ISSN 1948-5204 (online)

World Journal of *Gastrointestinal Oncology*

World J Gastrointest Oncol 2018 March 15; 10(3): 71-95

Published by Baishideng Publishing Group Inc

WJGO

World Journal of Gastrointestinal Oncology

Contents

Monthly Volume 10 Number 3 March 15, 2018

Contents

World Journal of Gastrointestinal Oncology
Volume 10 Number 3 March 15, 2018

ABOUT COVER

Editorial Board Member of *World Journal of Gastrointestinal Oncology*, C O'Morain, MD, Doctor, Department of Gastroenterol, Adelaide & Meath Hospital, Dublin 24, Ireland

AIM AND SCOPE

World Journal of Gastrointestinal Oncology (*World J Gastrointest Oncol*, *WJGO*, online ISSN 1948-5204, DOI: 10.4251) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJGO covers topics concerning carcinogenesis, tumorigenesis, metastasis, diagnosis, prevention, prognosis, clinical manifestations, nutritional support, molecular mechanisms, and therapy of benign and malignant tumors of the digestive tract. The current columns of *WJGO* include editorial, frontier, diagnostic advances, therapeutics advances, field of vision, mini-reviews, review, topic highlight, medical ethics, original articles, case report, clinical case conference (Clinicopathological conference), and autobiography. Priority publication will be given to articles concerning diagnosis and treatment of gastrointestinal oncology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJGO*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great clinical significance.

INDEXING/ABSTRACTING

World Journal of Gastrointestinal Oncology is now indexed in Science Citation Index Expanded (also known as SciSearch®), PubMed, and PubMed Central.

NAME OF JOURNAL
World Journal of Gastrointestinal Oncology

ISSN
ISSN 1948-5204 (online)

LAUNCH DATE
February 15, 2009

FREQUENCY
Monthly

EDITORIAL OFFICE
Xiu-Xia Song, Director
World Journal of Gastrointestinal Oncology
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive,
Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLICATION DATE
March 15, 2018

INSTRUCTIONS TO AUTHORS
http://www.wjgnet.com/bpg/gerinfo/204

ONLINE SUBMISSION
http://www.f6publishing.com

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2018 March 15; 10(3): 71-81

DOI: 10.4251/wjgo.v10.i3.71

ISSN 1948-5204 (online)

REVIEW

Fusobacterium nucleatum and colorectal cancer: A review

Fu-Mei Shang, Hong-Li Liu

Fu-Mei Shang, Hong-Li Liu, Cancer Center, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, Hubei Province, China

ORCID number: Fu-Mei Shang (0000-0002-9314-7110); Hong-Li Liu (0000-0002-5263-8108).

Author contributions: All authors equally contributed to this paper with conception and design of the study, literature review and analysis, drafting and critical revision and editing, and final approval of the final version.

Supported by the National Natural Science Foundation of China, No. 81472707.

Conflict-of-interest statement: No potential conflicts of interest.

Manuscript source: Unsolicited manuscript

Correspondence to: Hong-Li Liu, MD, PhD, Professor, Chief Physician, Cancer Center, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, No. 1277 Jiefang Avenue, Wuhan 430022, Hubei Province, China. hongli_liu@hust.edu.cn
Telephone: +86-27-85871962
Fax: +86-27-65650733

Received: December 8, 2017
Peer-review started: December 8, 2017
First decision: December 22, 2017
Revised: January 9, 2018
Accepted: March 6, 2018
Article in press: March 6, 2018
Published online: March 15, 2018

Abstract

Fusobacterium nucleatum (*F. nucleatum*) is a Gram-negative obligate anaerobe bacterium in the oral cavity and plays a role in several oral diseases, including periodontitis and gingivitis. Recently, several studies have reported that the level of *F. nucleatum* is significantly elevated in human colorectal adenomas and carcinomas compared to that in adjacent normal tissue. Several researchers have also demonstrated that *F. nucleatum* is obviously associated with colorectal cancer and promotes the development of colorectal neoplasms. In this review, we have summarized the recent reports on *F. nucleatum* and its role in colorectal cancer and have highlighted the methods of detecting *F. nucleatum* in colorectal cancer, the underlying mechanisms of pathogenesis, immunity status, and colorectal cancer prevention strategies that target *F. nucleatum*.

Key words: *Fusobacterium nucleatum*; Carcinoma; Colon and rectal carcinoma; Host immunity; Gut microbiome

Core tip: *Fusobacterium nucleatum* (*F. nucleatum*) promotes the progress of colorectal adenomas involving in multiple potential mechanisms. *F. nucleatum* positivity in colorectal cancer (CRC) is different in different research groups. Some potential biomarkers may be regarded as a criterion for judging CRC prognosis. Some chemoprevention and immunotherapy strategies on *F. nucleatum*-positive colorectal cancer need to be further explored in the future.

Shang FM, Liu HL. *Fusobacterium nucleatum* and colorectal cancer: A review. *World J Gastrointest Oncol* 2018; 10(3): 71-81 Available from: URL: http://www.wjgnet.com/1948-5204/full/v10/i3/71.htm DOI: http://dx.doi.org/10.4251/wjgo.v10.i3.71

INTRODUCTION

Colorectal cancer (CRC) is the third most prevalent malignant neoplasm and the fourth most frequent cause of cancer death in the world, and the five-year survival rate is nearly 65%[1]. For a long time, the mortality rate of CRC has declined in areas where medical resources are abundant, while the mortality rate has risen in areas with poor medical conditions[2]. CRC is a complex disease that is influenced by both genetic and environmental factors such as dietary habits and lifestyle. Recently, increasing evidence has indicated an association between the intestinal microbiota and CRC[3-5].

More than 100 trillion (10^{14}) microorganisms reside in the intestinal tract and play an extremely important role in human health. These microbes maintain intestinal homeostasis by regulating various biological activities such as mucosal barrier, immune and metabolic functions[6,7]. Once the intestinal balance is damaged, it may cause numerous intestinal diseases including inflammatory bowel diseases (IBD) and colorectal neoplasms[8-10]. There is accumulating evidence to suggest that the gut microbiota is associated with colorectal neoplasms[11-18]. Several studies have validated that the levels of *Bacteroides*, *Prevotella, Escherichia coli*, *Bacteroides fragilis* (ETBF), *Streptococcus gallolyticus*, *Enterococcus faecalis*, and *Streptococcus bovis* are significantly higher in CRC tissue compared to those in adjacent normal tissue[4,11-16,18]. ETBF has been confirmed to selectively stimulate *STATA3* in the colon, induce inflammation infiltrates of T helper type 17 and promote the development of CRC[19]. *Enterococcus faecalis* has been reported to facilitate tumorigenesis through activating the DNA damage pathways[20]. Furthermore, the abundance of both *Fusobacterium nucleatum* (*F. nucleatum*) and *C. difficile* was found to be significantly higher in CRCs compared to the healthy control group[21]. Additional studies have also confirmed that *F. nucleatum* associates with some Gram-negative bacteria, including *Streptococcus*, *Campylobacter spp.* and *Leptotrichia, and* synergistically promotes the occurrence of CRC[22,23].

F. nucleatum, a common Gram-negative anaerobic bacterium, is one of the most prevalent species in the oral cavity, and several studies have demonstrated that *F. nucleatum* is associated with oral inflammation diseases, such as periodontitis and gingivitis[24-26]. It has also been associated with pancreatic cancer, oral cancer, and premature and term stillbirths[27-30]. In addition, *F. nucleatum* is closely connected with liver abscess[9,31], appendicitis and infections of the head and neck, including mastoiditis, tonsillitis and maxillary sinusitis[32-35]. Increasing evidence has indicated that the levels of *F. nucleatum* are significantly elevated in tumor tissues and stool specimens of CRC patients relative to those in normal controls[36-42]. Researchers have reported that *F. nucleatum* may contribute to the development of CRC and that it is considered to be a potential risk factor for CRC progression[17,43]. Investigators have demonstrated that a higher abundance of *F. nucleatum* in CRC is associated with a shorter survival time[44]. Several researchers have also shown that a high-abundance of *F. nucleatum* induces a series of specific tumor molecular events, including CpG island methylator phenotype (CIMP), microsatellite instability (MSI), and genetic mutations in *BRAF*, *CHD7, CHD8* and *TP53*[44,45]. However, *F. nucleatum* was previously regarded as a passenger bacterium in human intestinal tract[46,47]. Recently, it has been considered to be a potential initiator of CRC susceptibility[37,45]. Kostic *et al*[48] have confirmed that *F. nucleatum* promotes colorectal tumorigenesis in $Apc^{min/+}$ mice. Rubinstein *et al*[43] have reported that *F. nucleatum* stimulates tumor cell growth in CRC by activating β-catenin signaling and inducing oncogenic gene expression *via* the FadA adhesion virulence factor. Together, these studies show that *F. nucleatum* plays an important role in the initiation of CRC and promoting tumor cell growth in CRC*,* supporting that *F. nucleatum* is a cause of CRC rather than a consequence. In this review, we have summarized the recent reports on *F. nucleatum* and its role in CRC and have highlighted the methods of detecting *F. nucleatum* in CRC, the underlying mechanisms of pathogenesis, immunity status, and colorectal prevention strategies that target *F. nucleatum*.

F. nucleatum invades human epithelial cells, activates β-catenin signaling, induces oncogenic gene expression and promotes growth of CRC cells through the FadA adhesion virulence factor.

METHODS FOR DETECTING *F. NUCLEATUM* IN CRC

To detect *F. nucleatum* in CRC, investigators have used several different methods, including fluorescent quantitative polymerase chain reaction (FQ-PCR), fluorescence in situ hybridization (FISH), quantitative real-time polymerase chain reaction (qPCR), and droplet digital polymerase chain reaction (ddPCR). Furthermore, sample collection methods also vary among studies, some of which are derived from formalin-fixed paraffin-embedded (FFPE) CRC tissues, CRC frozen tissues, genomic DNA, and feces collected from CRC patients.

As shown in Table 1, the detection method and the detection rate of *F. nucleatum* in CRC differ among studies. In one Chinese study, the *F. nucleatum* abundance was measured in frozen tissues from 101 CRC patients by FQ-PCR, and FISH analysis was conducted on 22 CRC FFPE tissues with the highest abundance of *F. nucleatum* to confirm the FQ-PCR results, and the positive rate of *F. nucleatum* was detected to be 87.13% (88/101)[40]. Analyzing 598 CRC patients in 2 American nationwide prospective cohort studies, researchers detected the abundance of *F. nucleatum* in FFPE tissue samples obtained from CRC patients by qPCR and found that the positive percentage of *F. nucleatum*

Table 1 Positive detection rates of *Fusobacterium nucleatum* in colorectal cancer reported by different research groups

Author (publish date)	Total cases (*n*)	Positive cases (*n*)	Positive percentage	Detection method	Detection samples
Li *et al*[40] (3/2016)	101	88	87.13%	FISH and FQ-PCR	Frozen tissue and FFPE tissue
Mima *et al*[38] (8/2015)	598	76	13%	qPCR	FFPE tissue
Nosho *et al*[49] (1/2016)	511	44	8.6%	qPCR	FFPE tissue
Tahara *et al*[45] (1/2014)	149	111	74%	qPCR	Genomic DNA
Ito *et al*[39] (2/2015)	511	286	56%	qPCR	FFPE tissue
Suehiro *et al*[50] (3/2017)	158	85	54%	ddPCR	Feces

qPCR: Quantitative real-time polymerase chain reaction; FQ-PCR: Fluorescent quantitative polymerase chain reaction; ddPCR: Droplet digital polymerase chain reaction; FISH: Fluorescence in situ hybridization; FFPE: Formalin-fixed paraffin-embedded.

accounted for 13% (76/598) of the CRC samples. This detection rate was significantly lower than that reported in the Chinese study (87.13%)[38]. In one Japanese study, the experimental specimens were obtained from CRC FFPE tissues from 511 Japanese patients, and the abundance of *F. nucleatum* was detected by qPCR. *F. nucleatum* was detected in 8.6% (44/511) of the CRC tissue samples, which was similar, albeit slightly lower, to that reported in the USA (13%)[49]. In another study, the richness of *F. nucleatum* was evaluated by qPCR, and the samples were prepared from genomic DNA extracted from 149 primary CRC tissue samples; *F. nucleatum* was detected in 74% (111/149) of the CRC tissue samples[45]. In a recent study, the samples consisted of FFPE tissues from 511 CRC patients, and *F. nucleatum* was detected in 56% (286/511) of the CRC patients by qPCR[39]. In another study, *F. nucleatum* was detected in the stool samples collected from CRC patients, and the sensitivity and specificity were found to be 72.1% (75/104) and 91.0%, respectively, while the high-abundance of *F. nucleatum* in patients exhibited a false positive rate of 7.0%[42]. In another study, the levels of *F. nucleatum* were measured in fecal specimens from Japanese CRC patients by ddPCR, and *F. nucleatum* was found to be present in 54% (85/158) of the specimens[50]. Furthermore, some researchers used a qPCR assay to detect *F. nucleatum* in FFPE tissue from CRC patients and revealed that *F. nucleatum* was present in 2.5% (4/157) of rectal cancers and 11% (19/178) of cecum cancers, with a significant linear trend along all subsites[51]. The percentage of *F. nucleatum*-enriched CRC gradually increases from rectum to cecum[51], suggesting that the rate at which *F. nucleatum* is present may also differ among intestinal sites.

Common specimens for detecting *F. nucleatum* in CRC include frozen tissues, FFPE tissues, genomic DNA and feces. The use of both frozen tissue and FFPE tissue specimens are limited by surgery or colonoscopy. Specimens derived from the feces of CRC patients are easy to obtain, but they often result in high false positive detection rates. As mentioned above, qPCR, ddPCR, FQ-PCR and FISH are applied to detect the levels of *F. nucleatum.* While the qPCR assay is the most popular technique to measure the abundance of *F. nucleatum* in CRC tissues, it is difficult to detect *F. nucleatum* in the feces[52]; in addition, a higher false positive rate is seen in the high abundance group of *F. nucleatum*[42]. It has been reported that ddPCR improved the sensitivity of *F. nucleatum* detection in the feces compared to qPCR, and ddPCR was demonstrated to be 1000 times more sensitive than qPCR[53]. In addition, ddPCR resulted in a higher detection rate of low concentrations of microorganisms compared with qPCR[54]. FQ-PCR is a convenient and rapid method for detecting pathogens and displays a higher sensitivity and specificity than qPCR[55]. In addition, it is difficult to contaminate FQ-PCR during experimental operation compared with qPCR[55].

UNDERLYING MECHANISMS OF *F.NUCLEATUM* PATHOGENESIS IN CRC

A previous study has shown that lymph node metastases are present in 52 out of 88 (59.1%) cases with a high-abundance of *F. nucleatum* and in 0 out of 13 (0%) subjects with a low-abundance of *F. nucleatum*, which indicates that a high abundance of *F. nucleatum* is associated with CRC progression and metastasis[40]. It has been suggested that high levels of *F. nucleatum* may be associated with poor outcomes of CRC. Some researchers have also reported that the load of *F. nucleatum* DNA in CRC tissue is correlated with higher colorectal cancer-specific mortality[44] and that *F. nucleatum* DNA may serve as a potential poor prognostic biomarker[44]. *Fusobacterium* was shown to be enriched in the mucosa-adherent microbiota and have the ability to adhere to and invade human epithelial and endothelial cells[27,52,56]. Recently, several researchers have suggested that *F. nucleatum* is a pathogenic bacterium rather than a bacterium that promotes colorectal carcinogenesis[43,57]. Several studies have shown that its virulence factors are closely linked with colorectal lesions. It has been demonstrated that *F. nucleatum* invades human epithelial cells, activates β-catenin signaling, induces oncogenic gene expression and promotes growth of CRC cells *via* the FadA adhesion virulence factor[43]. A second virulence factor, an autotransporter protein, Fap2, has been shown to potentiate the progress of CRC *via* inhibiting immune cell activity[58].

As shown in Figure 1, *F. nucleatum* attaches and

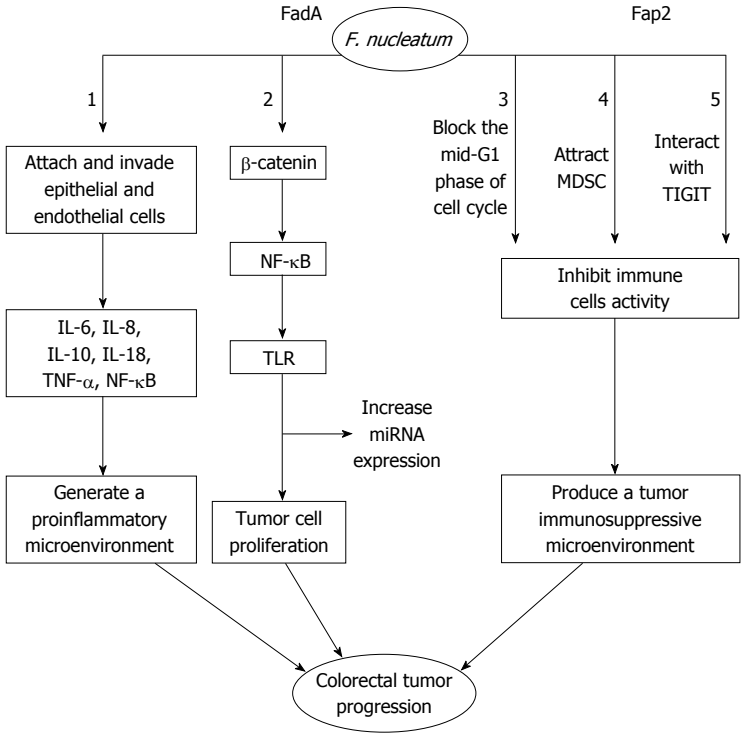

Figure 1 Underlying mechanism of *Fusobacterium nucleatum* pathogenesis in colorectal cancer. (1) In pathway 1, the FadA in *Fusobacterium nucleatum* (*F. nucleatum*) adheres to and invades human epithelial cells and endothelial cells, and inflammatory cytokine (IL-6, IL-8, IL-10, IL-18, TNF-α and NF-κB) levels increase in a proinflammatory microenvironment that accelerates the progression of colorectal tumors; (2) In pathway 2, FadA interacts with E-cadherin on the epithelial cell, activates β-catenin signaling, increases NF-κB inflammatory gene expression and promotes tumor cells proliferation. However, *F. nucleatum*-infected cells increase the expression of miRNA by activating Toll-like receptor and further promote the release of miRNA; (3) In pathway 3 and 4, *F. nucleatum* dampens human T-cell activation in a tumor immunosuppressive microenvironment that supports tumor cell growth by blocking the mid-G1 phase of cell cycle and attracting myeloid-derived suppressor cells; and (4) In pathway 5, the interaction between Fap2 of *F. nucleatum* and the human inhibitory receptor TIGIT induce human lymphocytes cell death and generate a tumor immunosuppressive microenvironment that promotes colorectal tumor progression. MDSC: Myeloid-derived suppressor cell; TLR: Toll-like receptor.

invades human epithelial and endothelial cells[27,56]. This attachment and invasion depends on the *F. nucleatum* FadA adhesion protein[59,60]. The FadA protein exists in two main forms. The first form is the intact pre-FadA consisting of 129 amino that is anchored to the membrane, and the second form is the secreted mature FadA (mFadA) consisting of 111 amino acids that are secreted outside of *F. nucleatum*[61]. When mFadA combines with pre-FadA, the pre-FadA-mFadA is internalized, and FadAc is activated[61]. The internalization of the pre-FadA and mFadA complex ensures that *F. nucleatum* binds to and invades host epithelial cells[61]. The host endothelial receptor for FadA is the vascular endothelial cadherin (CDH5), which is a member of the cadherin family[59]. The CDH5 receptor is required for *F. nucleatum* to adhere to and invade endothelial cells[59]. *F. nucleatum* invasion induces the production of cytokines such as interleukin-8 (IL-8), which is regulated by the p38 MAPK signaling pathway but independent of Toll-like receptor (TLR), NOD-1, NOD-2 and Nuclear Factor-kappaB (NF-κB) signaling[62]. *F. nucleatum* promotes the expression of several inflammatory genes such as NF-κB and cytokines, including IL-6, IL-8 and IL-18[43]. *F. nucleatum* also promotes the release of inflammatory cytokines particularly IL-8, IL-10 and tumor necrosis factor-α (TNF-α) in a proinflammatory microenvironment that accelerates colorectal tumor progression[37,62,63]. Another receptor of FadA is the cell-adhesion molecule E-cadherin expressed on non-CRC and CRC cells[43]. E-cadherin is a strong tumor suppressor that inhibits tumor growth and development[64].

FadA binding to wnt7b E-cadherin on CRC cells promotes *F. nucleatum* adhesion and invasion of host epithelial cells, activates β-catenin signaling that leads to increased expression of *Wnt* genes, oncogenes, transcription factors, and inflammatory genes, and promotes tumor cells proliferation[43]. FadAc, but not mFadA, binds specifically to the E-cadherin-5, the cytoplasmic or the transmembrane domains of E-cadherin, and results in E-cadherin phosphorylation and internalization[43,65]. As a result, a series of events, which include a decrease in β-catenin phosphorylation, an accumulation of β-catenin in the cytoplasm, and translocation toward the nucleus, leads to the activation of β-catenin-regulated transcription (CRT)[43]. CRT increases the expression of *wnt* signaling genes such as *wnt7a*, *wnt7b* and *wnt9a*, the oncogenes *myc* and cyclin D1, transcription factors such as the lymphoid enhancer factor (LEF-1), NF-κB such as NF-κB2, T cell factor

such as TCF1, TCF3 and TCF4, and proinflammatory cytokines including IL-6, IL-8 and IL-18[43]. On the other hand, *F. nucleatum* infected cells increase the expression of microRNA-21 (miR21) by activating TLR4 signaling to MYD88, which leads to the activation of NF-κB[41]. Subsequently, hyperactive NF-κB attaches to the promoter of miR21 and induces the oncogenic cascade in CRC[41]. Moreover, *F. nucleatum* reduces $CD3^+$ T-cell density in CRC tissue[38]. A previous study has shown that FDC364, sonic extract of *F. nucleatum*, inhibits human T-cell responses to antigens and mitogens[66]. By blocking the mid-G1 phase of cell cycle, the *F. nucleatum* inhibitory protein suppresses human T-cell activity[67]. This effect may promote an immunosuppressive microenvironment that allows tumor cell growth[67]. By releasing short-chain fatty acids (acetate, propionate, and butyrate) and short-peptides (formylmethionyl-leucyl-phenylalanine), *F. nucleatum* also selectively attracts myeloid-derived suppressor cells (MDSCs)[48,68]. MDSCs, a group of heterogeneous cells, show strong T-cell suppressive activity in the immune response[69]. MDSCs and their effectors are key components of the neoplasm and promote tumor progression[48,70]. *F. nucleatum*-associated tumors increase the myeloid-lineage infiltrating cells, including $CD11b^+$, tumor-associated macrophages (TAMs), M2-like TAMs, tumor-associated neutrophils, conventional myeloid dendritic cells (DCs) and $CD103^+$ regulatory DCs[48]. These cells play an important role in dampening antitumor immunity and promoting tumor progression[69,71-73]. Collectively, these studies have shown that *F. nucleatum* produces a tumor immunosuppressive microenvironment and promotes CRC progression. Fap2, a galactose-sensitive adhesion protein, plays an important role in coaggregation and cell adhesion[74]. In *F. nucleatum*, the virulence factor Fap2 protein suppresses immune cell activities through interacting with TIGIT[58]. The interaction between Fap2 and TIGIT protects tumors containing *F. nucleatum* from host immune cell attack[58]. TIGIT is an inhibitory receptor in humans that is expressed on T cells and natural killer (NK) cells[75]. The Fap2 has also been reported to induce human lymphocyte cell death[57]. In addition, Fap2 mediates *F. nucleatum* enrichment by interacting with Gal-GalNAc overexpressed in colorectal tumors[76]. Gal-GalNAc is a host polysaccharide overexpressed in CRC[76]. In summary, *F. nucleatum* produces a tumor immunosuppressive microenvironment that promotes CRC progression.

F. NUCLEATUM AND IMMUNITY STATUS IN CRC

Some researchers have demonstrated that *F. nucleatum* modulates the tumor immune microenvironment while promoting CRC development[48]. Recently, it has been confirmed that biomarkers such as immune antibodies, miRNA, TAMs, and tumor-infiltrating T-cell subsets play a significant role in *F. nucleatum*-associated CRC[44,48,77,78].

Several studies have shown that *F. nucleatum* infection causes high levels of serum *F. nucleatum*-IgA antibodies in CRC patients[77]. Researchers have confirmed that serum anti-*F. nucleatum*-IgA combined with CA19-9 and CEA has a higher sensitivity than CA19-9 and CEA alone in screening early CRC[77]. This study suggests that serum *F. nucleatum*-IgA antibodies may be regarded as a potential diagnosing biomarker for early CRC[77]. In addition, some researchers have found that the levels of the *fadA* gene in colon tissue from CRC patients are > 10-100 times higher in comparison with normal subjects[43]. This study also reveals a gradual increase in *fadA* gene copies in normal individuals compared to CRC patients[43]. The *fadA* gene has become a potential ideal diagnostic marker to identify individuals with CRC risk[43]. The *miR-21* gene has been demonstrated to promote tumor cell growth and migration *via* inhibiting sec23a protein expression[79]. The data also indicated that *F. nucleatum* induces a high level of miR-21 expression in advanced CRC[41]. The amount of miR-21 in CRC tissues has been shown to be associated with poor clinical outcomes[41]. Studies have reported that non-coding RNAs (lncRNAs) play a crucial role in the diagnosis and prognosis of CRC[80]. One study has found that low levels of NR_034119 and NR_029373 are associated with a short survival rate of CRC[80]. These researchers suggested that several lncRNAs (NR_034119, NR_029373, NR_026817, and BANCR) are potential diagnostic biomarkers for CRC and that NR_034119 and NR_029373 are potential prognostic indicators for CRC[80]. Another study reported that the level of lncRNA PANDAR was higher in CRC cells and tissues relative to adjacent normal tissues[81], and high levels of PANDAR expression were associated with short overall survival[81]. The authors suggested that the amount of PANDAR expression may be a prognostic indicator for CRC.

A previous study reported that *F. nucleatum*-positive tumors increased TAM infiltration[48]. TAMs play an important role in innate immunity, and subpopulations of regulatory T-lymphocytes (Tregs) are a component of the acquired immunity. A recent study has found that intense infiltration of TAMs in colorectal tumor tissue is associated with shorter disease-free survival and overall survival of CRC[78]. Infiltration of TAMs $CD68^+/iNOS^-$ in colorectal tumor stroma is confirmed to be related to the poor prognosis of CRC[78]. Some researchers have reported that tumor-infiltrating T-cell subpopulations distinctly regulate the prognosis of CRC[82]. For instance, in tumor-infiltrating T-cell subsets, $CD45RO^+$-cell density, but not that of $FOXP3^+$-cell, is significantly associated with a long survival of CRC patients[82]. $CD45RO^+$-cell is considered to be a potential good prognostic biomarker for CRC[82]. The $FOXP3^+$ transcription factor, which plays an important role in regulating the immune system, is regarded as an immunosuppressive factor. Some scholars have reported that infiltration of $FOXP3^+$ in colorectal tumor

stroma is associated with a poor prognosis in CRC[78]. However, several researchers also suggest that FOXP3$^+$-cells are generally associated with a good prognosis of CRC[83]. An article recently published in Nature Medicine has shown that distinct tumor-infiltrating FOXP3$^+$-T cell subpopulations have an opposite approach to determining CRC prognosis. The development of inflammatory FOXP3$^\pm$ (lo) non-T_{reg} cells was shown to be associated with tumor invasion by intestinal bacteria, particularly *F. nucleatum*[84]. In this study, CRC patients with a high infiltration of FOXP3$^\pm$ (lo) T cells exhibit a significantly better prognosis, compared to those with a FOXP3$^\pm$ (hi) T_{reg} cell infiltration[84]. When FOXP3$^\pm$ (hi) T_{reg} cells are depleted from CRC tissues, antitumor immunity is augmented[84]. The elimination of FOXP3$^\pm$ (hi) T_{reg} cells has been suggested to play a crucial role in suppressing CRC formation[84]. Recent research has also found that prudent diets such as whole grain and dietary fiber reduce the risk of *F nucleatum*-positive CRC[85].

In conclusion, anti-*F. nucleatum*-IgA, the *fadA* gene, and lncRNAs may be considered as potential diagnostic biomarkers during the early stage of *F. nucleatum*-positive CRC. The CD45RO$^+$-cell and FOXP3$^\pm$ (lo) T cell biomarkers are associated with a favorable prognosis in *F. nucleatum*-positive CRC, while the miR-21, LncRNA PANDAR, and TAMs CD68$^+$/iNOS$^-$ biomarkers are associated with a poor clinical prognosis of *F. nucleatum*-positive CRC.

PREVENTION STRATEGIES THAT TARGET *F. NUCLEATUM* IN CRC

Currently, cancer prevention strategies have been mainly focused on chemoprevention and immunotherapy. Chemoprevention, which involves the use of aspirin, cyclo-oxygenase-2 (*COX-2*) inhibitors, and selective EP2 antagonists, plays an important role in *F. nucleatum*-associated CRC. Immunotherapies, such as antibody treatment, immune-checkpoint blockade therapy and adoptive cell transfer therapies, may aid in the prevention of *F. nucleatum*-positive CRC.

Chemoprevention, including the use of aspirin, *COX-2* inhibitors, and selective EP2 antagonists, plays a significant role in the mechanisms of *F. nucleatum*-positive CRC. For instance, some researchers have reported that regular aspirin use lowers CRC incidence and mortality and reduces the risk of distant metastasis of CRC[85,86]. Regular doses of aspirin were also associated with a lower risk of CRC and low levels of CD45RO (PTPRC)$^+$T cells, CD3$^+$T cells or CD8$^+$ T cells[87]. Aspirin induces neutrophils apoptosis[88] and triggers a lipoxin-driven immune-regulatory effect[89]. Aspirin directly inhibits T-cell activation and proliferation and suppresses cytokine production involved in the T cell-mediated adaptive immune response[90]. Tumor-infiltrating immune cells have been associated with a good prognosis in CRC[91,92]. The amount of *F. nucleatum* is inversely proportional to CD3$^+$ T-cell density in colorectal carcinoma tissue[38]. These data indicate that aspirin may support the host immune system and prevent the development of *F. nucleatum*-associated CRC.

In addition, FadA in *F. nucleatum* specifically binds to E-cadherin and activates *Wnt* signaling[43]. *F. nucleatum* increases expression of inflammatory genes and *Wnt* genes[43]. A recent study has reported that EP2 enhances the expression of NF-κB-targeted proinflammatory genes induced by TNF-α in neutrophils[93]. The levels of cytokines such as TNF-α and IL-6, COX-2, chemokine CXCL1, and *Wnt* are significantly higher in tumor lesions of EP2-abundant mice than those in EP2- deficient mice[93]. This study revealed that EP2 promotes colon tumorigenesis by means of expanding inflammation and shaping a tumor microenvironment[93]. PF-04418948, a selective EP2 antagonist, significantly inhibits the formation of colon tumors[93]. This suggests that selective EP2 antagonists may be promising drugs for the chemoprevention of *F. nucleatum*-associated CRC.

Furthermore, COX expression in Braf^{V600E} cells may prevent CD103$^+$ DC activation and accumulation in tumors[94]. By suppressing local T-cell effector, COX-2 also promotes immune evasion and resistance to antigen-specific cancer immunity[95]. COX-2 is also considered an inhibitor of antigen-specific tumor immunotherapy[95]. This is powerful evidence that supports that COX inhibitors reduce the risk of CRC by inhibiting inflammatory pathways, and COX inhibitors may be important for immune-based therapy in CRC patients. In conclusion, aspirin, EP2 antagonists, and COX-2 inhibitors may be important tools for preventing *F. nucleatum*-associated CRC.

Immunotherapies, including antibody treatment, immune-checkpoint blockade therapy and adoptive cell transfer therapies, may be effective strategies for preventing *F. nucleatum*-positive CRC. For example, the interaction between Fap2 and TIGIT receptor protects tumors against immune cell attack and, accordingly, inhibits antitumor immunity and supports tumor cells growth[58]. Fap2 also induces lymphocyte cell death[57]. Fap2 mediates *F. nucleatum* enrichment *via* its interaction with Gal-GalNAc that is overexpressed in CRC, which may exacerbate the inhibition of antitumor immunity[76]. Therefore, anti-Fap2 antibody development may favor antitumor immune response and be a potential immunotherapy in *F. nucleatum*-positive CRC. *F. nucleatum* inhibits T-cell activity and stimulates lymphocyte cell death, which protects tumors from immune cell attack. *F. nucleatum* may have immunosuppressive function in the tumor immune microenvironment.

Recently, the approach to cancer immunotherapy involves immune-checkpoint blockade, such as cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and programmed death protein 1 (PD-1). CTLA-4 and PD-1 have been reported to be involved in T cell-mediated antitumor immunity[96,97]. It was speculated that blockade of CTLA-4 and PD-1 may shape the antitumor immunity response and be an effective immunotherapy

for *F. nucleatum*-associated CRC. Other CRC treatment strategies involving *F. nucleatum,* such as *miR-21* blockade may play a significant role in *F. nucleatum*-positive CRC, as *F. nucleatum* increases expression of *miR-21* by activating TLR4 signaling to NF-κB[41]. It has been demonstrated that *miR-21* promotes tumor cells proliferation and migration by down-regulating the expression of the sec23a protein[79]. The inhibition of *miR-21* suppresses the metastasis of colorectal tumor cells by regulating programmed cell death 4[98]. In a *miR-21* knockout mouse model, expression of proinflammatory and procarcinogenic cytokines was decreased, suggesting that *miR-21* deficiency promotes the apoptosis of tumor cells by suppressing STATA3 and Bcl-2 activation[99]. It has been suggested that the *miR-21* blockade may be a potential treatment strategy for *F. nucleatum*-associated CRC. Some adoptive cell transfer therapies, such as NK cells[100], cytokine-induced killer cells[101], and tumor-infiltrating lymphocytes[102], are also being used to strengthen antitumor immunity in clinical practice. These adoptive cell transfer therapies may also be considered as an immunotherapy approach in CRC associated with *F. nucleatum*.

In sum, CRC prevention strategies that target *F. nucleatum* are mainly focused on chemoprevention, which includes the use of aspirin, *COX-2* inhibitors and selective EP2 antagonists, and immunotherapy, which includes anti-Fap2 antibody treatment, CTLA-4, PD-1, *miR-21* blockade therapies and adoptive cell transfer therapies.

CONCLUSION

In summary, the gut microbiota, especially *F. nucleatum,* has been extensively associated with CRC. *F. nucleatum* promotes the progression of CRC *via* multiple potential mechanisms. The positive detection rate of *F. nucleatum* in CRC samples varies among different studies. FadA combined with anti-*F. nucleatum*-IgA may improve the diagnosis of CRC. Several potential biomarkers, such as miR-21, LncRNA PANDAR, TAMs $CD68^{+}/iNOS^{-}$, $FOXP3^{\pm}$ (lo) T cells and $CD45RO^{+}$ cells, may be considered as criteria for determining CRC prognosis. Furthermore, chemoprevention and immunotherapy strategies should be further explored in the future.

REFERENCES

1 **Siegel RL**, Miller KD, Jemal A. Cancer Statistics, 2017. *CA Cancer J Clin* 2017; **67**: 7-30 [PMID: 28055103 DOI: 10.3322/caac.21387]

2 **Center MM**, Jemal A, Smith RA, Ward E. Worldwide variations in colorectal cancer. *CA Cancer J Clin* 2009; **59**: 366-378 [PMID: 19897840 DOI: 10.3322/caac.20038]

3 **Jobin C**. Colorectal cancer: looking for answers in the microbiota. *Cancer Discov* 2013; **3**: 384-387 [PMID: 23580283 DOI: 10.1158/2159-8290.CD-13-0042]

4 **Sobhani I**, Tap J, Roudot-Thoraval F, Roperch JP, Letulle S, Langella P, Corthier G, Tran Van Nhieu J, Furet JP. Microbial dysbiosis in colorectal cancer (CRC) patients. *PLoS One* 2011; **6**: e16393 [PMID: 21297998 DOI: 10.1371/journal.pone.0016393]

5 **Mira-Pascual L**, Cabrera-Rubio R, Ocon S, Costales P, Parra A, Suarez A, Moris F, Rodrigo L, Mira A, Collado MC. Microbial mucosal colonic shifts associated with the development of colorectal cancer reveal the presence of different bacterial and archaeal biomarkers. *J Gastroenterol* 2015; **50**: 167-179 [PMID: 24811328 DOI: 10.1007/s00535-014-0963-x]

6 **Holmes E**, Li JV, Marchesi JR, Nicholson JK. Gut microbiota composition and activity in relation to host metabolic phenotype and disease risk. *Cell Metab* 2012; **16**: 559-564 [PMID: 23140640 DOI: 10.1016/j.cmet.2012.10.007]

7 **Xu J**, Gordon JI. Honor thy symbionts. *Proc Natl Acad Sci USA* 2003; **100**: 10452-10459 [PMID: 12923294 DOI: 10.1073/pnas.1734063100]

8 **Arthur JC**, Jobin C. The complex interplay between inflammation, the microbiota and colorectal cancer. *Gut Microbes* 2013; **4**: 253-258 [PMID: 23549517 DOI: 10.4161/gmic.24220]

9 **Ahmed Z**, Bansal SK, Dhillon S. Pyogenic liver abscess caused by Fusobacterium in a 21-year-old immunocompetent male. *World J Gastroenterol* 2015; **21**: 3731-3735 [PMID: 25834342 DOI: 10.3748/wjg.v21.i12.3731]

10 **Candela M**, Turroni S, Biagi E, Carbonero F, Rampelli S, Fiorentini C, Brigidi P. Inflammation and colorectal cancer, when microbiota-host mutualism breaks. *World J Gastroenterol* 2014; **20**: 908-922 [PMID: 24574765 DOI: 10.3748/wjg.v20.i4.908]

11 **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]

12 **Viljoen KS**, Dakshinamurthy A, Goldberg P, Blackburn JM. Quantitative profiling of colorectal cancer-associated bacteria reveals associations between fusobacterium spp., enterotoxigenic Bacteroides fragilis (ETBF) and clinicopathological features of colorectal cancer. *PLoS One* 2015; **10**: e0119462 [PMID: 25751261 DOI: 10.1371/journal.pone.0119462]

13 **Boleij A**, van Gelder MM, Swinkels DW, Tjalsma H. Clinical Importance of Streptococcus gallolyticus infection among colorectal cancer patients: systematic review and meta-analysis. *Clin Infect Dis* 2011; **53**: 870-878 [PMID: 21960713 DOI: 10.1093/cid/cir609]

14 **Zhou Y**, He H, Xu H, Li Y, Li Z, Du Y, He J, Zhou Y, Wang H, Nie Y. Association of oncogenic bacteria with colorectal cancer in South China. *Oncotarget* 2016; **7**: 80794-80802 [PMID: 27821805 DOI: 10.18632/oncotarget.13094]

15 **Tsai CE**, Chiu CT, Rayner CK, Wu KL, Chiu YC, Hu ML, Chuah SK, Tai WC, Liang CM, Wang HM. Associated factors in Streptococcus bovis bacteremia and colorectal cancer. *Kaohsiung J Med Sci* 2016; **32**: 196-200 [PMID: 27185602 DOI: 10.1016/j.kjms.2016.03.003]

16 **Krishnan S**, Eslick GD. Streptococcus bovis infection and colorectal neoplasia: a meta-analysis. *Colorectal Dis* 2014; **16**: 672-680 [PMID: 24824513 DOI: 10.1111/codi.12662]

17 **Flanagan L**, Schmid J, Ebert M, Soucek P, Kunicka T, Liska V, Bruha J, Neary P, Dezeeuw N, Tommasino M, Jenab M, Prehn JH, Hughes DJ. Fusobacterium nucleatum associates with stages of colorectal neoplasia development, colorectal cancer and disease outcome. *Eur J Clin Microbiol Infect Dis* 2014; **33**: 1381-1390 [PMID: 24599709 DOI: 10.1007/s10096-014-2081-3]

18 **Gagnière J**, Raisch J, Veziant J, Barnich N, Bonnet R, Buc E, Bringer MA, Pezet D, Bonnet M. Gut microbiota imbalance and colorectal cancer. *World J Gastroenterol* 2016; **22**: 501-518 [PMID: 26811603 DOI: 10.3748/wjg.v22.i2.501]

19 **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]

20 **Wang X**, Allen TD, May RJ, Lightfoot S, Houchen CW, Huycke

MM. Enterococcus faecalis induces aneuploidy and tetraploidy in colonic epithelial cells through a bystander effect. *Cancer Res* 2008; **68**: 9909-9917 [PMID: 19047172 DOI: 10.1158/0008-5472.CAN-08-1551]

21 **Fukugaiti MH**, Ignacio A, Fernandes MR, Ribeiro Júnior U, Nakano V, Avila-Campos MJ. High occurrence of Fusobacterium nucleatum and Clostridium difficile in the intestinal microbiota of colorectal carcinoma patients. *Braz J Microbiol* 2015; **46**: 1135-1140 [PMID: 26691472 DOI: 10.1590/S1517-838246420140665]

22 **Warren RL,** Freeman DJ, Pleasance S, Watson P, Moore RA, Cochrane K, Allen-Vercoe E, Holt RA. Co-occurrence of anaerobic bacteria in colorectal carcinomas. *Microbiome* 2013; **1**: 16 [PMID: 24450771 DOI: 10.1186/2049-2618-1-16]

23 **Yu J**, Feng Q, Wong SH, Zhang D, Liang QY, Qin Y, Tang L, Zhao H, Stenvang J, Li Y, Wang X, Xu X, Chen N, Wu WK, Al-Aama J, Nielsen HJ, Kiilerich P, Jensen BA, Yau TO, Lan Z, Jia H, Li J, Xiao L, Lam TY, Ng SC, Cheng AS, Wong VW, Chan FK, Xu X, Yang H, Madsen L, Datz C, Tilg H, Wang J, Brünner N, Kristiansen K, Arumugam M, Sung JJ, Wang J. Metagenomic analysis of faecal microbiome as a tool towards targeted non-invasive biomarkers for colorectal cancer. *Gut* 2017; **66**: 70-78 [PMID: 26408641 DOI: 10.1136/gutjnl-2015-309800]

24 **Yang NY**, Zhang Q, Li JL, Yang SH, Shi Q. Progression of periodontal inflammation in adolescents is associated with increased number of Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythensis, and Fusobacterium nucleatum. *Int J Paediatr Dent* 2014; **24**: 226-233 [PMID: 24025042 DOI: 10.1111/ipd.12065]

25 **Kistler JO**, Booth V, Bradshaw DJ, Wade WG. Bacterial community development in experimental gingivitis. *PLoS One* 2013; **8**: e71227 [PMID: 23967169 DOI: 10.1371/journal.pone.0071227]

26 **Fujii R**, Saito Y, Tokura Y, Nakagawa KI, Okuda K, Ishihara K. Characterization of bacterial flora in persistent apical periodontitis lesions. *Oral Microbiol Immunol* 2009; **24**: 502-505 [PMID: 19832803 DOI: 10.1111/j.1399-302X.2009.00534.x]

27 **Han YW,** Redline RW, Li M, Yin L, Hill GB, McCormick TS. Fusobacterium nucleatum induces premature and term stillbirths in pregnant mice: implication of oral bacteria in preterm birth. *Infect Immun* 2004; **72**: 2272-2279 [PMID: 15039352 DOI: 10.1128/iai.72.4.2272-2279.2004]

28 **Mitsuhashi K**, Nosho K, Sukawa Y, Matsunaga Y, Ito M, Kurihara H, Kanno S, Igarashi H, Naito T, Adachi Y, Tachibana M, Tanuma T, Maguchi H, Shinohara T, Hasegawa T, Imamura M, Kimura Y, Hirata K, Maruyama R, Suzuki H, Imai K, Yamamoto H, Shinomura Y. Association of Fusobacterium species in pancreatic cancer tissues with molecular features and prognosis. *Oncotarget* 2015; **6**: 7209-7220 [PMID: 25797243 DOI: 10.18632/oncotarget.3109]

29 **Binder Gallimidi A**, Fischman S, Revach B, Bulvik R, Maliutina A, Rubinstein AM, Nussbaum G, Elkin M. Periodontal pathogens Porphyromonas gingivalis and Fusobacterium nucleatum promote tumor progression in an oral-specific chemical carcinogenesis model. *Oncotarget* 2015; **6**: 22613-22623 [PMID: 26158901 DOI: 10.18632/oncotarget.4209]

30 **Krejs GJ**. Pancreatic cancer: epidemiology and risk factors. *Dig Dis* 2010; **28**: 355-358 [PMID: 20814212 DOI: 10.1159/000319414]

31 **Yoneda M**, Kato S, Mawatari H, Kirikoshi H, Imajo K, Fujita K, Endo H, Takahashi H, Inamori M, Kobayashi N, Kubota K, Saito S, Tohnai I, Watanuki K, Wada K, Maeda S, Nakajima A. Liver abscess caused by periodontal bacterial infection with Fusobacterium necrophorum. *Hepatol Res* 2011; **41**: 194-196 [PMID: 21269389 DOI: 10.1111/j.1872-034X.2010.00748.x]

32 **Yarden-Bilavsky H**, Raveh E, Livni G, Scheuerman O, Amir J, Bilavsky E. Fusobacterium necrophorum mastoiditis in children - emerging pathogen in an old disease. *Int J Pediatr Otorhinolaryngol* 2013; **77**: 92-96 [PMID: 23102657 DOI: 10.1016/j.ijporl.2012.10.003]

33 **Jensen A**, Hagelskjaer Kristensen L, Prag J. Detection of Fusobacterium necrophorum subsp. funduliforme in tonsillitis in young adults by real-time PCR. *Clin Microbiol Infect* 2007; **13**: 695-701 [PMID: 17403128 DOI: 10.1111/j.1469-0691.2007.01719.x]

34 **Finegold SM**, Flynn MJ, Rose FV, Jousimies-Somer H, Jakielaszek C, McTeague M, Wexler HM, Berkowitz E, Wynne B. Bacteriologic findings associated with chronic bacterial maxillary sinusitis in adults. *Clin Infect Dis* 2002; **35**: 428-433 [PMID: 12145727 DOI: 10.1086/341899]

35 **Salö M**, Marungruang N, Roth B, Sundberg T, Stenström P, Arnbjörnsson E, Fåk F, Ohlsson B. Evaluation of the microbiome in children's appendicitis. *Int J Colorectal Dis* 2017; **32**: 19-28 [PMID: 27613729 DOI: 10.1007/s00384-016-2639-x]

36 **Castellarin M**, Warren RL, Freeman JD, Dreolini L, Krzywinski M, Strauss J, Barnes R, Watson P, Allen-Vercoe E, Moore RA, Holt RA. Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma. *Genome Res* 2012; **22**: 299-306 [PMID: 22009989 DOI: 10.1101/gr.126516.111]

37 **McCoy AN**, Araújo-Pérez F, Azcárate-Peril A, Yeh JJ, Sandler RS, Keku TO. Fusobacterium is associated with colorectal adenomas. *PLoS One* 2013; **8**: e53653 [PMID: 23335968 DOI: 10.1371/journal.pone.0053653]

38 **Mima K**, Sukawa Y, Nishihara R, Qian ZR, Yamauchi M, Inamura K, Kim SA, Masuda A, Nowak JA, Nosho K, Kostic AD, Giannakis M, Watanabe H, Bullman S, Milner DA, Harris CC, Giovannucci E, Garraway LA, Freeman GJ, Dranoff G, Chan AT, Garrett WS, Huttenhower C, Fuchs CS, Ogino S. Fusobacterium nucleatum and T Cells in Colorectal Carcinoma. *JAMA Oncol* 2015; **1**: 653-661 [PMID: 26181352 DOI: 10.1001/jamaoncol.2015.1377]

39 **Ito M**, Kanno S, Nosho K, Sukawa Y, Mitsuhashi K, Kurihara H, Igarashi H, Takahashi T, Tachibana M, Takahashi H, Yoshii S, Takenouchi T, Hasegawa T, Okita K, Hirata K, Maruyama R, Suzuki H, Imai K, Yamamoto H, Shinomura Y. Association of Fusobacterium nucleatum with clinical and molecular features in colorectal serrated pathway. *Int J Cancer* 2015; **137**: 1258-1268 [PMID: 25703934 DOI: 10.1002/ijc.29488]

40 **Li YY**, Ge QX, Cao J, Zhou YJ, Du YL, Shen B, Wan YJ, Nie YQ. Association of Fusobacterium nucleatum infection with colorectal cancer in Chinese patients. *World J Gastroenterol* 2016; **22**: 3227-3233 [PMID: 27004000 DOI: 10.3748/wjg.v22.i11.3227]

41 **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]

42 **Wong SH**, Kwong TNY, Chow TC, Luk AKC, Dai RZW, Nakatsu G, Lam TYT, Zhang L, Wu JCY, Chan FKL, Ng SSM, Wong MCS, Ng SC, Wu WKK, Yu J, Sung JJY. Quantitation of faecal Fusobacterium improves faecal immunochemical test in detecting advanced colorectal neoplasia. *Gut* 2017; **66**: 1441-1448 [PMID: 27797940 DOI: 10.1136/gutjnl-2016-312766]

43 **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]

44 **Mima K**, Nishihara R, Qian ZR, Cao Y, Sukawa Y, Nowak JA, Yang J, Dou R, Masugi Y, Song M, Kostic AD, Giannakis M, Bullman S, Milner DA, Baba H, Giovannucci EL, Garraway LA, Freeman GJ, Dranoff G, Garrett WS, Huttenhower C, Meyerson M, Meyerhardt JA, Chan AT, Fuchs CS, Ogino S. Fusobacterium nucleatum in colorectal carcinoma tissue and patient prognosis. *Gut* 2016; **65**: 1973-1980 [PMID: 26311717 DOI: 10.1136/gutjnl-2015-310101]

45 **Tahara T**, Yamamoto E, Suzuki H, Maruyama R, Chung W, Garriga J, Jelinek J, Yamano HO, Sugai T, An B, Shureiqi I, Toyota M, Kondo Y, Estécio MR, Issa JP. Fusobacterium in colonic flora and molecular features of colorectal carcinoma. *Cancer Res* 2014; **74**: 1311-1318 [PMID: 24385213 DOI: 10.1158/0008-5472.CAN-13-1865]

46 **Tjalsma H**, Boleij A, Marchesi JR, Dutilh BE. A bacterial driver-passenger model for colorectal cancer: beyond the usual suspects.

Nat Rev Microbiol 2012; **10**: 575-582 [PMID: 22728587 DOI: 10.1038/nrmicro2819]
47 **Allen-Vercoe E**, Strauss J, Chadee K. Fusobacterium nucleatum: an emerging gut pathogen? *Gut Microbes* 2011; **2**: 294-298 [PMID: 22067936 DOI: 10.4161/gmic.2.5.18603]
48 **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]
49 **Nosho K**, Sukawa Y, Adachi Y, Ito M, Mitsuhashi K, Kurihara H, Kanno S, Yamamoto I, Ishigami K, Igarashi H, Maruyama R, Imai K, Yamamoto H, Shinomura Y. Association of Fusobacterium nucleatum with immunity and molecular alterations in colorectal cancer. *World J Gastroenterol* 2016; **22**: 557-566 [PMID: 26811607 DOI: 10.3748/wjg.v22.i2.557]
50 **Suehiro Y,** Sakai K, Nishioka M, Hashimoto S, Takami T, Higaki S, Shindo Y, Hazama S, Oka M, Nagano H, Sakaida I, Yamasaki T. Highly sensitive stool DNA testing of Fusobacterium nucleatum as a marker for detection of colorectal tumours in a Japanese population. *Ann Clin Biochem* 2017; **54**: 86-91 [PMID: 27126270 DOI: 10.1177/0004563216643970]
51 **Mima K**, Cao Y, Chan AT, Qian ZR, Nowak JA, Masugi Y, Shi Y, Song M, da Silva A, Gu M, Li W, Hamada T, Kosumi K, Hanyuda A, Liu L, Kostic AD, Giannakis M, Bullman S, Brennan CA, Milner DA, Baba H, Garraway LA, Meyerhardt JA, Garrett WS, Huttenhower C, Meyerson M, Giovannucci EL, Fuchs CS, Nishihara R, Ogino S. Fusobacterium nucleatum in Colorectal Carcinoma Tissue According to Tumor Location. *Clin Transl Gastroenterol* 2016; **7**: e200 [PMID: 27811909 DOI: 10.1038/ctg.2016.53]
52 **Chen W**, Liu F, Ling Z, Tong X, Xiang C. Human intestinal lumen and mucosa-associated microbiota in patients with colorectal cancer. *PLoS One* 2012; **7**: e39743 [PMID: 22761885 DOI: 10.1371/journal.pone.0039743]
53 **Hindson BJ**, Ness KD, Masquelier DA, Belgrader P, Heredia NJ, Makarewicz AJ, Bright IJ, Lucero MY, Hiddessen AL, Legler TC, Kitano TK, Hodel MR, Petersen JF, Wyatt PW, Steenblock ER, Shah PH, Bousse LJ, Troup CB, Mellen JC, Wittmann DK, Erndt NG, Cauley TH, Koehler RT, So AP, Dube S, Rose KA, Montesclaros L, Wang S, Stumbo DP, Hodges SP, Romine S, Milanovich FP, White HE, Regan JF, Karlin-Neumann GA, Hindson CM, Saxonov S, Colston BW. High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. *Anal Chem* 2011; **83**: 8604-8610 [PMID: 22035192 DOI: 10.1021/ac202028g]
54 **Singh G**, Sithebe A, Enitan AM, Kumari S, Bux F, Stenström TA. Comparison of droplet digital PCR and quantitative PCR for the detection of Salmonella and its application for river sediments. *J Water Health* 2017; **15**: 505-508 [PMID: 28771147 DOI: 10.2166/wh.2017.259]
55 **Jiao H**, Weng WC, Wang FJ, Cheng G, Wang W, Xie J. [Faster detection of Vibrio parahaemolyticus in foods by FQ-PCR technique]. *Wei Sheng Yan Jiu* 2005; **34**: 457-460 [PMID: 16229276]
56 **Han YW**, Shi W, Huang GT, Kinder Haake S, Park NH, Kuramitsu H, Genco RJ. Interactions between periodontal bacteria and human oral epithelial cells: Fusobacterium nucleatum adheres to and invades epithelial cells. *Infect Immun* 2000; **68**: 3140-3146 [PMID: 10816455 DOI: 10.1128/IAI.68.6.3140-3146.2000]
57 **Kaplan CW**, Ma X, Paranjpe A, Jewett A, Lux R, Kinder-Haake S, Shi W. Fusobacterium nucleatum outer membrane proteins Fap2 and RadD induce cell death in human lymphocytes. *Infect Immun* 2010; **78**: 4773-4778 [PMID: 20823215 DOI: 10.1128/IAI.00567-10]
58 **Gur C**, Ibrahim Y, Isaacson B, Yamin R, Abed J, Gamliel M, Enk J, Bar-On Y, Stanietsky-Kaynan N, Coppenhagen-Glazer S, Shussman N, Almogy G, Cuapio A, Hofer E, Mevorach D, Tabib A, Ortenberg R, Markel G, Miklić K, Jonjic S, Brennan CA, Garrett WS, Bachrach G, Mandelboim O. Binding of the Fap2 protein of Fusobacterium nucleatum to human inhibitory receptor TIGIT protects tumors from immune cell attack. *Immunity* 2015; **42**: 344-355 [PMID: 25680274 DOI: 10.1016/j.immuni.2015.01.010]
59 **Fardini Y,** Wang X, Témoin S, Nithianantham S, Lee D, Shoham M, Han YW. Fusobacterium nucleatum adhesin FadA binds vascular endothelial cadherin and alters endothelial integrity. *Mol Microbiol* 2011; **82**: 1468-1480 [PMID: 22040113 DOI: 10.1111/j.1365-2958.2011.07905.x]
60 **Han YW**, Ikegami A, Rajanna C, Kawsar HI, Zhou Y, Li M, Sojar HT, Genco RJ, Kuramitsu HK, Deng CX. Identification and characterization of a novel adhesin unique to oral fusobacteria. *J Bacteriol* 2005; **187**: 5330-5340 [PMID: 16030227 DOI: 10.1128/JB.187.15.5330-5340.2005]
61 **Xu M**, Yamada M, Li M, Liu H, Chen SG, Han YW. FadA from Fusobacterium nucleatum utilizes both secreted and nonsecreted forms for functional oligomerization for attachment and invasion of host cells. *J Biol Chem* 2007; **282**: 25000-25009 [PMID: 17588948 DOI: 10.1074/jbc.M611567200]
62 **Quah SY**, Bergenholtz G, Tan KS. Fusobacterium nucleatum induces cytokine production through Toll-like-receptor-independent mechanism. *Int Endod J* 2014; **47**: 550-559 [PMID: 24102075 DOI: 10.1111/iej.12185]
63 **Dharmani P**, Strauss J, Ambrose C, Allen-Vercoe E, Chadee K. Fusobacterium nucleatum infection of colonic cells stimulates MUC2 mucin and tumor necrosis factor alpha. *Infect Immun* 2011; **79**: 2597-2607 [PMID: 21536792 DOI: 10.1128/IAI.05118-11]
64 **Bryant DM**, Stow JL. The ins and outs of E-cadherin trafficking. *Trends Cell Biol* 2004; **14**: 427-434 [PMID: 15308209 DOI: 10.1016/j.tcb.2004.07.007]
65 **Gumbiner BM**. Regulation of cadherin-mediated adhesion in morphogenesis. *Nat Rev Mol Cell Biol* 2005; **6**: 622-634 [PMID: 16025097 DOI: 10.1038/nrm1699]
66 **Shenker BJ**, DiRienzo JM. Suppression of human peripheral blood lymphocytes by Fusobacterium nucleatum. *J Immunol* 1984; **132**: 2357-2362 [PMID: 6715883]
67 **Shenker BJ**, Datar S. Fusobacterium nucleatum inhibits human T-cell activation by arresting cells in the mid-G1 phase of the cell cycle. *Infect Immun* 1995; **63**: 4830-4836 [PMID: 7591143]
68 **Bashir A**, Miskeen AY, Hazari YM, Asrafuzzaman S, Fazili KM. Fusobacterium nucleatum, inflammation, and immunity: the fire within human gut. *Tumour Biol* 2016; **37**: 2805-2810 [PMID: 26718210 DOI: 10.1007/s13277-015-4724-0]
69 **Gabrilovich DI**, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol* 2012; **12**: 253-268 [PMID: 22437938 DOI: 10.1038/nri3175]
70 **Sun HL**, Zhou X, Xue YF, Wang K, Shen YF, Mao JJ, Guo HF, Miao ZN. Increased frequency and clinical significance of myeloid-derived suppressor cells in human colorectal carcinoma. *World J Gastroenterol* 2012; **18**: 3303-3309 [PMID: 22783056 DOI: 10.3748/wjg.v18.i25.3303]
71 **Mantovani A**, Sica A. Macrophages, innate immunity and cancer: balance, tolerance, and diversity. *Curr Opin Immunol* 2010; **22**: 231-237 [PMID: 20144856 DOI: 10.1016/j.coi.2010.01.009]
72 **Josefowicz SZ**, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. *Annu Rev Immunol* 2012; **30**: 531-564 [PMID: 22224781 DOI: 10.1146/annurev.immunol.25.022106.141623]
73 **Gulubova MV**, Ananiev JR, Vlaykova TI, Yovchev Y, Tsoneva V, Manolova IM. Role of dendritic cells in progression and clinical outcome of colon cancer. *Int J Colorectal Dis* 2012; **27**: 159-169 [PMID: 22065108 DOI: 10.1007/s00384-011-1334-1]
74 **Coppenhagen-Glazer S**, Sol A, Abed J, Naor R, Zhang X, Han YW, Bachrach G. Fap2 of Fusobacterium nucleatum is a galactose-inhibitable adhesin involved in coaggregation, cell adhesion, and preterm birth. *Infect Immun* 2015; **83**: 1104-1113 [PMID: 25561710 DOI: 10.1128/IAI.02838-14]
75 **Stanietsky N**, Simic H, Arapovic J, Toporik A, Levy O, Novik A, Levine Z, Beiman M, Dassa L, Achdout H, Stern-Ginossar N, Tsukerman P, Jonjic S, Mandelboim O. The interaction of TIGIT with PVR and PVRL2 inhibits human NK cell cytotoxicity. *Proc Natl Acad Sci USA* 2009; **106**: 17858-17863 [PMID: 19815499 DOI: 10.1073/pnas.0903474106]
76 **Abed J**, Emgård JE, Zamir G, Faroja M, Almogy G, Grenov A, Sol

A, Naor R, Pikarsky E, Atlan KA, Mellul A, Chaushu S, Manson AL, Earl AM, Ou N, Brennan CA, Garrett WS, Bachrach G. Fap2 Mediates Fusobacterium nucleatum Colorectal Adenocarcinoma Enrichment by Binding to Tumor-Expressed Gal-GalNAc. *Cell Host Microbe* 2016; **20**: 215-225 [PMID: 27512904 DOI: 10.1016/j.chom.2016.07.006]

77 **Wang HF**, Li LF, Guo SH, Zeng QY, Ning F, Liu WL, Zhang G. Evaluation of antibody level against Fusobacterium nucleatum in the serological diagnosis of colorectal cancer. *Sci Rep* 2016; **6**: 33440 [PMID: 27678333 DOI: 10.1038/srep33440]

78 **Waniczek D**, Lorenc Z, Śnietura M, Wesecki M, Kopec A, Muc-Wierzgoń M. Tumor-Associated Macrophages and Regulatory T Cells Infiltration and the Clinical Outcome in Colorectal Cancer. *Arch Immunol Ther Exp* (Warsz) 2017; **65**: 445-454 [PMID: 28343267 DOI: 10.1007/s00005-017-0463-9]

79 **Li C**, Zhao L, Chen Y, He T, Chen X, Mao J, Li C, Lyu J, Meng QH. MicroRNA-21 promotes proliferation, migration, and invasion of colorectal cancer, and tumor growth associated with down-regulation of sec23a expression. *BMC Cancer* 2016; **16**: 605 [PMID: 27495250 DOI: 10.1186/s12885-016-2628-z]

80 **Wang R**, Du L, Yang X, Jiang X, Duan W, Yan S, Xie Y, Zhu Y, Wang Q, Wang L, Yang Y, Wang C. Identification of long noncoding RNAs as potential novel diagnosis and prognosis biomarkers in colorectal cancer. *J Cancer Res Clin Oncol* 2016; **142**: 2291-2301 [PMID: 27591862 DOI: 10.1007/s00432-016-2238-9]

81 **Lu M**, Liu Z, Li B, Wang G, Li D, Zhu Y. The high expression of long non-coding RNA PANDAR indicates a poor prognosis for colorectal cancer and promotes metastasis by EMT pathway. *J Cancer Res Clin Oncol* 2017; **143**: 71-81 [PMID: 27629879 DOI: 10.1007/s00432-016-2252-y]

82 **Nosho K**, Baba Y, Tanaka N, Shima K, Hayashi M, Meyerhardt JA, Giovannucci E, Dranoff G, Fuchs CS, Ogino S. Tumour-infiltrating T-cell subsets, molecular changes in colorectal cancer, and prognosis: cohort study and literature review. *J Pathol* 2010; **222**: 350-366 [PMID: 20927778 DOI: 10.1002/path.2774]

83 **deLeeuw RJ**, Kost SE, Kakal JA, Nelson BH. The prognostic value of FoxP3+ tumor-infiltrating lymphocytes in cancer: a critical review of the literature. *Clin Cancer Res* 2012; **18**: 3022-3029 [PMID: 22510350 DOI: 10.1158/1078-0432.CCR-11-3216]

84 **Saito T**, Nishikawa H, Wada H, Nagano Y, Sugiyama D, Atarashi K, Maeda Y, Hamaguchi M, Ohkura N, Sato E, Nagase H, Nishimura J, Yamamoto H, Takiguchi S, Tanoue T, Suda W, Morita H, Hattori M, Honda K, Mori M, Doki Y, Sakaguchi S. Two FOXP3(+)CD4(+) T cell subpopulations distinctly control the prognosis of colorectal cancers. *Nat Med* 2016; **22**: 679-684 [PMID: 27111280 DOI: 10.1038/nm.4086]

85 **Mehta RS**, Nishihara R, Cao Y, Song M, Mima K, Qian ZR, Nowak JA, Kosumi K, Hamada T, Masugi Y, Bullman S, Drew DA, Kostic AD, Fung TT, Garrett WS, Huttenhower C, Wu K, Meyerhardt JA, Zhang X, Willett WC, Giovannucci EL, Fuchs CS, Chan AT, Ogino S. Association of Dietary Patterns With Risk of Colorectal Cancer Subtypes Classified by Fusobacterium nucleatum in Tumor Tissue. *JAMA Oncol* 2017; **3**: 921-927 [PMID: 28125762 DOI: 10.1001/jamaoncol.2016.6374]

86 **Rothwell PM**, Wilson M, Elwin CE, Norrving B, Algra A, Warlow CP, Meade TW. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. *Lancet* 2010; **376**: 1741-1750 [PMID: 20970847 DOI: 10.1016/S0140-6736(10)61543-7]

87 **Cao Y**, Nishihara R, Qian ZR, Song M, Mima K, Inamura K, Nowak JA, Drew DA, Lochhead P, Nosho K, Morikawa T, Zhang X, Wu K, Wang M, Garrett WS, Giovannucci EL, Fuchs CS, Chan AT, Ogino S. Regular Aspirin Use Associates With Lower Risk of Colorectal Cancers With Low Numbers of Tumor-Infiltrating Lymphocytes. *Gastroenterology* 2016; **151**: 879-892.e4 [PMID: 27475305 DOI: 10.1053/j.gastro.2016.07.030]

88 **Negrotto S**, Malaver E, Alvarez ME, Pacienza N, D'Atri LP, Pozner RG, Gómez RM, Schattner M. Aspirin and salicylate suppress polymorphonuclear apoptosis delay mediated by proinflammatory stimuli. *J Pharmacol Exp Ther* 2006; **319**: 972-979 [PMID: 16936242 DOI: 10.1124/jpet.106.109389]

89 **El Kebir D**, József L, Khreiss T, Pan W, Petasis NA, Serhan CN, Filep JG. Aspirin-triggered lipoxins override the apoptosis-delaying action of serum amyloid A in human neutrophils: a novel mechanism for resolution of inflammation. *J Immunol* 2007; **179**: 616-622 [PMID: 17579083 DOI: 10.4049/jimmunol.179.1.616]

90 **Hussain M**, Javeed A, Ashraf M, Zhao Y, Mukhtar MM, Rehman MU. Aspirin and immune system. *Int Immunopharmacol* 2012; **12**: 10-20 [PMID: 22172645 DOI: 10.1016/j.intimp.2011.11.021]

91 **Galon J**, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, Tosolini M, Camus M, Berger A, Wind P, Zinzindohoué F, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Pagès F. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* 2006; **313**: 1960-1964 [PMID: 17008531 DOI: 10.1126/science.1129139]

92 **Mei Z**, Liu Y, Liu C, Cui A, Liang Z, Wang G, Peng H, Cui L, Li C. Tumour-infiltrating inflammation and prognosis in colorectal cancer: systematic review and meta-analysis. *Br J Cancer* 2014; **110**: 1595-1605 [PMID: 24504370 DOI: 10.1038/bjc.2014.46]

93 **Ma X**, Aoki T, Tsuruyama T, Narumiya S. Definition of Prostaglandin E2-EP2 Signals in the Colon Tumor Microenvironment That Amplify Inflammation and Tumor Growth. *Cancer Res* 2015; **75**: 2822-2832 [PMID: 26018088 DOI: 10.1158/0008-5472.CAN-15-0125]

94 **Zelenay S**, van der Veen AG, Böttcher JP, Snelgrove KJ, Rogers N, Acton SE, Chakravarty P, Girotti MR, Marais R, Quezada SA, Sahai E, Reis e Sousa C. Cyclooxygenase-Dependent Tumor Growth through Evasion of Immunity. *Cell* 2015; **162**: 1257-1270 [PMID: 26343581 DOI: 10.1016/j.cell.2015.08.015]

95 **Göbel C**, Breitenbuecher F, Kalkavan H, Hähnel PS, Kasper S, Hoffarth S, Merches K, Schild H, Lang KS, Schuler M. Functional expression cloning identifies COX-2 as a suppressor of antigen-specific cancer immunity. *Cell Death Dis* 2014; **5**: e1568 [PMID: 25501829 DOI: 10.1038/cddis.2014.531]

96 **Vétizou M**, Pitt JM, Daillère R, Lepage P, Waldschmitt N, Flament C, Rusakiewicz S, Routy B, Roberti MP, Duong CP, Poirier-Colame V, Roux A, Becharef S, Formenti S, Golden E, Cording S, Eberl G, Schlitzer A, Ginhoux F, Mani S, Yamazaki T, Jacquelot N, Enot DP, Bérard M, Nigou J, Opolon P, Eggermont A, Woerther PL, Chachaty E, Chaput N, Robert C, Mateus C, Kroemer G, Raoult D, Boneca IG, Carbonnel F, Chamaillard M, Zitvogel L. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science* 2015; **350**: 1079-1084 [PMID: 26541610 DOI: 10.1126/science.aad1329]

97 **Tumeh PC**, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, Chmielowski B, Spasic M, Henry G, Ciobanu V, West AN, Carmona M, Kivork C, Seja E, Cherry G, Gutierrez AJ, Grogan TR, Mateus C, Tomasic G, Glaspy JA, Emerson RO, Robins H, Pierce RH, Elashoff DA, Robert C, Ribas A. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 2014; **515**: 568-571 [PMID: 25428505 DOI: 10.1038/nature13954]

98 **Nedaeinia R**, Sharifi M, Avan A, Kazemi M, Nabinejad A, Ferns GA, Ghayour-Mobarhan M, Salehi R. Inhibition of microRNA-21 via locked nucleic acid-anti-miR suppressed metastatic features of colorectal cancer cells through modulation of programmed cell death 4. *Tumour Biol* 2017; **39**: 1010428317692261 [PMID: 28347230 DOI: 10.1177/1010428317692261]

99 **Shi C**, Yang Y, Xia Y, Okugawa Y, Yang J, Liang Y, Chen H, Zhang P, Wang F, Han H, Wu W, Gao R, Gasche C, Qin H, Ma Y, Goel A. Novel evidence for an oncogenic role of microRNA-21 in colitis-associated colorectal cancer. *Gut* 2016; **65**: 1470-1481 [PMID: 25994220 DOI: 10.1136/gutjnl-2014-308455]

100 **Cerwenka A**, Lanier LL. Natural killer cell memory in infection, inflammation and cancer. *Nat Rev Immunol* 2016; **16**: 112-123 [PMID: 26806484 DOI: 10.1038/nri.2015.9]

101 **Pan K**, Guan XX, Li YQ, Zhao JJ, Li JJ, Qiu HJ, Weng DS, Wang QJ, Liu Q, Huang LX, He J, Chen SP, Ke ML, Zeng YX, Xia JC. Clinical activity of adjuvant cytokine-induced killer cell immunotherapy in patients with post-mastectomy triple-negative breast cancer. *Clin Cancer Res* 2014; **20**: 3003-3011 [PMID: 24668644 DOI: 10.1158/1078-0432.CCR-14-0082]

102 **Pilon-Thomas S**, Kuhn L, Ellwanger S, Janssen W, Royster E, Marzban S, Kudchadkar R, Zager J, Gibney G, Sondak VK, Weber J, Mulé JJ, Sarnaik AA. Efficacy of adoptive cell transfer of tumor-infiltrating lymphocytes after lymphopenia induction for metastatic melanoma. *J Immunother* 2012; **35**: 615-620 [PMID: 22996367 DOI: 10.1097/CJI.0b013e31826e8f5f]

P- Reviewer: El-Tawil AM, Roncucci L **S- Editor**: Cui LJ
L- Editor: A **E- Editor**: Li RF

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2018 March 15; 10(3): 82-90

DOI: 10.4251/wjgo.v10.i3.82

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Clinical Practice Study

Sessile serrated adenoma detection rate is correlated with adenoma detection rate

Daisuke Ohki, Yosuke Tsuji, Tomohiro Shinozaki, Yoshiki Sakaguchi, Chihiro Minatsuki, Hiroto Kinoshita, Keiko Niimi, Satoshi Ono, Yoku Hayakawa, Shuntaro Yoshida, Atsuo Yamada, Shinya Kodashima, Nobutake Yamamichi, Yoshihiro Hirata, Tetsuo Ushiku, Mitsuhiro Fujishiro, Masashi Fukayama, Kazuhiko Koike

Daisuke Ohki, Yosuke Tsuji, Yoshiki Sakaguchi, Chihiro Minatsuki, Hiroto Kinoshita, Keiko Niimi, Satoshi Ono, Yoku Hayakawa, Shuntaro Yoshida, Atsuo Yamada, Shinya Kodashima, Nobutake Yamamichi, Yoshihiro Hirata, Mitsuhiro Fujishiro, Kazuhiko Koike, Department of Gastroenterology, Graduate School of Medicine, the University of Tokyo, Tokyo 113-0033, Japan

Shuntaro Yoshida, Mitsuhiro Fujishiro, Department of Endoscopy and Endoscopic Surgery, Graduate School of Medicine, the University of Tokyo, Tokyo 113-0033, Japan

Keiko Niimi, Center for Epidemiology and Preventive Medicine, Graduate School of Medicine, the University of Tokyo, Tokyo 113-0033, Japan

Tomohiro Shinozaki, Department of Biostatistics, School of Public Health, the University of Tokyo, Tokyo 113-0033, Japan

Tetsuo Ushiku, Masashi Fukayama, Department of Pathology, Graduate School of Medicine, the University of Tokyo, Tokyo 113-0033, Japan

ORCID number: Daisuke Ohki (0000-0002-7400-0914); Yosuke Tsuji (0000-0001-9537-4993); Tomohiro Shinozaki (0000-0003-3395-9691); Yoshiki Sakaguchi (0000-0001-5078-3750); Chihiro Minatsuki (0000-0002-9727-4504); Hiroto Kinoshita (0000-0002-8179-6017); Keiko Niimi (0000-0001-6813-5583); Satoshi Ono (0000-0002-9864-348X); Yoku Hayakawa (0000-0002-3988-2499); Shuntaro Yoshida (0000-0002-9437-9132); Atsuo Yamada (0000-0003-4314-7777); Shinya Kodashima (0000-0002-3125-317X); Nobutake Yamamichi (0000-0002-5741-9887); Yoshihiro Hirata (0000-0002-8324-366X); Tetsuo Ushiku (0000-0002-1763-8380); Mitsuhiro Fujishiro (0000-0002-4074-1140); Masashi Fukayama (0000-0002-0460-064X); Kazuhiko Koike (0000-0002-9739-9243).

Author contributions: Ohki D and Tsuji Y contributed to planning the study protocol and manuscript writing; Shinozaki T provided advice on the statistical analysis of the data; Sakaguchi Y, Kinoshita H, Niimi K, Ono S, Yoshida S, Yamada A, Kodashima S, Yamamichi N and Hirata Y performed endoscopic procedures, checked the manuscript and suggested improvement ; Hayakawa Y and Minatsuki M checked the manuscript and suggested improvement; Ushiku T and Fukayama M provided advice on the histopathological diagnoses, checked the manuscript and suggested improvement; Koike K gave the final approval of the manuscript.

Institutional review board statement: In our institution, the comprehensive retrospective analysis of each patient's medical record was approved by our ethics committee (No. 2058); this study is included in that category.

Informed consent statement: All study participants provided informed written consent prior to study enrollment.

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

Data sharing statement: No additional data are available.

Manuscript source: Unsolicited manuscript

Correspondence to: Yosuke Tsuji, MD, PhD, Assistant Professor, Department of Gastroenterology, Graduate School of Medicine, the University of Tokyo, 7-3-1, Hongo, Tokyo 113-8655, Japan. ytsuji-tky@umin.ac.jp
Telephone: +81-3-38155411
Fax: +81-3-34486544

Received: December 20, 2017
Peer-review started: December 21, 2017
First decision: January 15, 2018
Revised: February 5, 2018
Accepted: March 6, 2018
Article in press: March 6, 2018
Published online: March 15, 2018

Abstract

AIM

To investigated the association between adenoma detection rate (ADR) and sessile serrated ADR (SSADR) and significant predictors for sessile serrated adenomas (SSA) detection.

METHODS

This study is a retrospective, single-center analysis. Total colonoscopies performed by the gastroenterologists at the University of Tokyo Hospital between January and December 2014 were retrospectively identified. Polyps were classified as low-grade or high-grade adenoma, cancer, SSA, or SSA with cytological dysplasia, and the prevalence of each type of polyp was investigated. Predictors of adenoma and SSA detection were examined using logistic generalized estimating equation models. The association between ADR and SSADR for each gastroenterologist was investigated by calculating a correlation coefficient weighted by the number of each gastroenterologist's examination.

RESULTS

A total of 3691 colonoscopies performed by 35 gastroenterologists were assessed. Overall, 978 (26.5%) low- and 84 (2.2%) high-grade adenomas, 81 (2.2%) cancers, 66 (1.8%) SSAs, and 2 (0.1%) SSAs with cytological dysplasia were detected. Overall ADR was 29.5% (men 33.2%, women 23.8%) and overall SSADR was 1.8% (men 1.7%, women 2.1%). In addition, 672 low-grade adenomas (68.8% of all the detected low-grade adenomas), 58 (69.9%) high-grade adenomas, 29 (34.5%) cancers, 52 (78.8%) SSAs, and 2 (100%) SSAs with cytological dysplasia were found in the proximal colon. Adenoma detection was the only significant predictor of SSA detection (adjusted OR: 2.53, 95%CI: 1.53-4.20; $P < 0.001$). The correlation coefficient between ADR and SSADR weighted by the number of each gastroenterologist's examinations was 0.606 ($P < 0.001$).

CONCLUSION

Our results demonstrated that ADR is correlated to SSADR. In addition, patients with adenomas had a higher prevalence of SSAs than those without adenomas.

Key words: Sessile serrated adenoma; Sessile serrated adenoma detection rate; Adenoma detection rate; Colonoscopy; Interval colorectal cancer

Core tip: Sessile serrated adenomas (SSAs) are difficult to detect and are associated with interval colorectal cancer (CRC). To reduce interval CRC and CRC death, SSA detection is important, and evaluation of the sessile serrated adenoma detection rate (SSADR) is crucial. In Western countries, there have been some reports showing the correlation of adenoma detection rate (ADR) and SSADR. However, in Asian countries, little is known about the correlation between ADR and SSADR. We investigated the association between ADR and SSADR and significant predictors for SSA detection in Japanese population. We found that ADR is correlated with SSADR, and patients with adenomas have a higher prevalence of SSAs than those without adenomas.

Ohki D, Tsuji Y, Shinozaki T, Sakaguchi Y, Minatsuki C, Kinoshita H, Niimi K, Ono S, Hayakawa Y, Yoshida S, Yamada A, Kodashima S, Yamamichi N, Hirata Y, Ushiku T, Fujishiro M, Fukayama M, Koike K. Sessile serrated adenoma detection rate is correlated with adenoma detection rate. *World J Gastrointest Oncol* 2018; 10(3): 82-90 Available from: URL: http://www.wjgnet.com/1948-5204/full/v10/i3/82.htm DOI: http://dx.doi.org/10.4251/wjgo.v10.i3.82

INTRODUCTION

Colorectal cancer (CRC) is one of the major causes of cancer mortality in the world[1]. Incidence of CRC has been increasing in Japan, and it is now the second leading cause of cancer-related death[2]. Colonoscopy currently plays a central role in CRC screening[3-5]. Total colonoscopy has been shown to reduce the risk of death from CRC by removing precancerous adenomas[5]. Total colonoscopy and detection of adenomas are imperative for preventing CRC. The adenoma detection rate (ADR) has been reported to be an excellent quality indicator of total colonoscopy[6,7]. ADR is also associated with the risk of interval CRC and death[8,9].

However, there have been some reports indicating that total colonoscopy is less effective in reducing the risk of cancer in the proximal colon[10,11]. The presence of sessile serrated adenomas (SSAs) in the right colon, which would progress *via* the serrated pathway to CRC, is thought to be a potential reason. A serrated pathway is an alternative pathway in which serrated polyps replace the traditional adenoma as precursor lesions to CRC[12]. CRCs derived from serrated pathways account for 20%-30% of all CRCs[13,14]. SSAs are usually flat or sessile, and are occasionally covered by a mucous cap[13]. They are difficult to detect because of their subtle morphology, and even when detected, are often incompletely resected. In addition, some SSAs are reported to progress to invasive cancer in a short period of time[15,16]. Therefore, SSAs are thought

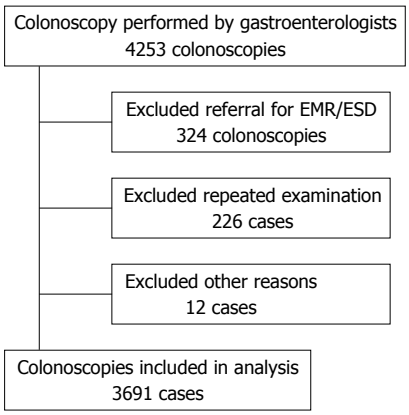

Figure 1 Study flow chart. EMR: Endoscopic mucosal resection; ESD: Endoscopic submucosal dissection.

to be strongly associated with interval CRC[16,17]. To reduce interval CRC and CRC-related death, detection of SSAs is important, and evaluation of the SSA detection rate (SSADR) is crucial. Recently, there have been few reports suggesting that SSADR is associated with ADR[17,18]. However, to the best of our knowledge, there has been no report in Asian countries showing a correlation between ADR and SSADR. In this context, we investigated the association between ADR and SSADR with significant predictors for SSA detection in total colonoscopy screening or surveillance in the Japanese population.

MATERIALS AND METHODS

Patients

This study is a retrospective, single-center analysis. We extracted data on total colonoscopies performed at the University of Tokyo Hospital between January and December 2014 by reviewing electronic medical records. All total colonoscopies performed by gastroenterologists were included in this analysis. Indications for total colonoscopy were classified as surveillance total colonoscopy, positive fecal occult blood test, screening for other symptoms (*e.g.*, abdominal pain, anemia, and chronic diarrhea), and others. The following colonoscopies were excluded: repeated examinations during the study period and referral colonoscopies for endoscopic mucosal resection/endoscopic submucosal dissection (Figure 1). All gastroenterologists involved in this study had more than 5 years of experience in total colonoscopy.

In this study, we classified the pathology of each resected polyp into the following categories: low- or high-grade adenoma, cancer (including intramucosal cancer), SSA, or SSA with cytological dysplasia (Figures 2 and 3). Polyps that were resected but not histologically evaluated, and endoscopically detected polyps that were not resected, were determined to be non-neoplastic. The histological definition for SSAs was in accordance with the definition of the Japanese Society for Cancer of the Colon and Rectum[19]. SSAs had two or more of the following features in more than 10% of the serrated area: (1) Dilated crypt; (2) irregularly branching crypt; and/or (3) dilation of the base of the crypt which often has a boot, L, or inverted T shape. SSA with cytological dysplasia was defined as a dysplastic area, similar to conventional adenoma[19,20]. In our institution, the comprehensive retrospective analysis of each patient's medical record was approved by our ethics committee (No. 2058); this study is included in that category. The present study was performed in accordance with the Declaration of Helsinki.

Procedure

The bowel preparation method in our institution was as follows: (1) 10 mL of 0.75% sodium picosulfate the day before endoscopy; and (2) 2-4 L of polyethylene glycol (Niflec: EA Pharma, Tokyo, Japan) on the morning of the endoscopy.

Video processor unit EVIS LUCERA SPECTRUM or EVIS LUCERA ELITE (Olympus Corporation, Tokyo, Japan) and single-channel lower gastrointestinal endoscope (PCF-Q260AZI, PCF-Q260AI, PCF-PQL, CF-240AI; Olympus Co.) were used. The choice of the endoscope was left to the discretion of each endoscopist.

Almost all colonoscopies were performed without sedation, but in some special cases where patients could not tolerate the colonoscopy procedure, conscious sedation using diazepam with or without pentazocine was administered.

Examination items

The polyp detection rate and location of each polyp were investigated. The proximal colon was defined as the area proximal to the splenic flexure (transverse colon, ascending colon, and cecum), while the distal colon was defined as the area distal to the splenic flexure (descending colon, sigmoid colon, and rectum). ADR was calculated as described in previous literature[6,21]: the proportion of colonoscopies where at least one colorectal low- or high-grade adenoma or cancer was detected. SSADR was calculated in the same way: the proportion of colonoscopies where at least one SSA or SSA with cytological dysplasia was detected.

Factors possibly related to adenoma detection and SSA detection was assessed: (1) Patients' age; (2) patients' sex; (3) years of colonoscopy experience of the endoscopist; (4) withdrawal time; (5) cecal intubation rate; and (6) bowel cleansing level. Withdrawal time was defined as the time from identification of cecum to identification of anus in colonoscopy cases where no polyps were removed. The bowel cleansing level was classified as "adequate" or "non-adequate" according to the ASGE/ACG task force recommendations. "Adequate" was defined as the examination allowed for the detection of polyps > 5 mm in size[6,22].

Statistical analysis

Characteristics of patients were summarized and compared between the presence (+) or absence (-)

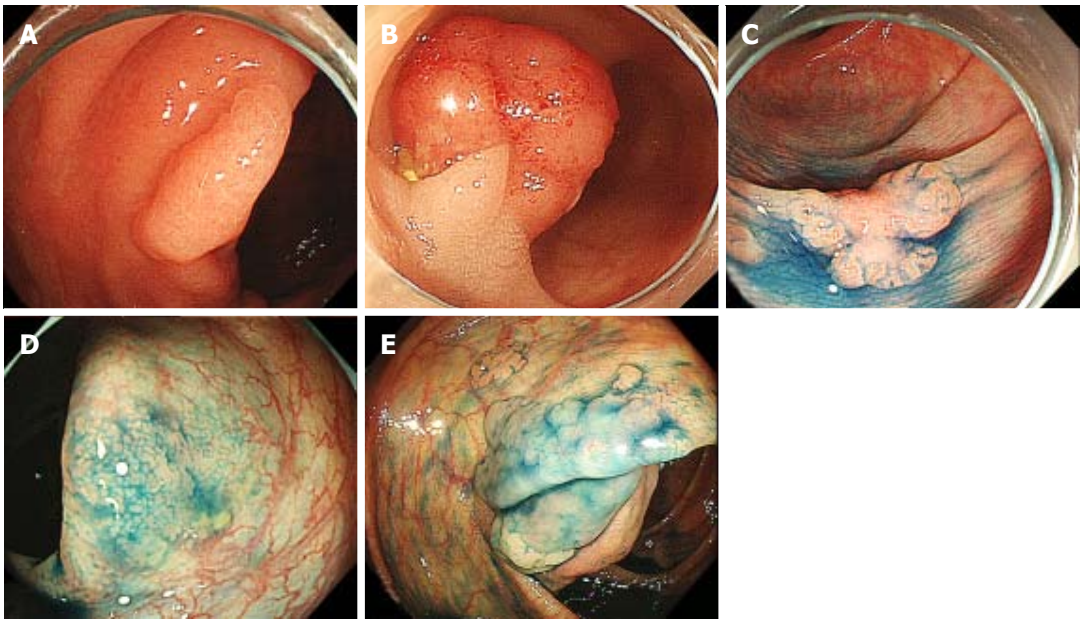

Figure 2 Typical endoscopic pictures of each polyp. A: Low grade adenoma; B: High grade adenoma; C: Cancer; D: Sessile serrated adenoma; E: Sessile serrated adenoma with cytological dysplasia.

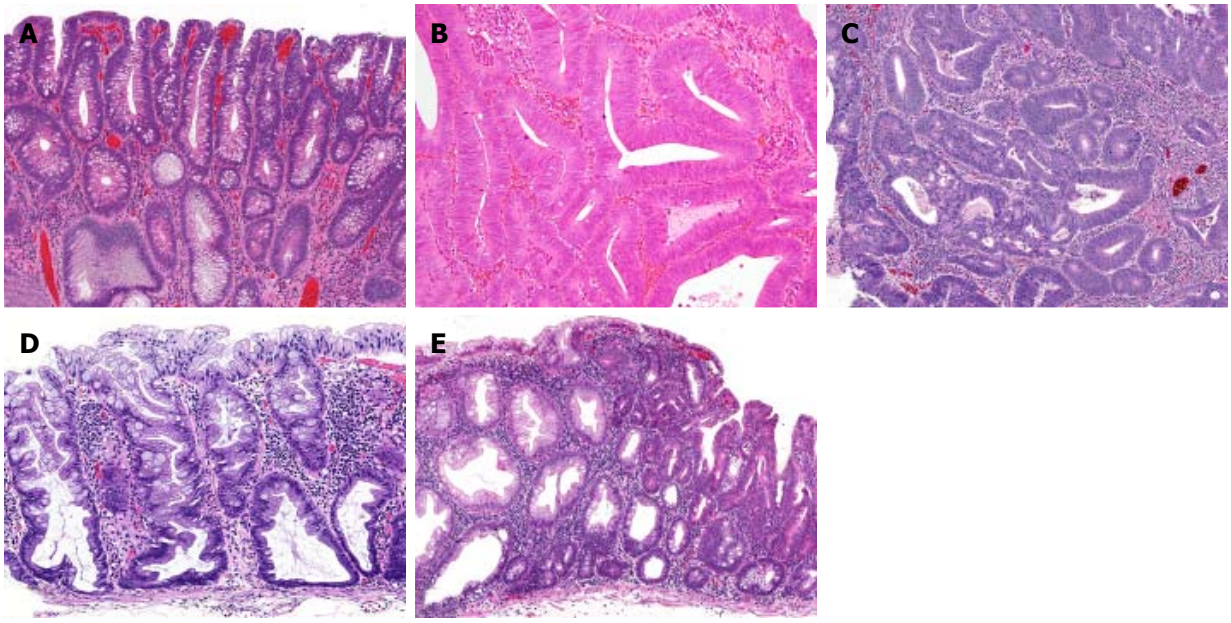

Figure 3 Histopathological pictures of each polyp. A: Low grade adenoma; B: High grade adenoma; C: Cancer; D: Sessile serrated adenoma; E: Sessile serrated adenoma with cytological dysplasia.

of adenoma detection using *t*-test or chi-squared test. Gastroenterologists' experience and their average withdrawal time that was calculated after excluding polypectomy were also summarized. Predictors of ADR were examined using logistic generalized estimating equation models, which explain the adenoma detection probability of each total colonoscopy by patient-and gastroenterologist-level variables. We used robust sandwich variance estimators that specified each gastroenterologist as a cluster to compute 95% confidence intervals (CI) and *P*-values. Predictors of SSADR were similarly examined, but adenoma detection of corresponding total colonoscopy was added as a predictor. The bivariate association of SSADR and ADR of each gastroenterologist were illustrated by a scatter plot and correlation coefficient that were weighted by the number of performed total colonoscopies. All analyses were conducted using SAS version 9.4 (Cary, NC, United States).

RESULTS

Study group and characteristics of colonoscopies

A total of 4253 colonoscopies were performed by

Table 1 Patient characteristics

	Total (n = 3691)
Age, mean ± SD (yr)	63.5 ± 13.3
Sex: Male (%)	2224 (60.3)
Adequate bowel cleansing (%)	3585 (97.1)
Cecal intubation rate (%)	3636 (98.5)
Indications for colonoscopy (%)	
Surveillance	1314 (35.6)
Fecal occult blood test	538 (14.6)
Screening for other symptoms	544 (14.7)
Others	1295 (35.1)

Others include screening before surgery or chemotherapy, patients' desire, and so on.

Table 2 Gastroenterologist characteristics

	n = 35
Sex: Male (%)	25/35 (71.4)
Years of experience in colonoscopy (%)	
5-9	24/35 (68.6)
10-14	6/35 (17.1)
⩾ 15	5/35 (14.3)
Number of colonoscopies performed (%)	
⩽ 100	19/35 (54.3)
100-200	10/35 (28.6)
⩾ 200	6/35 (17.1)
Withdrawal time: Mean (SD), m	10.1 ± 6.9

gastrenterologists during the study period. Overall, 562 colonoscopies were excluded based on the predetermined criteria, and 3691 colonoscopies were included in the analysis (Figure 1). Baseline characteristics of colonoscopies are shown in Table 1. Adequate bowel cleansing and cecal intubation rate were observed in 3585 (97.1%) cases and 3636 (98.5%) cases, respectively.

Characteristics of gastroenterologist

Baseline characteristics of gastroenterologists are shown in Table 2. All gastroenterologists had at least 5 years of colonoscopy experience; 16 (45.7%) gastroenterologists performed more than 100 cases a year.

Detection of each polyp

Low- and high-grade adenomas, and cancers were found in 978 (26.5%) cases, 84 (2.2%) cases and 81 (2.2%) cases, respectively. Overall ADR was 29.5% (men 33.2%, women 23.8%). SSAs and SSAs with cytological dysplasia were found in 66 (1.8%) cases and 2 (0.1%) cases, respectively. Overall SSADR was 1.8% (men 1.7%, women 2.1%).

The location of each polyp was also investigated. Altogether, 672 low-grade adenomas (68.8% of all the detected low-grade adenomas), 58 (69.9%) high-grade adenomas, 29 (34.5%) cancers, 52 (78.8%) SSAs, and 2 (100%) SSAs with cytological dysplasia were found in the proximal colon.

Predictors for adenoma detection

Univariable and multivariable analyses were performed to evaluate factors associated with adenoma detection (Table 3). In our institution, the cecal intubation rate was almost 100%, but could not be used in the analyses. Mean withdrawal time was 10 min, and there were only 2 gastroenterologists whose withdrawal time was less than 6 min. According to the scatter diagram plotting each endoscopist's ADR against their mean withdrawal time, as previously reported[7], the recommended ADR level of 25%[6] corresponded to a withdrawal time of 8 min. All factors, except for years of colonoscopy experience, were significantly associated with adenoma detection in both analyses with a 5% significance level. Being a woman (adjusted OR: 0.61, 95%CI: 0.54-0.70; P < 0.001) and those with non-adequate bowel cleansing (adjusted OR: 0.32, 95%CI: 0.19-0.52; P < 0.001) had a statistically inverse relationship with adenoma detection. Mean withdrawal time ⩾ 8 min had statistically significant correlation with adenoma detection (adjusted OR: 1.77, 95%CI: 1.28-2.46; P < 0.001).

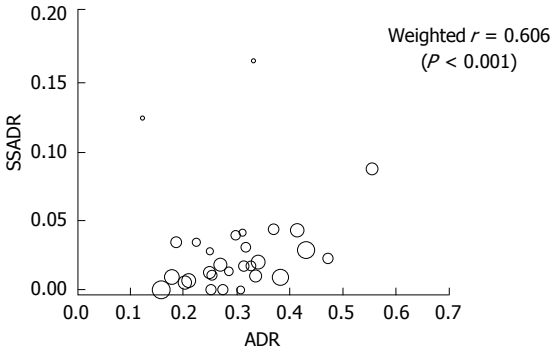

Figure 4 Weighted scatter plot and correlation coefficient for detection rates of sessile serrated adenomas and adenomas of each gastroenterologist. The area of the circle is proportional to the number of colonoscopies performed. SSADR: Sessile serrated adenomas; ADR: Adenomas.

Predictors for sessile serrated adenoma detection

Univariable and multivariable analyses were performed to evaluate factors associated with SSA detection (Table 4). Both analyses revealed that adenoma detection was the only significant predictor for SSA detection (adjusted OR: 2.53, 95%CI: 1.53-4.20; P < 0.001). Mean withdrawal time ⩾ 8 min tended to be associated with SSA detection, but was not statistically significant (adjusted OR 1.53; 95%CI: 0.62-3.75; P = 0.35).

Correlation between ADR and SSADR

As for the correlation between ADR and SSADR, a scatter diagram of ADR and SSADR is shown in Figure 4. The correlation coefficient between ADR and SSADR weighted by the number of each gastroenterologist's examinations was 0.606 (P < 0.001).

DISCUSSION

In the present study, a relatively strong association between ADR and SSADR was observed. Some reports

Table 3 Odds ratio estimates from logistic generalized estimating equations for adenoma detection

Variable	Univariable model		Multivariable model	
	OR (95%CI)	*P*	OR (95%CI)	*P*
Patient-level variable				
Age (yr)	1.02 (1.02, 1.03)	< 0.001	1.02 (1.02, 1.03)	< 0.001
Female	0.63 (0.55, 0.71)	< 0.001	0.61 (0.54, 0.70)	< 0.001
Non-adequate bowel cleansing	0.36 (0.22, 0.57)	< 0.001	0.32 (0.19, 0.52)	< 0.001
Endoscopist-level variable				
Endoscopist's experiment (yr)	0.98 (0.94, 1.02)	0.36	0.99 (0.96, 1.02)	0.55
Mean withdrawal time ⩾ 8 min (*vs* < 8 min)	1.72 (1.23, 2.41)	0.0015	1.77 (1.28, 2.46)	< 0.001

Multivariable model simultaneously adjusted for listed variables. Confidence intervals and *P*-values were calculated by robust variance specifying a gastroenterologist as a cluster.

Table 4 Odds ratio estimates from logistic generalized estimating equations for sessile serrated adenoma detection

Variable	Univariable model		Multivariable model	
	OR (95%CI)	*P*	OR (95%CI)	*P*
Patient-level variable				
Adenoma detection (*vs* none)	2.44 (1.45, 4.09)	< 0.001	2.53 (1.53, 4.20)	< 0.001
Age (yr)	0.99 (0.98, 1.01)	0.27	0.99 (0.98, 1.00)	0.07
Female	1.28 (0.77, 2.11)	0.34	1.40 (0.85, 2.29)	0.19
Non-adequate bowel cleansing	0.50 (0.07, 3.47)	0.48	0.60 (0.08, 4.28)	0.61
Endoscopist-level variable				
Endoscopist's experiment (yr)	0.99 (0.89, 1.10)	0.86	1.00 (0.91, 1.09)	0.96
Mean withdrawal time ⩾ 8 min (*vs* < 8 min)	1.74 (0.70, 4.29)	0.23	1.53 (0.62, 3.75)	0.35

Multivariable model simultaneously adjusted for listed variables.

have described the correlation of ADR and SSADR in Western countries patients[17,18]; however, to our knowledge, the prevalence of SSAs or SSADR in Asian populations has not yet been fully investigated and appropriate SSADR has not been determined. Therefore, our study holds importance, as it is the first report to demonstrate the correlation between SSADR and ADR in Asian populations.

There is controversy regarding the prevalence of SSAs, which differs among previously published studies, varying from 2%-10%[13,17,18,23,24]. In our institution, the prevalence of SSAs was approximately 2%, which is lower than previously reported results in Western populations. Each endoscopist's cognitive capability to detect SSAs may differ in degree. Payne *et al*[25] reported that the prevalence of SSAs varied among endoscopy centers. In addition, Abdeljawad K *et al*[26] reported that a review of pathology slides by an experienced gastrointestinal pathologist increased the prevalence of SSAs, and the prevalence of SSAs increased over the study period, suggesting that each endoscopist improved his detection skills over time. However, the gastroenterologist's ADR in this study was approximately 30%, which is within the standard of quality indicators for colonoscopy specified by ASGE[6]. Therefore, the quality of the present study is assured. The quality of the pathological evaluation was also high, because the experienced gastrointestinal pathologist (U.T.), who was acquainted with the definition of the Japanese Society for Cancer of the Colon and Rectum, reassessed the pathology slides. As previously mentioned, the prevalence of SSAs in Asian populations has not been determined, as there may be a difference between races. It is mandatory to investigate the true prevalence of SSAs in Asian populations in the future.

The factors associated with SSA detection were investigated, and our study demonstrated that adenoma detection at the patient level was the only independent significant factor associated with SSA detection. Previous reports have shown that when a patient presented with serrated lesions, especially SSAs, he/she was also more likely to have advanced neoplasia[23,27-29]. These results were compatible with previous reports and suggested that ADR is correlated with SSADR.

A withdrawal time of ⩾ 8 min was not a statistically significant factor for SSA detection, although it was significantly related to adenoma detection. However, considering that ADR and SSADR are correlated, a longer duration of inspection seems to improve ADR and SSADR. In this study, the total number of SSAs was quite small. This may be a reason why a significant association between withdrawal time and SSA detection was not found.

We acknowledge that there were several limitations in our study. First, this study was a retrospective single center study, and the number of SSA cases was small. Second, there were many cases of total colonoscopy surveillance in the present study in addition to total colonoscopy screening. As previously stated, the target ADR should be changed according to patient risk[30]. However, factors associated with adenoma detection in this study were similar to those in previous reports.

Moreover, Anderson JC reported that the serrated polyp detection rate was similar for screening or surveillance indications, suggesting that both indications could be used to derive the serrated polyp detection rate in practice[31].

Rex *et al*[32] has also recently reported that using overall ADR to calculate ADR from screening, surveillance, and diagnostic colonoscopies would be just as effective as a screening-only ADR. Taking this into account, the current findings can be applied to clinical practice to some extent. Finally, the ratio of adequate bowel cleansing in this study was much higher than in previous studies. The ASGE guidelines recommend that the quality of bowel cleansing should be evaluated after retained fluid or stool has been suctioned[6]. In our institution, if fluid and stool were retained, gastroenterologists suctioned as much as possible to identify polyps $\geqslant$ 5 mm in size. Such cases were considered adequate in our study, and therefore, the ratio of the "adequate" level was high.

In conclusion, our study suggests that ADR is correlated with SSADR. In addition, patients with adenomas may have a higher prevalence of SSAs than those without adenomas. A large-scale prospective study will be needed to validate these findings.

ARTICLE HIGHLIGHTS

Research background

Sessile serrated adenomas (SSAa) are difficult to detect and strongly associated with interval colorectal cancer (CRC). It is necessary to investigate the factors which influence SSA detection and to evaluate the SSA detection rate (SSADR).

Research motivation

In Western countries, some reports have described the correlation of ADR and SSADR. However, to the best of our knowledge, there has been no report in Asian countries showing a correlation between ADR and SSADR. In this context, we investigated the association between ADR and SSADR with significant predictors for SSA detection in total colonoscopy screening or surveillance in the Japanese population.

Research objectives

The main objectives were as follows; the prevalence of each polyp (low-grade or high-grade adenoma, cancer, SSA, or SSA with cytological dysplasia), each gastoroenterologist's ADR and SSADR, the association between ADR and SSADR for each gastroenterologist and predictors of adenoma and SSA detection.

Research methods

Total colonoscopies performed by the gastroenterologists at the University of Tokyo Hospital between January and December 2014 were retrospectively identified. The prevalence of each type of polyp was investigated. Predictors of adenoma and SSA detection were examined using logistic generalized estimating equation models. The association between ADR and SSADR for each gastroenterologist was investigated by calculating a correlation coefficient weighted by the number of each gastroenterologist's examination.

Research results

A total of 3691 colonoscopies by 35 gastroenterologists were assessed. 978 low grade adenomas (26.5%), 84 high grade adenomas (2.2%), 81 cancers (2.2%), 66 SSAs (1.8%) and 2 SSAs with cytological dysplasia (0.1%) were detected. Adenoma detection was the only significant predictor of SSA detection (adjusted OR: 2.53, 95%CI: 1.53-4.20; $P < 0.001$). The correlation coefficient between ADR and SSADR weighted by the number of each gastroenterologist's examinations was 0.606 ($P < 0.001$).

Research conclusions

Our study suggests that ADR is correlated with SSADR. Some reports have described the correlation of ADR and SSADR in Western countries patients; however, to our knowledge, the prevalence of SSAs or SSADR in Asian populations has not yet been fully investigated and appropriate SSADR has not been determined. Therefore, our study holds importance, as it is the first report to demonstrate the correlation between SSADR and ADR in Asian populations. In addition, patients with adenomas may have a higher prevalence of SSAs than those without adenomas.

Research perspectives

This study was a retrospective single center study, and the number of SSA cases was small. Therefore, a large-scale prospective study will be needed to validate these findings.

ACKNOWLEDGMENTS

This paper was presented at the Digestive Disease Week (DDW), May 21-24, 2016, in San Diego, United States of America.

REFERENCES

1 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin* 2016; **66**: 7-30 [PMID: 26742998 DOI: 10.3322/caac.21332]

2 **Matsuda A**, Matsuda T, Shibata A, Katanoda K, Sobue T, Nishimoto H; Japan Cancer Surveillance Research Group. Cancer incidence and incidence rates in Japan in 2008: a study of 25 population-based cancer registries for the Monitoring of Cancer Incidence in Japan (MCIJ) project. *Jpn J Clin Oncol* 2014; **44**: 388-396 [PMID: 24503029 DOI: 10.1093/jjco/hyu003]

3 **Lieberman DA**, Rex DK, Winawer SJ, Giardiello FM, Johnson DA, Levin TR. Guidelines for colonoscopy surveillance after screening and polypectomy: a consensus update by the US Multi-Society Task Force on Colorectal Cancer. *Gastroenterology* 2012; **143**: 844-857 [PMID: 22763141 DOI: 10.1053/j.gastro.2012.06.001]

4 **European Colorectal Cancer Screening Guidelines Working Group**, von Karsa L, Patnick J, Segnan N, Atkin W, Halloran S, Lansdorp-Vogelaar I, Malila N, Minozzi S, Moss S, Quirke P, Steele RJ, Vieth M, Aabakken L, Altenhofen L, Ancelle-Park R, Antoljak N, Anttila A, Armaroli P, Arrossi S, Austoker J, Banzi R, Bellisario C, Blom J, Brenner H, Bretthauer M, Camargo Cancela M, Costamagna G, Cuzick J, Dai M, Daniel J, Dekker E, Delicata N, Ducarroz S, Erfkamp H, Espinàs JA, Faivre J, Faulds Wood L, Flugelman A, Frkovic-Grazio S, Geller B, Giordano L, Grazzini G, Green J, Hamashima C, Herrmann C, Hewitson P, Hoff G, Holten I, Jover R, Kaminski MF, Kuipers EJ, Kurtinaitis J, Lambert R, Launoy G, Lee W, Leicester R, Leja M, Lieberman D, Lignini T, Lucas E, Lynge E, Mádai S, Marinho J, Maučec Zakotnik J, Minoli G, Monk C, Morais A, Muwonge R, Nadel M, Neamtiu L, Peris Tuser M, Pignone M, Pox C, Primic-Zakelj M, Psaila J, Rabeneck L, Ransohoff D, Rasmussen M, Regula J, Ren J, Rennert G, Rey J, Riddell RH, Risio M, Rodrigues V, Saito H, Sauvaget C, Scharpantgen A, Schmiegel W, Senore C, Siddiqi M, Sighoko D, Smith R, Smith S, Suchanek S, Suonio E, Tong W, Törnberg S, Van Cutsem E, Vignatelli L, Villain P, Voti L, Watanabe H, Watson J, Winawer S, Young G, Zaksas V, Zappa M, Valori R. European guidelines for quality assurance in colorectal cancer screening and diagnosis: overview and introduction to the full supplement publication. *Endoscopy* 2013; **45**: 51-59 [PMID: 23212726 DOI: 10.1055/s-0032-1325997]

5 **Zauber AG**, Winawer SJ, O'Brien MJ, Lansdorp-Vogelaar I, van Ballegooijen M, Hankey BF, Shi W, Bond JH, Schapiro M, Panish

JF, Stewart ET, Waye JD. Colonoscopic polypectomy and long-term prevention of colorectal-cancer deaths. *N Engl J Med* 2012; **366**: 687-696 [PMID: 22356322 DOI: 10.1056/NEJMoa1100370]

6 **Rex DK**, Schoenfeld PS, Cohen J, Pike IM, Adler DG, Fennerty MB, Lieb JG 2nd, Park WG, Rizk MK, Sawhney MS, Shaheen NJ, Wani S, Weinberg DS. Quality indicators for colonoscopy. *Gastrointest Endosc* 2015; **81**: 31-53 [PMID: 25480100 DOI: 10.1016/j.gie.2014.07.058]

7 **Barclay RL**, Vicari JJ, Doughty AS, Johanson JF, Greenlaw RL. Colonoscopic withdrawal times and adenoma detection during screening colonoscopy. *N Engl J Med* 2006; **355**: 2533-2541 [PMID: 17167136 DOI: 10.1056/NEJMoa055498]

8 **Corley DA**, Levin TR, Doubeni CA. Adenoma detection rate and risk of colorectal cancer and death. *N Engl J Med* 2014; **370**: 2541 [PMID: 24963577 DOI: 10.1056/NEJMc1405329]

9 **Kaminski MF**, Regula J, Kraszewska E, Polkowski M, Wojciechowska U, Didkowska J, Zwierko M, Rupinski M, Nowacki MP, Butruk E. Quality indicators for colonoscopy and the risk of interval cancer. *N Engl J Med* 2010; **362**: 1795-1803 [PMID: 20463339 DOI: 10.1056/NEJMoa0907667]

10 **Brenner H**, Hoffmeister M, Arndt V, Stegmaier C, Altenhofen L, Haug U. Protection from right- and left-sided colorectal neoplasms after colonoscopy: population-based study. *J Natl Cancer Inst* 2010; **102**: 89-95 [PMID: 20042716 DOI: 10.1093/jnci/djp436]

11 **Baxter NN**, Warren JL, Barrett MJ, Stukel TA, Doria-Rose VP. Association between colonoscopy and colorectal cancer mortality in a US cohort according to site of cancer and colonoscopist specialty. *J Clin Oncol* 2012; **30**: 2664-2669 [PMID: 22689809 DOI: 10.1200/JCO.2011.40.4772]

12 **Yamane L**, Scapulatempo-Neto C, Reis RM, Guimarães DP. Serrated pathway in colorectal carcinogenesis. *World J Gastroenterol* 2014; **20**: 2634-2640 [PMID: 24627599 DOI: 10.3748/wjg.v20.i10.2634]

13 **Rex DK**, Ahnen DJ, Baron JA, Batts KP, Burke CA, Burt RW, Goldblum JR, Guillem JG, Kahi CJ, Kalady MF, O'Brien MJ, Odze RD, Ogino S, Parry S, Snover DC, Torlakovic EE, Wise PE, Young J, Church J. Serrated lesions of the colorectum: review and recommendations from an expert panel. *Am J Gastroenterol* 2012; **107**: 1315-1329; quiz 1314, 1330 [PMID: 22710576 DOI: 10.1038/ajg.2012.161]

14 **Saiki H**, Nishida T, Yamamoto M, Hayashi S, Shimakoshi H, Shimoda A, Amano T, Sakamoto A, Otake Y, Sugimoto A, Takahashi K, Mukai K, Matsubara T, Nakajima S, Fukui K, Inada M, Yamamoto K, Tokuda R, Adachi S. Frequency of coexistent carcinoma in sessile serrated adenoma/polyps and traditional serrated adenomas removed by endoscopic resection. *Endosc Int Open* 2016; **4**: E451-E458 [PMID: 27092327 DOI: 10.1055/s-0042-103239]

15 **Oono Y**, Fu K, Nakamura H, Iriguchi Y, Yamamura A, Tomino Y, Oda J, Mizutani M, Takayanagi S, Kishi D, Shinohara T, Yamada K, Matumoto J, Imamura K. Progression of a sessile serrated adenoma to an early invasive cancer within 8 months. *Dig Dis Sci* 2009; **54**: 906-909 [PMID: 18688718 DOI: 10.1007/s10620-008-0407-7]

16 **Omori K**, Yoshida K, Tamiya S, Daa T, Kan M. Endoscopic Observation of the Growth Process of a Right-Side Sessile Serrated Adenoma/Polyp with Cytological Dysplasia to an Invasive Submucosal Adenocarcinoma. *Case Rep Gastrointest Med* 2016; **2016**: 6576351 [PMID: 27437153 DOI: 10.1155/2016/6576351]

17 **Ross WA**, Thirumurthi S, Lynch PM, Rashid A, Pande M, Shafi MA, Lee JH, Raju GS. Detection rates of premalignant polyps during screening colonoscopy: time to revise quality standards? *Gastrointest Endosc* 2015; **81**: 567-574 [PMID: 25583558 DOI: 10.1016/j.gie.2014.07.030]

18 **Zorzi M**, Senore C, Da Re F, Barca A, Bonelli LA, Cannizzaro R, de Pretis G, Di Furia L, Di Giulio E, Mantellini P, Naldoni C, Sassatelli R, Rex DK, Zappa M, Hassan C; Equipe Working Group. Detection rate and predictive factors of sessile serrated polyps in an organised colorectal cancer screening programme with immunochemical faecal occult blood test: the EQuIPE study (Evaluating Quality Indicators of the Performance of Endoscopy). *Gut* 2017; **66**: 1233-1240 [PMID: 26896459 DOI: 10.1136/gutjnl-2015-310587]

19 **Yao T,** Sugai T, Iwashita A, Fujimori T, Kushima R, Nobuki M, Mitomi H, Ajioka Y, Konishi F. Histopathological characteristics and diagnostic criteria of SSA/P. Project research ''potential of cancerization of colorectal serrated lesions'' of Japanese Society for Cancer of the Colon and Rectum. *Stomach Intest* 2011; **46**: 442-448

20 **Wada R**, Morimoto T, Inayoshi T. Pathological features of the sessile serrated adenoma/polyp with special references of its carcinogenesis. *Med Mol Morphol* 2014; **47**: 123-129 [PMID: 24748273 DOI: 10.1007/s00795-014-0075-y]

21 **Rex DK**, Petrini JL, Baron TH, Chak A, Cohen J, Deal SE, Hoffman B, Jacobson BC, Mergener K, Petersen BT, Safdi MA, Faigel DO, Pike IM; ASGE/ACG Taskforce on Quality in Endoscopy. Quality indicators for colonoscopy. *Am J Gastroenterol* 2006; **101**: 873-885 [PMID: 16635231 DOI: 10.1111/j.1572-0241.2006.00673.x]

22 **Rex DK**, Bond JH, Winawer S, Levin TR, Burt RW, Johnson DA, Kirk LM, Litlin S, Lieberman DA, Waye JD, Church J, Marshall JB, Riddell RH; U.S. Multi-Society Task Force on Colorectal Cancer. Quality in the technical performance of colonoscopy and the continuous quality improvement process for colonoscopy: recommendations of the U.S. Multi-Society Task Force on Colorectal Cancer. *Am J Gastroenterol* 2002; **97**: 1296-1308 [PMID: 12094842 DOI: 10.1111/j.1572-0241.2002.05812.x]

23 **IJspeert JE**, de Wit K, van der Vlugt M, Bastiaansen BA, Fockens P, Dekker E. Prevalence, distribution and risk of sessile serrated adenomas/polyps at a center with a high adenoma detection rate and experienced pathologists. *Endoscopy* 2016; **48**: 740-746 [PMID: 27110696 DOI: 10.1055/s-0042-105436]

24 **Rex DK**, Boland CR, Dominitz JA, Giardiello FM, Johnson DA, Kaltenbach T, Levin TR, Lieberman D, Robertson DJ. Colorectal cancer screening: Recommendations for physicians and patients from the U.S. Multi-Society Task Force on Colorectal Cancer. *Gastrointest Endosc* 2017; **86**: 18-33 [PMID: 28600070 DOI: 10.1016/j.gie.2017.04.003]

25 **Payne SR**, Church TR, Wandell M, Rösch T, Osborn N, Snover D, Day RW, Ransohoff DF, Rex DK. Endoscopic detection of proximal serrated lesions and pathologic identification of sessile serrated adenomas/polyps vary on the basis of center. *Clin Gastroenterol Hepatol* 2014; **12**: 1119-1126 [PMID: 24333512 DOI: 10.1016/j.cgh.2013.11.034]

26 **Abdeljawad K**, Vemulapalli KC, Kahi CJ, Cummings OW, Snover DC, Rex DK. Sessile serrated polyp prevalence determined by a colonoscopist with a high lesion detection rate and an experienced pathologist. *Gastrointest Endosc* 2015; **81**: 517-524 [PMID: 24998465 DOI: 10.1016/j.gie.2014.04.064]

27 **Álvarez C**, Andreu M, Castells A, Quintero E, Bujanda L, Cubiella J, Salas D, Lanas Á, Carballo F, Morillas JD, Hernández C, Jover R, Sarasqueta C, Enriquéz-Navascués JM, Hernández V, Estévez P, Macenlle R, Sala T, Balaguer F, Pellisé M, Moreira L, Gil I, Peris A, González-Rubio F, Ferrández A, Poves C, Ponce M, Grau J, Serradesanferm A, Ono A, Cruzado J, Pérez-Riquelme F, Alonso-Abreu I, Carrillo-Palau M, Santander C, Díaz Tasende J, Herreros A, Cacho G, Barranco LE, Bessa X; ColonPrev study investigators. Relationship of colonoscopy-detected serrated polyps with synchronous advanced neoplasia in average-risk individuals. *Gastrointest Endosc* 2013; **78**: 333-341.e1 [PMID: 23623039 DOI: 10.1016/j.gie.2013.03.003]

28 **Gao Q**, Tsoi KK, Hirai HW, Wong MC, Chan FK, Wu JC, Lau JY, Sung JJ, Ng SC. Serrated polyps and the risk of synchronous colorectal advanced neoplasia: a systematic review and meta-analysis. *Am J Gastroenterol* 2015; **110**: 501-509; quiz 510 [PMID: 25756237 DOI: 10.1038/ajg.2015.49]

29 **Schreiner MA**, Weiss DG, Lieberman DA. Proximal and large hyperplastic and nondysplastic serrated polyps detected by colonoscopy are associated with neoplasia. *Gastroenterology* 2010; **139**: 1497-1502 [PMID: 20633561 DOI: 10.1053/j.gastro.2010.06.074]

30 **Sanaka MR**, Rai T, Navaneethan U, Gohel TD, Podugu A, Thota PN, Lopez R, Kiran RP, Burke CA. Adenoma detection rate in high-risk patients differs from that in average-risk patients. *Gastrointest Endosc* 2016; **83**: 172-178 [PMID: 26024584 DOI: 10.1016/j.gie.2015.04.019]

31 **Anderson JC**, Butterly LF, Weiss JE, Robinson CM. Providing data for serrated polyp detection rate benchmarks: an analysis of the New Hampshire Colonoscopy Registry. *Gastrointest Endosc* 2017; **85**: 1188-1194 [PMID: 28153571 DOI: 10.1016/j.gie.2017.01.020]

32 **Rex DK**, Ponugoti PL. Calculating the adenoma detection rate in screening colonoscopies only: Is it necessary? Can it be gamed? *Endoscopy* 2017; **49**: 1069-1074 [PMID: 28753699 DOI: 10.1055/s-0043-113445]

P- Reviewer: Ahluwalia NK, De Silva AP, Dutta AK, Herszenyi L, Teramoto-Matsubara OT **S- Editor**: Cui LJ **L- Editor**: A **E- Editor**: Li RF

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2018 March 15; 10(3): 91-95

DOI: 10.4251/wjgo.v10.i3.91

ISSN 1948-5204 (online)

CASE REPORT

Case of pancreatic metastasis from colon cancer in which cell block using the Trefle® endoscopic scraper enables differential diagnosis from pancreatic cancer

Akihisa Kato, Itaru Naitoh, Hiroyuki Kato, Kazuki Hayashi, Katsuyuki Miyabe, Michihiro Yoshida, Yasuki Hori, Makoto Natsume, Naruomi Jinno, Takeshi Yanagita, Shuji Takiguchi, Satoru Takahashi, Takashi Joh

Akihisa Kato, Itaru Naitoh, Kazuki Hayashi, Katsuyuki Miyabe, Michihiro Yoshida, Yasuki Hori, Makoto Natsume, Naruomi Jinno, Takashi Joh, Department of Gastroenterology and Metabolism, Nagoya City University Graduate School of Medical Sciences, Nagoya 467-8601, Japan

Hiroyuki Kato, Satoru Takahashi, Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences, Nagoya 467-8601, Japan

Takeshi Yanagita, Shuji Takiguchi, Department of Gastroenterological Surgery, Nagoya City University Graduate School of Medical Sciences, Nagoya 467-8601, Japan

ORCID number: Akihisa Kato (0000-0002-7733-7854); Itaru Naitoh (0000-0001-8342-886X); Hiroyuki Kato (0000-0002-5888-6217); Kazuki Hayashi (0000-0001-5217-2873); Katsuyuki Miyabe (0000-0002-4915-9835); Michihiro Yoshida (0000-0003-4151-6225); Yasuki Hori (0000-0001-9510-2568); Makoto Natsume (0000-0001-7818-512X); Naruomi Jinno (0000-0002-2634-2338); Takeshi Yanagita (0000-0002-7503-6978); Shuji Takiguchi (0000-0002-1339- 354X); Satoru Takahashi (0000-0002-8139-8158); Takashi Joh (0000-0002-3624-0597).

Author contributions: Kato A and Naitoh I mainly designed this concept and drafted the article; Miyabe K, Yoshida M, Hori Y, Natsume M, Jinno N and Yanagita T performed the treatment for the patient and provided data involved in clinical course; Kato H and Takahashi S performed histological evaluation and provided data involved in immunohistochemistry; Hayashi K and Takiguchi S revised the article for important intellectual content; Joh T gave a final approval of the article.

Informed consent statement: Written informed consent was obtained from the patient.

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

Manuscript source: Unsolicited manuscript

Correspondence to: Itaru Naitoh, MD, PhD, Assistant Professor, Department of Gastroenterology and Metabolism, Nagoya City University Graduate School of Medical Sciences, 1 Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan. inaito@med.nagoya-cu.ac.jp
Telephone: +81-52-8538211
Fax: +81-52-8520952

Received: December 5, 2017
Peer-review started: December 5, 2017
First decision: January 3, 2018
Revised: January 9, 2018
Accepted: February 3, 2018
Article in press: February 3, 2018
Published online: March 15, 2018

Abstract

Endoscopic transpapillary brush cytology and forceps biopsy during endoscopic retrograde cholangiopancreatology are generally used to obtain pathological evidence of biliary strictures. Recently, the new endoscopic scraper Trefle® has been reported and demonstrated high cancer detectability in malignant biliary strictures. This device is used to scrape the stricture over the guidewire, and, in the original method, the tissue and/or cell samples obtained are subjected

to histological and/or cytological analysis separately. However, discrimination of chunks of tissue is hampered by the opacity of the surrounding fluid. We have developed a cell block technique for the Trefle® device without dividing obtained specimens into tissue and cellular components, which is the simplest method and enables immunohistochemical analysis. We present a case of obstructive jaundice diagnosed immunohistochemically as pancreatic metastasis from colon cancer using cell block sections obtained with the Trefle® device, which procedure is as easy as conventional brush cytology.

Key words: Trefle®; Cell block; Endoscopic scraper; Pancreatic metastasis; Biliary strictures

Core tip: We described a case of pancreatic metastasis from colon cancer in which cell block technique with the specimens obtained by the new endoscopic device Trefle® was useful in the differential diagnosis from pancreatic cancer. The combination of cell block technique and Trefle® might be a promising method in the diagnosis of biliary strictures because this procedure is as easy as conventional brush cytology.

Kato A, Naitoh I, Kato H, Hayashi K, Miyabe K, Yoshida M, Hori Y, Natsume M, Jinno N, Yanagita T, Takiguchi S, Takahashi S, Joh T. Case of pancreatic metastasis from colon cancer in which cell block using the Trefle® endoscopic scraper enables differential diagnosis from pancreatic cancer. *World J Gastrointest Oncol* 2018; 10(3): 91-95 Available from: URL: http://www.wjgnet.com/1948-5204/full/v10/i3/91.htm DOI: http://dx.doi.org/10.4251/wjgo.v10.i3.91

INTRODUCTION

Accurate diagnosis of biliary strictures is challenging, despite development of various imaging modalities. It is essential to diagnose the cause of biliary strictures using pathological evidence prior to selection of the appropriate therapy. Endoscopic transpapillary brush cytology during endoscopic retrograde cholangiopancreatology (ERCP) is conventionally used to obtain specimens for pathological diagnosis of biliary strictures, because it is technically easy and rapid. To provide larger tissue samples and improve sensitivity, endoscopic transpapillary forceps biopsy is also frequently performed[1,2]. However, forceps biopsy is technically more difficult and time-consuming than brush cytology[3,4]. Benign and malignant lesions can be diagnosed using cytology specimens, but these cannot be subjected to immunohistochemical analysis, despite its utility for differential diagnosis. The Trefle® endoscopic scraper (Piolax Medical Devices, Yokohama, Japan) enables detection of cancer in malignant biliary strictures[5]. This device has three scraping loops and was designed to access biliary strictures over the guidewire and obtain tissues and/or cell samples for histology or cytology. The procedure using the Trefle® device is almost identical to that for conventional brush cytology; scraped tissues and/or cell samples, together with bile juice, are aspirated from the side port of the outer sheath into a syringe. In the original method, after allowing the aspirate to settle in a sterile tube, specimens were divided into tissue and fluid components for histological and cytological analyses, respectively. However, discrimination of chunks of tissue is hampered by the opacity of the surrounding fluid. Therefore, a simpler method of processing specimens obtained using the Trefle® device is required.

The cell block technique improves diagnostic yield and facilitates immunohistochemical analysis[6-9]. We typically use the Trefle® device to obtain specimens from biliary strictures, which, together with aspirated bile juice and affixed tissues, are poured into a sterile tube. The tube is sent to the Pathology Department for evaluation by the cell block method, which enables differentiation of benign from malignant lesions, as well as immunohistochemical analysis during any time of need. We report here a case of obstructive jaundice with pancreatic metastasis from colon cancer, differential diagnosis of which from pancreatic cancer was performed by immunohistochemical examination of cell block sections obtained using the Trefle® device.

CASE REPORT

A 69-year-old male underwent laparoscopic low anterior resection for rectal adenocarcinoma (stage IV; pT4N2M1 according to the American Joint Committee on Cancer 7th Edition Cancer Staging Manual) 18 mo prior and received adjuvant chemotherapy [FOLFOX (folinic acid, 5-fluorouracil, and oxaliplatin) plus panitumumab as the first line and FOLFIRI (folinic acid, 5-fluorouracil, and irinotecan) plus bevacizumab as the second line]. Metastases to the liver and lung occurred despite administration of second-line chemotherapy, and the patient presented with epigastric pain and jaundice. Laboratory evaluation revealed high aspartate/alanine transaminase levels (777/394 IU/L) and bilirubin/direct bilirubin levels (11.4/7.3 mg/dL) (Table 1).

Contrast-enhanced computed tomography (CT) revealed a defined 1.5 cm × 1.5 cm mass, which was poorly enhanced in both the early and late phases, at the pancreatic head, dilated common bile duct and upstream main pancreatic duct, as well as masses in both lobes of the liver and both lungs (Figure 1A and B). The patient was diagnosed with obstructive jaundice due to primary pancreatic ductal adenocarcinoma or pancreatic metastasis from colon cancer. Therefore, we planned to perform endoscopic biliary drainage to treat the obstructive jaundice and obtain histopathological evidence.

An ERCP demonstrated a biliary stricture of the lower common bile duct approximately 2 cm in length, as well as dilatation of the proximal bile duct (Figure

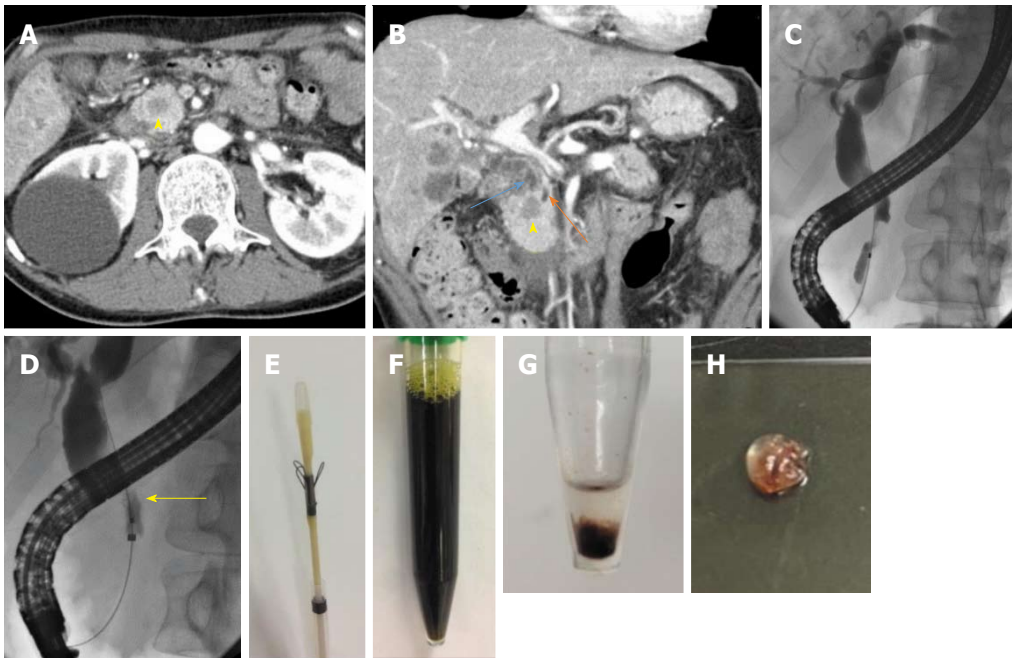

Figure 1 Imaging findings and samples obtained using the Trefle® device. A and B: Abdominal computed tomography indicated a poorly enhanced region (yellow arrowhead), dilated common bile duct (blue arrow), and upstream main pancreatic duct (orange arrow); C: Endoscopic retrograde cholangiopancreatology demonstrated a biliary stricture; D: The Trefle® device was inserted and opened, and the scraping loops were identified under fluoroscopic guidance (yellow arrow); E: Appearance of the Trefle® device; F: Appearance of samples obtained; G and H: Appearance of the centrifuged deposit.

Table 1 Laboratory data

Variable	Value	Reference range
White blood cell	10.6	3.6-9.6 × 10^3/μL
Hemoglobin	12.1	13.2-17.2 g/dL
Platelet	541	148-339 × 10^3/μL
C-reactive protein	5.55	⩽ 0.30 mg/dL
Aspartate transaminase	777	13-33 IU/L
Alanine transaminase	394	6-30 IU/L
Lactate dehydrogenase	405	119-229 IU/L
Alkaline phosphatase	4861	115-359 IU/L
γ-glutamyl transpeptidase	1347	10-47 IU/L
Amylase	404	37-125 IU/L
Total bilirubin	6.3	0.3-1.2 mg/dL
Direct bilirubin	4.3	0.0-0.3 mg/dL
Carcinoembryonic antigen	12.5	< 5.0 ng/mL
Carbohydrate antigen 19-9	13280.0	< 37.0 U/mL

1C). After performing endoscopic sphincterotomy (EST), the Trefle® device was inserted into the bile duct over the guidewire. Next, the scraping loops of the device were opened and passed through the stricture in the proximal-to-distal direction under fluoroscopic guidance (Figure 1D). All specimens including aspirated bile juice and tissues were transferred to a sterile tube; the scraping loops were cut using scissors (Figure 1E and F). The centrifuged deposit was fixed in formalin overnight. Next, the deposit was washed in saline, mixed with 1% sodium aspartate, and centrifuged again. Finally, the deposit was put a few drop of 1 M calcium chloride and embedded in paraffin, yielding a cell block (Figure 1G and H). The cell block was sectioned for hematoxylin-and-eosin (HE) and immunohistochemical staining. The lesion was confirmed to be moderately differentiated adenocarcinoma, which by immunohistochemical staining was focally positive for cytokeratin 7 (CK 7) and positive for CK 20 and caudal type homeobox 2 (CDX 2). These findings were consistent with those of previous resected specimens, confirming the final diagnosis of pancreatic metastasis from colon cancer (Figure 2).

A covered self-expanding metal stent (SEMS) was inserted to resolve the symptoms and establish biliary drainage. The third-line chemotherapy regimen, FOLFIRI plus ramucirumab, was administered based on the results of immunohistochemical examination, and the patient is alive at the time of writing. The combination of the cell-block technique and the Trefle® device was useful for making decisions regarding management of this patient.

DISCUSSION

This case demonstrated contrast-enhanced CT findings compatible with typical pancreatic ductal adenocarcinoma with hypovascular tumor and a dilated upstream main pancreatic duct. In this case, differential diagnosis of pancreatic metastasis of colon cancer was necessary, because the patient had a medical history of colon cancer with distant metastasis. However, pancreatic metastasis from colon cancer is rare in clinical practice. Pancreatic metastases from non-pancreatic primary tumors are rare, accounting for approximately 2% of all pancreatic neoplasms[10], and arise most commonly from primary tumors of the kidney, lung, breast, and colon. Immunohistochemistry is essential for identifying the primary site of metastatic neoplasms using molecular markers. Determination of CK 7, CK 20, and CDX 2

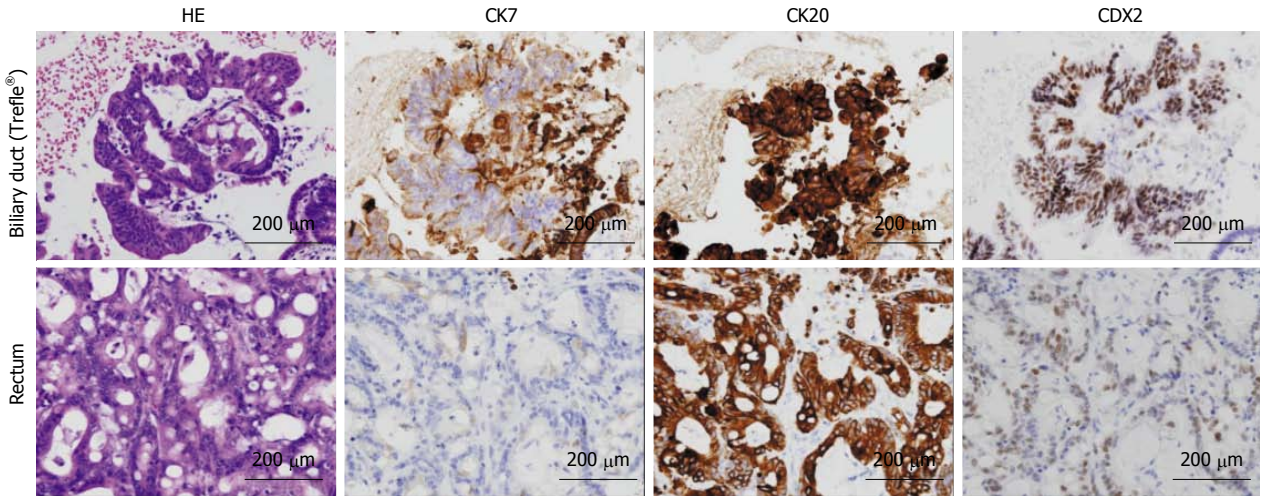

Figure 2 Histological findings. Immunohistochemical staining revealed that cancer cells in cell block specimens obtained using the Trefle® device were focally positive for cytokeratin 7 (CK 7), and positive for CK 20 and caudal type homeobox 2 (CDX 2). These findings were consistent with those of rectal resection specimens.

expression is useful for distinguishing colon cancer. CK 7 is expressed by various cancers, including that of the pancreas, but not the gastrointestinal tract. In contrast, CK 20 is expressed by most gastrointestinal tumors-including primary colonic, pancreatic, and gastric cancers, but is non-specific. CDX 2 is also expressed by colon adenocarcinoma, but at very low levels in most gastric and pancreatic tumors[11-13]. Biopsy specimens are generally required for immunohistochemical analysis; cytology specimens are unsuitable for this purpose. However, the cell block method is appropriate for immunohistochemical analysis. Use of sodium aspartate as a fixative increases the cellularity, increasing morphological detail and improving the diagnostic sensitivity. The cell block method can also generate multiple sections for staining and immunohistochemistry[14]. The efficacy of cell block method has been reported in the bile duct cytology and endoscopic ultrasound-guided fine needle aspiration of pancreas and gastrointestinal solid neoplastic lesions[7-9].

The amount of tissue collected from the biliary tract by brush cytology is insufficient for immunohistochemical analysis, despite the need for immunohistochemical analysis to diagnose various diseases of the biliary tract, such as IgG4-SC or metastasis from cancer in other organs. Although endoscopic transpapillary forceps biopsy can be performed to obtain larger tissue samples, its success is dependent on operator skill because it is technically more difficult than brush cytology. Hence, alternative techniques that yield tissue samples of adequate size are required. In this case, we used the Trefle® endoscopic device, which has been demonstrated to be superior to forceps biopsy in terms of histologic/cytologic sample yield (93.5% *vs* 83.7%) and cancer detection (64.7% *vs* 51.3%)[5]. Specimens obtained using the Trefle® device are divided into tissue and fluid components for histological and cytological analyses, respectively. However, distinguishing chunks of tissue is hampered by the opacity of the surrounding fluid. In addition, some tissue may remain in the fluid component. Therefore, a simpler and more efficient specimen-processing method is needed. We typically subject specimens obtained using the Trefle® device to the cell block method to enable differentiation of benign and malignant lesions, as well as immunohistochemical examination. In the case presented herein, the cell block method with the Trefle® device facilitated differential diagnosis of a biliary stricture. Further studies involving larger populations are needed to confirm the efficacy of this method.

In conclusion, we describe a case of pancreatic metastasis from colon cancer in which the cell block technique, together with immunohistochemistry, enabled differential diagnosis from pancreatic cancer. The combination of the cell block technique and the Trefle® device shows promise for diagnosis of biliary strictures as it is as easy as conventional brush cytology.

ARTICLE HIGHLIGHTS

Case characteristics

The patient underwent resection for rectal adenocarcinoma presented metastases to the liver and lung with epigastric pain and jaundice.

Clinical diagnosis

The patient was diagnosed with obstructive jaundice.

Differential diagnosis

Primary pancreatobiliary carcinoma or pancreatic metastasis from colon cancer.

Laboratory diagnosis

Laboratory evaluation revealed the findings of obstructive jaundice.

Imaging diagnosis

The patient was diagnosed with obstructive jaundice due to primary pancreatic ductal adenocarcinoma.

Pathological diagnosis

Immunohistochemical findings of the cell block sections obtained using the

Trefle® endoscopic scraper were consistent with those of previous resected specimens, confirming the final diagnosis of pancreatic metastasis from colon cancer.

Treatment

A covered self-expanding metal stent was inserted to resolve the symptoms and establish biliary drainage and the third-line chemotherapy regimen for colon cancer was administered.

Related reports

There have been few reports dealing with the combination of a scraper Trefle® and cell block method for histocytological diagnosis of malignant biliary strictures.

Experiences and lessons

The combination of the cell block technique and the Trefle® device shows promise for diagnosis of biliary strictures as it is as easy as conventional brush cytology.

REFERENCES

1 **Yasuda I,** Enya M, Moriwaki H, Tomita E, Kato T, Mukai T, Adachi S, Kasahara S, Asano T. Diagnostic value of transpapillary biopsy using double lumen introducer for determination of mucosal extent in extrahepatic bile duct cancer. *Digestive endoscopy: official journal of the Japan Gastroenterological Endoscopy Society* 2003; **15**: 200-205 [DOI: 10.1046/j.1443-1661.2003.00245.x]

2 **Noda Y,** Fujita N, Kobayashi G, Ito K, Horaguchi J, Takazawa O, Obana T, Nakahara K, Ishida K, Suzuki T, Hirasawa D, Sugawara T, Ohira T, Onochi K, Harada Y, Tsuchiya T, Sawai T, Uzuki M, Kariya Y. Introductal ultrasonography before biliary drainage and transpapillary biopsy in assessment of the longitudinal extent of bile duct cancer. *Digestive endoscopy: official journal of the Japan Gastroenterological Endoscopy Society* 2008; **20**: 73-78 [DOI: 10.1111/j.1443-1661.2008.00779.x]

3 **Navaneethan U**, Njei B, Lourdusamy V, Konjeti R, Vargo JJ, Parsi MA. Comparative effectiveness of biliary brush cytology and intraductal biopsy for detection of malignant biliary strictures: a systematic review and meta-analysis. *Gastrointest Endosc* 2015; **81**: 168-176 [PMID: 25440678 DOI: 10.1016/j.gie.2014.09.017]

4 **Naitoh I**, Nakazawa T, Kato A, Hayashi K, Miyabe K, Shimizu S, Kondo H, Nishi Y, Yoshida M, Umemura S, Hori Y, Kuno T, Takahashi S, Ohara H, Joh T. Predictive factors for positive diagnosis of malignant biliary strictures by transpapillary brush cytology and forceps biopsy. *J Dig Dis* 2016; **17**: 44-51 [PMID: 26717051 DOI: 10.1111/1751-2980.12311]

5 **Sakuma Y**, Kodama Y, Sogabe Y, Nakai Y, Yamashita Y, Mikami S, Kajimura K, Ikeda K, Tamaki H, Iwamoto S, Matsuda F, Fujita K, Uza N, Kawamura T, Uemoto S, Seno H, Chiba T, Yazumi S; Kyoto Pancreatobiliary Study Group. Diagnostic performance of a new endoscopic scraper for malignant biliary strictures: a multicenter prospective study. *Gastrointest Endosc* 2017; **85**: 371-379 [PMID: 27497604 DOI: 10.1016/j.gie.2016.07.060]

6 **Grunze H**. The comparative diagnostic accuracy, efficiency and specificity of cytologic technics used in the diagnosis of malignant neoplasm in serous effusions of the pleural and pericardial cavities. *Acta Cytol* 1964; **8**: 150-163 [PMID: 14154149]

7 **Noda Y**, Fujita N, Kobayashi G, Ito K, Horaguchi J, Hashimoto S, Koshita S, Ishii S, Kanno Y, Ogawa T, Masu K, Tsuchiya T, Oikawa M, Honda H, Sawai T, Uzuki M, Fujishima F. Prospective randomized controlled study comparing cell block method and conventional smear method for bile cytology. *Dig Endosc* 2013; **25**: 444-452 [PMID: 23808950 DOI: 10.1111/j.1443-1661.2012.01404.x]

8 **Ieni A**, Barresi V, Todaro P, Caruso RA, Tuccari G. Cell-block procedure in endoscopic ultrasound-guided-fine-needle-aspiration of gastrointestinal solid neoplastic lesions. *World J Gastrointest Endosc* 2015; **7**: 1014-1022 [PMID: 26322154 DOI: 10.4253/wjge.v7.i11.1014]

9 **Ieni A**, Barresi V, Tuccari G. Diagnostic relevance of cell block procedure in secondary tumors of the pancreas. *Cytojournal* 2016; **13**: 19 [PMID: 27651821 DOI: 10.4103/1742-6413.189638]

10 **Z'graggen K**, Fernández-del Castillo C, Rattner DW, Sigala H, Warshaw AL. Metastases to the pancreas and their surgical extirpation. *Arch Surg* 1998; **133**: 413-417; discussion 418-419 [PMID: 9565122 DOI: 10.1001/archsurg.133.4.413]

11 **Dennis JL**, Hvidsten TR, Wit EC, Komorowski J, Bell AK, Downie I, Mooney J, Verbeke C, Bellamy C, Keith WN, Oien KA. Markers of adenocarcinoma characteristic of the site of origin: development of a diagnostic algorithm. *Clin Cancer Res* 2005; **11**: 3766-3772 [PMID: 15897574 DOI: 10.1158/1078-0432.CCR-04-2236]

12 **Chu P**, Wu E, Weiss LM. Cytokeratin 7 and cytokeratin 20 expression in epithelial neoplasms: a survey of 435 cases. *Mod Pathol* 2000; **13**: 962-972 [PMID: 11007036 DOI: 10.1038/modpathol.3880175]

13 **Werling RW**, Yaziji H, Bacchi CE, Gown AM. CDX2, a highly sensitive and specific marker of adenocarcinomas of intestinal origin: an immunohistochemical survey of 476 primary and metastatic carcinomas. *Am J Surg Pathol* 2003; **27**: 303-310 [PMID: 12604886 DOI: 10.1097/00000478-200303000-00003]

14 **Dey S**, Nag D, Nandi A, Bandyopadhyay R. Utility of cell block to detect malignancy in fluid cytology: Adjunct or necessity? *J Cancer Res Ther* 2017; **13**: 425-429 [PMID: 28862203 DOI: 10.4103/0973-1482.177501]

P- Reviewer: Noda Y, Tuccari G **S- Editor**: Cui LJ **L- Editor**: A **E- Editor**: Li RF

Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com