: 1050 [PMID: 32340362 DOI: 10.3390/cancers12041050]

30 **Tubbs A**, Nussenzweig A. Endogenous DNA Damage as a Source of Genomic Instability in Cancer. *Cell* 2017; **168**: 644-656 [PMID: 28187286 DOI: 10.1016/j.cell.2017.01.002]

31 **Mazouzi A**, Velimezi G, Loizou JI. DNA replication stress: causes, resolution and disease. *Exp Cell Res* 2014; **329**: 85-93 [PMID: 25281304 DOI: 10.1016/j.yexcr.2014.09.030]

32 **Shortt J**, Johnstone RW. Oncogenes in cell survival and cell death. *Cold Spring Harb Perspect Biol* 2012; **4**: a009829 [PMID: 23209150 DOI: 10.1101/cshperspect.a009829]

33 **Grady WM**, Carethers JM. Genomic and epigenetic instability in colorectal cancer pathogenesis. *Gastroenterology* 2008; **135**: 1079-1099 [PMID: 18773902 DOI: 10.1053/j.gastro.2008.07.076]

34 **Ray Chaudhuri A**, Nussenzweig A. The multifaceted roles of PARP1 in DNA repair and chromatin remodelling. *Nat Rev Mol Cell Biol* 2017; **18**: 610-621 [PMID: 28676700 DOI: 10.1038/nrm.2017.53]

35 **Wei H**, Yu X. Functions of PARylation in DNA Damage Repair Pathways. *Genomics Proteomics Bioinformatics* 2016; **14**: 131-139 [PMID: 27240471 DOI: 10.1016/j.gpb.2016.05.001]

36 **Sun S**, Osterman MD, Li M. Tissue specificity of DNA damage response and tumorigenesis. *Cancer Biol Med* 2019; **16**: 396-414 [PMID: 31565474 DOI: 10.20892/j.issn.2095-3941.2019.0097]

37 **Reilly NM**, Novara L, Di Nicolantonio F, Bardelli A. Exploiting DNA repair defects in colorectal cancer. *Mol Oncol* 2019; **13**: 681-700 [PMID: 30714316 DOI: 10.1002/1878-0261.12467]

38 **Konstantinopoulos PA**, Ceccaldi R, Shapiro GI, D'Andrea AD. Homologous Recombination Deficiency: Exploiting the Fundamental Vulnerability of Ovarian Cancer. *Cancer Discov* 2015; **5**: 1137-1154 [PMID: 26463832 DOI: 10.1158/2159-8290.CD-15-0714]

39 **Liu H**, Liu X, Zhang C, Zhu H, Xu Q, Bu Y, Lei Y. Redox Imbalance in the Development of Colorectal Cancer. *J Cancer* 2017; **8**: 1586-1597 [PMID: 28775778 DOI: 10.7150/jca.18735]

40 **Kurutas EB**. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutr J* 2016; **15**: 71 [PMID: 27456681 DOI: 10.1186/s12937-016-0186-5]

41 **Wu T**, Wang XJ, Tian W, Jaramillo MC, Lau A, Zhang DD. Poly(ADP-ribose) polymerase-1 modulates Nrf2-dependent transcription. *Free Radic Biol Med* 2014; **67**: 69-80 [PMID: 24140708 DOI: 10.1016/j.freeradbiomed.2013.10.806]

42 **Ma Q**. Role of nrf2 in oxidative stress and toxicity. *Annu Rev Pharmacol Toxicol* 2013; **53**: 401-426 [PMID: 23294312 DOI: 10.1146/annurev-pharmtox-011112-140320]

43 **Panieri E**, Telkoparan-Akillilar P, Suzen S, Saso L. The NRF2/KEAP1 Axis in the Regulation of Tumor Metabolism: Mechanisms and Therapeutic Perspectives. *Biomolecules* 2020; **10**: 791 [PMID: 32443774 DOI: 10.3390/biom10050791]

44 **Rezazadeh S**, Yang D, Tombline G, Simon M, Regan SP, Seluanov A, Gorbunova V. SIRT6 promotes transcription of a subset of NRF2 targets by mono-ADP-ribosylating BAF170. *Nucleic Acids Res* 2019; **47**: 7914-7928 [PMID: 31216030 DOI: 10.1093/nar/gkz528]

45 **Gallyas F Jr**, Sumegi B, Szabo C. Role of Akt Activation in PARP Inhibitor Resistance in Cancer. *Cancers (Basel)* 2020; **12**: 532 [PMID: 32106627 DOI: 10.3390/cancers12030532]

46 **Vishwakarma R**, McManus KJ. Chromosome Instability; Implications in Cancer Development, Progression, and Clinical Outcomes. *Cancers (Basel)* 2020; **1** 2: 824 [PMID: 32235397 DOI: 10.3390/cancers12040824]

47 **Pino MS**, Chung DC. The chromosomal instability pathway in colon cancer. *Gastroenterology* 2010; **138**: 2059-2072 [PMID: 20420946 DOI: 10.1053/j.gastro.2009.12.065]

48 **Ferguson LR**, Chen H, Collins AR, Connell M, Damia G, Dasgupta S, Malhotra M, Meeker AK, Amedei A, Amin A, Ashraf SS, Aquilano K, Azmi AS, Bhakta D, Bilsland A, Boosani CS, Chen S, Ciriolo MR, Fujii H, Guha G, Halicka D, Helferich WG, Keith WN, Mohammed SI, Niccolai E, Yang X, Honoki K, Parslow VR, Prakash S, Rezazadeh S, Shackelford RE, Sidransky D, Tran PT, Yang ES, Maxwell CA. Genomic instability in human cancer: Molecular insights and opportunities for therapeutic attack and prevention through diet and nutrition. *Semin Cancer Biol* 2015; **35** Suppl: S5-S24 [PMID: 25869442 DOI: 10.1016/j.semcancer.2015.03.005]

49 **Jubin T**, Kadam A, Jariwala M, Bhatt S, Sutariya S, Gani AR, Gautam S, Begum R. The PARP family: insights into functional aspects of poly (ADP-ribose) polymerase-1 in cell growth and survival. *Cell Prolif* 2016; **49**: 421-437 [PMID: 27329285 DOI: 10.1111/cpr.12268]

50 **Schiewer MJ**, Knudsen KE. Transcriptional roles of PARP1 in cancer. *Mol Cancer Res* 2014; **12**: 1069-1080 [PMID: 24916104 DOI: 10.1158/1541-7786.MCR-13-0672]

51 **Fyodorov DV**, Zhou BR, Skoultchi AI, Bai Y. Emerging roles of linker histones in regulating

chromatin structure and function. *Nat Rev Mol Cell Biol* 2018; **19**: 192-206 [PMID: 29018282 DOI: 10.1038/nrm.2017.94]

52 **Smith R**, Lebeaupin T, Juhász S, Chapuis C, D'Augustin O, Dutertre S, Burkovics P, Biertümpfel C, Timinszky G, Huet S. Poly(ADP-ribose)-dependent chromatin unfolding facilitates the association of DNA-binding proteins with DNA at sites of damage. *Nucleic Acids Res* 2019; **47**: 11250-11267 [PMID: 31566235 DOI: 10.1093/nar/gkz820]

53 **Matveeva E**, Maiorano J, Zhang Q, Eteleeb AM, Convertini P, Chen J, Infantino V, Stamm S, Wang J, Rouchka EC, Fondufe-Mittendorf YN. Involvement of PARP1 in the regulation of alternative splicing. *Cell Discov* 2016; **2**: 15046 [PMID: 27462443 DOI: 10.1038/celldisc.2015.46]

54 **Muthurajan UM**, Hepler MR, Hieb AR, Clark NJ, Kramer M, Yao T, Luger K. Automodification switches PARP-1 function from chromatin architectural protein to histone chaperone. *Proc Natl Acad Sci USA* 2014; **111**: 12752-12757 [PMID: 25136112 DOI: 10.1073/pnas.1405005111]

55 **Messner S**, Hottiger MO. Histone ADP-ribosylation in DNA repair, replication and transcription. *Trends Cell Biol* 2011; **21**: 534-542 [PMID: 21741840 DOI: 10.1016/j.tcb.2011.06.001]

56 **Koriakov DE**. [Histone modification and regulation of chromatin function]. *Genetika* 2006; **42**: 1170-1185 [PMID: 17100086]

57 **Javaid N**, Choi S. Acetylation- and Methylation-Related Epigenetic Proteins in the Context of Their Targets. *Genes (Basel)* 2017; **8**: 196 [PMID: 28783137 DOI: 10.3390/genes8080196]

58 **Jin B**, Robertson KD. DNA methyltransferases, DNA damage repair, and cancer. *Adv Exp Med Biol* 2013; **754**: 3-29 [PMID: 22956494 DOI: 10.1007/978-1-4419-9967-2_1]

59 **Migliore L**, Migheli F, Spisni R, Coppedè F. Genetics, cytogenetics, and epigenetics of colorectal cancer. *J Biomed Biotechnol* 2011; **2011**: 792362 [PMID: 21490705 DOI: 10.1155/2011/792362]

60 **Belhadj S**, Moutinho C, Mur P, Setien F, Llinàs-Arias P, Pérez-Salvia M, Pons T, Pineda M, Brunet J, Navarro M, Capellá G, Esteller M, Valle L. Germline variation in O^6-methylguanine-DNA methyltransferase (MGMT) as cause of hereditary colorectal cancer. *Cancer Lett* 2019; **447**: 86-92 [PMID: 30677446 DOI: 10.1016/j.canlet.2019.01.019]

61 **O'Sullivan CC**, Moon DH, Kohn EC, Lee JM. Beyond Breast and Ovarian Cancers: PARP Inhibitors for BRCA Mutation-Associated and BRCA-Like Solid Tumors. *Front Oncol* 2014; **4**: 42 [PMID: 24616882 DOI: 10.3389/fonc.2014.00042]

62 **Soll JM**, Sobol RW, Mosammaparast N. Regulation of DNA Alkylation Damage Repair: Lessons and Therapeutic Opportunities. *Trends Biochem Sci* 2017; **42**: 206-218 [PMID: 27816326 DOI: 10.1016/j.tibs.2016.10.001]

63 **Cisyk AL**, Nugent Z, Wightman RH, Singh H, McManus KJ. Characterizing Microsatellite Instability and Chromosome Instability in Interval Colorectal Cancers. *Neoplasia* 2018; **20**: 943-950 [PMID: 30121009 DOI: 10.1016/j.neo.2018.07.007]

64 **Potapova TA**, Zhu J, Li R. Aneuploidy and chromosomal instability: a vicious cycle driving cellular evolution and cancer genome chaos. *Cancer Metastasis Rev* 2013; **32**: 377-389 [PMID: 23709119 DOI: 10.1007/s10555-013-9436-6]

65 **Pihan GA**. Centrosome dysfunction contributes to chromosome instability, chromoanagenesis, and genome reprograming in cancer. *Front Oncol* 2013; **3**: 277 [PMID: 24282781 DOI: 10.3389/fonc.2013.00277]

66 **Fujita Y**, Hayashi T, Kiyomitsu T, Toyoda Y, Kokubu A, Obuse C, Yanagida M. Priming of centromere for CENP-A recruitment by human hMis18alpha, hMis18beta, and M18BP1. *Dev Cell* 2007; **12**: 17-30 [PMID: 17199038 DOI: 10.1016/j.devcel.2006.11.002]

67 **Katsha A**, Belkhiri A, Goff L, El-Rifai W. Aurora kinase A in gastrointestinal cancers: time to target. *Mol Cancer* 2015; **14**: 106 [PMID: 25987188 DOI: 10.1186/s12943-015-0375-4]

68 **Li GQ**, Zhang HF. Mad2 and p27 expression profiles in colorectal cancer and its clinical significance. *World J Gastroenterol* 2004; **10**: 3218-3220 [PMID: 15457580 DOI: 10.3748/wjg.v10.i21.3218]

69 **Sun Z**, Liu J, Jing H, Dong SX, Wu J. The diagnostic and prognostic value of *CHFR* hypermethylation in colorectal cancer, a meta-analysis and literature review. *Oncotarget* 2017; **8**: 89142-89148 [PMID: 29179506 DOI: 10.18632/oncotarget.19408]

70 **Slade D**. Mitotic functions of poly(ADP-ribose) polymerases. *Biochem Pharmacol* 2019; **167**: 33-43 [PMID: 30910692 DOI: 10.1016/j.bcp.2019.03.028]

71 **Bartlett E**, Bonfiglio JJ, Prokhorova E, Colby T, Zobel F, Ahel I, Matic I. Interplay of Histone Marks with Serine ADP-Ribosylation. *Cell Rep* 2018; **24**: 3488-3502. e5 [PMID: 30257210 DOI: 10.1016/j.celrep.2018.08.092]

72 **Chin CF**, Yeong FM. Safeguarding entry into mitosis: the antephase checkpoint. *Mol Cell Biol* 2010; **30**: 22-32 [PMID: 19841063 DOI: 10.1128/MCB.00687-09]

73 **Kashima L**, Idogawa M, Mita H, Shitashige M, Yamada T, Ogi K, Suzuki H, Toyota M, Ariga H, Sasaki Y, Tokino T. CHFR protein regulates mitotic checkpoint by targeting PARP-1 protein for ubiquitination and degradation. *J Biol Chem* 2012; **287**: 12975-12984 [PMID: 22337872 DOI: 10.1074/jbc.M111.321828]

74 **Privette LM**, Petty EM. CHFR: A Novel Mitotic Checkpoint Protein and Regulator of Tumorigenesis. *Transl Oncol* 2008; **1**: 57-64 [PMID: 18633460 DOI: 10.1593/tlo.08109]

75 **Boland CR**, Goel A. Microsatellite instability in colorectal cancer. *Gastroenterology* 2010; **138**: 2073-2087. e3 [PMID: 20420947 DOI: 10.1053/j.gastro.2009.12.064]

76 **Sullivan KM**, Kozuch PS. Impact of KRAS Mutations on Management of Colorectal Carcinoma. *Patholog Res Int* 2011; **2011**: 219309 [PMID: 21437184 DOI: 10.4061/2011/219309]

77 **Salovaara R**, Roth S, Loukola A, Launonen V, Sistonen P, Avizienyte E, Kristo P, Järvinen H, Souchelnytskyi S, Sarlomo-Rikala M, Aaltonen LA. Frequent loss of SMAD4/DPC4 protein in colorectal cancers. *Gut* 2002; **51**: 56-59 [PMID: 12077092 DOI: 10.1136/gut.51.1.56]

78 **Cheng X**, Xu X, Chen D, Zhao F, Wang W. Therapeutic potential of targeting the Wnt/β-catenin signaling pathway in colorectal cancer. *Biomed Pharmacother* 2019; **110**: 473-481 [PMID: 30530050 DOI: 10.1016/j.biopha.2018.11.082]

79 **Shang S**, Hua F, Hu ZW. The regulation of β-catenin activity and function in cancer: therapeutic opportunities. *Oncotarget* 2017; **8**: 33972-33989 [PMID: 28430641 DOI: 10.18632/oncotarget.15687]

80 **Kastenhuber ER**, Lowe SW. Putting p53 in Context. *Cell* 2017; **170**: 1062-1078 [PMID: 28886379 DOI: 10.1016/j.cell.2017.08.028]

81 **Benchabane H**, Ahmed Y. The adenomatous polyposis coli tumor suppressor and Wnt signaling in the regulation of apoptosis. *Adv Exp Med Biol* 2009; **656**: 75-84 [PMID: 19928354 DOI: 10.1007/978-1-4419-1145-2_7]

82 **Hankey W**, Frankel WL, Groden J. Functions of the APC tumor suppressor protein dependent and independent of canonical WNT signaling: implications for therapeutic targeting. *Cancer Metastasis Rev* 2018; **37**: 159-172 [PMID: 29318445 DOI: 10.1007/s10555-017-9725-6]

83 **Idogawa M**, Yamada T, Honda K, Sato S, Imai K, Hirohashi S. Poly(ADP-ribose) polymerase-1 is a component of the oncogenic T-cell factor-4/beta-catenin complex. *Gastroenterology* 2005; **128**: 1919-1936 [PMID: 15940627 DOI: 10.1053/j.gastro.2005.03.007]

84 **Dziaman T**, Ludwiczak H, Ciesla JM, Banaszkiewicz Z, Winczura A, Chmielarczyk M, Wisniewska E, Marszalek A, Tudek B, Olinski R. PARP-1 expression is increased in colon adenoma and carcinoma and correlates with OGG1. *PLoS One* 2014; **9**: e115558 [PMID: 25526641 DOI: 10.1371/journal.pone.0115558]

85 **Lee MH**, Na H, Kim EJ, Lee HW, Lee MO. Poly(ADP-ribosyl)ation of p53 induces gene-specific transcriptional repression of MTA1. *Oncogene* 2012; **31**: 5099-5107 [PMID: 22286760 DOI: 10.1038/onc.2012.2]

86 **Porru M**, Pompili L, Caruso C, Biroccio A, Leonetti C. Targeting KRAS in metastatic colorectal cancer: current strategies and emerging opportunities. *J Exp Clin Cancer Res* 2018; **37**: 57 [PMID: 29534749 DOI: 10.1186/s13046-018-0719-1]

87 **Jinesh GG**, Sambandam V, Vijayaraghavan S, Balaji K, Mukherjee S. Molecular genetics and cellular events of K-Ras-driven tumorigenesis. *Oncogene* 2018; **37**: 839-846 [PMID: 29059163 DOI: 10.1038/onc.2017.377]

88 **Cinque G**, Ferino A, Pedersen EB, Xodo LE. Role of Poly [ADP-ribose] Polymerase 1 in Activating the *Kirsten ras* (*KRAS*) Gene in Response to Oxidative Stress. *Int J Mol Sci* 2020; **21**: 6237 [PMID: 32872305 DOI: 10.3390/ijms21176237]

89 **Nishikawa T**, Kuwano Y, Takahara Y, Nishida K, Rokutan K. HnRNPA1 interacts with G-quadruplex in the TRA2B promoter and stimulates its transcription in human colon cancer cells. *Sci Rep* 2019; **9**: 10276 [PMID: 31311954 DOI: 10.1038/s41598-019-46659-x]

90 **Malyuchenko NV**, Kotova EY, Kulaeva OI, Kirpichnikov MP, Studitskiy VM. PARP1 Inhibitors: antitumor drug design. *Acta Naturae* 2015; **7**: 27-37 [PMID: 26483957]

91 **Castiel A**, Visochek L, Mittelman L, Dantzer F, Izraeli S, Cohen-Armon M. A phenanthrene derived PARP inhibitor is an extra-centrosomes de-clustering agent exclusively eradicating human cancer cells. *BMC Cancer* 2011; **11**: 412 [PMID: 21943092 DOI: 10.1186/1471-2407-11-412]

92 **Delaney CA**, Wang LZ, Kyle S, White AW, Calvert AH, Curtin NJ, Durkacz BW, Hostomsky Z, Newell DR. Potentiation of temozolomide and topotecan growth inhibition and cytotoxicity by novel poly(adenosine diphosphoribose) polymerase inhibitors in a panel of human tumor cell lines. *Clin Cancer Res* 2000; **6**: 2860-2867 [PMID: 10914735]

93 **Calabrese CR**, Batey MA, Thomas HD, Durkacz BW, Wang LZ, Kyle S, Skalitzky D, Li J, Zhang C, Boritzki T, Maegley K, Calvert AH, Hostomsky Z, Newell DR, Curtin NJ. Identification of potent nontoxic poly(ADP-Ribose) polymerase-1 inhibitors: chemopotentiation and pharmacological studies. *Clin Cancer Res* 2003; **9**: 2711-2718 [PMID: 12855651]

94 **Calabrese CR**, Almassy R, Barton S, Batey MA, Calvert AH, Canan-Koch S, Durkacz BW, Hostomsky Z, Kumpf RA, Kyle S, Li J, Maegley K, Newell DR, Notarianni E, Stratford IJ, Skalitzky D, Thomas HD, Wang LZ, Webber SE, Williams KJ, Curtin NJ. Anticancer chemosensitization and radiosensitization by the novel poly(ADP-ribose) polymerase-1 inhibitor AG14361. *J Natl Cancer Inst* 2004; **96**: 56-67 [PMID: 14709739 DOI: 10.1093/jnci/djh005]

95 **Zhou P**, Wang J, Mishail D, Wang CY. Recent advancements in PARP inhibitors-based targeted cancer therapy. *Precis Clin Med* 2020; **3**: 187-201 [PMID: 32983586 DOI: 10.1093/pcmedi/pbaa030]

96 **Saleh N**, Copur MS. Overview: FDA Approval of Olaparib Maintenance for BRCA-Mutated. *Oncology (Williston Park)* 2019; **33** [PMID: 31365755]

97 **Weil MK**, Chen AP. PARP inhibitor treatment in ovarian and breast cancer. *Curr Probl Cancer* 2011; **35**: 7-50 [PMID: 21300207 DOI: 10.1016/j.currproblcancer.2010.12.002]

98 **Colombo I**, Lheureux S, Oza AM. Rucaparib: a novel PARP inhibitor for *BRCA* advanced ovarian cancer. *Drug Des Devel Ther* 2018; **12**: 605-617 [PMID: 29606854 DOI: 10.2147/DDDT.S130809]

99 **Boussios S**, Abson C, Moschetta M, Rassy E, Karathanasi A, Bhat T, Ghumman F, Sheriff M, Pavlidis N. Poly (ADP-Ribose) Polymerase Inhibitors: Talazoparib in Ovarian Cancer and Beyond. *Drugs R D* 2020; **20**: 55-73 [PMID: 32215876 DOI: 10.1007/s40268-020-00301-8]

100 **Heo YA**, Duggan ST. Niraparib: A Review in Ovarian Cancer. *Target Oncol* 2018; **13**: 533-539 [PMID: 30073633 DOI: 10.1007/s11523-018-0582-1]

101 **Wang C**, Jette N, Moussienko D, Bebb DG, Lees-Miller SP. ATM-Deficient Colorectal Cancer Cells Are Sensitive to the PARP Inhibitor Olaparib. *Transl Oncol* 2017; **10**: 190-196 [PMID: 28182994 DOI: 10.1016/j.tranon.2017.01.007]

102 **Genther Williams SM**, Kuznicki AM, Andrade P, Dolinski BM, Elbi C, O'Hagan RC, Toniatti C. Treatment with the PARP inhibitor, niraparib, sensitizes colorectal cancer cell lines to irinotecan regardless of MSI/MSS status. *Cancer Cell Int* 2015; **15**: 14 [PMID: 25685067 DOI: 10.1186/s12935-015-0162-8]

103 **Augustine T**, Maitra R, Zhang J, Nayak J, Goel S. Sensitization of colorectal cancer to irinotecan therapy by PARP inhibitor rucaparib. *Invest New Drugs* 2019; **37**: 948-960 [PMID: 30612311 DOI: 10.1007/s10637-018-00717-9]

104 **Davidson D**, Wang Y, Aloyz R, Panasci L. The PARP inhibitor ABT-888 synergizes irinotecan treatment of colon cancer cell lines. *Invest New Drugs* 2013; **31**: 461-468 [PMID: 23054213 DOI: 10.1007/s10637-012-9886-7]

105 **McCann KE**, von Euw E, O'Brien N, Slamon D. Abstract 4124: The combination of PARP inhibitor talazoparib with low-dose temozolomide results in increased cell lethality in BRCA1/2 wild-type melanoma, small cell lung cancer, ovarian, and colon cancer cell lines and mouse xenografts *via* the formation of DNA double-strand breaks during S-phase. *Cancer Res* 2020; **80**: 4124-4124 [DOI: 10.1158/1538-7445.AM2020-4124]

106 **Gruber JJ**, Afghahi A, Hatton A, Scott D, McMillan A, Ford JM, Telli ML. Talazoparib beyond BRCA: A phase II trial of talazoparib monotherapy in BRCA1 and BRCA2 wild-type patients with advanced HER2-negative breast cancer or other solid tumors with a mutation in homologous recombination (HR) pathway genes. *J Clin Oncol* 2019; **37**: 3006-3006 [DOI: 10.1200/JCO.2019.37.15_suppl.3006]

107 **Gorbunova V**, Beck T, Hofheinz R, Garcia-Alfonso P, Nechaeva M, Gracian AC, Mangel L, Elez E, Deming DA, Ramanathan RK, Torres A, Sullivan DM, Komarnitsky PB, Berlin J. Phase 2 study of veliparib plus FOLFIRI ± bevacizumab *vs* placebo plus FOLFIRI ± bevacizumab in metastatic colorectal cancer. *J Clin Oncol* 2018; **36**: 3543-3543 [DOI: 10.1200/JCO.2018.36.15_suppl.3543]

108 **Mayo CA**, Gurary EB, Marinello P. Olaparib ± bevacizumab *vs* bevacizumab + fluorouracil in patients with unresectable or metastatic colorectal cancer not progressing on first-line FOLFOX + bevacizumab: Phase III LYNK-003 study. *J Clin Oncol* 2021; **39**: TPS156-TPS156 [DOI: 10.1200/JCO.2021.39.3_suppl.TPS156]

109 **Yale University**. TMZ + Olaparib for MGMT Hypermethylated Colorectal Cancer. In: ClinicalTrials.gov [Cited 20 March 2021]. Available from: https://ClinicalTrials.gov/show/NCT04166435

110 **Alese OB**, Shaib WL, Akce M, Wu C, Lesinski GB, El-Rayes BF. A phase II study of niraparib in combination with EGFR inhibitor panitumumab in patients with advanced colorectal cancer. *J Clin Oncol* 2020; **38**: TPS269-TPS269 [DOI: 10.1200/JCO.2020.38.4_suppl.TPS269]

111 **Academic and Community Cancer Research United**. Liposomal Irinotecan, Fluorouracil, Leucovorin Calcium, and Rucaparib in Treating Patients With Metastatic Pancreatic, Colorectal, Gastroesophageal, or Biliary Cancer. In: ClinicalTrials.gov [Cited 20 March 2021]. Available from: https://ClinicalTrials.gov/show/NCT03337087

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World J Gastrointest Oncol 2021 June 15; 13(6): 589-599

DOI: 10.4251/wjgo.v13.i6.589

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Retrospective Study

Yield of surgery in solid pseudopapillary neoplasms of the pancreas: A case series and literature review

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Author contributions: All authors contributed to review and writing the manuscript equally; Silano F, de Melo Amaral RB, Santana RC, Neves VC, Ardengh JC, do Amaral PCG all approved the final version.

Institutional review board statement: Approval Obtained from Institutional Reivew Board of "Hospital São Rafael/Rede D'Or Ethics and Research Committee; Approval No. 35555820.0.0000.0048; Approval Date: 28/07/2020."

Informed consent statement: This is not applicable. The authors' own database was used. There is no clinical trial, there is no drug test, and there is no exposure of patients.

Conflict-of-interest statement: None of the Authors has any conflict of interest related to the manuscript.

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Abstract

BACKGROUND

Solid pseudopapillary neoplasms (SPN) of the pancreas represents approximately 2% of non-endocrine tumors of the pancreas. It is described in the literature as a rare and predominant tumor in young women.

AIM

To report a case series with SPN and analyzing clinical, surgical, anatomopathological characteristics, as well as the prognosis and review of literature.

METHODS

Retrospective analysis of patients undergoing surgery, with histological diagnosis of SPN between 1998 and 2018, using standardized and prospectively completed forms, performed at the Surgery Service of the Upper Digestive System at Hospital São Rafael/Rede D'Or in Salvador - BA. Review of literature through a database search in MEDLINE/PubMed of retrospective articles.

Data sharing statement: No additional data available.

Manuscript source: Unsolicited manuscript

Specialty type: Surgery

Country/Territory of origin: Brazil

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Received: November 3, 2020
Peer-review started: November 3, 2020
First decision: January 29, 2021
Revised: March 24, 2021
Accepted: May 7, 2021
Article in press: May 7, 2021
Published online: June 15, 2021

P-Reviewer: Anastasiou I, Eysselein VE
S-Editor: Yan JP
L-Editor: A
P-Editor: Yuan YY

RESULTS

Fourteen female patients with the average age of 31.6 years (range min-max) were selected. Twelve patients (85.7%) were asymptomatic, being an incidental diagnosis or due to screening for other reasons. One patient had abdominal pain due to gastric compression and another patient had jaundice. The 14 patients were staged with computerized tomography or magnetic resonance imaging. None had evidence of metastasis. In 8 patients (57.1%), the tumor was in the tail and body. The average size was 6.7 cm (range min-18). The type of surgery was according to the anatomical location of the tumor. There was no lymph node involvement. In two cases, vascular resection with the use of a prosthesis was required for reconstruction. The surgical margins were free. In all cases, postoperative immunohistochemistry confirmed that it was a solid pseudo-papillary neoplasia of the pancreas. There has been no disease recurrence in any case so far.

CONCLUSION

The tumors had a benign, indolent and histopathological behavior compatible with the literature. Curative surgery is recommended in all cases.

Key Words: Frantz tumor; Malignancy; Solid pseudo-papillary tumor; Surgical treatment; Survival; Pancreas

Core Tip: Surgery is the only curative treatment for solid papillary neoplasm of the pancreas. Even in cases of large tumors, wherein extensive resections of both the main tumor and the metastases are an absolute requirement, surgery can be curative and allow a long, disease-free survival. Some of the patients in the service studied underwent tumor resection more than 13 years ago, without relapse of the disease and maintaining a good quality of life. This type of neoplasm is considered rare, but in the present study most cases were discovered incidentally through imaging examinations, with the number of cases increasing since 2012.

Citation: Silano F, de Melo Amaral RB, Santana RC, Neves VC, Ardengh JC, do Amaral PCG. Yield of surgery in solid pseudopapillary neoplasms of the pancreas: A case series and literature review. *World J Gastrointest Oncol* 2021; 13(6): 589-599
URL: https://www.wjgnet.com/1948-5204/full/v13/i6/589.htm
DOI: https://dx.doi.org/10.4251/wjgo.v13.i6.589

INTRODUCTION

Solid pseudopapillary neoplasm (SPN) is a rare tumor of the pancreas, which was first described in 1959[1,2]. It represents approximately 1 to 2% of pancreatic tumors, but a higher incidence has been reported in recent years[3,4]. It occurs in young women, its natural history is unknown, and it exhibits an indolent behavior. However, SPN is potentially aggressive, particularly when large masses are present, and it may cause symptoms due to the invasion of nearby organs and vascular structures; it may even send distant metastases[3,5,6]. Surgery is the only curative treatment for SPN. Even for large tumors, for which extensive resection is imperative, including metastases, surgical treatment may be curative and allow long-term disease-free survival[5,7-9].

The authors present herein a case series from a single center with extensive experience in pancreatic surgery and assess the postoperative results in a long-term follow-up of SPN patients with curative intent.

MATERIALS AND METHODS

This study was approved by the Human Research Ethics Committee. The database from Surgery Service of the Upper Digestive System at the Hospital São Rafael/Rede

D'Or, in Salvador- State of Bahia, Brazil, was retrospectively studied but with prospective data collection in a subsequent treatment by surgery. The authors identified all patients undergoing pancreatic resection for SPN between 1998 and 2018.

The clinical characteristics, perioperative, anatomopathological data, and long-term follow-up were analyzed. The occurrence of adverse events (AE) and the treatment was analyzed. The review of the literature, composed of retrospective articles, was conducted through a database search in MEDLINE/PubMed.

The Karnofsky Performance Status Scale was used in the initial evaluation of the patients, correlating the disease symptoms with physical impairment and self-care, on a scale of 10%-100%. A score ≥ 80% indicates an ability to perform daily activities without special care, despite the presence of symptoms of the disease. All surgeries were performed by surgeons experienced in pancreatic surgery. Pylorus-preserving pancreaticoduodenectomy (PPD) was the surgical technique of choice for tumors of the head of the pancreas, whereas distal pancreatectomy (DP) was performed for tumors of the body and tail of the pancreas. All patients who underwent DP were vaccinated against encapsulated bacteria 15 d before the procedure, including those whose spleen was preserved.

The Enhanced Recovery After Surgery protocol was used, except in DPs that were systematically drained, because reliable predictors for pancreatic fistula are lacking in the literature. The drain was removed on the third day after surgery if amylase was ≤ 5000 U/L in the drained fluid[10]. The amount of pancreatic tissue removed was determined in the DPs and related to the appearance of diabetes mellitus (DM). The mean size of the surgical specimens collected in all the DPs performed in center (8 cm) was used. The occurrence of fistulas was classified according to the criteria of the International Study Group on Pancreatic Fistula[11]. A fistula was defined as an amylase level in the drainage fluid that is more than 3 times the upper normal limit of serum amylase persisting beyond 3 wk.

The Clavien–Dindo classification was used to classify AEs, whether related to the surgery site or not, with the aim of categorizing them into minor events, which required the use of analgesics and prokinetics (Clavien–Dindo I) or blood transfusion associated with parenteral nutrition (Clavien–Dindo II), and major events, which required intervention ranging from endoscopy to surgery (Clavien–Dindo III)[12]. Surgery-related mortality was considered up to 90 d after the procedure[13].

The microscopic criterion for malignancy, as defined by the World Health Organization (WHO), was the invasion of perineural, angiolymphatic, capsular, or peripancreatic adipose tissues[14].

The patients were followed up on an outpatient basis for at least 5 years, except one case, which was followed for only one year. The following parameters were analyzed: tumor relapse, appearance of DM, and pre- and postoperative quality of life as perceived by the patient (Karnofsky score).

RESULTS

Sample characteristics

Fourteen patients with SPN submitted to curative surgery over a 20-year period were identified, all of them female. The mean age was 31.5 years (range: 20-55); 11 patients (78.6%) were aged 35 years or younger. Twelve patients (85.7%) were asymptomatic. The diagnosis was made through abdominal ultrasound (US), which had been requested due to other causes. One patient (7.1%) had abdominal pain along with nausea and vomiting due to an 11 cm tumor that affected the body and head of the pancreas and caused gastric compression (Figure 1A). Another patient presented with jaundice and weight loss due to a 9 cm mass in the head of the pancreas (Figure 1B). All cases had a Karnofsky score ≥ 80%.

Imaging examinations and staging

All patients were staged using either computed tomography (CT) or abdominal magnetic resonance imaging (MRI). None had any evidence of liver, peritoneum, or lymph node metastases. Endosonography was performed in one of the patients (7.1%) and SPN was confirmed. The mean size of the tumors identified by the preoperative imaging examinations was 6.72 cm (range: 1.5-18 cm). The most common locations were the body and tail of the pancreas (Table 1).

Table 1 Preoperative demographic data and clinical radiological characteristics

Demographic data (*n* = 14)	*n* (%)
Gender	
Female/male	14 (100)/0 (0)
Mean age (yr); (range)	31.6 (20-55)
35 yr	11 (78.6)
Symptoms	
Asymptomatic	12 (85.7)
Abdominal pain and vomiting	1 (7.1)
Jaundice/weight loss	1 (7.1)
Radiological characteristics	
Largest injury (cm)	18
Minor injury (cm)	1.5
Average lesion size (cm); (range)	6.7 (1.5-18)
Tumor location	
Head	6 (42.8)
Body/tail	8 (57.1)
Metastasis	
Yes	0
No	14 (100)
Lymph node enlargement	0
Vascular invasion	2 (14.3)

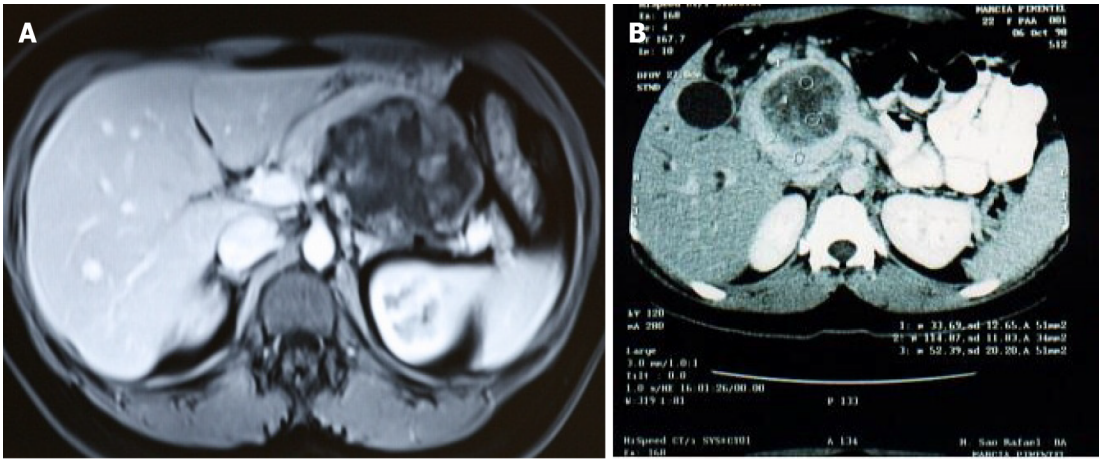

Figure 1 Heterogeneous tumor and massive lesion in the head of the pancreas. A: Heterogeneous tumor, with cystic areas, solid components, rejecting the gastric wall upwards; B: Massive lesion in the head of the pancreas.

Surgery

Nine patients (64.3%) underwent open surgery and 5 (35.7%) had laparoscopic surgery. Splenectomy was performed in 7 patients (50%), and one patient (7.1%) required a transverse colon resection and a partial gastrectomy due to SPN invasion of these organs. Because of the tumor location, DP was the most common procedure (8 patients, 57%), while PPD was performed in 6 patients (43%). The mean intraoperative time was 346.6 minutes for PPD and 228.12 minutes for DP (Table 2).

Table 2 Intraoperative surgical data

Intraoperative characteristics (*n* = 14)	*n* (%)
Access way	
Laproscopic	5 (38.4)
Open Laparotomy	9 (64.2)
Type of surgery	
Duodenopancreatectomy	6 (42.8)
Distal pancreatectomy	8 (57.1)
Vascular resection	
Yes	2 (14.2)
No	12 (85.7)
Resection of other organs	
Yes	8 (57.1)
Spleen	(7)
Transverse colon	(1)
Stomach	(1)
No	6 (42.8)
Blood transfusion	
Yes	2 (14.2)
No	12 (85.7)

AEs

One PPD procedure (7.1%) required intraoperative vascular resection of the hepatic artery and superior mesenteric vein (Figure 2A), with the surgery lasting 17 h and the need for blood transfusion. The patient developed ascites and liver failure, caused by massive hypoperfusion of the liver due to the long period of ischemia. This patient's treatment was conservative (Clavien–Dindo I). Another segmental resection of the superior mesenteric vein (Figure 2B) was necessary during a DP, with the surgery lasting 9 h and the need for blood transfusion. The patient had a satisfactory evolution.

Two patients (14.3%) submitted to PPD had AEs classified as Clavien–Dindo grade II (urinary and surgical wound infections, both treated with antibiotic therapy). Two patients (14.3%) who underwent DP developed fistulas, one had a gradeB fistula (Clavien–Dindo II) and the other had a gradeC fistula (Clavien–Dindo III) (Table 3).

Surgical specimen size

In 2 patients (14.3%) among the 8 patients submitted to DP, the surgical specimen measured less than 8 cm, and in 85.7% of the patients, the specimen measured more than 8 cm, with a 9-18 cm range. Two patients (14.3%) submitted to DP developed DM at 18 mo and 4 years after surgery. These two patients had 9 cm and 15 cm of the pancreas resected, respectively. They did not have comorbid conditions, including obesity.

Pathological characteristics

In the present study, the evaluation of the resected surgical specimens according to the WHO criterion did not show any features predictive of recurrence, except in one patient who had invasion of the peripancreatic adipose tissue and who was operated in 2012 with no subsequent relapse. In all surgeries, resection with free margins (R0) was achieved. No lymph node metastases were found in any case. The immunohistochemical analysis confirmed the SPN diagnosis in all surgical specimens (Table 4).

Follow up

The mean follow-up period was 56.6 mo (12-156 mo). Ten patients were followed up for 5 years; 3 patients with less than 5 years since the surgery are still under follow-up. There were no cases of relapse. The 5year survival and disease-free survival rates were

Table 3 Postoperative characteristics, adverse events (classification and treatment), readmission, and deaths

Postoperative characteristics (*n* = 14)	*n* (%)	Clavien-Dindo
Abdominal drain	14 (100)	-
Surgical site (adverse events)	4 (28.5)	-
Surgical wound infection (PD)	1 (7.1)	II (Antibiotic)
Transient liver failure/ascites (PD)	1 (7.1)	I
Grade C pancreatic fistula (CCP); Grade B pancreatic fistula (CCP)	1 (7.1); 1 (7.1)	III (EL); II (abdominal drain)
Surgical site (adverse events outside)	1 (7.1)	
Urinary infection (PD)	1 (7.1)	II (Antibiotic)
Readmission	1 (7.1)	Grade C fistula (EL)
Death up to 90 d	0	

EL: Exploratory laparotomy; PD: Pancreaticoduodenectomy.

Table 4 Pathological characteristics (*n* = 14)

Pathological characteristics	*n* (%)
Free surgical margins	14 (100)
Average of resected lymph nodes	6.5
Lymph node metastasis	0
Perineural invasion	0
Angiolymphatic invasion	0
Microscopic extension of the tumor	1
Peripancreatic adipose tissue	1
Invasion of the capsule	0

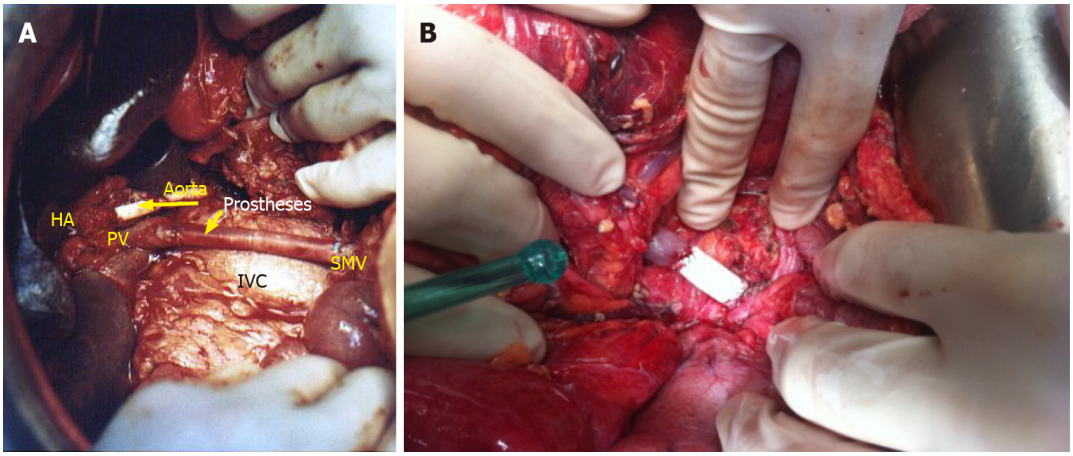

Figure 2 Resection of the hepatic artery and partial resection of the superior mesenteric vein. A: Resection of the hepatic artery and superior mesenteric vein with placement of polytetrafluoroethylene (PTFE) prosthesis; B: Partial resection of the superior mesenteric vein using a PTFE vascular prosthesis. HA: Hepatic artery; PV: Portal vein; SMV: Superior mesenteric vein; IVC: Inferior vena cava.

both 100% (Table 5). The mortality rate from causes related to the surgery was 0% in the group. There was one death from bronchopneumonia 12 mo postoperatively. However, one patient reported worsened quality of life, with a Karnofsky score < 80%.

Table 5 Follow-up of patients with solid pseudopapillary neoplasm of the pancreas

Year of surgery	*n*	Follow-up (yr)	Disease free	Conduct
1998	1	13	Yes	MD
1998	1	1	Yes	Death[1]
2012	4	5	Yes	MD
2013	1	5	Yes	MD
2014	2	5	Yes	MD
2015	2	5	Yes	MD
2017	1	3	Yes	SG
2018	2	2	Yes	SG

Mean follow-up: 4.7 years (range 1-13 years). Medical discharge: 10 patients; 3 patients still under follow-up, and one died a year after surgery.
[1]Patient remained free of disease and died 1 year later from pneumonia, unrelated to the underlying pathology.
CD: Conduct; SG: Follow-up; MD: Medical discharge.

Literature review

Table 6 shows several series of SPN cases described in the literature.

DISCUSSION

SPN occurs in young women, with a prevalence ratio of 9.7:1 relative to men[15-25]. The mean age varies from 21.9 to 41.2 years[6,15-21], which is similar to that observed in our case series (31.5 years). Two patients in our study were aged 47 years or older, a finding that corroborates the statement that SPN does not occur exclusively in young individuals of reproductive age. β-estrogen and progesterone receptors found in SPN may be the cause of the predominance of the disease among young women, including tumor growth in a favorable hormonal environment such as pregnancy[26,27]. In the present study, a 20-year-old woman in the 8th week of pregnancy with an incidental diagnosis of SPN (6 cm) in the head of the pancreas during an evaluation of urinary infection underwent PPD without harm to the pregnancy. However, the occurrence of SPN in men suggests that the disease is not exclusively hormone-dependent[27,28].

The presence of symptoms in patients with SPN is related to the size of the abdominal mass, which compresses adjacent organs, and with the location of the mass, a tumor in the head of the pancreas may cause abdominal pain and jaundice[15,17,20,21]. Two patients (14.3%) in the present study had epigastric pain and nausea (one patient) and jaundice associated with weight loss (one patient). The former patient's symptoms resulted from the compression of the stomach by an 11 cm SPN in the body of the pancreas, while the latter patient had a 9.5 cm SPN resting on the head of the pancreas. Most patients with SPN are asymptomatic and have an incidental diagnosis, similar to the cases of 85.7% of patients in the present study (Table 1)[20]. Torres *et al*[19], in a retrospective multicenter study of 16 patients with SPN located in the head of the pancreas, did not observe any case of jaundice, even for lesions ≥ 10 cm in diameter.

SPN is malignant but indolent when compared to other pancreatic tumors. Tumor aggressiveness occurs in 10%-15% of the cases and is detected either at diagnosis or during disease progression[16,25]. Characteristics of aggressiveness include invasion of nearby organs or of vascular structures, hepatic metastasis, and relapse after treatment[5,15,16,20,21]. There are reports in the literature that an incomplete capsule and solid components larger than cystic components on imaging exams are related to greater aggressiveness[25,29]. Tumor size appears to have a stronger association with aggressive SPNs. Butte *et al*[5] suggest that SPNs ≥ 7.8 cm have a greater correlation with malignancy ($P < 0.005$). Kang *et al*[23] studied 351 patients and multivariate analysis showed a correlation between tumors > 8 cm and relapse ($P < 0.0018$). In the present study, 42.8% of patients had SPN ≥ 9 cm but this characteristic was not found to be correlated with the presence of distant or lymph node metastases or with disease recurrence, including the two cases with vascular invasion.

Table 6 Incidental and symptoms in patients with solid pseudopapillary neoplasms (literature data)

Ref.	Year	*n*	Study	Incidental (%)	Ap (%)	Other findings (%)
Papavramidis *et al*[15]	2006	718	R	15.5	46.5	Mass 34.8
Lubezky *et al*[16]	2017	32	R	28	48	Unspecific 24
Song *et al*[17]	2017	53	R	39.6	37.7	Mass 30.2
Wright *et al*[6]	2019	78	R	30.7	42.3	Nauseas 14.1
Lin *et al*[18]	2019	60	R	65	31.7	Strain 2.2
Torres *et al*[19]	2019	16	R	-	87.5	Mass 12.5
Liu *et al*[20]	2019	243	R	63.4	19	Unspecific
Farhat *et al*[21]	2020	10	R	20	40	Mass 40

R: Retrospective study; Ap: Abdominal pain.

Surgery is the treatment of choice for SPN and over 95% of tumors can be resected (R0). Surgery can be curative even with distant metastases[20]. Despite the indolent behavior seen in the present study, this neoplasm can invade nearby structures and organs. All patients in our study were treated with primary surgery (R0 resection), even the cases that required extensive resection of blood vessels (common hepatic artery and superior mesenteric vein) and of organs such as the transverse colon, stomach, and spleen, and the surgical treatment was effective. In a meta-analysis of highly heterogeneous retrospective studies, Yepuri *et al*[30] observed an SPN recurrence of 2% after surgical resection. Additionally, it was found that factors such as R1 resection, positive lymph nodes, and the male gender were associated with increased recurrence risks. The mean time for recurrence was 41 mo, with a rate of 71% in the first 5 years, 25% between 5-10 years, and 4% (two cases) after 10 years. In the present study in which all patients were female, there was no disease recurrence during follow-up (Table 5), not even in the case of one patient who was followed up for 13 years. The disease-free survival of > 5 years in 100% of the patients may be attributed to the absence of compromised lymph nodes, affected lymphatic vessels or perineural involvement (Table 4), and particularly to the thorough preoperative staging, which made surgeries with R0 margins possible, even when those margins were extensive and included the resection of large vessels.

The development of DM after DP is debatable. Studies have shown a low incidence of DM in these patients, between 4.8 and 8%[30]. The risk for DM appears to increase 40%-50% seven years after surgery, which may be correlated with the amount of resected pancreas tissue[31]. However, obesity appears to also influence the risk. It is not known how much remaining pancreas tissue is required for normal glucose metabolism to be maintained. In our study, 25% of the patients who underwent DP had DM; they had 9 to 15 cm of their pancreas resected and were not obese.

For years SPN has been described as rare, representing approximately 3% of all pancreatic tumors[20]. An increase in the number of diagnosed cases has been reported in specialized centers. In the literature, the incidence varies from 2.5% to 5.1% [16,21,32]. In the present study, the incidence was 6.3%, *i.e.*, a significant increase in notified cases. In a series of cases from 1988 to 2008 at the Johns Hopkins Hospital, 41 out of the 78 patients were diagnosed in the last 10 years of the series[6]. Other studies indicate a recent increase in the number of diagnoses, with over 60% of cases reported in the last decade[15,22,23]. In the present study, 2 patients were diagnosed and treated before 1998 and the others were diagnosed after 2012 (Table 5). The authors indicate some factors that may contribute to the appearance of new cases, including greater access to specialist physicians, radiologists specialized in diseases of the pancreas, and advances in imaging methods such as CT, MRI, and endosonography. Social programs such as women's health programs — in which screening exams, *e.g.*, US — are performed, explain the finding of pancreatic tumors in asymptomatic patients.

CONCLUSION

In this study, SPN occurred in young women, most of which were asymptomatic. Surgery was curative for all patients and the tumors exhibited an indolent behavior in all cases.

ARTICLE HIGHLIGHTS

Research background

Study with 14 patients, operated in a single center, regardless of the size of the tumor, obtained Margins R0, disease-free survival in 100% of cases, with a follow-up longer than 5 years.

Research motivation

Surgical treatment is the main pillar of cure for solid papillary neoplasm of the pancreas. The challenge is to improve access to imaging exams, screening programs and surgeons specialized in pancreatic surgery. The importance is in early diagnosis, greater chance of R0 surgery with less resection of other structures and consequently greater survival.

Research objectives

Show that solid pseudopapillary neoplasms, even when in large volumes and with the need to dry other noble structures, such as large vessels, has the possibility of cure in surgery. We were able to demonstrate in this study, with R0 surgeries and evidencing deonca-free survival and quality of life.

Research methods

Retrospective study with analysis of a prospectively filled database.

Research results

This study was made up of female patients in its entirety, corroborating the literature on being a more frequent neoplasm in women. Approximately 85% asymptomatic, incidental discovery. In our series, we have seen an increase in cases since 2012. Perhaps due to greater accessibility to imaging methods. The fact that women have specific programs for women's health with periodic examinations and screening, may contribute to the discovery of tumors such as Solid papillary neoplasm of the pancreas in asymptomatic patients, and that we are facing an underreported neoplasm, but it is evident that we need more studies to affirm this.

Research conclusions

One theory would be that of an underdiagnosed neoplasm.

Research perspectives

Follow-up of operated patients to assess the natural history of the disease.

REFERENCES

1 **Frantz VK**. Atlas of tumor patology. Tumor of the pancreas. Washington, DC: US force Armed Force institute of pathology, 1959: 32-33

2 **Kosmahl M**, Seada LS, Jänig U, Harms D, Klöppel G. Solid-pseudopapillary tumor of the pancreas: its origin revisited. *Virchows Arch* 2000; **436**: 473-480 [PMID: 10881741 DOI: 10.1007/s004280050475]

3 **de Castro SM**, Singhal D, Aronson DC, Busch OR, van Gulik TM, Obertop H, Gouma DJ. Management of solid-pseudopapillary neoplasms of the pancreas: a comparison with standard pancreatic neoplasms. *World J Surg* 2007; **31**: 1130-1135 [PMID: 17429567 DOI: 10.1007/s00268-006-0214-2]

4 **Scholten L**, van Huijgevoort NCM, van Hooft JE, Besselink MG, Del Chiaro M. Pancreatic Cystic Neoplasms: Different Types, Different Management, New Guidelines. *Visc Med* 2018; **34**: 173-177 [PMID: 30182024 DOI: 10.1159/000489641]

5 **Butte JM**, Brennan MF, Gönen M, Tang LH, D'Angelica MI, Fong Y, Dematteo RP, Jarnagin WR, Allen PJ. Solid pseudopapillary tumors of the pancreas. Clinical features, surgical outcomes, and long-term survival in 45 consecutive patients from a single center. *J Gastrointest Surg* 2011; **15**: 350-

357 [PMID: 20824369 DOI: 10.1007/s11605-010-1337-1]
6 **Wright MJ**, Javed AA, Saunders T, Zhu Y, Burkhart RA, Yu J, He J, Cameron JL, Makary MA, Wolfgang CL, Weiss MJ. Surgical Resection of 78 Pancreatic Solid Pseudopapillary Tumors: a 30-Year Single Institutional Experience. *J Gastrointest Surg* 2020; **24**: 874-881 [PMID: 31073801 DOI: 10.1007/s11605-019-04252-7]
7 **Guo N**, Zhou QB, Chen RF, Zou SQ, Li ZH, Lin Q, Wang J, Chen JS. Diagnosis and surgical treatment of solid pseudopapillary neoplasm of the pancreas: analysis of 24 cases. *Can J Surg* 2011; **54**: 368-374 [PMID: 21939604 DOI: 10.1503/cjs.011810]
8 **Mao C**, Guvendi M, Domenico DR, Kim K, Thomford NR, Howard JM. Papillary cystic and solid tumors of the pancreas: a pancreatic embryonic tumor? *Surgery* 1995; **118**: 821-828 [PMID: 7482268 DOI: 10.1016/s0039-6060(05)80271-5]
9 **Reddy S**, Cameron JL, Scudiere J, Hruban RH, Fishman EK, Ahuja N, Pawlik TM, Edil BH, Schulick RD, Wolfgang CL. Surgical management of solid-pseudopapillary neoplasms of the pancreas (Franz or Hamoudi tumors): a large single-institutional series. *J Am Coll Surg* 2009; **208**: 950-7; discussion 957 [PMID: 19476869 DOI: 10.1016/j.jamcollsurg.2009.01.044]
10 **McMillan MT**, Malleo G, Bassi C, Allegrini V, Casetti L, Drebin JA, Esposito A, Landoni L, Lee MK, Pulvirenti A, Roses RE, Salvia R, Vollmer CM Jr. Multicenter, Prospective Trial of Selective Drain Management for Pancreatoduodenectomy Using Risk Stratification. *Ann Surg* 2017; **265**: 1209-1218 [PMID: 27280502 DOI: 10.1097/SLA.0000000000001832]
11 **Bassi C**, Marchegiani G, Dervenis C, Sarr M, Abu Hilal M, Adham M, Allen P, Andersson R, Asbun HJ, Besselink MG, Conlon K, Del Chiaro M, Falconi M, Fernandez-Cruz L, Fernandez-Del Castillo C, Fingerhut A, Friess H, Gouma DJ, Hackert T, Izbicki J, Lillemoe KD, Neoptolemos JP, Olah A, Schulick R, Shrikhande SV, Takada T, Takaori K, Traverso W, Vollmer CR, Wolfgang CL, Yeo CJ, Salvia R, Buchler M; International Study Group on Pancreatic Surgery (ISGPS). The 2016 update of the International Study Group (ISGPS) definition and grading of postoperative pancreatic fistula: 11 Years After. *Surgery* 2017; **161**: 584-591 [PMID: 28040257 DOI: 10.1016/j.surg.2016.11.014]
12 **Dindo D**, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg* 2004; **240**: 205-213 [PMID: 15273542 DOI: 10.1097/01.sla.0000133083.54934.ae]
13 **Vin Y**, Sima CS, Getrajdman GI, Brown KT, Covey A, Brennan MF, Allen PJ. Management and outcomes of postpancreatectomy fistula, leak, and abscess: results of 908 patients resected at a single institution between 2000 and 2005. *J Am Coll Surg* 2008; **207**: 490-498 [PMID: 18926450 DOI: 10.1016/j.jamcollsurg.2008.05.003]
14 **Tjaden C**, Hassenpflug M, Hinz U, Klaiber U, Klauss M, Büchler MW, Hackert T. Outcome and prognosis after pancreatectomy in patients with solid pseudopapillary neoplasms. *Pancreatology* 2019; **19**: 699-709 [PMID: 31227367 DOI: 10.1016/j.pan.2019.06.008]
15 **Papavramidis T**, Papavramidis S. Solid pseudopapillary tumors of the pancreas: review of 718 patients reported in English literature. *J Am Coll Surg* 2005; **200**: 965-972 [PMID: 15922212 DOI: 10.1016/j.jamcollsurg.2005.02.011]
16 **Lubezky N**, Papoulas M, Lessing Y, Gitstein G, Brazowski E, Nachmany I, Lahat G, Goykhman Y, Ben-Yehuda A, Nakache R, Klausner JM. Solid pseudopapillary neoplasm of the pancreas: Management and long-term outcome. *Eur J Surg Oncol* 2017; **43**: 1056-1060 [PMID: 28238521 DOI: 10.1016/j.ejso.2017.02.001]
17 **Song H**, Dong M, Zhou J, Sheng W, Zhong B, Gao W. Solid Pseudopapillary Neoplasm of the Pancreas: Clinicopathologic Feature, Risk Factors of Malignancy, and Survival Analysis of 53 Cases from a Single Center. *Biomed Res Int* 2017; **2017**: 5465261 [PMID: 29094047 DOI: 10.1155/2017/5465261]
18 **Lin X**, Lin R, Lu F, Chen Y, Huang H. Surgical Management of Solid Pseudopapillary Neoplasms of Pancreas: A Single-Center Experience of 60 Patients. *Dig Surg* 2020; **37**: 348-354 [PMID: 31958791 DOI: 10.1159/000505062]
19 **Torres OJM**, Rezende MB, Waechter FL, Neiva RF, Moraes-Junior JMA, Torres CCS, Fernandes ESM. Pancreatoduodenectomy for solid pseudopapillary tumor of the pancreas: a multi-institution study. *Arq Bras Cir Dig* 2019; **32**: e1442 [PMID: 31460602 DOI: 10.1590/0102-672020190001e1442]
20 **Liu M**, Liu J, Hu Q, Xu W, Liu W, Zhang Z, Sun Q, Qin Y, Yu X, Ji S, Xu X. Management of solid pseudopapillary neoplasms of pancreas: A single center experience of 243 consecutive patients. *Pancreatology* 2019; **19**: 681-685 [PMID: 31281058 DOI: 10.1016/j.pan.2019.07.001]
21 **Farhat W**, Ammar H, Amine Said M, Mizouni A, Bouazzi A, Abdessaied N, Ben Mabrouk M, Ben Ali A. Solid pseudopapillary neoplasm of the pancreas: a report of 10 cases and literature review. *ANZ J Surg* 2020; **90**: 1683-1688 [PMID: 31989788 DOI: 10.1111/ans.15701]
22 **Gordon-Dseagu VL**, Devesa SS, Goggins M, Stolzenberg-Solomon R. Pancreatic cancer incidence trends: evidence from the Surveillance, Epidemiology and End Results (SEER) population-based data. *Int J Epidemiol* 2018; **47**: 427-439 [PMID: 29149259 DOI: 10.1093/ije/dyx232]
23 **Kang CM**, Choi SH, Kim SC, Lee WJ, Choi DW, Kim SW; Korean Pancreatic Surgery Club. Predicting recurrence of pancreatic solid pseudopapillary tumors after surgical resection: a multicenter analysis in Korea. *Ann Surg* 2014; **260**: 348-355 [PMID: 24743622 DOI: 10.1097/SLA.0000000000000583]
24 **Law JK**, Ahmed A, Singh VK, Akshintala VS, Olson MT, Raman SP, Ali SZ, Fishman EK, Kamel I, Canto MI, Dal Molin M, Moran RA, Khashab MA, Ahuja N, Goggins M, Hruban RH, Wolfgang CL,

Lennon AM. A systematic review of solid-pseudopapillary neoplasms: are these rare lesions? *Pancreas* 2014; **43**: 331-337 [PMID: 24622060 DOI: 10.1097/MPA.0000000000000061]

25 **You L**, Yang F, Fu DL. Prediction of malignancy and adverse outcome of solid pseudopapillary tumor of the pancreas. *World J Gastrointest Oncol* 2018; **10**: 184-193 [PMID: 30079144 DOI: 10.4251/wjgo.v10.i7.184]

26 **Farrell JJ**. Prevalence, Diagnosis and Management of Pancreatic Cystic Neoplasms: Current Status and Future Directions. *Gut Liver* 2015; **9**: 571-589 [PMID: 26343068 DOI: 10.5009/gnl15063]

27 **Morales A**, Duarte-Rojo A, Angeles-Angeles A, Mery CM, Ruíz-Molina JM, Díaz-Sánchez V, Robles-Díaz G. The beta form of the estrogen receptor is predominantly expressed in the papillary cystic neoplasm of the pancreas. *Pancreas* 2003; **26**: 258-263 [PMID: 12657952 DOI: 10.1097/00006676-200304000-00009]

28 **Machado MC**, Machado MA, Bacchella T, Jukemura J, Almeida JL, Cunha JE. Solid pseudopapillary neoplasm of the pancreas: distinct patterns of onset, diagnosis, and prognosis for male versus female patients. *Surgery* 2008; **143**: 29-34 [PMID: 18154930 DOI: 10.1016/j.surg.2007.07.030]

29 **Reddy S**, Wolfgang CL. Solid pseudopapillary neoplasms of the pancreas. *Adv Surg* 2009; **43**: 269-282 [PMID: 19845185 DOI: 10.1016/j.yasu.2009.02.011]

30 **Yepuri N**, Naous R, Meier AH, Cooney RN, Kittur D, Are C, Jain A, Dhir M. A systematic review and meta-analysis of predictors of recurrence in patients with Solid Pseudopapillary Tumors of the Pancreas. *HPB (Oxford)* 2020; **22**: 12-19 [PMID: 31350105 DOI: 10.1016/j.hpb.2019.06.005]

31 **King J**, Kazanjian K, Matsumoto J, Reber HA, Yeh MW, Hines OJ, Eibl G. Distal pancreatectomy: incidence of postoperative diabetes. *J Gastrointest Surg* 2008; **12**: 1548-1553 [PMID: 18543045 DOI: 10.1007/s11605-008-0560-5]

32 **Lam KY**, Lo CY, Fan ST. Pancreatic solid-cystic-papillary tumor: clinicopathologic features in eight patients from Hong Kong and review of the literature. *World J Surg* 1999; **23**: 1045-1050 [PMID: 10512945 DOI: 10.1007/s002689900621]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2021 June 15; 13(6): 600-611

DOI: 10.4251/wjgo.v13.i6.600

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Observational Study

Serum vascular endothelial growth factor as a tumor marker for hepatocellular carcinoma in hepatitis C virus-related cirrhotic patients

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Author contributions: Alzamzamy A contributed to data acquisition, data analysis, interpretation, statistics, and drafting of the manuscript; Aboubakr A, Abd Elraouf M, Eltoukhy H edited the manuscript and supervised the study; Alzamzamy A, Elsayed H designed the study; Megahed T contributed to laboratory work and results analysis; all authors approved the final version of the manuscript.

Institutional review board statement: The study was reviewed and approved by (Egypt Center for research and regenerative medicine, and Al Azhar University) Institutional Review Board, No. IRB 00012517.

Informed consent statement: All study participants or their legal guardian provided informed written consent about personal and medical data collection prior to study enrolment.

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Abstract

BACKGROUND

Hepatocellular carcinoma (HCC) accounts for 8.2% of all cancer-related deaths worldwide. Being a vascular tumor, vascular endothelial growth factor (VEGF) plays a vital role in HCC pathogenesis, growth, and spread.

AIM

To determine the accuracy of serum VEGF and VEGF/platelet (PLT) as tumor markers in the early detection of HCC cases in patients with hepatitis C virus (HCV)-related liver cirrhosis.

METHODS

We conducted a case-control study with HCV patients from the outpatient and inpatient hepatology clinics. Patients were classified into three groups: (1) HCC group; (2) Cirrhosis group; and (3) HCV without cirrhosis (control group). Patients were clinically evaluated, and blood samples were drawn for the analysis; serum VEGF levels were measured by a specific VEGF human recombinant enzyme-linked immunosorbent assay kit. Data from the three study groups were compared by the one-way analysis of variance or Kruskal-Wallis test. Receivers operating characteristic curves were constructed to determine the

Conflict-of-interest statement: None to declare.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at [dr_zamzamy@hotmail.com]. Participants gave informed consent for data sharing and risk of identification is low. No additional data are available.

STROBE statement: The authors have read the STROBE statement—checklist of items, and the manuscript was prepared and revised according to the STROBE statement—checklist of items.

Manuscript source: Invited manuscript

Specialty type: Oncology

Country/Territory of origin: Egypt

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Received: January 27, 2021
Peer-review started: January 27, 2021
First decision: March 8, 2021
Revised: April 13, 2021
Accepted: May 22, 2021
Article in press: May 22, 2021
Published online: June 15, 2021

optimal cut-off values of alpha fetoprotein (AFP), VEGF, and VEGF/PLT that provided the best diagnostic accuracy. The sensitivity and specificity at the optimal cut-off value of each biomarker were then calculated.

RESULTS

This study included one hundred patients (HCC, cirrhosis, and control groups: n = 40, 30, 30, respectively). HCC patients had significantly higher serum VEGF and VEGF/PLT levels than the non-HCC groups (P = 0.001). Serum VEGF and VEGF/PLT showed significant positive correlations with and HCC tumor size, stage, vascular invasion, and Child-Pugh classification. Moreover, a VEGF cut-off the value of 250 pg/mL provided 80% sensitivity and 81.7% specificity for discriminating HCC patient from non-HCC patients. Similarly, the ratio of VEGF/PLT provided sensitivity and specificity of 77.5% and 80%, respectively which is higher than the accuracy provided by AFP. The combination of AFP, VEGF, and VEGF/PLT increases the accuracy of diagnosing HCC to > 95%.

CONCLUSION

In HCV patients, serum VEGF and VEGF/PLT separately or in combination with AFP are reliable biomarkers for early and accurate HCC diagnosis.

Key Words: Hepatocellular carcinoma; Vascular endothelial growth factor; Biomarkers; Diagnosis

Core Tip: We conducted an observational study with 100 patients to assess the usefulness of serum vascular endothelial growth factor (VEGF) and VEGF/platelet (PLT) as tumor markers for hepatocellular carcinoma (HCC) diagnosis in hepatitis C virus-related cirrhotic patients, and comparing them to serum alpha fetoprotein (AFP); the conventional marker of HCC. It was found that serum VEGF and VEGF/PLT appear to be additional diagnostic markers for HCC detection and prognostic markers during HCC patients follow up. Also, combined measurement of serum VEGF, VEGF/PLT and AFP significantly increase the sensitivity, specificity and accuracy in detection of HCC among cirrhotic patients rather than using of AFP, VEGF, or VEGF/PLT separately.

Citation: Alzamzamy A, Elsayed H, Abd Elraouf M, Eltoukhy H, Megahed T, Aboubakr A. Serum vascular endothelial growth factor as a tumor marker for hepatocellular carcinoma in hepatitis C virus-related cirrhotic patients. *World J Gastrointest Oncol* 2021; 13(6): 600-611
URL: https://www.wjgnet.com/1948-5204/full/v13/i6/600.htm
DOI: https://dx.doi.org/10.4251/wjgo.v13.i6.600

INTRODUCTION

Liver cancer is the sixth most prevalent cancer worldwide with about 841000 new cases in 2018 according to the recent Global Cancer Observatory estimates[1]. Also, liver cancer is the fourth leading cause of cancer deaths worldwide accounting for 782000 deaths annually. Hepatocellular carcinoma (HCC) comprises about 75% to 85% of liver cancers[1].

Most of HCC patients are diagnosed at a late stage which makes HCC associated with low survival and poor prognosis[2]. The available screening methods for early detection of HCC in patients with liver cirrhosis are inadequate. The most established and well-studied HCC biomarker is serum alpha fetoprotein (AFP), and abdominal ultrasound performed every six months. However, this screening method has several limitations: (1) The limited sensitivity of AFP; AFP is not secreted in all cases of HCC and may be normal in about 40% of patients with early HCC; and (2) Abdominal ultrasound is highly dependent on the skills and experience of the examiner[3,4]. Recently, an unmet need to find novel tumor markers with high sensitivity and specificity for early diagnosis of HCC and to differentiate HCC from benign lesions

P-Reviewer: Fernandes SA
S-Editor: Fan JR
L-Editor: A
P-Editor: Li JH

has been demonstrated.

Most of the current HCC biomarkers have limited sensitivity or specificity which can be explained by the substantial heterogeneity of HCC. Therefore, it is suggested that the combination of two or three biomarkers with high specificity might provide early HCC optimal diagnosis.

Vascular endothelial growth factor (VEGF) is responsible for angiogenesis, and it appears to be the most important angiogenic factor in HCC. Hypoxia was suggested as the central stimulus of angiogenesis and liver carcinogenesis *via* upregulation of VEGF gene expression.

In HCC patients, a positive correlation between platelet count and serum VEGF was found[5]. Platelets store and transport VEGF to target cells; therefore, they have been reported to play a substantial role in tumor angiogenesis, progression, and prognosis. Serum VEGF/platelet (PLT) was used to overcome the variations in serum VEGF levels in HCC patients with different platelet counts. Therefore, VEGF/PLT has been proposed as an indirect theoretical estimate of VEGF in platelets.

Because HCC is characterized by high vascularity and the tumor relies on the formation of new blood vessels to grow and spread, angiogenic factors, such as VEGF play an important role in HCC pathogenesis[6]. VEGF acts on the VEGF receptor-2 (VEGFR2) and leads to induction of cancer stem cells and formation of microscopic blood vessels that facilitate tumor growth, invasion, and spread[7,8]. The role of VEGF in tumor pathogenesis, invasion, and spread has been described in many types of cancer, including HCC. Several studies report VEGF overexpression in HCC. Matsui *et al*[9] found higher levels of VEGF in patients with more advanced stages of the disease, larger tumor sizes, and more vascular invasion. As a prognostic factor, overexpression of VEGF was associated with an increase in vascular invasion and poor overall survival of HCC patients[10].

Cumulative evidence from the literature supports the idea that VEGF might be useful as a tumor marker for early HCC detection. Although several studies have been conducted to measure VEGF levels in HCC patients in different clinical settings, to the best of our knowledge few data are available about the HCC diagnostic performance of VEGF. Therefore, we conducted this observational study to assess the usefulness of serum VEGF and VEGF/PLT as tumor markers in patients with hepatitis C virus (HCV)-related liver cirrhosis and HCC and compare them to serum AFP, the conventional marker of HCC. Also, this study aimed to verify the possibility of using combined measurements of serum VEGF, VEGF/PLT, and AFP for HCC diagnosis.

MATERIALS AND METHODS

We report this manuscript according to the Strengthening the Reporting of Observational Studies in Epidemiology statement guidelines[11]. The study was approved by the ethics committee of the Faculty of Medicine, Al Azhar University and Egypt center for research and regenerative medicine, Cairo, Egypt.

Study design, setting, and duration

We performed a case-control study at the inpatient and outpatient clinics of the Gastroenterology and Hepatology Department, Maadi Armed Forces Medical Complex, Cairo, Egypt. The study was conducted on patients attending the study setting from January 2015 to June 2017.

Eligibility criteria of the study population

Study subjects were selected according to several criteria: (1) Male and female patients aged between 18 and 60 years; and (2) Patients with chronic HCV infection confirmed by seropositive anti-HCV antibody detection using the third generation enzyme-linked immunosorbent assay (ELISA) and HCV-RNA seropositive histopathological criteria indicating chronic HCV liver disease.

We excluded patients with certain conditions: (1) Patients with any type of cancer other than HCC (such as breast, lung, brain, gastrointestinal, renal, bladder and ovarian); (2) Patients with collagen diseases (rheumatoid arthritis, psoriasis, and systemic sclerosis); (3) Patients with heart failure, chronic obstructive pulmonary disease, pulmonary hypertension and, acute respiratory distress syndrome; (4) Patients with diabetes mellitus, diabetic retinopathy, age-related macular degeneration; (5) Patients with sickle cell anaemia, pregnancy and preeclampsia; (6) Patients with co-infection with hepatitis B virus; and (7) Patients with alcoholic liver disease.

Study groups

The study included three groups: (1) HCC group: This group included patients with HCC secondary to liver cirrhosis and chronic HCV, confirmed by HCV RNA polymerase chain reaction (PCR), abdominal ultrasound (US), triphasic spiral computed tomography (CT) of the abdomen, and/or dynamic abdominal magnetic resonance imaging (MRI); (2) Cirrhosis group: This group included patients with HCV-related liver cirrhosis without HCC. The diagnosis of cirrhosis was based on the clinical picture, US and laboratory findings suggestive liver cirrhosis; and (3) This group included patients with chronic HCV without cirrhosis or HCC.

Recruitment of the control group

The control group consisted of HCV patients who were free from cirrhosis and HCC. We used the medical records of the participants to confirm that they did not have cirrhosis or HCC.

Baseline assessment of the study participants

Clinical assessment: All participants underwent a full clinical assessment, and medical histories were obtained. The clinical evaluation focused on the assessment of several factors: (1) Jaundice; (2) Ascites; (3) Palmar erythema; (4) Variceal bleeding; (5) Spider nevi; (6) Pallor; (7) Flapping tremors; and (8) Hepatic encephalopathy.

Laboratory assessment: For all participants, the following laboratory tests were done: (1) HCV viral markers: Anti-HCV antibody, HCV-RNA based on PCR, hepatitis B surface antigen, hepatitis B core antibody, and human immunodeficiency Ab complete blood picture; (2) Liver biochemical profile: Alanine aminotransferase, aspartate transaminase, alkaline phosphatase, gamma glutamyl transferase, serum albumin, serum bilirubin (total and direct), prothrombin time, and international normalized ratio; (3) Renal function tests: blood urea and serum creatinine; (4) Fasting blood glucose; (5) Postprandial blood glucose; (6) Glycosylated hemoglobin (normal < 6%); (7) Quantitative measurement of serum AFP; (8) Quantitative measurement of serum VEGF using ELISA kits; and (9) Quantitative measurement of serum VEGF/PLT by dividing serum VEGF concentration by the platelet count.

Imaging and radiographic assessment

For imaging, all participants underwent abdominal US with emphasis on the signs suggestive of liver cirrhosis: (1) Any focal lesion (its number, site, and size); (2) Portal vein (patency and its diameter); (3) Splenic size; and (4) The presence of ascites (mild, moderate, severe).

Also, abdominal triphasic spiral CT with or without dynamic abdomen MRI was done to confirm the diagnosis of any suspected focal lesions suspected based on the US.

Determination of VEGF serum levels

We measured the total serum VEGF with a human recombinant ELISA kit that is designed to measure human VEGF concentration in serum (Glory Science Co., Ltd, United States). We followed the established principals and the steps of VEGF kit assay according to the manufacturer`s instructions.

Statistical analysis

Categorical data were summarized as frequencies and percentages. While continuous data were initially tested for normality using the Kolmogorov-Smirnov test. Continuous data were presented as means and standard deviations. For comparison of categorical variables, we used chi-square and Fischer's exact tests. For comparison of two means, the student's *t*- and Mann-Whitney tests were used for the normally and non-normally distributed data, respectively. To compare the quantitative variables between the three groups, we used either a one-way analysis of variance (ANOVA) followed by Tukey`s post-hoc or the Kruskal-Wallis test according to the type and distribution of the data. To study the relationship between two variables, Pearson's correlation test was used, and the correlation coefficient (r) was calculated. To determine the optimal cut-off point for serum VEGF and VEGF/PLT ratio in which these measures achieved the highest sensitivity and specificity in diagnosing HCC, we constructed a receiver operating characteristic curve (ROC) which allowed us to plot the sensitivity against the 1-specificity at each point. An alpha level below 0.05 was considered statistically significant. All analyses were done using the Statistical Package for Social Science (SPSS software version 18 for Windows).

RESULTS

Characteristics of the study population

Our study included 100 participants (HCC group/group I: n = 40, cirrhosis group/group II: n = 30, and control group/group III: n = 30). Of them, 67 patients were males, and 33 were females. The demographic characteristics of the study groups are shown in Table 1. There was no statistically significant difference between the three studied groups as regard age and gender (Table 1). Moreover, there was no significant correlation between VEGF, AFP, and VEGF/PLT values and age and sex of the patients. Also, there was no significant correlation between tumor characteristics by Barcelona Clinic Liver Cancer (BCLC) and age and sex of the patients.

The Child-Pugh classification for group I (HCC group) was A for 24 patients, B for 11 patients, and C for five patients while the Child-Pugh classification in group II (cirrhosis group) was A for 19 patients, B for eight patients, and C for three patients (Table 1).

In HCC group, 20 patients were classified as stage I, five patients as stage II, three patients as stage IIIA, 11 patients as stage IIIB, and one patient as stage IVB with respect to tumor/node/metastasis (TNM). While six patients were classified as stage 0 disease, 13 patients as stage A, eight patients as stage B, 11 patients as stage C, and two patients as stage D with respect to the BCLC tumor stage. Also, 12 patients with vascular invasion were noted, and only one patient had distal metastasis.

Biomarker levels in the study groups

HCC group had significantly higher AFP levels than the two non-HCC groups (cirrhosis group and control group). Moreover, the VEGF levels were significantly higher in the HCC group when compared with the cirrhosis group (1409 pg/mL *vs* 233.3 pg/mL) and when compared with the control group (1409 pg/mL *vs* 204 pg/mL). The ratio of VEGF/PLT was 13.2 in the HCC group, which was much higher than the 1.68 reported in the cirrhosis group, and the 0.95 reported in the control group. The biomarker levels in the three study groups are shown in Table 2.

Statistically highly significant differences in the levels of the studied markers (AFP, VEGF, and VEGF/PLT) among the HCC, the cirrhotic, and the control groups were found.

Diagnostic performance of the AFP, VEGF, and VEGF/PLT for detection of HCC

At a cut-off value of 40 ng/mL, serum AFP provided an accuracy of 74.1% when diagnosing HCC (a sensitivity of 65% and specificity of 83.3%). At a cut-off value of 250 pg/mL, serum VEGF provided 80% and 81.7% sensitivity and specificity, respectively, for diagnosing HCC. At a cut-off value of 2.31, serum VEGF/PLT provided a sensitivity and specificity of 77.5% and 80%, respectively. When the three parameters were combined, the highest accuracy was achieved with a sensitivity of 92.5% and a specificity of 98.3%. The ROC curve of the three HCC diagnostic markers and their combinations is shown in Figure 1. The diagnostic performance of the three parameters is shown in Table 3.

VEGF showed the highest sensitivity among the serum tumor markers. Also, ROC curves for the three studied tumor markers indicated that the specificity of VEGF was very high and other serum tumor markers were similar.

The area under the ROC curve (AUC) for serum VEGF was 0.859, while the AUC values for serum AFP and VEGF/PLT were 0.754 and 0.842, respectively. In addition, the accuracy of VEGF was 80.84%, while that of AFP and VEGF/PLT was 74.17% and 78.75%, respectively.

For diagnosis of HCC, serum VEGF showed higher sensitivity than the other tumor markers and it had the largest AUC on ROC analysis in addition to the highest accuracy. These results indicate that the serum VEGF level was more useful for the diagnosis of HCC than the other two tumor markers (serum AFP and VEGF/PLT) in patients with HCV-related liver cirrhosis

In this study, a significant increase in the AUC, sensitivity, specificity, accuracy, and positive and negative predictive values for detection of HCC in cirrhotic patients was detected, when we use combined measurements of serum VEGF, VEGF/PLT, and AFP rather than using AFP, VEGF, or VEGF/PLT separately (Table 3).

Correlations between the tumor characteristics and the AFP, VEGF, and VEGF/PLT

AFP was significantly correlated with the size of the tumor and the BCLC stage of the tumor but not with the TNM staging. Both VEGF and VEGF/PLT showed significantly positive correlations with the size of the tumor, TNM staging, and BLCL tumor

Table 1 Demographic and clinical characteristics of the three study groups

		HCC group	Cirrhosis group	Control group	*P* value
Gender	Male	30	17	20	> 0.05
	Female	10	13	10	
Age (years)	mean (SD)	60.07 ± 5.34	58.33 ± 8.07	55.2 ± 7.6	> 0.05
	Range	50-68	37-67	34-62	
Child-Pugh score, *n* (%)	A	24 (60.0)	19 (63.3)	Not applicable	
	B	11 (27.5)	8 (26.7)	Not applicable	
	C	5 (12.5)	3 (10.0)	Not applicable	

HCC: Hepatocellular carcinoma; SD: Standard deviation.

Table 2 Biomarker levels in the three study groups

	HCC group			Cirrhosis group			Control group			*P* value
	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	
AFP (ng/mL)	694.6	885.38	169.5	29.71	38.71	12.95	23.58	32.15	9.5	< 0.001[1]
VEGF (pg/mL)	1409	917.40	1250	233.3	196.51	150	204	190.89	110	< 0.001[1]
VEGF/PLT	13.2	10.42	10.64	1.68	1.40	1.18	0.95	0.71	0.72	< 0.001[1]

[1]Kruskal Wallis test.
AFP: Alpha fetoprotein; VEGF: Vascular endothelial growth factor; PLT: Platelet; SD: Standard deviation; HCC: Hepatic cell carcinoma.

Table 3 Diagnostic performance of alpha fetoprotein, vascular endothelial growth factor, and vascular endothelial growth factor/platelet, separately and in combination, in the prediction of hepatocellular carcinoma

	Cut-off point	AUC	Sensitivity	Specificity	Accuracy	PPV	NPV	*P* value
AFP	40	0.754	65.00	83.33	74.17%	72.2	78.1	< 0.0001
VEGF	250	0.859	80.00	81.67	80.84	74.4	86.0	< 0.0001
VEGF/PLT	2.31	0.842	77.50	80.00	78.75	72.1	84.2	< 0.0001
Combination	--	0.953	92.50	98.33	95.42	97.4	95.2	< 0.0001

AUC: Area under the receiver operating characteristic curve; PPV: Positive predictive value; NPV: Negative predictive value; AFP: Alpha fetoprotein; VEGF: Vascular endothelial growth factor; PLT: Platelet.

staging (Table 4).

HCC cases with portal vein thrombosis had significantly higher VEGF (2056.3 *vs* 1231.6; $P = 0.021$) and VEGF/PLT levels (20.85 *vs* 11.35; $P = 0.019$) compared with those with patent portal vein (Table 5). Thus, statistically significant positive correlations between serum VEGF and serum VEGF/PLT levels; and the presence of portal vein thrombosis among HCC cases were found. On the other hand, no significant correlation between serum AFP and PVT was found.

In the patients with HCC, a statistically significant correlation between serum VEGF level and platelet count ($r = 0.668$; $P = 0.02$) was noted (Table 6 and Figure 2). However, no correlation between serum VEGF levels and platelet count in the patients with liver cirrhosis and the control group ($P = 0.970$; $P = 0.781$, respectively) was found (Table 6).

No correlation between serum AFP and VEGF levels among HCC cases was detected (Table 7). Also, no correlation between serum AFP and VEGF/PLT levels among HCC cases was found (Table 8). A statistically significant correlation between serum VEGF and VEGF/PLT levels among HCC cases was shown (Table 9).

Table 4 Correlations between tumor characteristics (tumor size and stage) and each of alpha fetoprotein, vascular endothelial growth factor and vascular endothelial growth factor/ platelet among hepatocellular carcinoma cases

		AFP (ng/mL)	VEGF (pg/mL)	VEGF/PLT
Size of tumor	Correlation coefficient	0.492	0.662	0.483
	P value	0.001	0.0001	0.002
TNM stage of tumor	Correlation coefficient	0.247	0.795	0.696
	P value	0.124	0.0001	0.0001
BCLC stage of tumor	Correlation coefficient	0.382	0.889	0.740
	P value	0.015	0.0001	0.0001

AFP: Alpha fetoprotein; VEGF: Vascular endothelial growth factor; PLT: Platelet; BCLC: Barcelona clinic liver; TNM: Tumor/node/metastasis.

Table 5 Correlations between the presence of portal vein thrombosis and each of serum alpha fetoprotein, vascular endothelial growth factor, and vascular endothelial growth factor/platelet levels among hepatocellular carcinoma cases

	No PVT		PVT		Independent *t*-test	
	Mean	SD	Mean	SD	*t*-value	*P* value
AFP (ng/mL)	580.64	854.69	1143.63	923.42	−1.641	0.109
VEGF (pg/mL)	1231.56	907.97	2056.25	661.94	−2.404	0.021
VEGF/PLT	11.35	9.98	20.85	9.12	−2.446	0.019

AFP: Alpha fetoprotein; VEGF: Vascular endothelial growth factor; PLT: Platelet; PVT: Portal vein thrombosis; SD: Standard deviation.

Table 6 Correlation between serum vascular endothelial growth factor and platelet count among the three study groups

	VEGF					
	HCC group		CLC group		Control group	
	r	*P* value	*r*	*P* value	*r*	*P* value
Platelets	0.668[1]	0.002	-0.007[1]	0.970	0.053[1]	0.781

[1]Spearman correlation coefficients.
VEGF: Vascular endothelial growth factor; CLC: Hepatitis C virus-related liver cirrhosis; HCC: Heptatocellular carcinoma.

Table 7 Correlation between serum alpha fetoprotein and vascular endothelial growth factor levels among hepatocellular carcinoma cases

	AFP (ng/mL)	
	r	*P* value
VEGF (pg/mL)	0.119	0.463[1]

[1]Spearman correlation coefficients.
AFP: Alpha fetoprotein; VEGF: Vascular endothelial growth factor.

DISCUSSION

Summary of the main findings

The main aim of this work was to assess the usefulness of serum VEGF and VEGF/PLT as tumor markers in patients with HCV-related liver cirrhosis and HCC and to compare them with the control group (patients with chronic HCV without cirrhosis or HCC) in order to detect their sensitivity and specificity as diagnostic

Table 8 Correlation between serum alpha fetoprotein and vascular endothelial growth factor/platelet levels among hepatocellular carcinoma cases

	AFP (ng/mL)	
	r	*P* value
VEGF/PLT	0.175	0.280[1]

[1]Spearman correlation coefficients.
AFP: Alpha fetoprotein; VEGF/PLT: Vascular endothelial growth factor/platelet.

Table 9 Positive correlation between serum vascular endothelial growth factor; and vascular endothelial growth factor/platelet levels among hepatocellular carcinoma cases

	VEGF (pg/mL)	
	r	*P* value
VEGF/PLT	0.925	0.000[1]

[1]Spearman correlation coefficients.
VEGF: Vascular endothelial growth factor; PLT: Platelet.

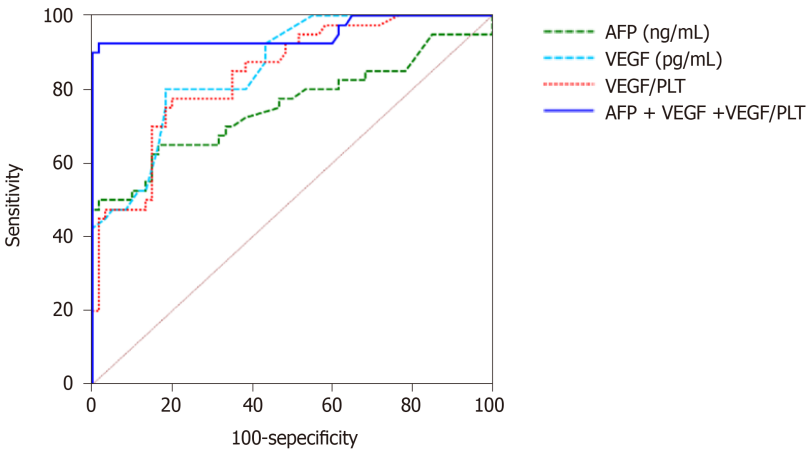

Figure 1 The receivers operating characteristic curve of the three hepatocellular carcinoma diagnostic markers and their combination.
VEGF: Vascular endothelial growth factor; AFP: Alpha fetoprotein; PLT: Platelet.

markers for HCC. These two markers were also compared to AFP, the conventional marker of HCC, and were then correlated with tumor size, stage, vascular invasion, and Child-Pugh classification. Also, this study aimed to verify the possibility of using combined measurement of serum VEGF, VEGF/PLT, and AFP for diagnosing HCC early and accurately.

Our findings showed that HCC patients had significantly higher serum VEGF and VEGF/PLT levels compared with non-HCC patients. Also, serum VEGF and VEGF/PLT levels were positively correlated with the tumor size, stage, vascular invasion and Child-Pugh classification. Serum VEGF and VEGF/PLT achieved higher accuracy (higher sensitivity and specificity) than AFP for diagnosing HCC. Also, the combined measurement of serum VEGF, VEGF/PLT and AFP significantly increased the sensitivity, specificity, accuracy, AUC, and positive and negative predictive values in detection of HCC among cirrhotic patients.

Explanation of the study results

Results of this study can be explained by the essential role of VEGF in HCC pathogenesis and spread. Therefore, high serum VEGF levels in HCC patients were associated with larger tumor size and more advanced disease stages. Moreover, a VEGF cut-off the value of 250 pg/mL provided 80% sensitivity and 81.7% specificity

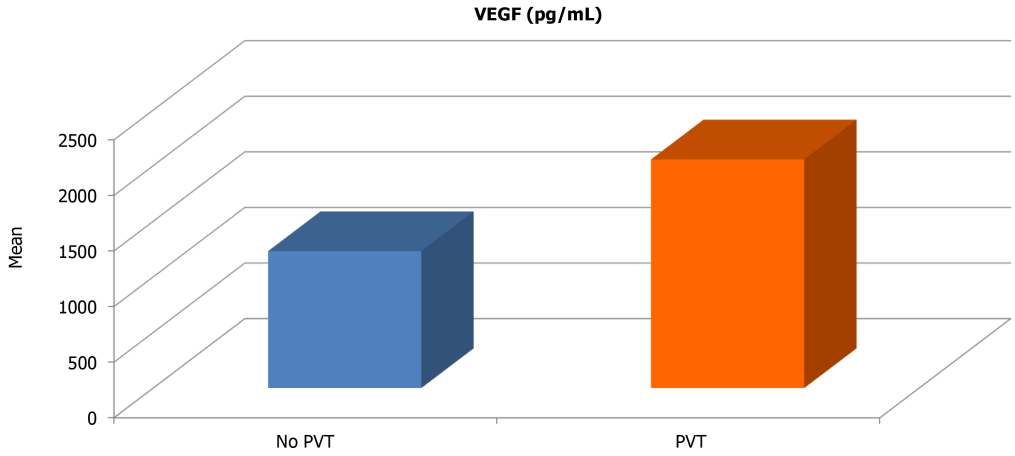

Figure 2 Positive correlation between serum vascular endothelial growth factor and the presence of performance validity testing among hepatocellular carcinoma cases. PVT: Performance validity testing; VEGF: Vascular endothelial growth factor.

for discriminating HCC patients from non-HCC patients. Similarly, the ratio of VEGF/PLT provided sensitivity and specificity of 77.5% and 80%, respectively, which is higher than the accuracy provided by AFP.

It is suggested that hypoxia stimulates angiogenesis and liver carcinogenesis *via* upregulation of *VEGF* gene expression. While the production of AFP occurs due to the de-differentiation of cancer cells, which does not usually occur in early stages since HCC often starts as a well-differentiated tumor and then undergoes de-differentiation as the tumor grows.

A positive correlation between serum VEGF levels and tumor VEGF expression as assessed by immunohistochemical study was reported and suggests that serum VEGF levels at least in part reflects the tumor VEGF expression.

In HCC cases, serum VEGF correlates with the platelet counts. VEGF is produced by tumor cells and is stored and transported by platelets[8,12]. The reservoir of VEGF in platelets might be indicative of HCC angiogenesis and invasion. Serum VEGF/PLT was shown to correlate with HCC, suggesting a role for VEGF/PLT as a standard measure of circulating VEGF[10].

A large body of evidence suggests that VEGF might be used as a tumor marker for HCC. First, HCC is a vascular tumor that depends on angiogenesis for tumor growth and survival. Second, high VEGF levels were associated with poor clinical characteristics of the tumor and poor prognosis; it was reported that HCC patients treated with sorafenib who have higher levels of VEGF had significantly poorer overall survival and less response to the treatment compared to those with low VEGF levels, suggesting that VEGF might be a useful tool for predicting patient response to sorafenib treatment[13]. Third, anti-VEGF agents, such as sorafenib, are effective HCC treatments that could improve patient survival, A recent meta-analysis of seven randomized controlled trials showed that tyrosine kinase inhibitors targeting VEGF were effective for the treatment of unresectable metastatic HCC and that anti-VEGF could extend the overall survival and time till disease progression in HCC patient[14]. Fourth, siRNA silencing of VEGF through hepatic artery perfusion could lead to suppression of HCC proliferation, induction of tumor cell apoptosis, and reduction in tumor angiogenesis[15].

It is evident that VEGF plays two roles in HCC pathogenesis: (1) Formation of blood vessels that lead to tumor growth and spread; and (2) Activation of VEGFR2 induces HCC cancer stem cells that lead to tumor recurrence[16], which makes it a reliable biomarker that is positively correlated with the clinical HCC stage, and is useful for predicting patient response to anti-VEGF treatment predicting tumor recurrence after HCC treatment.

Previous studies

Several previous studies have evaluated the accuracy of VEGF for diagnosing HCC.

According to our results, at a cut-off 250 pg/mL, the sensitivity was 80%, specificity was 81.67%, accuracy was 80.84%, the AUC was 0.859, positive predictive value was 74.4%, and negative predictive value was 86.0%. The present results were comparable to those of Mukozu *et al*[17] who reported a sensitivity of 86.4%, and specificity of

96.2% when the cut-off value was 108 pg/mL; in that study, accuracy was 89.4%, and the AUC was 0.988[17]. Also, our results were comparable to those of Sabry *et al*[18] who found that the sensitivity of VEGF was 88% and the specificity was 60% at a cut-off value of 228 pg/mL, accuracy was 82%, positive predictive value 90%, and negative predictive value 55%.

Another study of VEGF in HCC patients showed that VEGF had 90% sensitivity and specificity at a cut-off value of 271.85 pg/mL with an AUC of 0.972[19]. When combining serum VEGF with AFP, the accuracy increased to 100% and 98.7% for the sensitivity and specificity, respectively, which is in agreement with our findings[19]. In another study, the optimal AFP cut-off value of 15 ng/mL provided a sensitivity and specificity of 76% and 62%, respectively, while the serum VEGF cut-off value of 108 pg/mL achieved a sensitivity and specificity of 98% and 46%, respectively. They concluded that among the many studied serum biomarkers, VEGF had the highest sensitivity in diagnosing HCC[18]. Yvamoto *et al*[20] found that VEGF levels had a sensitivity and specificity of 65% and 85%, respectively, while AFP had sensitivity and specificity of 28% and 99% for diagnosing HCC, respectively.

Strength points and limitations

Our study expands the literature by providing information about the accuracy of serum VEGF and VEGF/PLT in diagnosing HCC. Both serum VEGF and VEGF/PLT achieved better diagnostic performance than the traditional AFP. Moreover, when the three parameters were combined, the highest accuracy (> 95%) was achieved with a sensitivity and specificity of 92.5%, 98.3%, respectively.

The strength points of our study include three main point: (1) Validation of VEGF diagnostic accuracy in the HCV population, rather than in the general population, which makes these results applicable in the clinical setting; (2) The study was powered enough to demonstrate the accuracy of serum VEGF and VEGF/PLT in diagnosing HCC; and (3) Inclusion of a third group of cirrhotic patients to validate the specificity of VEGF and exclude cirrhotic HCV patients without HCC.

Recommendations for future research

We recommend greater number of patients to gain greater insight into potential usefulness of serum VEGF and VEGF/PLT in patients with HCC. Also, we recommend more studies which are much more precise: (1) Serum or plasma VEGF; and (2) Serum VEGF or serum VEGF/PLT. Follow up of patients over several years is recommended to detect the association between serum VEGF levels and response to treatment in comparison to serum AFP levels. Future studies are recommended to explore the relationship between serum VEGF levels and the presence of HCC with or without portal vein invasion before and after treatment.

CONCLUSION

Serum VEGF and VEGF/PLT appear to be additional diagnostic markers for HCC detection and prognostic markers during the follow-up of HCC patients since these markers showed significant correlations with tumor size, and stage, and the presence of vascular invasion among HCC cases.

Combined measurements of serum VEGF, VEGF/PLT, and AFP significantly increase the sensitivity, specificity, accuracy, AUC, and positive and negative predictive values for detecting HCC among cirrhotic patients rather than using AFP, VEGF, or VEGF/PLT separately.

ARTICLE HIGHLIGHTS

Research background

Hepatocellular carcinoma (HCC) is diagnosed at a late stage. Therefore, the prognosis of patients with HCC is generally poor. The recommended screening strategy for patients with liver cirrhosis includes the determination of serum alpha fetoprotein (AFP) levels and an abdominal ultrasound every 6 mo to detect HCC at an earlier stage. AFP, however, is a marker characterized by poor sensitivity and specificity, and abdominal ultrasound is highly dependent on the operator's experience. Vascular endothelial growth factor (VEGF) is a primary driving force for both physiological and pathological angiogenesis, and its overexpression is observed in HCC. VEGF is one of

the most important angiogenic factors and it promotes angiogenesis in most human tumors. One of the notable features of most HCCs is hypervascularity and it has been reported that VEGF expression is correlated with tumor vascularity. The circulating VEGF level was reported to be correlated with the stage of HCC and the highest VEGF levels are found in patients with metastasis

Research motivation

Try to determine the accuracy of serum VEGF and VEGF/platelet (PLT) as tumor markers in the early detection of HCC cases in patients with hepatitis C virus (HCV)-related liver cirrhosis.

Research objectives

The present study provides important data for the early diagnosis of HCC, enabling an increase in the number of cases treated worldwide. The main aim of this work was to assess the usefulness of serum VEGF and VEGF/PLT as tumor markers in patients with HCV-related liver cirrhosis and HCC and to compare them with the control group (patients with chronic HCV without cirrhosis or HCC) in order to detect their sensitivity and specificity as diagnostic markers for HCC. These two markers were also compared to AFP, the conventional marker of HCC, and were then correlated with tumor size, stage, vascular invasion, and Child-Pugh classification. Also, this study aimed to verify the possibility of using combined measurement of serum VEGF, VEGF/PLT, and AFP for diagnosing HCC early and accurately.

Research methods

We conducted a case-control study with HCV patients from the outpatient and inpatient hepatology clinics. Patients were classified into three groups: (1) HCC group; (2) Cirrhosis group; and (3) HCV without cirrhosis (control group). Patients were clinically evaluated, and blood samples were drawn for the analysis; serum VEGF levels were measured by a specific VEGF human recombinant enzyme-linked immunosorbent assay kit. Data from the three study groups were compared by the one-way analysis of variance or Kruskal-Wallis test. Receivers operating characteristic curves (ROC) were constructed to determine the optimal cut-off values of AFP, VEGF, and VEGF/PLT that provided the best diagnostic accuracy. The sensitivity and specificity at the optimal cut-off value of each biomarker were then calculated.

Research results

This study included one hundred patients (HCC, cirrhosis, and control groups: n = 40, 30, 30, respectively). HCC patients had significantly higher serum VEGF and VEGF/PLT levels than the non-HCC groups (P = 0.001). Serum VEGF and VEGF/PLT showed significant positive correlations with and HCC tumor size, stage, vascular invasion, and Child Pugh classification. Moreover, a VEGF cut-off the value of 250 pg/mL provided 80% sensitivity and 81.7% specificity for discriminating HCC patient from non-HCC patients. Similarly, the ratio of VEGF/PLT provided sensitivity and specificity of 77.5% and 80%, respectively which is higher than the accuracy provided by AFP. The combination of AFP, VEGF, and VEGF/PLT increases the accuracy of diagnosing HCC to > 95%.

Research conclusions

Serum VEGF and VEGF/PLT appear to be additional diagnostic markers for HCC detection and prognostic markers during the follow-up of HCC patients since these markers showed significant correlations with tumor size, and stage, and the presence of vascular invasion among HCC cases. Combined measurements of serum VEGF, VEGF/PLT, and AFP significantly increase the sensitivity, specificity, accuracy, area under the ROC curve, and positive and negative predictive values for detecting HCC among cirrhotic patients rather than using AFP, VEGF, or VEGF/PLT separately.

Research perspectives

Most of the current biomarkers for early and accurate diagnosis of HCC have limited sensitivity or specificity, so patients with HCC are diagnosed at a late stage and have low survival and poor prognosis. Therefore, a combination of two or three biomarkers with high specificity might provide the optimal diagnosis of early HCC is suggested. In this study, we conducted an observational study with 100 patients to assess the usefulness of serum VEGF and VEGF/PLT as tumor markers for early and accurate diagnosis of HCC in patients with HCV-related liver cirrhosis, and comparing them to serum AFP, the conventional marker of HCC. Also, this study aimed to verify the

possibility of using combined measurement of serum VEGF, VEGF/PLT, and AFP for HCC diagnosis.

REFERENCES

1 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]

2 **Golabi P**, Fazel S, Otgonsuren M, Sayiner M, Locklear CT, Younossi ZM. Mortality assessment of patients with hepatocellular carcinoma according to underlying disease and treatment modalities. *Medicine (Baltimore)* 2017; **96**: e5904 [PMID: 28248853 DOI: 10.1097/MD.0000000000005904]

3 **El-Serag HB**, Davila JA. Surveillance for hepatocellular carcinoma: in whom and how? *Therap Adv Gastroenterol* 2011; **4**: 5-10 [PMID: 21317990 DOI: 10.1177/1756283X10385964]

4 **Frenette C**. Surveillance for Hepatocellular Carcinoma. *Gastroenterol Hepatol (NY)* 2016; **12**: 394-396

5 **George ML**, Eccles SA, Tutton MG, Abulafi AM, Swift RI. Correlation of plasma and serum vascular endothelial growth factor levels with platelet count in colorectal cancer: clinical evidence of platelet scavenging? *Clin Cancer Res* 2000; **6**: 3147-3152 [PMID: 10955796]

6 **Moon WS**, Rhyu KH, Kang MJ, Lee DG, Yu HC, Yeum JH, Koh GY, Tarnawski AS. Overexpression of VEGF and angiopoietin 2: a key to high vascularity of hepatocellular carcinoma? *Mod Pathol* 2003; **16**: 552-557 [PMID: 12808060 DOI: 10.1097/01.MP.0000071841.17900.69]

7 **Beck B**, Driessens G, Goossens S, Youssef KK, Kuchnio A, Caauwe A, Sotiropoulou PA, Loges S, Lapouge G, Candi A. A vascular niche and a VEGF-Nrp1 Loop regulate the initiation and stemness of skin tumors. *Nature* 2011; **478**: 399-403 [DOI: 10.1038/nature10525]

8 **Goel HL**, Mercurio AM. VEGF targets the tumour cell. *Nat Rev Cancer* 2013; **13**: 871-882 [PMID: 24263190 DOI: 10.1038/nrc3627]

9 **Matsui D**, Nagai H, Mukozu T, Ogino YU, Sumino Y. VEGF in patients with advanced hepatocellular carcinoma receiving intra-arterial chemotherapy. *Anticancer Res* 2015; **35**: 2205-2210 [PMID: 25862879]

10 **Choi SB**, Han HJ, Kim WB, Song TJ, Choi SY. VEGF Overexpression Predicts Poor Survival in Hepatocellular Carcinoma. *Open Med (Wars)* 2017; **12**: 430-439 [PMID: 29318189 DOI: 10.1515/med-2017-0061]

11 **von Elm E**, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP; STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *J Clin Epidemiol* 2008; **61**: 344-349 [PMID: 18313558 DOI: 10.1016/j.jclinepi.2007.11.008]

12 **Leung DW**, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* 1989; **246**: 1306-1309 [PMID: 2479986 DOI: 10.1126/science.2479986]

13 **Cao G**, Li X, Qin C, Li J. Prognostic Value of VEGF in Hepatocellular Carcinoma Patients Treated with Sorafenib: A Meta-Analysis. *Med Sci Monit* 2015; **21**: 3144-3151 [PMID: 26476711 DOI: 10.12659/MSM.894617]

14 **Chintalacheruvu LM**, Buddam A, Kanmanthareddy A, Ganti AK. Efficacy and safety of anti-VEGF therapy in metastatic unresectable hepatocellular carcinoma: A meta-analysis. *J Clin Oncol* 2017; **35**: e15632-e15632 [DOI: 10.1200/JCO.2017.35.15_suppl.e15632]

15 **Zou Y**, Guo CG, Zhang MM. Inhibition of human hepatocellular carcinoma tumor angiogenesis by siRNA silencing of VEGF *via* hepatic artery perfusion. *Eur Rev Med Pharmacol Sci* 2015; **19**: 4751-4761 [PMID: 26744866]

16 **Liu K**, Hao M, Ouyang Y, Zheng J, Chen D. $CD133^+$ cancer stem cells promoted by VEGF accelerate the recurrence of hepatocellular carcinoma. *Sci Rep* 2017; **7**: 41499 [PMID: 28134312 DOI: 10.1038/srep41499]

17 **Mukozu T**, Nagai H, Matsui D, Kanekawa T, Sumino Y. Serum VEGF as a tumor marker in patients with HCV-related liver cirrhosis and hepatocellular carcinoma. *Anticancer Res* 2013; **33**: 1013-1021 [PMID: 23482775]

18 **Sabry HS**, Nouh MA, Yoffe B, El-Sebaai HM, Mohamed HI, Mohamed SA. Study of the Diagostic Role of Vascular Endothelial Growth Factor in Hepatocellular Carcioma. *J Am Sci* 2012; **8**: 273-279

19 **Atta MM**, Atta HM, Gad MA, Rashed LA, Said EM, Hassanien Sel-S, Kaseb AO. Clinical significance of vascular endothelial growth factor in hepatitis C related hepatocellular carcinoma in Egyptian patients. *J Hepatocell Carcinoma* 2016; **3**: 19-24 [PMID: 27574588 DOI: 10.2147/JHC.S86708]

20 **Yvamoto EY**, Ferreira RF, Nogueira V, Pinhe MA, Tenani GD, Andrade JG, Baitello ME, Gregório ML, Fucuta PS, Silva RF, Souza DR, Silva RC. Influence of vascular endothelial growth factor and alpha-fetoprotein on hepatocellular carcinoma. *Genet Mol Res* 2015; **14**: 17453-17462 [PMID: 26782388 DOI: 10.4238/2015.December.21.16]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2021 June 15; 13(6): 612-624

DOI: 10.4251/wjgo.v13.i6.612

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Prospective Study

Gastrointestinal function testing model using a new laryngopharyngeal pH probe (Restech) in patients after Ivor-Lewis esophagectomy

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Author contributions: Fuchs HF contributed to the study concept and design; Fuchs H and Müller DT acquired the data; Fuchs HF, Babic B, Leers JM, Schröder W and Bruns CJ contributed to analysis and interpretation of data; Fuchs HF and Babic B drafted the manuscript; Fuchs HF, Babic B, Müller DT, Gebauer F, Schiffmann LM, Datta RR, and Bruns CJ critically revised the manuscript for important intellectual content; Fuchs HF performed statistical analysis; Fuchs HF was charge of administrative, technical, or material support; Leers JM and Fuchs HF supervised the study.

Supported by Cologne Fortune Scientific Grant Project, No. 176/2016.

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Abstract

BACKGROUND

There is no established correlation between 24-h esophageal pH-metry (Eso-pH) and the new laryngopharyngeal pH-monitoring system (Restech) as only small case series exist. Eso-pH was not designed to detect laryngopharyngeal reflux (LPR) and Restech may detect LPR better. We have previously published a dataset using the two techniques in a large patient collective with gastroesophageal reflux disease. Anatomically, patients after esophagectomy were reported to represent an ideal human reflux model as no reflux barrier exists.

AIM

To use a human reflux model to examine our previously published correlation in these patients.

METHODS

Patients after Ivor Lewis esophagectomy underwent our routine follow-up program with surveillance endoscopies, computed tomography scans and further exams following surgery. Only patients with a complete check-up program and reflux symptoms were offered inclusion into this prospective study and evaluated using Restech and simultaneous Eso-pH. Subsequently, the relationship between the two techniques was evaluated

RESULTS

A total of 43 patients from May 2016 - November 2018 were included. All patients presented with mainly typical reflux symptoms such as heartburn (74%), regurgitation (84%), chest pain (58%), and dysphagia (47%). Extraesophageal

symptoms such as cough, hoarseness, asthma symptoms, and globus sensation were also present. Esophageal 24-hour pH-metry was abnormal in 88% of patients with a mean DeMeester Score of 229.45 [range 26.4-319.5]. Restech evaluation was abnormal in 61% of cases in this highly selective patient cohort. All patients with abnormal supine LPR were also abnormal for supine esophageal reflux measured by conventional Eso-pH.

CONCLUSION

Patients following esophagectomy and reconstruction with gastric interposition can ideally serve as a human reflux model. Interestingly, laryngopharyngeal reflux phases occur mainly in the upright position. In this human volume-reflux model, results of simultaneous esophageal and laryngopharyngeal (Restech) pH-metry showed 100% correlation as being explicable by one of our reflux scenarios.

Key Words: Gastroesophageal reflux disease; Laryngopharyngeal reflux; Minimally invasive esophagectomy; Surgical technology; Restech; Esophageal pH-metry

Core Tip: There is no established correlation between 24-h esophageal pH-metry (Eso-pH) and the new laryngopharyngeal pH-monitoring system (Restech) as only small case series exist. Anatomically, patients after esophagectomy were reported to represent an ideal human reflux model as no reflux barrier exists. Patients after esophagectomy were evaluated using Restech and simultaneous Eso-pH. In this human volume-reflux model, Eso-pH correlated completely with laryngopharyngeal pH-metry (Restech).

Citation: Babic B, Müller DT, Gebauer F, Schiffmann LM, Datta RR, Schröder W, Bruns CJ, Leers JM, Fuchs HF. Gastrointestinal function testing model using a new laryngopharyngeal pH probe (Restech) in patients after Ivor-Lewis esophagectomy. *World J Gastrointest Oncol* 2021; 13(6): 612-624
URL: https://www.wjgnet.com/1948-5204/full/v13/i6/612.htm
DOI: https://dx.doi.org/10.4251/wjgo.v13.i6.612

INTRODUCTION

Gastroesophageal reflux disease (GERD) is a common disorder of the upper gastrointestinal tract with a high prevalence, especially in the western world[1]. Symptoms are usually defined as typical/esophageal and atypical/extraesophageal symptoms with the most common and typical symptoms being heartburn and regurgitation. Still, a significant number of patients suffers from atypical/extraesophageal symptoms such as chronic cough, hoarseness, sore throat, and pharyngeal burning. Other, more unspecific symptoms like a burning sensation of the tongue and mouth, a globus sensation, and dental erosions may also be present. A causal association of extraesophageal symptoms with GERD or nasopharyngeal etiologies remains a major diagnostic challenge in these patients. In consequence, a satisfying therapy of patients with extraesophageal symptoms is not easy to offer[2,3]. A positive response to a medical therapy with proton pump inhibitors seems to be a positive prognostic predictor for connecting GERD to extraesophageal symptoms. Still the level of evidence for respiratory diseases caused by GERD remains rather low[1,4].

Esophageal 24-h pH monitoring (Eso-pH) is the gold standard for the detection of GERD. Herewith, the acid exposure of the lower esophagus can be identified and quantified[1]. To improve measurement of episodes caused by proximal esophageal reflux, a dual-probe pH monitoring was introduced in the late 1990's[5,6]. With the necessity of a high esophageal positioning of the pH-probe for the detection of laryngopharyngeal reflux (LPR), existing pH-metry devices designed for lower esophageal pH-metry were not always reliable and valid. The development and implementation of pH-impedance monitoring made it possible to distinguish between acid and non-acid reflux and furthermore allowed a quantification of proximal

Institutional review board statement: The study was conducted with approval from the institutional review board at the University of Cologne (IRB reference 16-727).

Clinical trial registration statement: Our prospective clinical study has been registered in the "German Clinical Trial Register" and can be found under the following link: https://www.drks.de/drks_web/navigate.do?navigationId=trial.HTML&TRIAL_ID=DRKS00011123. The study number is DRKS00011123.

Informed consent statement: All study participants provided written consent prior to study enrollment.

Conflict-of-interest statement: The authors of this manuscript having no conflicts of interest to disclose.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

Specialty type: Research and experimental medicine

Country/Territory of origin: Germany

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Received: December 6, 2020
Peer-review started: December 6, 2020
First decision: January 29, 2021
Revised: February 11, 2021
Accepted: May 19, 2021
Article in press: May 19, 2021
Published online: June 15, 2021

P-Reviewer: Salvatore S
S-Editor: Gong ZM
L-Editor: A
P-Editor: Li JH

esophageal reflux. In addition, correlation between symptoms and episodes of reflux can be seen.

Recently a novel pH device (Restech pH measurement system, Respiratory Technology Corp., Houston, TX, United States = Restech) has been developed and normal values were published in 2009[7]. This device is designed to be positioned above the upper esophageal sphincter in the oropharynx. The teardrop design prevents drying of the catheter, a common problem of the Eso-pH catheter when placed high in the oropharynx. Restech was created to detect both liquid and acidic gas vapor, and the oropharyngeal placement may lead to more accurate results[8]. Worrell *et al*[9] published that Restech may help to achieve a better patient selection for a successful outcome for extraesophageal reflux symptoms after laparoscopic anti-reflux surgery. Our group recently published the largest case series and validation study using Restech and classic esophageal pH-metry simultaneously in more than 100 patients with GERD[10] showing that. various reflux scenarios exist in patients with reflux disease.

Other researchers developed a human reflux model in the early 2000's[11]. We further evolved this idea and developed the University of Cologne human reflux model. Patients that underwent esophagectomy were followed up thoroughly after surgery over a long period of time and data on those bothersome symptoms was collected[12]. Interestingly, patients who had undergone esophagectomy for adenocarcinoma of the esophagus showed a faster progression to so-called Neo-Barretts in the esophageal remnant.

Therefore, it is the aim of this current study to further validate the new technology (Restech) using a prospective cohort of patients after Ivor-Lewis esophagectomy clinically presenting with severe GERD.

MATERIALS AND METHODS

Patients

Our academic center is a certified center of excellence for surgery of the upper gastrointestinal tract. A prospective analysis of patient data during follow-up after hybrid minimally invasive Ivor Lewis esophagectomy for cancer was performed. To obtain a homogenous population, only patients at least 3 mo out of surgery and disease-free survival were included in this study to eliminate immediate postoperative effects. The study was conducted with approval from the institutional review board at the University of Cologne (IRB reference 16-727) and subjects gave written informed consent prior to participation in the study.

Demographics, endoscopic findings, biopsies at different follow-up time points, as well as tumor histology and stage were recorded in the prospective database. Additionally, symptoms were recorded at all times of follow-up. Only patients that presented with reflux-related symptoms or those showing endoscopic proof of mucosal damage in the esophageal remnant were offered inclusion in this study. To minimize the risks and inconvenience to our patients and to follow current ethical principles for research, no control group without symptoms or proof of reflux associated changes was investigated for this study.

Treatment and follow-up of patients with esophageal cancer

Esophageal cancer was treated according to previously published guidelines[13-15]. In a multimodal setting, surgery was scheduled 4 to 8 wk after neoadjuvant treatment and was typically performed as a hybrid or totally minimally invasive Ivor Lewis procedure with high intrathoracic esophagogastric anastomosis and two-field lymphadenectomy. We performed hybrid and totally minimally invasive procedures according to international guidelines[16-18]. All patients underwent our previously published risk assessment before surgery[19]. Esophagitis was recorded according to the Los Angeles classification during follow-up endoscopy[20]. The definition of Barrett's mucosa included both specialized and non-specialized columnar epithelium from the esophageal remnant and was only diagnosed when goblet cells were present [21]. We published our management if mucosal inflammation was encountered during follow-up of esophageal cancer before[12].

Further evaluation of patients with GERD symptoms during follow up

All patients with GERD symptoms were offered inclusion to our study. They were subsequently seen in a specialized surgical outpatient clinic with all laboratory instruments for up-to-date gastrointestinal function testing (High Resolution

Manometry (HRM), upper-gastrointestinal endoscopy, contrast radiography, and 24-h impedance-pH-monitoring with simultaneous 24-h Restech pH-monitoring). All patients underwent a standardized interview about quality of life (GIQLI, GERD-HRQL), the presence of heartburn, regurgitation, dysphagia, and atypical symptoms, as reported by others before[22,23]. Gastrointestinal function testing was performed according to the current EAES (European Association of Endoscopic Surgery) recommendations for management of GERD[1]. No HRM or barium swallow was performed in this study. We have published a detailed description of upper-gastrointestinal endoscopy, esophageal pH-monitoring (Eso-pH), and simultaneous laryngopharyngeal pH-monitoring as performed in our center before[10]. The esophagogastric anastomosis was defined as esophagogastric junction for placement of the pH-metry probe. Placement of both laryngopharyngeal and esophageal pH probes are shown in Figures 1-3. In addition, a standardized protocol was followed during the measurements to ensure valid study data. Patients were asked to maintain their regular diet and instructed to eat three meals per day with drinking allowed only at mealtimes. Mealtimes were then excluded from the analysis.

Subgroup analysis

Based on gastrointestinal function testing, and previous studies from our group[10], patients were subdivided in groups A-D (Table 1). Group A consists of patients with an abnormal esophageal but normal oropharyngeal acid exposure, Group B consists of patients with a normal esophageal but abnormal oropharyngeal acid exposure, Group C of patients with both abnormal esophageal and oropharyngeal acid exposure, and Group D of patients with no abnormal esophageal and oropharyngeal acid exposure.

Data collection and statistical analysis

Data were collected prospectively, including but not limited to, age, gender, Body Mass Index, esophageal pH-metry results, Restech pH-metry results, endoscopic findings, oncological parameters, and surgical therapy.

Main outcome of measure was the correlation of esophageal and laryngopharyngeal pH-metry results in this human reflux model. Continuous variables are presented as means and range. Categorical data are presented as numbers and percentages. The Student *t*-test, (for continuous variables), and Chi-square test, (for nominal or categorical variables), were used for all bivariate analyses. All tests were 2-sided, with statistical significance set at $P \leq 0.05$. Data were analyzed by GraphPad (GraphPad Software, San Diego, CA, United States). Statistical review of this study was performed by a biomedical statistician.

RESULTS

Demographics

A total of 413 patients underwent Ivor-Lewis esophagectomy at our institution between May 2016 and November 2018. In the same period, 43 patients after Ivor-Lewis esophagectomy (9 females) with a mean age of 61 years (range 39-79) consented for the present study and were completely followed up including gastrointestinal function testing and subsequently included in this study. Typical GERD symptoms such as regurgitation, dysphagia, and heartburn were present in a large proportion of our collective. Some patients also suffered from extraesophageal reflux symptoms such as chronic cough, hoarseness, sore throat and pharyngeal burning. Unwanted weight loss and retrosternal pain were other chief complaints of this study cohort. The detailed follow-up information is given in Table 2. Of the total 43 patients, 34 patients (79%) had adenocarcinoma and 9 patients (21%) had squamous cell carcinoma of the esophagus. All patients were routinely on proton pump inhibitors at a daily dose of 40mg and off PPIs for at least 7 d for the measurement. Mean level of intrathoracic anastomosis was at 24 cm (range 20-33 cm) from incisors.

Gastrointestinal function testing

Complete workup consisting of esophagogastroduodenoscopy, 24-h esophageal pH-metry and Restech pH-metry, was available for 33 patients. Two patients did not tolerate the pH probe and 8 patients did not have a complete data set available for analysis. A total of 29 (88%) patients had an abnormal pH-metry as defined by a DeMeester Score of > 14.7. Restech pH-metry was abnormal in 20 (61%) patients as defined by a RYAN Score of 9.4 in upright and/or 6.8 in supine position using

Table 1 Group assignment of patients with simultaneous laryngopharyngeal and esophageal pH-metry (*n* = 33)

Restech pH-metry	Esophageal pH-metry	
	Normal	Abnormal
Normal	D (*n* = 4)	A (*n* = 9)
Abnormal	B (*n* = 0)	C (*n* = 20)

Table 2 Follow up time, endoscopic findings and symptoms of patients' cohort

	n	%
Total	43	
Follow up time (d)	Mean 790 (median 574)	Range 106-3640
Mucosal disease		
Reflux esophagitis		
None	18	42
LA grade A	9	21
LA Grade B	7	16
LA Grade C	5	12
LA Grade D	4	9
Barrett's	1	2
Symptoms		
Heartburn	32	74
Regurgitation	36	84
Dysphagia	20	47
Chest pain	25	58
Atypical symptoms	10	23
Weight loss	20	47

DataView 3 for analysis of measured pH data. Restech pH-metry was more commonly abnormal in upright position (*n* = 20) than in supine (*n* = 4) position (61 % *vs* 12%; $P < 0.0001$). However, patients with an abnormal supine RYAN score also had an abnormal upright score. All patients with an abnormal supine RYAN score also had abnormal acid exposure in supine position measured with esophageal pH-metry. Endoscopic findings were esophagitis in the esophageal remnant in half of the included patients, and Barrett's esophagus in 1 patient.

Subgroup analyses

Group A (abnormal Eso-pH, normal Restech): A total of 9 patients, (1 female), fulfilled inclusion criteria of subgroup A. All patients complained of heartburn. Other symptoms reported were primarily regurgitation, (89%), and chest pain. Two patients also reported extraesophageal reflux symptoms (cough). Patients had a severely abnormal esophageal acid exposure with a mean DeMeester score of 202.9 (range 27-308.5) and unobtrusive Restech results. Further details are depicted in Tables 3 and 4.

Group B (normal Eso-pH, abnormal Restech): No patients fulfilling criteria for this group were found in this collective of heavy volume reflux patients (University of Cologne Human Reflux model).

Group C (abnormal Eso-pH, abnormal Restech): A total of 20 patients, (3 females), fulfilled inclusion criteria of subgroup C. Symptoms reported were primarily heartburn, (85%), and regurgitation as well as chest pain. Four patients also complained of extraesophageal reflux symptoms. Patients suffered from severe esophageal acid exposure with an abnormal mean DeMeester score of 242, (range 26.4-

Table 3 Results overview

	Group A		Group C		Group D	
	n	%	*n*	%	*n*	%
Total	9	100	20	100	4	100
Females	1	11	3	15	2	50
Heartburn	9	100	15	75	0	0
Regurgitation	8	89	17	85	1	25
Dysphagia	4	44	11	55	2	50
Chest Pain	8	89	11	55	0	0
Weight loss	5	56	10	50	2	50
Extraesophageal reflux symptoms	2	22	4	20	1	25
	Mean	Range	Mean	Range	Mean	Range
Age (yr)	59	48-66	61	46-77	69	58-77
BMI (kg/m^2)	24.9	19.2-29.1	25	20.5-29.6	23.1	17.6-26.3
Restech						
RYAN upright	2.36	2.12-4.26	84.8	10.6-381	2.12	-
RYAN supine	2.17	-	14.4	2.17 – 149.1	2.17	-
Eso-pH						
DeMeester score	202.9	27-308.5	242	26.4-319.5	0	-
% time pH < 4	36.4	6.2-71	29.1	3.7-90.7	0.55	0-1.9
Gastric pH	3.2	1.6-6.7	2.62	1.2-5.6	3.9	2-6.7

BMI: Body mass index.

Table 4 Endoscopic findings of subgroups

	Group A		Group C		Group D	
	n	%	*n*	%	*n*	%
Total	9	100	20	100	4	100
Anastomosis (cm) mean/range	25	20-29	25	20-33	22	21-23
Esophagitis						
LA Grade A	4	44	1	5	1	25
LA Grade B	1	11	4	20	0	0
LA Grade C	1	11	3	15	0	0
LA Grade D	1	11	2	10	0	0
Barrett's	0	0	1	5	0	0

319.5), and also abnormal Restech results with a mean RYAN score of 84.8, (range, 10.61-381), in upright position. Further details are depicted in Tables 3 and 4.

Group D (normal Eso-pH, normal Restech): A total of 4 patients, (2 females), fulfilled inclusion criteria of subgroup D. Symptoms reported were primarily dysphagia, (n = 2), and weight loss. Only one patient complained of extraesophageal reflux symptoms. Patients had a normal esophageal acid exposure with a mean DeMeester score of 0 and a normal Restech results with a mean RYAN score of 2.12 (range 2.12-2.17), in upright position. Further details are depicted in Tables 3 and 4.

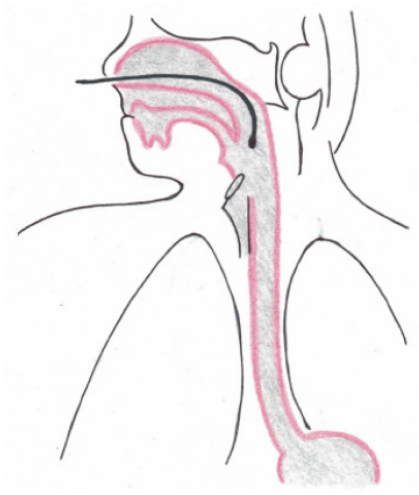

Figure 1 Probe placement Restech Dx-pH.

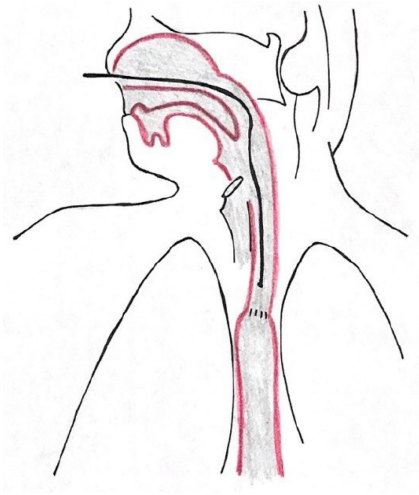

Figure 2 Probe placement esophageal pH-metry.

Critical comparison of groups: Subgroup analysis of this study was based on our previous study[10], showing that different reflux scenarios exist and that those can be represented by four groups. Group A (abnormal Eso-pH, normal Restech) can physiologically be explained by reflux episodes that do not reach the oropharynx and therefore do not get detected by oropharyngeal pH testing. All patients in this group showed primarily typical reflux symptoms such as heartburn and regurgitation. Group B (normal Eso-pH, abnormal Restech) was previously described in patients with suspected GERD that underwent simultaneous esophageal and oropharyngeal pH testing. However, no patients fulfilled criteria for this group in this collective of heavy volume reflux patients (University of Cologne Human Reflux model). Group C (eso-pH abnormal, Restech abnormal) and group D (Eso-pH normal, Restech normal) show correlating results. Patients in group D showed a significantly lower symptom load than patients with an abnormal pH test. A correlation between extraesophageal reflux symptoms and group assignment could not be found. Endoscopic findings differed in our subgroups but were in alignment with pH test results. Endoscopy revealed reflux esophagitis in 78% of patients in group A, compared to 50% in group C and only 25% in group D. Severe esophagitis (LA Grade C or D) was present in 22% of patients in group A and 25% of group C compared to no patient in group D. Demographic factors did not differ in our group comparison and only trends could be found ($P > 0.05$). Only 12% ($n = 4$) showed normal test results for both pH tests validating the use of this selected cohort of patients as a human reflux model.

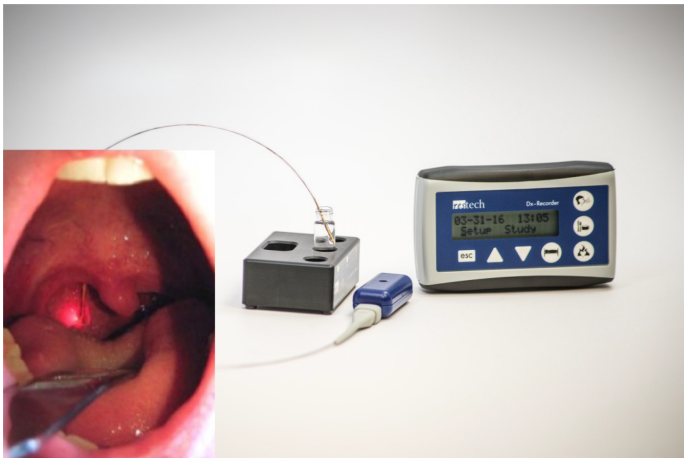

Figure 3 Restech device and intraoral placement of the pH probe.

Acidity of gastric conduit

Measurement of gastric conduit acidity was available for 35 patients of our cohort. This depicts patients that underwent esophageal pH measurement using the conventional system. Mean gastric pH overall was 2.97 (range 1.2-6.7, median 2.5). Patients were further grouped according to length of follow up. Already shortly after surgery (group 1, follow up 3-6 mo) acidity of the gastric conduit almost normalized (mean pH 2.4; range 2-2.8). Mean pH in group 2 (Follow up 6-24 mo) was 3.4 (range 1.6-6.7). Group 3 (Follow up > 24 mo) showed a mean gastric pH of 2.4 (range 1.2-4.8). A comparison of groups 2 and 3 showed a clear trend of normalization of gastric pH in correlation with length of follow up (P = 0.0608). Further details are depicted in Table 5.

Using the previously described subgroups A-D, a trend of correlation between pH test result and gastric conduit acidity can be seen between group C (abnormal Eso-pH, abnormal Restech) and group D (normal Eso-pH, normal Restech). Patients with an abnormal acid exposure showed a lower pH of their gastric conduit than patients with a normal test result (P = 0.073). Further details are depicted in Table 3 and 4.

DISCUSSION

As stated before, we believe that a variety of different reflux scenarios following Ivor-Lewis esophagectomy exist and that the 3 groups evaluated in our study explain these options in a logical way. It is in interesting to note that patients in all analyzed groups suffer from many different (including extraesophageal) reflux symptoms. Patients were informed upon inclusion into the study that only limited options for improvement of these symptoms exist, and that in contrast to patients that did not undergo esophagectomy for cancer, no surgical option such as fundoplication were possible. On the other hand, all included patients were happy to learn more about their altered anatomy and how to conservatively overcome reflux-related impairment of life.

Whereas we defined a group B (normal Eso-pH, abnormal Restech) in our previous study about the Restech device, no patients in this human reflux model fulfilled inclusion criteria for this group. Group B is not explainable from a pathophysiological standpoint and probably not valid for patients with volume reflux such as patients in this present collective. Patients without any anatomical alteration and mainly acidic gas vapor might be the ones that fall into this category.

Previous validation studies tried to prove corresponding results in oropharyngeal and esophageal pH-metry[24-26]. As stated with our four different groups from our previous study, we do not believe that Restech and eso-pH necessarily need to correspond. This thinking resulted from two groups with non-corresponding results: Group A, (abnormal Eso-pH, normal Restech), and Group B, (normal Eso-pH, abnormal Restech). Group A is physiologically explicable with reflux episodes that do not reach the oropharynx. Group B is more difficult to explain with esophageal reflux alone from a physiological standpoint. Results from our last paper indicated that acidic vapor or other factors may cause abnormal Restech results with simultaneous

Table 5 Demographic information, endoscopic findings and pH data

	Group 1 (3-6 mo follow up)		Group 2 (6-24 mo follow up)		Group 3 (> 24 mo follow up)	
	n	%	*n*	%	*n*	%
Total	3	100	19	100	13	100
Females	2	67	5	26	0	0
Esophagitis						
LA Grade A	0	0	5	26	1	8
LA Grade B	0	0	2	11	3	23
LA Grade C	0	0	1	5	3	23
LA Grade D	0	0	4	21	0	0
	Mean	Range	Mean	Range	Mean	Range
Follow up time (d)	127	106-167	414	209-652	1322	725-1966
Gastric pH	2.4	2 – 2.8	3.4	1.6-6.7	2.4	1.2-4.8
DeMeester score	159.5	0-273.5	197	0-313.4	281.3	116.5-319.5

normal eso-pH results.

Previous studies showed only a weak correlation between esophageal and laryngopharyngeal pH measurement resulting in the conclusion that the Restech device adds no or little value as a diagnostic device in the evaluation of GERD and LPR[24-26]. However, only small numbers of patients were included in those studies (n = 10-36) and esophageal pH monitoring was either not performed simultaneously or not performed at all, leading to an insufficient comparison of results. In their critical report about laryngopharyngeal pH testing, Wilhelm at al. concluded from abnormal results of 6 out of 10 patients after gastrectomy that "pH values assessed by the Dx-pH device […] are obviously dissociated from gastric acid production". Yet, no simultaneous esophageal pH testing for validation was performed. Another study focused on the correlation results of laryngopharyngeal pH testing and clinical findings during laryngoscopy. A significant correlation could not be found, however again only a small number of patients (n = 33) was included in the study and trends approaching statistical significance were noted[27]. In addition, Yadlapati *et al*[28] investigated the correlation between laryngopharyngeal pH testing and PPI response and found no significant correlation. Interestingly, only 35% of patients with atypical symptoms and a positive reflux symptom index showed a completed response to PPIs and 50% of patients showed no response at all. Another study of the same group showed that neither laryngopharyngeal pH testing nor salivary pepsin analysis are able to distinguish between reflux patients and healthy ones. Like many other studies, no esophageal pH testing was performed to validate the results[29]. In comparison, Vailati *et al*[30] have previously shown the Restech Dx-pH device to have a 69% sensitivity and 100% specificity for the responsiveness to medical therapy in patients with LPR, making it a valuable tool for those. The same group later showed a poor correlation between esophageal and laryngopharyngeal pH testing resulting in a currently rather low level of evidence for the use of the Restech device in patient evaluation[26]. The current normal values and discriminating pH thresholds of Restech were initially validated by a study group at University of Southern California in 2009 and 2010 using 55 and 81 normal subjects[7,8]. Later, the same institution published data suggesting that patients with abnormal results in pharyngeal pH monitoring might benefit from antireflux surgery[9]. The latter report is again limited by sample size, (n = 20), and the fact that that esophageal pH-metry and Restech were not performed simultaneously and may therefore not represent the same reflux scenario.

Another important issue addressed in this study focuses on the concern that current literature shows up to 50% of patients developing Neo-Barrett's Esophagus above the anastomosis after Ivor-Lewis esophagectomy[31]. In addition, as we observed in a previous study, patients with known Barrett's esophagus and adenocarcinoma have a higher risk of developing reflux-associated lesions in the remnant esophagus than patients with SCC. This group showed significantly less mucosal damage in the remnant esophagus given the same surgical approach[12]. Our established reflux model was based upon patients that underwent esophagectomy with gastric tube

reconstruction resulting in limited esophageal motility and no reflux barrier being present. Hardly any studies examine the functional changes after esophagectomy with gastric tube reconstruction that can lead to mucosal damage in the remnant esophagus. Due to bilateral vagotomy during transthoracic resection, gastric acidity was thought to be reduced permanently. However, acidity of the gastric conduit can quickly recover even though bilateral vagotomy is performed[32]. Our data shows a normalization of gastric pH as early as 106 d after surgery. This phenomenon seems to heavily contribute to the occurrence of mucosal damage in the esophageal remnant and has important implications on the pathogenesis of Barrett's esophagus and esophageal cancer.

Overall, the current level of evidence using the Restech device is limited by rather small case studies concluding that more research regarding the new reflux measurement device needs to be done. We have already added to the literature our large series of 101 patients with benign disease that were simultaneously measured by a standardized and validated system, and also by the Restech system. This present research using the University of Cologne Reflux model was needed and helped to better understand the different existing reflux scenarios as well as purely validate Restech in volume refluxers. In addition, one of our previous projects focused on the validation of the new software version DataView 4 for analysis of measured pH data with the Restech device, suggesting that improvements made to the new software version might increase quality of results and correlation with esophageal pH measurement[33].

Our study has some limitations that may be related to partly retrospective data analysis. Also, the limited sample size as a result of incomplete datasets might limit the conclusions that can be made. In addition, non-acid reflux episodes were not analyzed as no impedance pH-monitoring was performed. On the other hand, our collective of patients that undergo 24-h pH-monitoring with simultaneous 24-h Restech pH-monitoring after Ivor Lewis esophagectomy is to our knowledge quite unique in literature. Of significance, our study has several features and important implications on treatment of GERD patients with atypical reflux symptoms. Our study again emphasizes that important conclusions can be made from a Restech evaluation. Our key message as demonstrated by our 3 comparison groups in this human reflux model, representing different reflux scenarios, is that Eso-pH and Restech do not necessarily need to correspond. Nevertheless, all patients with an abnormal Restech evaluation also showed abnormal Eso-pH showing an evident relationship between both measurements. The Restech Dx-pH may therefore, in combination with upper gastrointestinal endoscopy, be a sufficient tool for evaluation of this patient group.

CONCLUSION

Patients following esophagectomy and reconstruction with gastric interposition can ideally serve as a human reflux model, as a large proportion suffers from severe postoperative GERD. Interestingly, laryngopharyngeal reflux phases occur mainly in the upright position, and acidity of the gastric conduit is already nearly normalized shortly after surgery.

In this human volume-reflux model, esophageal pH-metry correlated precisely with an abnormal laryngopharyngeal pH-metry (Restech).

ARTICLE HIGHLIGHTS

Research background

There is no established correlation between 24-h esophageal pH-metry (Eso-pH) and the new laryngopharyngeal pH-monitoring system (Restech) as only small case series exist. Eso-pH was not designed to detect laryngopharyngeal reflux (LPR) and Restech may detect LPR better. We have previously published a dataset using the two techniques in a large patient collective with Gastroesophageal Reflux Disease. Anatomically, patients after esophagectomy were reported to represent an ideal human reflux model as no reflux barrier exists.

Research motivation

Patients after esophagectomy ideally serve as a human reflux model, as they show an impaired esophageal motility and no reflux barrier. This study aims to use this human

reflux model to examine a previously established correlation between esophageal and laryngopharyngeal pH testing and to further validate laryngopharyngeal pH testing.

Research objectives

Previous validation studies tried to prove corresponding results in laryngopharyngeal and esophageal pH-metry. We, however, believe that a variety of different reflux scenarios exist and that those can be logically explained by our human reflux model in patients after Ivor-Lewis esophagectomy. Group A (abnormal Eso-pH, normal Restech) can easily be explained by reflux episodes that do not reach the oropharynx and are therefore not measured by laryngopharyngeal pH testing. Group B (normal Eso-pH, abnormal Restech) is not explainable from a pathophysiological standpoint. Results from our last paper indicated that acidic vapor or other factors may cause abnormal Restech results with simultaneous normal Eso-pH results. Previous studies showed only a weak correlation between esophageal and laryngopharyngeal pH measurement resulting in the conclusion that the Restech device adds no or little value as a diagnostic device in the evaluation of Gastroesophageal reflux disease (GERD) and LPR. However, only small numbers of patients were included in those studies and esophageal pH monitoring was either not performed simultaneously or not performed at all, leading to an insufficient comparison of results.

Research methods

A prospective analysis of patient data during follow-up after hybrid minimally invasive Ivor Lewis esophagectomy for cancer was performed. To obtain a homogenous population, only patients at least 3 mo out of surgery and disease-free survival were included in this study to eliminate immediate postoperative effects. Demographics, endoscopic findings, biopsies at different follow-up time points, as well as tumor histology and stage were recorded in the prospective database. Additionally, symptoms were recorded at all times of follow-up. Only patients that presented with reflux-related symptoms or those showing endoscopic proof of mucosal damage in the esophageal remnant were offered inclusion in this study. Gastrointestinal function testing (simultaneous esophageal and laryngopharyngeal pH testing) as well as upper GI endoscopy was completed. No HRM or barium swallow was performed in this study. Subsequently, the relationship between the two techniques was evaluated.

Research results

A total of 43 patients from May 2016 - November 2018 were included. All patients presented with mainly typical reflux symptoms such as heartburn (74%), regurgitation (84%), chest pain (58%), and dysphagia (47%). Extraesophageal symptoms such as cough, hoarseness, asthma symptoms, and globus sensation were also present. Esophageal 24-hour pH-metry was abnormal in 88% of patients with a mean DeMeester Score of 229.45 [range 26.4-319.5]. Restech evaluation was abnormal in 61% of cases in this highly selective patient cohort. All patients with abnormal supine LPR were also abnormal for supine esophageal reflux measured by conventional eso-pH.

Research conclusions

Patients following esophagectomy and reconstruction with gastric interposition can ideally serve as a human reflux model, as a large proportion suffers from severe postoperative GERD. Interestingly, laryngopharyngeal reflux phases occur mainly in the upright position, and acidity of the gastric conduit is already nearly normalized shortly after surgery. In this human volume-reflux model, esophageal pH-metry correlated precisely with an abnormal laryngopharyngeal pH-metry (Restech).

Research perspectives

Overall, the current level of evidence using the Restech device is limited by rather small case studies concluding that more research regarding the new reflux measurement device needs to be done. In addition, patients after esophagectomy can ideally serve as a human reflux model for further investigations and validation studies.

REFERENCES

1 **Fuchs KH**, Babic B, Breithaupt W, Dallemagne B, Fingerhut A, Furnee E, Granderath F, Horvath P,

Kardos P, Pointner R, Savarino E, Van Herwaarden-Lindeboom M, Zaninotto G; European Association of Endoscopic Surgery (EAES). EAES recommendations for the management of gastroesophageal reflux disease. *Surg Endosc* 2014; **28**: 1753-1773 [PMID: 24789125 DOI: 10.1007/s00464-014-3431-z]

2 **Vakil N**, van Zanten SV, Kahrilas P, Dent J, Jones R; Global Consensus Group. The Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus. *Am J Gastroenterol* 2006; **101**: 1900-1920; quiz 1943 [PMID: 16928254 DOI: 10.1111/j.1572-0241.2006.00630.x]

3 **Hom C**, Vaezi MF. Extra-esophageal manifestations of gastroesophageal reflux disease: diagnosis and treatment. *Drugs* 2013; **73**: 1281-1295 [PMID: 23881666 DOI: 10.1007/s40265-013-0101-8]

4 **Naik RD**, Vaezi MF. Extra-esophageal manifestations of GERD: who responds to GERD therapy? *Curr Gastroenterol Rep* 2013; **15**: 318 [PMID: 23435747 DOI: 10.1007/s11894-013-0318-4]

5 **Schnatz PF**, Castell JA, Castell DO. Pulmonary symptoms associated with gastroesophageal reflux: use of ambulatory pH monitoring to diagnose and to direct therapy. *Am J Gastroenterol* 1996; **91**: 1715-1718 [PMID: 8792686]

6 **Issing WJ**, Karkos PD, Perreas K, Folwaczny C, Reichel O. Dual-probe 24-hour ambulatory pH monitoring for diagnosis of laryngopharyngeal reflux. *J Laryngol Otol* 2004; **118**: 845-848 [PMID: 15638969 DOI: 10.1258/0022215042703660]

7 **Ayazi S**, Lipham JC, Hagen JA, Tang AL, Zehetner J, Leers JM, Oezcelik A, Abate E, Banki F, DeMeester SR, DeMeester TR. A new technique for measurement of pharyngeal pH: normal values and discriminating pH threshold. *J Gastrointest Surg* 2009; **13**: 1422-1429 [PMID: 19421822 DOI: 10.1007/s11605-009-0915-6]

8 **Ayazi S**, Hagen JA, Zehetner J, Oezcelik A, Abate E, Kohn GP, Sohn HJ, Lipham JC, Demeester SR, Demeester TR. Proximal esophageal pH monitoring: improved definition of normal values and determination of a composite pH score. *J Am Coll Surg* 2010; **210**: 345-350 [PMID: 20193899 DOI: 10.1016/j.jamcollsurg.2009.12.006]

9 **Worrell SG**, DeMeester SR, Greene CL, Oh DS, Hagen JA. Pharyngeal pH monitoring better predicts a successful outcome for extraesophageal reflux symptoms after antireflux surgery. *Surg Endosc* 2013; **27**: 4113-4118 [PMID: 23836124 DOI: 10.1007/s00464-013-3076-3]

10 **Fuchs HF**, Müller DT, Berlth F, Maus MK, Fuchs C, Dübbers M, Schröder W, Bruns CJ, Leers JM. Simultaneous laryngopharyngeal pH monitoring (Restech) and conventional esophageal pH monitoring-correlation using a large patient cohort of more than 100 patients with suspected gastroesophageal reflux disease. *Dis Esophagus* 2018; **31** [PMID: 29534167 DOI: 10.1093/dote/doy018]

11 **Dresner SM**, Griffin SM, Wayman J, Bennett MK, Hayes N, Raimes SA. Human model of duodenogastro-oesophageal reflux in the development of Barrett's metaplasia. *Br J Surg* 2003; **90**: 1120-1128 [PMID: 12945080 DOI: 10.1002/bjs.4169]

12 **Fuchs HF**, Schmidt HM, Meissner M, Brinkmann S, Maus M, Bludau M, Schröder W, Hölscher AH, Leers JM. Endoscopic and histopathologic reflux-associated mucosal damage in the remnant esophagus following transthoracic esophagectomy for cancer-5-year long-term follow-up. *Dis Esophagus* 2018; **31**: 1-6 [PMID: 29036607 DOI: 10.1093/dote/dox115]

13 **Moehler M**, Al-Batran SE, Andus T, Anthuber M, Arends J, Arnold D, Aust D, Baier P, Baretton G, Bernhardt J, Boeing H, Böhle E, Bokemeyer C, Bornschein J, Budach W, Burmester E, Caca K, Diemer WA, Dietrich CF, Ebert M, Eickhoff A, Ell C, Fahlke J, Feussner H, Fietkau R, Fischbach W, Fleig W, Flentje M, Gabbert HE, Galle PR, Geissler M, Gockel I, Graeven U, Grenacher L, Gross S, Hartmann JT, Heike M, Heinemann V, Herbst B, Herrmann T, Höcht S, Hofheinz RD, Höfler H, Höhler T, Hölscher AH, Horneber M, Hübner J, Izbicki JR, Jakobs R, Jenssen C, Kanzler S, Keller M, Kiesslich R, Klautke G, Körber J, Krause BJ, Kuhn C, Kullmann F, Lang H, Link H, Lordick F, Ludwig K, Lutz M, Mahlberg R, Malfertheiner P, Merkel S, Messmann H, Meyer HJ, Mönig S, Piso P, Pistorius S, Porschen R, Rabenstein T, Reichardt P, Ridwelski K, Röcken C, Roetzer I, Rohr P, Schepp W, Schlag PM, Schmid RM, Schmidberger H, Schmiegel WH, Schmoll HJ, Schuch G, Schuhmacher C, Schütte K, Schwenk W, Selgrad M, Sendler A, Seraphin J, Seufferlein T, Stahl M, Stein H, Stoll C, Stuschke M, Tannapfel A, Tholen R, Thuss-Patience P, Treml K, Vanhoefer U, Vieth M, Vogelsang H, Wagner D, Wedding U, Weimann A, Wilke H, Wittekind C; AWMF; AWMF. [German S3-guideline "Diagnosis and treatment of esophagogastric cancer"]. *Z Gastroenterol* 2011; **49**: 461-531 [PMID: 21476183 DOI: 10.1055/s-0031-1273201]

14 **Moehler M**, Baltin CT, Ebert M, Fischbach W, Gockel I, Grenacher L, Hölscher AH, Lordick F, Malfertheiner P, Messmann H, Meyer HJ, Palmqvist A, Röcken C, Schuhmacher C, Stahl M, Stuschke M, Vieth M, Wittekind C, Wagner D, Mönig SP. International comparison of the German evidence-based S3-guidelines on the diagnosis and multimodal treatment of early and locally advanced gastric cancer, including adenocarcinoma of the lower esophagus. *Gastric Cancer* 2015; **18**: 550-563 [PMID: 25192931 DOI: 10.1007/s10120-014-0403-x]

15 **Hölscher AH**, Stahl M, Messmann H, Stuschke M, Meyer HJ, Porschen R. [New S3 guideline for esophageal cancer : Important surgical aspects]. *Chirurg* 2016; **87**: 865-872 [PMID: 27406251 DOI: 10.1007/s00104-016-0214-1]

16 **Egberts JH**, Biebl M, Perez DR, Mees ST, Grimminger PP, Müller-Stich BP, Stein H, Fuchs H, Bruns CJ, Hackert T, Lang H, Pratschke J, Izbicki J, Weitz J, Becker T. Robot-Assisted Oesophagectomy: Recommendations Towards a Standardised Ivor Lewis Procedure. *J Gastrointest Surg* 2019; **23**: 1485-1492 [PMID: 30937716]

17 **Straatman J**, van der Wielen N, Cuesta MA, Daams F, Roig Garcia J, Bonavina L, Rosman C, van Berge Henegouwen MI, Gisbertz SS, van der Peet DL. Minimally Invasive Versus Open Esophageal Resection: Three-year Follow-up of the Previously Reported Randomized Controlled Trial: the TIME Trial. *Ann Surg* 2017; **266**: 232-236 [PMID: 28187044 DOI: 10.1097/sla.0000000000002171]

18 **Briez N**, Piessen G, Bonnetain F, Brigand C, Carrere N, Collet D, Doddoli C, Flamein R, Mabrut JY, Meunier B, Msika S, Perniceni T, Peschaud F, Prudhomme M, Triboulet JP, Mariette C. Open versus laparoscopically-assisted oesophagectomy for cancer: a multicentre randomised controlled phase III trial - the MIRO trial. *BMC Cancer* 2011; **11**: 310 [PMID: 21781337 DOI: 10.1186/1471-2407-11-310]

19 **Fuchs HF**, Harnsberger CR, Broderick RC, Chang DC, Sandler BJ, Jacobsen GR, Bouvet M, Horgan S. Simple preoperative risk scale accurately predicts perioperative mortality following esophagectomy for malignancy. *Dis Esophagus* 2017; **30**: 1-6 [PMID: 26727414 DOI: 10.1111/dote.12451]

20 **Lundell LR**, Dent J, Bennett JR, Blum AL, Armstrong D, Galmiche JP, Johnson F, Hongo M, Richter JE, Spechler SJ, Tytgat GN, Wallin L. Endoscopic assessment of oesophagitis: clinical and functional correlates and further validation of the Los Angeles classification. *Gut* 1999; **45**: 172-180 [PMID: 10403727 DOI: 10.1136/gut.45.2.172]

21 **Baretton GB**, Aust DE. [Barrett's esophagus. An update]. *Pathologe* 2012; **33**: 5-16 [PMID: 22293785 DOI: 10.1007/s00292-011-1541-0]

22 **Velanovich V**. Comparison of generic (SF-36) vs. disease-specific (GERD-HRQL) quality-of-life scales for gastroesophageal reflux disease. *J Gastrointest Surg* 1998; **2**: 141-145 [PMID: 9834409 DOI: 10.1016/s1091-255x(98)80004-8]

23 **Eypasch E**, Williams JI, Wood-Dauphinee S, Ure BM, Schmülling C, Neugebauer E, Troidl H. Gastrointestinal Quality of Life Index: development, validation and application of a new instrument. *Br J Surg* 1995; **82**: 216-222 [PMID: 7749697 DOI: 10.1002/bjs.1800820229]

24 **Becker V**, Graf S, Schlag C, Schuster T, Feussner H, Schmid RM, Bajbouj M. First agreement analysis and day-to-day comparison of pharyngeal pH monitoring with pH/impedance monitoring in patients with suspected laryngopharyngeal reflux. *J Gastrointest Surg* 2012; **16**: 1096-1101 [PMID: 22450948 DOI: 10.1007/s11605-012-1866-x]

25 **Wilhelm D**, Jell A, Feussner H, Schmid RM, Bajbouj M, Becker V. Pharyngeal pH monitoring in gastrectomy patients - what do we really measure? *United European Gastroenterol J* 2016; **4**: 541-545 [PMID: 27536364]

26 **Mazzoleni G**, Vailati C, Lisma DG, Testoni PA, Passaretti S. Correlation between oropharyngeal pH-monitoring and esophageal pH-impedance monitoring in patients with suspected GERD-related extra-esophageal symptoms. *Neurogastroenterol Motil* 2014; **26**: 1557-1564 [PMID: 25208949 DOI: 10.1111/nmo.12422]

27 **Agrawal N**, Yadlapati R, Shabeeb N, Price CP, Lidder A, Shintani-Smith S, Bové M, Pandolfino J, Tan B. Relationship between extralaryngeal endoscopic findings, proton pump inhibitor (PPI) response, and pH measures in suspected laryngopharyngeal reflux. *Dis Esophagus* 2019; **32** [PMID: 30101358 DOI: 10.1093/dote/doy072]

28 **Yadlapati R**, Pandolfino JE, Lidder AK, Shabeeb N, Jaiyeola DM, Adkins C, Agrawal N, Cooper A, Price CP, Ciolino JD, Gawron AJ, Smith SS, Bove M, Tan BK. Oropharyngeal pH Testing Does Not Predict Response to Proton Pump Inhibitor Therapy in Patients with Laryngeal Symptoms. *Am J Gastroenterol* 2016; **111**: 1517-1524 [PMID: 27091320 DOI: 10.1038/ajg.2016.145]

29 **Yadlapati R**, Adkins C, Jaiyeola DM, Lidder AK, Gawron AJ, Tan BK, Shabeeb N, Price CP, Agrawal N, Ellenbogen M, Smith SS, Bove M, Pandolfino JE. Abilities of Oropharyngeal pH Tests and Salivary Pepsin Analysis to Discriminate Between Asymptomatic Volunteers and Subjects With Symptoms of Laryngeal Irritation. *Clin Gastroenterol Hepatol* 2016; **14**: 535-542. e2 [PMID: 26689899 DOI: 10.1016/j.cgh.2015.11.017]

30 **Vailati C**, Mazzoleni G, Bondi S, Bussi M, Testoni PA, Passaretti S. Oropharyngeal pH monitoring for laryngopharyngeal reflux: is it a reliable test before therapy? *J Voice* 2013; **27**: 84-89 [PMID: 23159026 DOI: 10.1016/j.jvoice.2012.08.006]

31 **Dunn LJ**, Burt AD, Hayes N, Griffin SM. Columnar Metaplasia in the Esophageal Remnant After Esophagectomy: A Common Occurrence and a Valuable Insight Into the Development of Barrett Esophagus. *Ann Surg* 2016; **264**: 1016-1021 [PMID: 26756755 DOI: 10.1097/SLA.0000000000001591]

32 **Gutschow C**, Collard JM, Romagnoli R, Salizzoni M, Hölscher A. Denervated stomach as an esophageal substitute recovers intraluminal acidity with time. *Ann Surg* 2001; **233**: 509-514 [PMID: 11303132 DOI: 10.1097/00000658-200104000-00005]

33 **Müller DT**, Schulte E, Babic B, Knepper L, Fuchs C, Schröder W, Bruns CJ, Leers JM, Fuchs HF. Software improvement for evaluation of laryngopharyngeal pH testing (Restech) - a comparison between DataView 3 and 4. *World J Gastrointest Surg* 2020; **12**: 236-246 [PMID: 32551029 DOI: 10.4240/wjgs.v12.i5.236]

World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2021 June 15; 13(6): 625-637

DOI: 10.4251/wjgo.v13.i6.625

ISSN 1948-5204 (online)

SYSTEMATIC REVIEWS

Current role of hepatopancreatoduodenectomy for the management of gallbladder cancer and extrahepatic cholangiocarcinoma: A systematic review

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Author contributions: Fancellu A, and Porcu A contributed to this paper with conception and study design, manuscript writing and critical revision; Sanna V contributed with manuscript drafting and critical revision of oncologic outcomes; Deiana G, Ninniri C, and Perra T contributed with literature review and analysis, drafting and critical revision/editing; Turilli D contributed to tables and figures drafting; all authors have read and approved the final manuscript.

Conflict-of-interest statement: The authors do not have any conflicts of interest relevant to this article.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist statement, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist statement.

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Abstract

BACKGROUND

Hepatopancreatoduodenectomy (HPD) is the simultaneous combination of hepatic resection, pancreaticoduodenectomy, and resection of the entire extrahepatic biliary system. HPD is not a universally accepted due to high mortality and morbidity rates, as well as to controversial survival benefits.

AIM

To evaluate the current role of HPD for curative treatment of gallbladder cancer (GC) or extrahepatic cholangiocarcinoma (ECC) invading both the hepatic hilum and the intrapancreatic common bile duct.

METHODS

A systematic literature search using the PubMed, Web of Science, and Scopus databases was performed to identify studies reporting on HPD, using the following keywords: 'Hepatopancreaticoduodenectomy', 'hepatopancreatoduodenectomy', 'hepatopancreatectomy', 'pancreaticoduodenectomy', 'hepatectomy', 'hepatic resection', 'liver resection', 'Whipple procedure', 'bile duct cancer', 'gallbladder cancer', and 'cholangiocarcinoma'.

RESULTS

This updated systematic review, focusing on 13 papers published between 2015 and 2020, found that rates of morbidity for HPD have remained high, ranging between 37.0% and 97.4%, while liver failure and pancreatic fistula are the most serious complications. However, perioperative mortality for HPD has decreased compared to initial experiences, and varies between 0% and 26%, although in

Manuscript source: Invited manuscript

Specialty type: Surgery

Country/Territory of origin: Italy

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Received: March 5, 2021
Peer-review started: March 5, 2021
First decision: March 29, 2021
Revised: April 3, 2021
Accepted: May 25, 2021
Article in press: May 25, 2021
Published online: June 15, 2021

P-Reviewer: Khachfe H
S-Editor: Zhang H
L-Editor: A
P-Editor: Li JH

selected center it is well below 10%. Long term survival outcomes can be achieved in selected patients with R0 resection, although 5–year survival is better for ECC than GC.

CONCLUSION

The present review supports the role of HPD in patients with GC and ECC with horizontal spread involving the hepatic hilum and the intrapancreatic bile duct, provided that it is performed in centers with high experience in hepatobiliary-pancreatic surgery. Extensive use of preoperative portal vein embolization, and preoperative biliary drainage in patients with obstructive jaundice, represent strategies for decreasing the occurrence and severity of postoperative complications. It is advisable to develop internationally-accepted protocols for patient selection, preoperative assessment, operative technique, and perioperative care, in order to better define which patients would benefit from HPD.

Key Words: Hepatopancreatoduodenectomy; Extrahepatic cholangiocarcinoma; Gallbladder cancer; Survival; Morbidity; Mortality

Core Tip: Hepatopancreatoduodenectomy (HPD) is a complex operation that may achieve curative treatment for selected patients with locally advanced gallbladder cancer and extrahepatic cholangiocarcinoma. However, it represents a surgical procedure with high morbidity and mortality rates, that should be performed in centers with high experience in hepatobiliary-pancreatic surgery. Internationally-accepted protocols on selection criteria, preoperative assessment, operative technique, and perioperative care, are needed in order to better define which patients would benefit from HPD.

Citation: Fancellu A, Sanna V, Deiana G, Ninniri C, Turilli D, Perra T, Porcu A. Current role of hepatopancreatoduodenectomy for the management of gallbladder cancer and extrahepatic cholangiocarcinoma: A systematic review. *World J Gastrointest Oncol* 2021; 13(6): 625-637
URL: https://www.wjgnet.com/1948-5204/full/v13/i6/625.htm
DOI: https://dx.doi.org/10.4251/wjgo.v13.i6.625

INTRODUCTION

Gallbladder cancer (GC) and extrahepatic cholangiocarcinoma (ECC) are tumours with dismal prognosis. Resection provides the only chance of cure, although this kind of surgery is technically challenging due to the complexity of biliary and vascular anatomy of the hepatobiliary-pancreatic region, and the necessity to perform extended hepatic resection[1-4]. In general, biliary cancers have various modes of local extension, including a 'horizontal spread' involving the entire extrahepatic biliary tree. Hepatopancreatoduodenectomy (HPD) is the simultaneous combination of hepatic resection, pancreaticoduodenectomy (PD), and resection of the entire extrahepatic biliary system that has been used for curative treatment of selected patients with GC and ECC invading both the hepatic hilum and the intrapancreatic common bile duct, historically considered as unresectable tumours[1]. The combination in the same operation of hepatic resection and PD, both of which belong to the category of major surgical oncology procedures, has known a limited spread due to high mortality and morbidity rates registered in the initial experiences, as well as to controversial survival benefits. A systematic review of safety and efficacy of HPD for biliary cancer published in 2015 from Zhou *et al*[5] just found 18 studies including 397 patients.

To date, HPD is not universally recognized as a surgical option in patients with locally advanced GC and ECC. However, although it remains a debated surgical operation currently performed in few centers with high expertise in hepatobiliary-pancreatic surgery, perioperative mortality has gradually decreased and encouraging survival outcomes have been observed in recent years.

Drawing on a recent case of HPD carried out at our institution (illustrated in Figure 1), the present paper aims to make a review of new insights on the use of this surgical intervention, focusing on current indications, mortality, morbidity, and survival outcomes of patients who received HPD for GC or ECC.

Historical perspective

HPD was described for the first time in 1974 from Kasumi *et al*[6], for treatment of a patient with GC involving the duodenum. The patient overcame the operation but died for cancer recurrence 5 months later. Subsequently, Takasaki *et al*[7], described 5 cases of extended right lobectomy combined with PD for gallbladder carcinoma. During the 80es and the 90es, HPD was performed in some institutions in Japan mostly for advanced GC and ECC, with reported high mortality and morbidity rates, and poor survival outcomes. In general, it should be recognized that Japanese surgeons have contributed significantly to the evolution of extended surgery for hepatobiliary-pancreatic malignancies[1,2]. Since the results of those procedures had been published essentially in Japanese journals, HPD had for years limited diffusion in the rest of the world[2]. It was not until the start of the 2000's that limited patient series on the use of that procedure were published also from American, European, and Asian institutions other than Japanese ones[8-11]. Looking at review articles, two main papers reported on the results of HPD published until the year 2015[1,5]. The appearance in the literature of new cohort studies in the last six years partially prompted the present review (Table 1).

MATERIALS AND METHODS

Literature search and review design

A systematic literature search using the PubMed, Web of Science, and Scopus databases was performed in January 2021 to identify studies in English reporting on HPD during the time-frame 2015-2020, with the aim of focusing on the most recent insights in the use of this complex procedure. The following keywords were used and combined for the search: 'Hepatopancreaticoduodenectomy', 'hepatopancreatoduodenectomy', 'hepatopancreatectomy', 'pancreaticoduodenectomy', 'hepatectomy', 'hepatic resection', 'liver resection', 'Whipple procedure', 'bile duct cancer', 'gallbladder cancer', and 'cholangiocarcinoma'. Reference lists were searched manually to identify further studies. To be included in the present review, the articles had to report on at least 10 cases of HPD intended as simultaneous hepatic resection and PD. Case reports, small case-series, and articles in which HPD was not used for biliary cancer were excluded. The flowchart of the study search and selection in this review was reported in Figure 2.

Statistical analysis

In contrast to classic meta-analyses, the outcomes were defined as the percentages of outcomes of interest without comparison (morbidity and mortality) in cohorts of patients receiving HPD for GC or ECC. Overall proportions can be estimated from the weighted mean of percentages measured in each study. The weight in this case is derived from the number of subjects included in the studies (resumed in Table 1) out of the total number of subjects in all studies, which is inverse of the variance in the classic meta-analyses.

RESULTS

Current indications for HPD

At the time of this review, a total of 13 studies were found in which HPD was used for treatment of either GC or ECC. HPD represents the only curative treatment for GC and ECC (the latter also known as 'Klatskin tumour', or 'hilar cholangiocarcinoma' or 'peri-hilar cholangiocarcinoma'), having extensive horizontal tumor spread with infiltration of the hepatic hilum and the intrapancreatic bile duct, due to the tissue invasion *via* the lymphatics and perineural spaces[3,4]. While CG and ECC represent the main indication for HPD, in a minority of cases this surgical approach has been used also in patients having benign disease, liver cancer, neuroendocrine tumours (especially pancreatic neuroendocrine tumor metastatic to the liver) and other malignancies[8,9,12-14]. However, for the purposes of this study, survival outcomes of HPD only for the

Table 1 Recent studies reporting on the use of HPD (published between 2015 and 2020)[1]

Ref.	Country	No. of patients submitted to HPD	Time frame	Inclusion criteria	Main conclusions
Tran *et al*[23], 2015	United States	107[2]	2005-2013	ECC, GC, pancreatic cancer, benign pancreatic disease NET, secondary liver cancer	A synchronous hemihepatectomy (or trisectionectomy) with PD remains a high morbid combination and should be reserved for patients who have undergone extremely cautious selection.
Fukami *et al*[15], 2016	Japan	38	1994-2014	ECC, GC	Major HPD with resection of the hepatic artery can be a preferable option for ECC with acceptable perioperative morbidity and mortality, as well as long-term survival. This procedure for GC should not be performed.
Fernandes *et al*[8], 2016	Brazil	35	2004-2014	ECC, GC, NET, secondary liver cancer/liver direct infiltration	Major liver resection with PD is associated to very high mortality. Efforts to ensure a remnant liver over 40%-50% of the total liver volume is the key to obtain patient survival.
Aoki *et al*[21], 2016	Japan	52	1994-2014	ECC, GC	HPD can be safely performed using the presently reported surgical strategies with acceptable short and long-term outcomes.
Dai *et al*[13], 2017	China	12	1998-2014	ECC, GC, HCC, liver sarcoma	Morbidity and mortality after HPD were significant. With R0 resection, the 5-year OS and DFS rates were 27.8% and 29.6%, respectively.
Lee *et al*[41], 2018	Korea	22	2004-2013	ECC, GC	HPD for GC and ECC can be performed with acceptable mortality and morbidity rates. GC patients who underwent HPD showed comparable survival rates compared with ECC patients.
Welch *et al*[9], 2019	United States	23	2014-2016	ECC, GC, pancreatic cancer, NET, liver cancer, other malignancy, benign disease	The morbidity and mortality after HPD are significantly higher than after major hepatectomy or PD alone. Centralization of HPD to a very few centers may be a strategy to improve outcomes.
Mizuno *et al*[37], 2019	Japan	38	1996-2016	GC	HPD for GC is associated with poor OS, high morbidity and mortality rates compared to hepatic resection. Although HPD may eradicate locally spreading GC, the procedure is questioned from an oncological view.
D'Souza *et al*[10], 2019	Sweden[3]	66	2003-2018	ECC, GC	HPD, although associated with substantial perioperative mortality, can offer a survival benefit in patient subgroups with ECC and GC. To achieve negative resection margins is paramount for an improved survival.
Toyoda *et al*[43], 2019	Japan	100	2001-2017	ECC	Presurgical cholangiographic classification, diffuse or localized type, is a tumor-related factor closely associated with survival; therefore, it may be a useful feature for patient selection prior to HPD for ECC.
Liu *et al*[11], 2020	China	16	2007-2017	ECC	The radical resection of ECC combined with the partial resection of the pancreatic head in some selected patients can actually replace HPD as a surgical treatment for ECC with distal bile duct involvement.
Shimizu *et al*[28], 2020	Japan	37	1990-2019	ECC	HPD is a valid treatment option for extensive cholangiocarcinoma, offering long-term survival benefit at the cost of relatively high but acceptable morbidity and mortality. HPD is advocated in selected patients provided that it is considered possible to achieve R0 resection.
Oba *et al*[42], 2020	Japan	36	1998-2018	ECC	Invasive tumor thickness could be measured using simple methods and may be used to stratify postoperative prognosis in patients with ECC.

[1]Only articles reporting on at least 10 cases of HPD were included.

[2]The paper was focused on patients receiving "hepatopancreatectomy", of whom 107 received HPD and 373 hepatic resection plus distal pancreatectomy.

[3]This is a multicentric study from 19 European countries.

GC: Gallbladder cancer; ECC: Extrahepatic cholangiocarcinoma; HCC: Hepatocarcinoma; HPD: Hepatopancreatoduodenectomy; NET: Neuroendocrine tumour; OS: Overall survival; DFS: Disease-free survival.

treatment of GC and ECC were considered.

Surgical considerations

HPD undoubtedly represents the most complex operation in the hepatobiliary-pancreatic region, and to date still remains a controversial procedure[5,9]. In the majority of cases, HPD includes a major hepatic resection (at least three Coinaud's segments), being right hepatectomy with simultaneous PD the most common combination in HPD[1,8,15]. Usually, also the segment I is included in the liver resection during HPD in order to increase the rate of R0 resection, especially in cases of ECC of Bismuth-Corlette type III–IV extending to the pancreato-duodenum, since the caudate lobe is involved by tumour[3,10]. Segmental hepatic resection or metastasectomy associated to PD (like in cases of PD for neuroendocrine tumours with limited hepatic metastases), or PD associated to hepatic resection without extirpation of the hilar bile duct (like in cases of GC with retropancreatic lymph node involvement) should not be considered as pure HPD. In fact, genuine HPD consists in removal of the entire extrahepatic biliary system with the adjacent liver and the pancreatoduodenum [1]. Also a two-stage procedure in which the pancreatic and liver resections were performed at two different occasions not separated more than 2 months in time, can be barely defined as pure HPD[10].

Variations in surgical steps of HPD have been described. Nonetheless, meticulous preparation of the hepatic inflow vessels represents the first step, in order to achieve preservation of the future liver remnant after hepatic resection. Usually, pylorus-preserving (or subtotal stomach-preserving) PD precedes the hepatic resection, and the tumor is removed en bloc by HPD[1,8]. A frozen section histologic examination at the proximal bile duct margin and distal ductal stump is performed like in standard PD. Clearance of the lymph nodes of the hepatoduodenal ligament and pancreaticoduodenal region is necessary in all cases. Reconstruction of the digestive tract is carried out with a Roux-en-Y jejunal limb.

Other authors prefer a 'liver first' approach for HPD, in which liver transection precedes PD, because this method may facilitate a curability assessment of the liver side, especially when doubts exist about the proximal extension of the tumour, allowing for an extended hepatectomy to be planned[12,16,17].

To note, reconstruction of the portal vein or hepatic artery or both is required in 20%–30% of cases during HPD[18]. Vascular resection/reconstruction during PD or hepatic resection is a complex procedure performed in centers with expertise in hepatobiliary-pancreatic surgery. In particular, venous resection has increased the number of resectable patients with pancreatic cancer[19]. Infiltration of the portal-mesenteric axis is no longer a contraindication for PD, and portal resection/reconstruction can be effectively carried out with direct suture, or using autologous or synthetic graft. The results of a recent meta-analysis demonstrated that PD plus venous resection has inferior survival outcomes and higher 30-d mortality when compared with standard PD, nonetheless that operation can obtain better survival outcomes when compared to nonoperative treatments in patients with portal-mesenteric invasion from pancreatic head adenocarcinoma[19]. For extension, venous resection has been used when necessary also during HPD[10,14]. On the other hand, the role arterial resection in surgical treatment of pancreatic and bile duct cancer remains controversial, although the prognostic value of hepatectomy with simultaneous resections of the portal vein and hepatic artery in patients with advanced ECC has been reported by some authors[2]. In this regard, Fukami *et al*[15] and Ota *et al*[20] performed HPD with hepatic artery resection/reconstruction (the so-called hepato-ligamento-pancreatoduodenectomy) in patients with ECC having macroscopic hepatoduodenal ligament invasion. Fukama e al did not observe any significant difference in 2-year survival between the patients with (12) and without (26) hepatic artery resection (P = 0.465). The same authors advised against the use of that procedure for GC[15].

Ideally, such a complex operation like HPD should be carefully planned preoperatively, taking into account the risk/benefit balance. In the European experience described by D'souza *et al*[10], in 46% of the patients, the decision to perform HPD was taken intraoperatively, while in the series from Aoki *et al*[21] the operative procedure was switched to an HPD in 25% of cases. Not surprisingly, intraoperative switch to HPD has been associated to a decreased recurrence-free survival.

Mortality and morbidity

HBP is a skill-demanding procedure with high morbidity and mortality rates. In the review of Zhou *et al*[5], the perioperative mortality associated to HPD was 10.3%. However, recent studies published between 2015 and 2020 showed significant

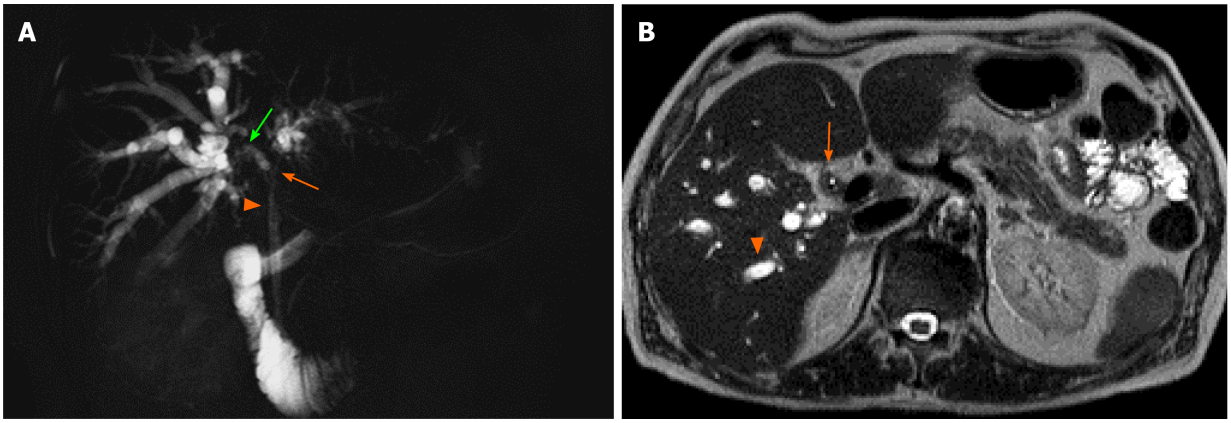

Figure 1 Patient was a 67-year-old man who was admitted to our hospital due to obstructive jaundice. A: Cholangio-MRI showed severe and long stricture of the common (arrowhead) and both right (green arrow) and left (orange arrow) hepatic ducts and, to a lesser extent, of distal branching of both right anterior and posterior segmental duct, with secondary upstream intrahepatic bile duct dilatation; B: Axial T2-weighted magnetic resonance image showed intrahepatic biliary dilatation (arrowhead) due to a T2 isointense intraductal mass (arrow). Preoperative imaging was consistent with an extrahepatic cholangiocarcinoma of Bismuth-Corlette type IV. Endoscopic preoperative biliary drainage was performed to relieve the obstruction. After multidisciplinary discussion, extended right hepatectomy was planned. Portal vein embolization of the right liver was carried out three weeks before the operation. Then, the patient underwent right hepatectomy extended to segment I, complete extirpation of the extrahepatic biliary system, and simultaneous pancreatoduodenectomy due to tumour involvement of the distal common bile duct at intraoperative frozen section. Thus, hepatopancreatoduodenectomy was the final surgical procedure. Final pathology showed a moderately differentiated cholangiocarcinoma with mucinous component, with 14 negative lymph nodes. Postoperative course was complicated by development of transient liver failure with ascites, electrolyte imbalance, and delayed gastric emptying with nausea and vomiting. The patient was discharged in postoperative day 58 and did not undergo chemotherapy. After 12 months, the patient is doing well, in stable health condition.

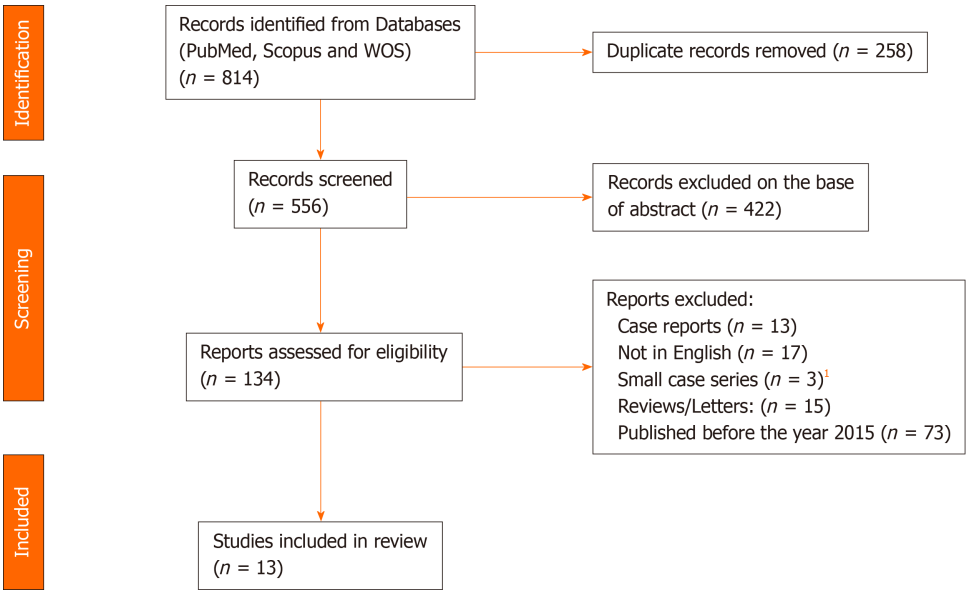

Figure 2 Flowchart of the study search and selection in this review. [1]Articles not reporting on at least 10 cases of hepatopancreatoduodenectomy.

differences among Eastern and Western countries, also reflecting the existing differences in mortality rates (12% *vs* 3%) after resection of ECC without HPD[10] (Table 2).

In a recent study investigating the safety-related outcomes of hepatobiliary-pancreatic surgeries performed in Japan after establishment of the 'Japanese Society of Hepato-Biliary-Pancreatic Surgery board certification system for expert surgeons', a mortality rate of 7.6% for HPD was registered[22]. Higher mortality rates for HPD were observed in United States (18.2%), Brasil (34.2%), and Europe (15%)[8,10,13,23]. However, it should be recognized that rates of mortality in selected centers from Japan were well below 5%. In fact, recent reports documented a mortality of 2.4%[24] or even no mortality in patients who underwent HPD for GC or ECC[14,21].

The morbidity rates associated to HPD were historically around 80%[5]. The largest single center report of 85 HPD cases for cholangiocarcinoma at the University of Nagoya published in 2012 found a high morbidity (76% of patients with Clavien-

Table 2 Studies reporting on morbidity and mortality outcomes for gallbladder cancer and extrahepatic cholangiocarcinoma after hepatopancreatoduodenectomy (years 2015-2020)

Ref.	Total *n* of patients	Morbidity (%)	Perioperative mortality (%)
Tran *et al*[23], 2015	107	87.5[3]	18.2[4]
Fukami *et al*[15], 2016	38	44.7[1]	13.5
Fernandes *et al*[8], 2016	35	97.4	34.2
Aoki *et al*[21], 2016	52	37.0[1]	0
Dai *et al*[13], 2017	12	83.3	25.0
Lee *et al*[41], 2018	22	68.2	4.5
Welch *et al*[9], 2019	23	87.0	26.0
Mizuno *et al*[37], 2019	38	87.0[1]	18.0
D'Souza *et al*[10], 2019	66	50.0[1]	15.0
Toyoda *et al*[43], 2019	100	81.0[1]	0%[2]
Liu *et al*[11], 2020	16	62.5	12.5
Shimizu *et al*[28], 2020	37	51.4[1]	5.4

[1]≥ 3 Clavien grade 3 morbidity.
[2]100 patients were enrolled in the study after excluding 4 patients who died of surgical.
[3]Lobectomy or trisectionectomy with pancreaticoduodenectomy.
[4]In-hospital mortality complications.

Dindo 3 or higher complications), in spite of considerable low operative mortality (2.4%)[24]. Similar results were reported from Utsumi *et al*[14] in a study on 17 patients, where morbidity rate was 88.3% and mortality rate 0%. D'souza *et al*[10] found postoperative complications Clavien-Dindo 3–4 in 50% of patients, with a higher rate in patients with ECC (63%) than in those with GC (35%). Welch *et al*[9], in their study promoted from the American College of Surgeons-National Surgical Quality Improvement Program, reported an overall morbidity and mortality for HPD of 87% and 26%, respectively. To note, morbidity and mortality rates were significantly higher when compared to both major hepatectomy (51% and 7.6%) and PD (52% and 1.4%), respectively (Table 2).

Hepatic failure, pancreatic fistula, biliary fistula and sepsis are the most common and serious postoperative complications of HPD, and also are important predictors of mortality[5,8,9]. The conspicuous blood losses associated to HPD undoubtedly play an important role in the occurrence of perioperative complications[2].

Interestingly, hepatic failure is the most common cause of perioperative death[5,9], although different definitions of that condition were encountered in the studies. Most HPDs include a major hepatectomy with removal of a large amount of hepatic mass, which exposes to the risk of leaving an insufficient liver remnant. An effective strategy for improving the safety and feasibility of major hepatectomy has become the preoperative portal vein embolization, that induces atrophy of the segments to be resected and compensatory contralateral hypertrophy of the remnant liver[17,23]. Ebata *et al*[24], among 85 patients receiving HPD, performed preoperative portal vein embolization in 78.8% of cases. In the experience of Fukami *et al*[15], criteria for preoperative portal vein embolization before HPD were right hepatectomy with a future remnant liver volume less than 40%. In spite of preoperative portal vein embolization, in some cases a desirable future liver remnant cannot be achieved, and volume increases and rapid tumour progression can occur while waiting for surgery. In those cases, HPD including liver parenchymal sparing surgery such as mesohepatectomy or central liver resection, may be used instead of typical major hepatectomy [25,26]. It should be taken into account that postoperative performance of the remnant liver is not only a matter of volume, in fact it is related also to the underlying liver function that need to be assessed with clinical examination, biochemistry, and other liver function tests[3]. The technique of Associating Liver Partition and Portal vein for Staged hepatectomy (ALLPS)[27], that has been used to rapidly enhance the volume of the liver remnant, is associated to considerable mortality and morbidity rates, and has no place in patients candidates to HPD[2,25].

According to Shimuzu *et al*[28], the indications for HPD in patients 70 years or older should be carefully considered, because they may require greater liver remnant volume in order to avoid the occurrence of postoperative liver failure.

In the pathogenesis of hepatic failure after HPD, also preoperative hyperbilirubinemia plays an important role[14]. The effects of the biliary stasis on the liver remnant include impaired function of hepatocyte mitochondria, impaired activity of microsomial mixed function oxidase, and in general increased predisposition to endotoxemia[5,29]. The role of preoperative biliary drainage of jaundiced patients scheduled for PD remains questioned[11]. However, authors suggested that biliary drainage may be appropriate before HPD, especially when major hepatectomy is planned[10,11,30,31].

Another primary concern in patients undergoing HPD is the occurrence of pancreatic fistula[32-34]. Postoperative pancreatic fistula is associated with other serious complications (especiallly intraabdominal hemorrhage and formation of abscesses) and mortality after PD[10,17,34]. Hepatic hilar clamping during liver resection, that usually follows PD, may induce venous congestion in the remnant pancreas that might facilitate pancreatic fistula formation[15]. To prevent a pancreatic fistula after HPD different methods have been used such external drainage of pancreatic juice by inserting a tube into the main pancreatic duct[35], that can also be followed by second-stage pancreatojejunostomy[36], and wrapping an omental flap around the dissected gastroduodenal artery[17]. Fukami *et al*[15] routinely employed an external pancreatojejunostomy stent in their series including 38 HPDs. Other possible complications, which can originate from the combination of hepatic resection and PD were delayed gastric emptying, hemorrhage, multi-organ failure, liver abscess, suppurative cholangitis, peritonitis, metabolic acidosis, portal vein thrombosis, sepsis, and hepaticojejunostomy leakage[5,8-10,20]. Some authors have proposed technical variants like 'pancreatic sparing resection' during HPD with the aim to reduce mortality and morbidity linked to HPD[11,20], but no conclusions can be drawn at this stage due to the paucity of reports.

High body mass index is a known independent risk factor for morbidity after HPD [22]. Since body mass index of Japanese people is lower than Western people, this finding might partially explain the better outcomes observed in Japanese series.

A careful patient selection and a multidisciplinary approach are essential issues to limit the occurrence and severity of complications of HPD[37]. An accurate assessment of nutritional status can be useful to stratify the perioperative risk of complications in order to optimize preoperative conditions as much as possible[8].

In summary, from the recent literature one can argue that HPD including simultaneous major hepatic resection and PD remains an intervention with a high risk of complications, although low perioperative mortality rates can be reached in institutions with high expertise. Centralization in centers of excellence of patients who can benefit from HPD may be a strategy to improve outcomes[9,38].

Survival outcomes

While patients with GC and ECC have in general a poor prognosis, long survival outcomes can be achieved in selected patients with R0 resection, since it has been demonstrated that negative margin is the most prognostic factor influencing long term survival after resection[11]. HPD carried out with curative intent with free margins has been reported to obtain acceptable survival outcomes, although important differences exist between GC and ECC, having the former a worst prognosis. For that reason, some authors have underscored that HPD can be considered an acceptable option for ECC, but have questioned its utility in patients with GC[1,2]. In fact, some authors underscored that no patients who received HPD for advanced GC survived after 5 years in their experience[39,40]. On the contrary, Mizuno *et al* among 38 patients with GC submitted to HPD reported a 5-year survival of 11%[37] . To note, two study reported comparable survival between patients who underwent HPD for GD or ECC [21,41].

In general, advancement in multimodality treatment of biliary cancer has led to improvement in survival after HPD in both GC and ECC in the last ten years. Zhou *et al*[5] in a review including studies published until 2014, reported that the 5-year overall survival in patients who underwent HPD with R0 resection ranged between 18% and 68.8% (median 51.3%), while it was 0% in those with R1 or R2 resection. The median 5-year survival rate of patients receiving HPD was 33% and 10.4% for patients with ECC and GC, respectively. In another review from Ebata *et al*[1], including the studies published between 2000 and 2013, the 5-year survival rates were 12%-64% for ECC and 0%-25% for GC[1]. It is important to look with attention at more recent cohort studies on HPD, in that better survival outcomes were observed. In a multicenter

study from Europe published in 2019, 3-year overall survival after HPD was cholangiocarcinoma 80% for ECC and 30% for GC (P = 0.018). The authors argued that more advanced T-stage for the GC might partially explain the worse survival[10]. Fukami *et al*[15] observed a 2-year overall survival of 71% and 39%, with a median survival time 42.3 and 13,5 months (P = 0.465) between patients with GC and ECC who underwent HPD plus hepatic artery resection and HPD without hepatic artery resection, respectively. The survival of the patients with CG was significantly worse than patients with ECC (P = 0.001). One of the most important reports on the use of HPD for advanced ECC was that form the Shinshu University (Japan) on 37 consecutive patients. The 1-, 3-, and 5-year overall survival rates were 83%, 48%, and 37%, respectively. Interestingly, in patients with R0 resection, 5-year overall survival was comparable between patients who had undergone major HPD and major hepatectomy alone (41% *vs* 40%)[28].

The survival outcomes of papers published in the time frame 2015-2020 were resumed in Table 3.

In summary, recent reports have noted good survival results, provided that R0 resection was achieved, although survival for GC remains worse than that for ECC.

Prognostic factors in patients with biliary cancer undergoing HPD remain to be clarified, and may somehow differ from those receiving major hepatectomy[42]. In a recent study including 100 patients, pathologic vascular invasion, pancreatic invasion, nodal metastasis, and margin status were not prognostic factors from the standpoint of long-term survival. Instead, presurgical cholangiographic classification, differentiating between "diffuse" or "localized" type, seems to be a tumor-related factor closely associated with survival probability. According to Toyoda *et al*[43], that cholangiographic classification may be effective to stratify patients candidates to HPD according to long-term survival probability.

DISCUSSION

Surgical resection with free margins remains the only possibility of cure able to achieve significant survival outcomes in patients with biliary cancer. In fact, systemic therapy and/or local treatments alternative to surgery demonstrated limited efficacy. The present review supports the role of HPD in patients with GC and ECC with horizontal spread involving the hepatic hilum and the intrapancreatic bile duct, although several aspects need to be clarified. HPD has had a limited diffusion, mainly due to the limited number of patients operated on with high mortality rates, and also because of questionable survival benefit. However, recent reports have showed improved operative results in centers with expertise in hepatobiliary-pancreatic surgery, due to advances in surgical techniques and perioperative patient care. Mortality rates in patients operated on in centers of excellence for this procedure were less than 10%, although morbidity rates remained high[11,21]. Indubitably, the team's expertise in advanced hepatobiliary-pancreatic surgery, and specifically in HPD procedure, plays a pivotal role in obtaining satisfactory results in terms of perioperative outcomes. As for oncological outcomes, recent reports have showed acceptable 5-year survival of 25% and 18%-40%, for GC and ECC, respectively. It is our view that the survival outcomes of patients receiving HPD should not be compared with those patients who had standard hepatic resection, but rather with those who receive nonoperative or palliative treatments. In this regard, authors observed a significantly better prognosis of patients receiving HPD for GC than those of the unresectable group[44].

The improved results in terms of perioperative morbidity and mortality, as well as the encouraging survival outcomes, have led to attach importance to HPD as a curative treatment in selected patients with biliary cancer, although it is not currently considered a standard procedure worldwide. Meticulous patients' selection is fundamental in order to obtain a R0 resection, that should represent the oncological objective of the procedure. From a risk/benefit perspective, we believe that R1 or R2 resection should not be an option in such a complex procedure as HPD. Prevention of hepatic failure with precise preoperative evaluation of the remnant liver function plays a key role in the success of HPD. According to centers' practice, methods such as 99mTc labeled galactosyl human serum albumin liver scintigraphy, computed tomography volumetry, or indocyanine green kinetics, can be used to quantitatively assess hepatic function. Probably, a remnant liver over 40%-50% of the total liver volume should be maintained to ensure patient survival[8].Extensive use of preoperative portal vein embolization, and preoperative biliary drainage in patients

Table 3 Studies reporting on survival outcomes after hepatopancreatoduodenectomy for gallbladder cancer and extrahepatic cholangiocarcinoma (2015-2020)

Ref.	Total *n* of patients	GC	ECC	Survival outcomes	
				GC	ECC
Aoki *et al*[21], 2016	52	13	39	NR[3]	NR[3]
Lee *et al*[41], 2018	22	8	14	25.0%[2]	17.9%[2]
D'Souza *et al*[10], 2019	66	31	35	30.0%[1]	80.0%[1]
Toyoda *et al*[43], 2019	100	0	100	-	49.2%[2]
Liu *et al*[11], 2020	16	0	16	-	20.0%[2]
Shimizu *et al*[28], 2020	37	0	37	-	36.8%[2]

[1]3-year overall survival.
[2]5-year overall survival.
[3]The study reported a 5-year survival of 44.5% for the entire cohort, with no significant difference between patients with gallbladder cancer and those with extrahepatic cholangiocarcinoma (P = 0.54).
GC: Gallbladder cancer; ECC: Extrahepatic cholangiocarcinoma; NR: Not reported.

with obstructive jaundice, represent strategies for decreasing the occurrence and severity of postoperative complications[25,26].

We recognize that the present review has some limitations, in that it includes only articles published in English in the time-lapse 2015-2020. Moreover, in the included studies, the indications for the HPD procedure were heterogeneous. However, this work has some points of strength since it addresses the insights from the most recent experiences in the use of HPD, thus it may be useful as an update review for best practices in the clinical setting.

It is plausible that the growing experience in HPD in selected centers will give impetus to further research on the use of that approach in the near future. To note, the exact role of HPD in patients with locally extended biliary cancer still remains to be defined and the combination of HPC with a multimodality approach with adjuvant/neoadjuvant treatments needs to be explored[31,45]. The indications for HPD slightly differ between Western and Eastern countries, and need to be standardized. Differences also exist in preoperative work up and operative technique among the institutions. Furthermore, survival outcomes for both GC and ECC in the different studies are difficult to compare due to heterogeneous methodologies and patients' inclusion criteria; also the results of the present review suggest that the role HPD may differ in the treatment of those two conditions. It is advisable to develop internationally-accepted protocols on selection criteria, preoperative assessment, operative technique, perioperative care, information sharing and data collection in order to better define which patients would benefit from HPD.

CONCLUSION

In conclusion, the present study suggests that HPD does have a definite role in the treatment of patients with GC and ECC with horizontal spread, although some aspects of the procedure remain to be elucidated. Surgeons' experience and careful patients' selection have a pivotal role in achieving R0 resection and acceptable oncological outcomes.

ARTICLE HIGHLIGHTS

Research background

Hepatopancreatoduodenectomy (HPD) is a challenging procedure that can be used for treatment of gallbladder cancer or extrahepatic cholangiocarcinoma invading the hepatic hilum and the intrapancreatic common bile duct. Due to high mortality and morbidity rates, as well as to controversial survival benefits, HPD is not a universally accepted procedure.

Research motivation

The aim of this review was to consolidate the evidence currently available on HPD for the treatment of gallbladder cancer and extrahepatic cholangiocarcinoma in a systematic fashion.

Research objectives

The main outcomes of interest were morbidity rates, mortality rates and survival outcomes after HPD for treatment of gallbladder cancer or extrahepatic cholangiocarcinoma.

Research methods

A systematic literature search was performed in PubMed, Web of Science, and Scopus databases to identify studies reporting on HPD during the time-frame 2015-2020.

Research results

Thirteen studies were included in this systematic review. Mortality rates varied among studies from Eastern and Western countries. In selected centers from Japan with high expertise in the hepatobiliary surgery, mortality rates were below 10%. Morbidity rates, albeit variable, were reported in more than 50% of patients. Five-year survival after HPD was higher in patients with extrahepatic cholangiocarcinoma than gallbladder carcinoma, and can be considered acceptable in cases were a R0 resection was obtained.

Research conclusions

The present review supports the role of hepatopancreaticoduodenectomy in selected patients with gallbladder cancer and extrahepatic cholangiocarcinoma, provided that a R0 resection is achieved. Preoperative portal vein embolization and preoperative biliary drainage in jaundiced patients represent strategies for decreasing the occurrence and severity of postoperative complications.

Research perspectives

The present review may be useful as a reference for best practices in the clinical setting, since it addresses the insights from the most recent experiences in the use of heptopancreaticoduodenectomy. Internationally-accepted protocols on selection criteria, preoperative assessment, operative technique, and perioperative care, are warranted to identify patients who would benefit from HPD.

REFERENCES

1 **Ebata T**, Yokoyama Y, Igami T, Sugawara G, Mizuno T, Nagino M. Review of hepatopancreatoduodenectomy for biliary cancer: an extended radical approach of Japanese origin. *J Hepatobiliary Pancreat Sci* 2014; **21**: 550-555 [PMID: 24464987 DOI: 10.1002/jhbp.80]

2 **Nagino M**. Fifty-year history of biliary surgery. *Ann Gastroenterol Surg* 2019; **3**: 598-605 [PMID: 31788648 DOI: 10.1002/ags3.12289]

3 **Capobianco I**, Rolinger J, Nadalin S. Resection for Klatskin tumors: technical complexities and results. *Transl Gastroenterol Hepatol* 2018; **3**: 69 [PMID: 30363698 DOI: 10.21037/tgh.2018.09.01]

4 **Feo CF**, Ginesu GC, Barmina M, Pinna A, Scanu AM, Cossu ML, Fancellu A, Porcu A. Curative Resection for Hilar Cholangiocarcinoma: Single-Center Experience with Long-Term Follow-Up. *Am Surg* 2018; **84**: 9-10 [PMID: 29642977]

5 **Zhou Y**, Zhang Z, Wu L, Li B. A systematic review of safety and efficacy of hepatopancreatoduodenectomy for biliary and gallbladder cancers. *HPB (Oxford)* 2016; **18**: 1-6 [PMID: 26776844 DOI: 10.1016/j.hpb.2015.07.008]

6 **Kasumi F**, Takagi K, Konishi T, Sakamoto G. Treatment of gallbladder cancer. *Jpn J Gastroenterol Surg* 1976; **9**: 170-177

7 **Takasaki K**, Kobayashi S, Muto S, Akimoto K, Toda S, Asado Y. (1980) Our experience (5 cases) of extended right lobectomy combined with pancreatoduodenectomy for carcinoma of the gallbladder. *Tan to Sui (J Bil Pancr)* 1980; **1**: 923-932

8 **Fernandes Ede S**, Mello FT, Ribeiro-Filho J, Monte-Filho AP, Fernandes MM, Coelho RJ, Matos MC, Souza AA, Torres OJ. THE LARGEST WESTERN EXPERIENCE WITH HEPATOPANCREATODUODENECTOMY: LESSONS LEARNED WITH 35 CASES. *Arq Bras Cir Dig* 2016; **29**: 17-20 [PMID: 27120733 DOI: 10.1590/0102-6720201600010005]

9 **Welch JC**, Gleeson EM, Karachristos A, Pitt HA. Hepatopancreatoduodenectomy in North America: are the outcomes acceptable? *HPB (Oxford)* 2020; **22**: 360-367 [PMID: 31519357 DOI: 10.1016/j.hpb.2019.08.010]

10 **D'Souza MA**, Valdimarsson VT, Campagnaro T, Cauchy F, Chatzizacharias NA, D'Hondt M, Dasari B, Ferrero A, Franken LC, Fusai G, Guglielmi A, Hagendoorn J, Hidalgo Salinas C, Hoogwater FJH, Jorba R, Karanjia N, Knoefel WT, Kron P, Lahiri R, Langella S, Le Roy B, Lehwald-Tywuschik N, Lesurtel M, Li J, Lodge JPA, Martinou E, Molenaar IQ, Nikov A, Poves I, Rassam F, Russolillo N, Soubrane O, Stättner S, van Dam RM, van Gulik TM, Serrablo A, Gallagher TM, Sturesson C; E-AHPBA scientific and research committee. Hepatopancreatoduodenectomy -a controversial treatment for bile duct and gallbladder cancer from a European perspective. *HPB (Oxford)* 2020; **22**: 1339-1348 [PMID: 31899044 DOI: 10.1016/j.hpb.2019.12.008]

11 **Liu F**, Hu HJ, Ma WJ, Wang JK, Ran CD, Regmi P, Li FY. Is radical resection of hilar cholangiocarcinoma plus partial resection of pancreatic head justified for advanced hilar cholangiocarcinoma? *ANZ J Surg* 2020; **90**: 1666-1670 [PMID: 32452116 DOI: 10.1111/ans.15955]

12 **Iacono C**, De Bellis M, Guglielmi A. ASO Author Reflections: Hepatopancreatoduodenectomy: Why, When, and How? *Ann Surg Oncol* 2020; **27**: 3358-3359 [PMID: 32382894 DOI: 10.1245/s10434-020-08569-5]

13 **Dai WC**, Chok KS, Cheung TT, Chan AC, Chan SC, Lo CM. Hepatopancreatoduodenectomy for advanced hepatobiliary malignancies: a single-center experience. *Hepatobiliary Pancreat Dis Int* 2017; **16**: 382-386 [PMID: 28823368 DOI: 10.1016/S1499-3872(17)60039-0]

14 **Utsumi M**, Sadamori H, Shinoura S, Umeda Y, Yoshida R, Nobuoka D, Takagi K, Fujiwara T, Yagi T. Risk factors of morbidity and predictors of long-term survival after hepatopancreatoduodenectomy for biliary cancer. *Hepatogastroenterology* 2014; **61**: 2167-2172 [PMID: 25699343]

15 **Fukami Y**, Kaneoka Y, Maeda A, Takayama Y, Onoe S. Major hepatopancreatoduodenectomy with simultaneous resection of the hepatic artery for advanced biliary cancer. *Langenbecks Arch Surg* 2016; **401**: 471-478 [PMID: 27023217 DOI: 10.1007/s00423-016-1413-4]

16 **Ishii T**, Seo S, Ito T, Ogiso S, Fukumitsu K, Masui T, Taura K. Liver Transection-First Approach in Hepatopancreatoduodenectomy for Hilar Cholangiocarcinoma: A Safe and Secure Technique for the Early Assessment of Curable Resection and Vascular Reconstruction. *Ann Surg Oncol* 2020 [PMID: 33169301 DOI: 10.1245/s10434-020-09303-x]

17 **Chiba N**, Abe Y, Yokozuka K, Hikita K, Kobayashi T, Sano T, Tomita K, Tsutsui R, Kawachi S. Surgical Technique of Pancreatic Parenchyma Transection-Delayed Approach (PPTDA) in Hepatopancreatoduodenectomy for Hilar Cholangiocarcinoma. *J Gastrointest Surg* 2019; **23**: 613-616 [PMID: 30187328 DOI: 10.1007/s11605-018-3923-6]

18 **Hemming AW**. Hepatopancreatoduodenectomy in North America: acceptable outcomes? *HPB (Oxford)* 2020; **22**: 358-359 [PMID: 31607638 DOI: 10.1016/j.hpb.2019.09.011]

19 **Fancellu A**, Petrucciani N, Porcu A, Deiana G, Sanna V, Ninniri C, Perra T, Celoria V, Nigri G. The Impact on Survival and Morbidity of Portal-Mesenteric Resection During Pancreaticoduodenectomy for Pancreatic Head Adenocarcinoma: A Systematic Review and Meta-Analysis of Comparative Studies. *Cancers (Basel)* 2020; **12** [PMID: 32698500 DOI: 10.3390/cancers12071976]

20 **Ota T**, Araida T, Yamamoto M, Takasaki K. Operative outcome and problems of right hepatic lobectomy with pancreatoduodenectomy for advanced carcinoma of the biliary tract. *J Hepatobiliary Pancreat Surg* 2007; **14**: 155-158 [PMID: 17384906 DOI: 10.1007/s00534-006-1110-8]

21 **Aoki T**, Sakamoto Y, Kohno Y, Akamatsu N, Kaneko J, Sugawara Y, Hasegawa K, Makuuchi M, Kokudo N. Hepatopancreaticoduodenectomy for Biliary Cancer: Strategies for Near-zero Operative Mortality and Acceptable Long-term Outcome. *Ann Surg* 2018; **267**: 332-337 [PMID: 27811506 DOI: 10.1097/SLA.0000000000002059]

22 **Otsubo T**, Kobayashi S, Sano K, Misawa T, Ota T, Katagiri S, Yanaga K, Yamaue H, Kokudo N, Unno M, Fujimoto J, Miura F, Miyazaki M, Yamamoto M. Safety-related outcomes of the Japanese Society of Hepato-Biliary-Pancreatic Surgery board certification system for expert surgeons. *J Hepatobiliary Pancreat Sci* 2017; **24**: 252-261 [PMID: 28258614 DOI: 10.1002/jhbp.444]

23 **Tran TB**, Dua MM, Spain DA, Visser BC, Norton JA, Poultsides GA. Hepato-pancreatectomy: how morbid? *HPB (Oxford)* 2015; **17**: 763-769 [PMID: 26058463 DOI: 10.1111/hpb.12426]

24 **Ebata T**, Yokoyama Y, Igami T, Sugawara G, Takahashi Y, Nimura Y, Nagino M. Hepatopancreatoduodenectomy for cholangiocarcinoma: a single-center review of 85 consecutive patients. *Ann Surg* 2012; **256**: 297-305 [PMID: 22750757 DOI: 10.1097/SLA.0b013e31826029ca]

25 **Nagaraj K**, Goto Y, Kojima S, Sakai H, Hisaka T, Akagi Y, Okuda K. Central hepatopancreatoduodenectomy-oncological effectiveness and parenchymal sparing option for diffusely spreading bile duct cancer: report of two cases. *BMC Surg* 2021; **21**: 23 [PMID: 33407366 DOI: 10.1186/s12893-020-01012-2]

26 **Mizuno T**, Kanemoto H, Sugiura T, Okamura Y, Uesaka K. Central hepatectomy with pancreatoduodenectomy for diffusely spread bile duct cancer. *J Hepatobiliary Pancreat Sci* 2015; **22**: 287-293 [PMID: 25488828 DOI: 10.1002/jhbp.197]

27 **Schnitzbauer AA**, Lang SA, Goessmann H, Nadalin S, Baumgart J, Farkas SA, Fichtner-Feigl S, Lorf T, Goralcyk A, Hörbelt R, Kroemer A, Loss M, Rümmele P, Scherer MN, Padberg W, Königsrainer A, Lang H, Obed A, Schlitt HJ. Right portal vein ligation combined with in situ splitting induces rapid left lateral liver lobe hypertrophy enabling 2-staged extended right hepatic resection in small-for-size settings. *Ann Surg* 2012; **255**: 405-414 [PMID: 22330038 DOI: 10.1097/SLA.0b013e31824856f5]

28 **Shimizu A**, Motoyama H, Kubota K, Notake T, Fukushima K, Ikehara T, Hayashi H, Yasukawa K, Kobayashi A, Soejima Y. Safety and Oncological Benefit of Hepatopancreatoduodenectomy for Advanced Extrahepatic Cholangiocarcinoma with Horizontal Tumor Spread: Shinshu University

Experience. *Ann Surg Oncol* 2021; **28**: 2012-2025 [PMID: 33044629 DOI: 10.1245/s10434-020-09209-8]

29 **Maguchi H**, Takahashi K, Katanuma A, Osanai M, Nakahara K, Matuzaki S, Urata T, Iwano H. Preoperative biliary drainage for hilar cholangiocarcinoma. *J Hepatobiliary Pancreat Surg* 2007; **14**: 441-446 [PMID: 17909711 DOI: 10.1007/s00534-006-1192-3]

30 **Torres OJM**, Alikhanov R, Li J, Serrablo A, Chan AC, de Souza M Fernandes E. Extended liver surgery for gallbladder cancer revisited: Is there a role for hepatopancreatoduodenectomy? *Int J Surg* 2020; **82S**: 82-86 [PMID: 32535266 DOI: 10.1016/j.ijsu.2020.05.085]

31 **Mizuno T**, Ebata T, Nagino M. Advanced hilar cholangiocarcinoma: An aggressive surgical approach for the treatment of advanced hilar cholangiocarcinoma: Perioperative management, extended procedures, and multidisciplinary approaches. *Surg Oncol* 2020; **33**: 201-206 [PMID: 31301935 DOI: 10.1016/j.suronc.2019.07.002]

32 **Gouillat C**, Gigot JF. Pancreatic surgical complications--the case for prophylaxis. *Gut* 2001; **49** Suppl 4: iv32-iv39 [PMID: 11878792 DOI: 10.1136/gut.49.suppl_4.iv29]

33 **Fancellu A**, Ginesu GC, Feo CF, Cossu ML, Puledda M, Pinna A, Porcu A. Pancreatic head excavation for tissue diagnosis may reduce unnecessary pancreaticoduodenectomies in the setting of chronic pancreatitis. *Hepatobiliary Pancreat Dis Int* 2017; **16**: 315-322 [PMID: 28603101 DOI: 10.1016/s1499-3872(17)60015-8]

34 **Nigri GR**, Rosman AS, Petrucciani N, Fancellu A, Pisano M, Zorcolo L, Ramacciato G, Melis M. Metaanalysis of trials comparing minimally invasive and open distal pancreatectomies. *Surg Endosc* 2011; **25**: 1642-1651 [PMID: 21184115 DOI: 10.1007/s00464-010-1456-5]

35 **Zhou Y**, Yang C, Wang S, Chen J, Li B. Does external pancreatic duct stent decrease pancreatic fistula rate after pancreatic resection? *Pancreatology* 2011; **11**: 362-370 [PMID: 21876365 DOI: 10.1159/000330222]

36 **Miwa S**, Kobayashi A, Akahane Y, Nakata T, Mihara M, Kusama K, Ogawa S, Soeda J, Miyagawa S. Is major hepatectomy with pancreatoduodenectomy justified for advanced biliary malignancy? *J Hepatobiliary Pancreat Surg* 2007; **14**: 136-141 [PMID: 17384903 DOI: 10.1007/s00534-006-1107-3]

37 **Mizuno T**, Ebata T, Yokoyama Y, Igami T, Yamaguchi J, Onoe S, Watanabe N, Ando M, Nagino M. Major hepatectomy with or without pancreatoduodenectomy for advanced gallbladder cancer. *Br J Surg* 2019; **106**: 626-635 [PMID: 30762874 DOI: 10.1002/bjs.11088]

38 **Endo I**, Hirahara N, Miyata H, Yamamoto H, Matsuyama R, Kumamoto T, Homma Y, Mori M, Seto Y, Wakabayashi G, Kitagawa Y, Miura F, Kokudo N, Kosuge T, Nagino M, Horiguchi A, Hirano S, Yamaue H, Yamamoto M, Miyazaki M. Mortality, morbidity, and failure to rescue in hepatopancreatoduodenectomy: An analysis of patients registered in the National Clinical Database in Japan. *J Hepatobiliary Pancreat Sci* 2021; **28**: 305-316 [PMID: 33609319 DOI: 10.1002/jhbp.918]

39 **Sakamoto Y**, Nara S, Kishi Y, Esaki M, Shimada K, Kokudo N, Kosuge T. Is extended hemihepatectomy plus pancreaticoduodenectomy justified for advanced bile duct cancer and gallbladder cancer? *Surgery* 2013; **153**: 794-800 [PMID: 23415082 DOI: 10.1016/j.surg.2012.11.024]

40 **Kaneoka Y**, Yamaguchi A, Isogai M. Hepatopancreatoduodenectomy: its suitability for bile duct cancer versus gallbladder cancer. *J Hepatobiliary Pancreat Surg* 2007; **14**: 142-148 [PMID: 17384904 DOI: 10.1007/s00534-006-1108-2]

41 **Lee EC**, Han SS, Lee SD, Park SJ. Is Hepatopancreatoduodenectomy an Acceptable Operation for Biliary Cancer? *Am Surg* 2018; **84**: 703-711 [PMID: 29966572]

42 **Oba M**, Nakanishi Y, Amano T, Okamura K, Tsuchikawa T, Nakamura T, Noji T, Asano T, Tanaka K, Hirano S. Stratification of Postoperative Prognosis by Invasive Tumor Thickness in Perihilar Cholangiocarcinoma. *Ann Surg Oncol* 2021; **28**: 2001-2009 [PMID: 33040247 DOI: 10.1245/s10434-020-09135-9]

43 **Toyoda Y**, Ebata T, Mizuno T, Yokoyama Y, Igami T, Yamaguchi J, Onoe S, Watanabe N, Nagino M. Cholangiographic Tumor Classification for Simple Patient Selection Prior to Hepatopancreatoduodenectomy for Cholangiocarcinoma. *Ann Surg Oncol* 2019; **26**: 2971-2979 [PMID: 31102092 DOI: 10.1245/s10434-019-07457-x]

44 **Yamamoto Y**, Sugiura T, Okamura Y, Ito T, Ashida R, Uemura S, Miyata T, Kato Y, Uesaka K. Is combined pancreatoduodenectomy for advanced gallbladder cancer justified? *Surgery* 2016; **159**: 810-820 [PMID: 26506566 DOI: 10.1016/j.surg.2015.09.009]

45 **Nagino M**, Ebata T, Yokoyama Y, Igami T, Mizuno T, Yamaguchi J, Onoe S, Watanabe N. Hepatopancreatoduodenectomy with simultaneous resection of the portal vein and hepatic artery for locally advanced cholangiocarcinoma: Short- and long-term outcomes of superextended surgery. *J Hepatobiliary Pancreat Sci* 2021; **28**: 376-386 [PMID: 33587829 DOI: 10.1002/jhbp.914]

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