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EDITORIAL

Classificatory updates in verrucous and cuniculatum carcinomas: Insights from the 5th edition of WHO-IARC head and neck tumor classification

Felipe Martins Silveira, Lauren Frenzel Schuch, Ronell Bologna-Molina

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Abstract

The International Agency for Research on Cancer (IARC) and World Health Organization (WHO) collaboratively produce the 'WHO Blue Books' essential tools standardizing the diagnostic process for human cancers. Regular updates in this classification accommodate emerging molecular discoveries, advances in immunohistochemical techniques, and evolving clinical insights. The 5th edition of the WHO/IARC classification of head and neck tumors refines the 'Oral Cavity and Mobile Tongue' chapter, including sections for non-neoplastic lesions, epithelial tumors, and tumors of uncertain histogenesis. Notably, the epithelial tumors section is rearranged by tumor behavior, starting with benign squamous papillomas and progressing through potentially malignant oral disorders to oral squamous cell carcinoma (OSCC). The section on OSCC reflects recent information on epidemiology, pathogenesis, and histological prognostic factors. Noteworthy is the specific categorization of verrucous carcinoma (VC) and carcinoma cuniculatum (CC), both associated with the oral cavity and distinct in clinical and histologic characteristics. This classification adjustment emphasizes the oral cavity as their predominant site in the head and neck. Designating specific sections for VC and CC aims to provide comprehensive insights into these unique subtypes, elucidating their clinical features, distinct histological characteristics, prevalence, significance, and clinical relevance. By categorizing these subtypes into specific sections, the 5th edition of the WHO classification aims to provide a more nuanced and detailed account, enhancing our understanding of these specific variants within the broader spectrum of head and neck tumors.

Key Words: World Health Organization; Squamous cell carcinoma of head and neck;

Verrucous carcinoma; Mouth neoplasms

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Core Tip: The collaboration between the International Agency for Research on Cancer (IARC) and the World Health Organization (WHO) has produced indispensable 'WHO Blue Books' crucial for standardizing cancer diagnostics. In the 5th edition of the WHO/IARC classification of head and neck tumors, the 'Oral Cavity and Mobile Tongue' chapter refines its structure, introducing sections for non-neoplastic lesions, epithelial tumors, and tumors with uncertain histogenesis. Notable adjustments in the epithelial tumors section highlight a reorganization based on tumor behavior, offering comprehensive insights into distinct subtypes.

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INTRODUCTION

The classification of tumors performed by the International Agency for Research on Cancer (IARC)/World Health Organization (WHO), commonly known as "WHO Blue Books", serves as a tool to standardize the diagnostic process by establishing a consistent nomenclature for human cancers. This classification is based on the application of analytic criteria supported by evidence critically assessed by experts in the field. Regular updates of this classification facilitate the enhancement of tumor classifications and the inclusion of novel entities, driven by advancements in molecular discoveries, progress in immunohistochemical techniques, and evolving clinical insights. Traditionally, cancer classification predominantly relied on histopathological consensus, with minimal consideration for molecular pathology. However, recent technological advancements have significantly accelerated the evolution of pathology. Understanding cancer's molecular intricacies has reached a pivotal stage, underscoring the imperative inclusion of this knowledge for precise diagnostic evaluations. The content of this classification has recently been updated to its 5th edition, comprising a total of 14 volumes in the series. In addition, the latest update includes two volumes dedicated to cytopathology, titled WHO Reporting Systems for Cytopathology[1].

In the 5th edition of the WHO/IARC classification of head and neck tumors, the 6th chapter titled "Oral Cavity and Mobile Tongue" is now divided into non-neoplastic lesions, epithelial tumors, and tumors of uncertain histogenesis[1]. This classification is more concise compared to the previous version, which encompassed categories such as malignant surface epithelial tumors, oral potentially malignant disorders and oral epithelial dysplasia, papillomas, tumors of uncertain histogenesis, soft tissue and neural tumors, oral mucosal melanoma, salivary type tumors, and hematolymphoid tumors[2]. These changes are linked to modifications in this new edition, notably the collective grouping of tumors common to multiple systems. In the previous edition, various soft tissue neoplasms were included in this section, but they now find their place in the soft tissue tumor chapter. The relocation of oral melanoma to the melanocytic tumors chapter and the addressing of intraoral salivary gland lesions in the salivary gland chapter exemplifies the reorganization in this edition.

Epithelial tumors

Squamous cell carcinomas (SCC), previously collectively described in the 4th edition, along with all its histological subtypes under the subsection titled "Malignant Surface Epithelial Tumors"[2], now receive specific attention in the latest edition of the WHO/IARC classification of head and neck tumors. The epithelial tumors section is reorganized based on tumor behavior starting, with squamous papillomas, followed by oral potentially malignant disorders, oral epithelial dysplasia, proliferative verrucous leukoplakia, submucosal fibrosis, and HPV-associated dysplasia. Oral squamous cell carcinoma (OSCC) is now positioned last in this section, following a presentation based on biological behavior. In this classification[1], OSCC is defined as "a malignant neoplasm arising from the mucosal epithelium of the oral cavity and showing variable squamous differentiation" and is identified as the 16th most prevalent cancer, registering an annual incidence surpassing 377000 cases, data based on the Global Cancer Statistics/2020[3]. This definition of OSCC is more objective compared to the one presented in the 4th edition of the WHO Blue Book, which also included demographic characteristics and the presentation of associated risk factors. These alterations involve a classification reorganization. Considering the behavior of pathological entities, it is logical to begin with the presentation of squamous papilloma, a benign epithelial neoplastic lesion, then progress to oral potentially malignant disorders, and finally, address OSCC, a malignant epithelial neoplasm.

The alterations in the new section on OSCC have been updated to reflect the most recent information regarding the epidemiology, pathogenesis, and histological prognostic factors of this entity. Regarding its clinical features, in the 5th Edition of the Blue Book, the occurrence of OSCC is reported to predominantly affects male individuals with the potential to manifest at any location within the oral mucosa, presenting as lesions characterized by diverse colorations (white, red,

or mixed) and varied configurations (flat, nodular, or mass) in terms of size. The pathogenesis of OSCC is characterized to distinguished by most cases arising in regions featuring pre-existing epithelial dysplasia or in correlation with oral potentially malignant disorders. OSCC, for the most part, exhibits genetic instability, marked by notable chromosomal alterations and a heightened burden of somatic mutations. In this latest edition, the described chromosomal losses are observed in 3p, 8p, 9p, and 17p, and the gains in 3q, 5p, 8q, and 11q. The somatic mutations reported are documented in the following genes: *TP53*, *CDKN2A*, *FAT1*, *NOTCH1*, *KMT2D*, *CASP8*, *AJUBA*, *NSD1*, *HLA-A*, *TGFBR2*, *USP9X*, *MLL4*, *HRAS*, *UNC13C*, *ARID2*, and *TRPM3*. It is noteworthy to observe that in the penultimate version of this classification[2], under the Etiology section, several etiological factors of OSCC were well described, including tobacco use, alcohol consumption, smokeless tobacco, HPV, ultraviolet radiation, and the association with poor oral health – this last one being reported as not proved as an independent risk factor. A diet rich in fruits and vegetables was also cited as a protective effect against oral cancer.

Histopathology and subtypes of oral squamous cell carcinoma

In latest WHO classification of head and neck tumors[1], it is specified that the majority of cancers affecting the oral cavity and mobile tongue belong to the category of conventional keratinizing SCC. The classification also recognizes the potential occurrence of rare histological subtypes within this malignant epithelial neoplasia. The histological subtypes of OSCC comprise the following six distinct variants according to the WHO: Spindle cell carcinoma variant manifests as a biphasic tumor, comprising a SCC and a malignant spindle cell component with a mesenchymal appearance. This subtype is recognized for its aggressive behavior and rapid growth[4]. The Basaloid SCC variant is typified by a basaloid cell population and a diminished presence of squamous cells. This type tends to demonstrate a propensity for local relapse and regional lymph node metastasis[5]. The Acantholytic SCC variant is characterized by a disruption of cellular cohesion. This subtype may present with a clinical course marked by increased aggressiveness[6]. Adenosquamous carcinoma variant exhibits dual differentiations-both squamous and glandular. This particular subtype is acknowledged for its heightened aggressiveness[7]. The Papillary SCC variant is identified by finger-like projections or papillae. In contrast to the other subtypes, it may carry a more favorable prognosis[8]. The Lymphoepithelial Carcinoma variant showcases a pronounced lymphoid stroma and is comparatively rare within the oral cavity[9].

As noted, in the 5th edition of the WHO Blue Book, the histological subtypes of OSCC remain unchanged being specified in the Subtype(s) section as: Spindle cell, basaloid, acantholytic, adenosquamous, papillary, and lymphoepithelial. Notably, an alteration from the previous classification is here observed, wherein verrucous carcinoma (VC) and carcinoma cuniculatum (CC) variants are now described in specific sections. VC is often linked to prolonged tobacco use, presents with a well-differentiated, warty appearance, and tends to display a less aggressive nature[10]. CC stands out as a distinct subtype, characterized by a gradual, endophytic growth pattern featuring crypt-like structures[11]. This adjustment recognizes the oral cavity as the primary site in the head and neck for both entities, each with distinct clinical and histologic characteristics that set them apart from the conventional type. The choice to designate specific sections for these entities likely originates from the imperative to provide comprehensive and focused information about these particular subtypes. This involves elucidating their unique clinical features, distinct histological characteristics, prevalence, and significance, as well as their clinical relevance.

Verrucous carcinoma and carcinoma cuniculatum

In the previous edition, VC was discussed in the chapter covering the hypopharynx, larynx, trachea, and parapharyngeal space. Given the distinctive manifestation of VC in the oral cavity, accounting for more than half of all VC cases in the head and neck, a specialized section has been included in the most recent edition. The current classification[1] defines VC as a well-differentiated, non-metastasizing SCC with a warty keratinized surface and distinctive architecture, lacking significant cytologic features of malignancy. VC is reported as a rare entity, comprising 2%-16% of oral carcinomas, predominantly affecting older individuals. The use of terminology such as "Ackerman tumor" and "verrucous hyperplasia" is not endorsed in the latest classification. The oral mucosa is the primary site, representing 50%-75% of cases in the head and neck. Etiologically linked to the use of chewing tobacco or snuff, VC clinically presents as a slowly growing, slightly exophytic white tumor. Left untreated, VC can lead to bone erosion and extensive destruction. While the pathogenesis is not fully understood, the molecular signature distinguishes VC from other oral SCCs. Morphologically, VC is characterized by a well-differentiated, broad-based squamous epithelial proliferation with marked keratinization. Invasion into the stroma is uniform with well-defined borders, lacking substantial cytologic features of malignancy. VC has an excellent prognosis, with surgery as the preferred treatment; irradiation is considered for select cases.

CC is defined an infrequent, well-differentiated squamous cell carcinoma characterized by local invasiveness without metastatic potential[1]. Typically observed in individuals aged seven to eight decades, CC shows no gender bias, with the gingival-alveolar complex of the mandible being the primary site, followed by the maxilla. Clinical manifestations include pain, swelling, mucosal ulceration, tooth mobility, and induration. The etiology of CC remains undefined in this classification, and its pathogenic mechanisms and potential association with HPV are unknown. Morphologically, CC exhibits an endophytic growth pattern, forming a labyrinthine network of well-differentiated squamous epithelium with interconnecting keratin-filled crypts reaching the surface. Cytological atypia is mild, and the presence of intraepithelial and stromal neutrophils, along with keratin microabscesses, is common. Microsequestra are frequently associated with bone invasion. Prognosis following complete excision is excellent, with local recurrence uncommon following accurate preoperative diagnosis. Notably, metastases do not develop in cases of CC despite multiple interventions.

CONCLUSION

Recent advances in unraveling the molecular pathogenesis of oral squamous cell carcinoma have significantly enhanced the understanding of the tumor's genesis and evolutionary processes, influencing diagnostic criteria. Due to the dynamic nature of this field, classifications and terminologies undergo continuous refinement. Therefore, keeping abreast of the most up-to-date insights is essential, requiring regular reference to the latest World Health Organization classification edition and contemporary literature. Anticipating future discoveries, ongoing updates, and reclassifications within the Blue Books will be imperative to maintain the precision and relevance of oral cancer diagnostics.

FOOTNOTES

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EDITORIAL

Understanding the role of transmembrane 9 superfamily member 1 in bladder cancer pathogenesis

Venkata Krishna Vamsi Gade, Budhi Singh Yadav

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Abstract

In this editorial we comment on the article by Wei *et al*, published in the recent issue of the *World Journal of Clinical Oncology*. The authors investigated the role of Transmembrane 9 superfamily member 1 (TM9SF1) protein in bladder cancer (BC) carcinogenesis. Lentiviral vectors were used to achieve silencing or overexpression of TM9SF1 gene in three BC cell lines. These cell lines were then subject to cell counting kit 8, wound-healing assay, transwell assay, and flow cytometry. Proliferation, migration, and invasion of BC cells were increased in cell lines subjected to TM9SF1 overexpression. TM9SF1 silencing inhibited proliferation, migration and invasion of BC cells. The authors conclude that TM9SF1 may be an oncogene in bladder cancer pathogenesis.

Key Words: Urinary bladder cancer; Transmembrane 9 superfamily member 1 gene cell line; Lentiviral vectors; Wound healing assay; Oncogene; Proliferation; Migration

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Core Tip: The scratch wound healing assay and transwell assay showed significantly improved cellular migration in the Transmembrane 9 superfamily member 1 (TM9SF1) overexpression group. TM9SF1 silencing inhibited proliferation, migration and invasion of bladder cancer (BC) cells. TM9SF1 can be used as a therapeutic molecular target. The importance of TM9SF1 as an oncogene and its use as a therapeutic target would ultimately depend on the prevalence of the mutation in BC tissues and replication of *in vitro* activity in tumour tissue.

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INTRODUCTION

In a novel basic study, Wei *et al*[1] investigated the role of Transmembrane 9 superfamily member 1 (TM9SF1) protein in bladder cancer (BC) carcinogenesis[1]. Lentiviral vectors were used to achieve silencing or overexpression of TM9SF1 gene in three BC cell lines. These cell lines were then subject to Cell counting kit 8 (CCK8), wound-healing assay, transwell assay, and flow cytometry. Proliferation, migration, and invasion of BC cells were increased in cell lines subjected to TM9SF1 overexpression. Whereas TM9SF1 silencing inhibited proliferation, migration and invasion of BC cells. The authors concluded that TM9SF1 may be an oncogene in BC pathogenesis.

BC is the most common cancer of the urinary tract with more than 500000 cases diagnosed in 2020 worldwide[2]. Non muscle invasive BC (NMIBC) comprise around 60% of all cases. Although NMIBC has an estimated 5-year overall survival rate of 71%-90%, they have a 15%-30% recurrence rate and up to a 10% rate of progression to muscle invasive BC (MIBC)[3]. MIBC has a 5 year survival rate of 60%-70% with patients having an aggressive clinical course as compared to NMIBC[4]. Patients of MIBC develop distant metastases in up to 29% of cases[5]. However, systemic therapy protocols in BC have remained largely unchanged in the last 2 decades[6,7]. The development of immune checkpoint inhibitors has led to their use in advanced BC. Randomised trials however, have only demonstrated a modest survival advantage with the use of immune checkpoint inhibitors in advanced BC[8,9]. Therefore, there is a pressing need to identify new molecular targets in BC through basic research.

Increasing age, tobacco smoke, Schistosoma infection, exposure to aromatic amines and polycyclic hydrocarbons, ionizing radiation and phenacetin-containing analgesics are all proven risk factors for BC development[10]. A review of the genomic landscape of urinary BC shows that polymorphisms of N-acetyltransferase 2 and glutathione S-transferase-μ1 genes confer an increased risk of BC[11,12]. Other genes suspected to play a role in the pathogenesis include MYC, TP63, TERT, FGFR3, PSCA, UGT1A1, TACC3 and APOBEC3A[13]. TM9SF1, first identified in 1997, is a transmembrane protein localised to the autophagosomal and lysosomal membranes in the cytoplasm. TM9SF1 was identified to be one of the 17 common differentially expressed genes in BC samples but its precise role in BC pathogenesis was unclear.

In this study by Wei *et al*[1], stable transfectants overexpressing TM9SF1 were successfully constructed in all three BC cell lines which was detected by quantitative real-time polymerase chain reaction. The CCK8 assay showed that the proliferation rate of BC cells in the TM9SF1 overexpression group was significantly higher than in the control group. The scratch wound healing assay and transwell assay showed significantly improved cellular migration in the TM9SF1 overexpression group. Matrix gel testing and flow cytometry showed TM9SF1 cells were more likely to demonstrate cell invasion and transition to G2/M phase. When these tests were performed in transfectants with silenced TM9SF1, the authors noted reduced cellular proliferation, invasion, migration and G1 cell block. With these findings TM9SF1 has been proposed to be a novel oncogene in BC pathogenesis.

In addition to BC, TM9SF1 has been found to be overexpressed in esophageal and cervical cancer with a speculated link to poorer survival and recurrence rates in some preclinical studies[14,15]. Its role as an oncogene might be due to its effects on the G1 phase. The precise molecular interaction of TM9SF1 with cell cycle proteins needs further investigation. The synergistic interaction of TM9SF1 with proteins regulating the epithelial mesenchymal transition such as EBAG9 might explain the increased invasion and migration in TM9SF1 overexpressed cells[16].

The inhibition of cellular proliferation, migration and invasion caused by TM9SF1 silencing hints at its pro-oncogenic role. Future directions might involve correlation of stage, grade and histology of BC patients with TM9SF1 overexpression. The prognostic and predictive value of TM9SF1 overexpression in BC would first need to be established in a retrospective study. For instance, while point mutation of the FGFR3 gene is observed in 60%-70% of NMIBC cases, it is only detected in 5%-10% of MIBC cases[17]. The upregulation of EGFR is observed in 20% of NMIBC cases, but it can be seen in up to 50% of MIBC cases[18]. Response of TM9SF1 overexpressing BC to standard chemotherapy regimens and radiation needs investigation.

The study also highlights the possible utility of TM9SF1 as a therapeutic molecular target. Since transfectants with silenced TM9SF1 had reduced cellular proliferation, invasion, migration and G1 arrest; therapeutic molecules inhibiting TM9SF1 might improve BC outcomes. The use of targeted therapy and immunotherapy is quickly gaining acceptance in BC treatment. Bacillus Calmette Guerin (BCG) is one of the oldest forms of immunotherapy used in BC treatment. Its intravesical use is recommended in intermediate and high-risk NMIBC after transurethral resection of bladder tumour. BCG acts on BC cells *via* direct and indirect effects. Direct cytotoxicity of BC cells occurs due to apoptosis mediated by TLR7 and cellular necrosis mediated by HMGB7. Indirect effects occur due to the internalization of BCG followed by signal transduction leading to cytokine release that ultimately results in modulation of innate and acquired immune response[19].

This study by Wei *et al*[1] was based on *in vitro* cell line experiments. In a large meta-analysis of genomic hybridisation studies, there was a high degree of correlation between mutation patterns in tissue and cell line groups of similar histology. However, quantitatively, cell lines showed higher locus-specific and cell line-specific aberrations when compared with tissue samples[20]. Microarray studies in other tumour types such as in cervical cancer have shown that though major pathogenic mutations are reflected in cell lines, there were also several notable discordant genes forming

major clusters. The reason for such discordance has not been definitively established and has been speculated to be due to changes in the tumour microenvironment[21]. Therefore, TM9SF1 expression patterns and behaviour in BC tissue samples warrants further investigation.

The importance of TM9SF1 as an oncogene and its use as a therapeutic target would ultimately depend on the prevalence of the mutation in BC tissues and replication of invitro activity in tumour tissue.

CONCLUSION

The importance of TM9SF1 as an oncogene and its use as a therapeutic target would ultimately depend on the prevalence of the expression in BC tissues and replication of *in vitro* activity in tumour tissue.

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EDITORIAL

Management of lateral pelvic lymph nodes in rectal cancer: Is it time to reach an Agreement?

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Abstract

In this editorial, we proceed to comment on the article by Chua *et al*, addressing the management of metastatic lateral pelvic lymph nodes (mLLN) in stage II/III rectal cancer patients below the peritoneal reflection. The treatment of this nodal area sparks significant controversy due to the strategic differences followed by Eastern and Western physicians, albeit with a higher degree of convergence in recent years. The dissection of lateral pelvic lymph nodes without neoadjuvant therapy is a standard practice in Eastern countries. In contrast, in the West, preference leans towards opting for neoadjuvant therapy with chemoradiotherapy or radiotherapy, that would cover the treatment of this area without the need to add the dissection of these nodes to the total mesorectal excision. In the presence of high-risk nodal characteristics for mLLN related to radiological imaging and lack of response to neoadjuvant therapy, the risk of lateral local recurrence increases, suggesting the appropriate selection of strategies to reduce the risk of recurrence in each patient profile. Despite the heterogeneous and retrospective nature of studies addressing this area, an international consensus is necessary to approach this clinical scenario uniformly.

Key Words: Rectal cancer; Lateral pelvic lymph node metastases; Pelvic lymph node dissection; Total neoadjuvant therapy; Selective management of the lateral pelvic nodes; Prophylactic management of the lateral pelvic nodes; Chemoradiotherapy; Total mesorectal excision

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Core Tip: The lack of consensus in managing metastatic lateral pelvic lymph nodes in stage II/III rectal cancer patients below the peritoneal reflection, with differing medical strategies between East and West, generates uncertainty due to limited available evidence. Characteristics such as lymph node size, neoadjuvant treatment, and selective dissection of lateral pelvic lymph nodes are part of the strategies, but the first steps toward a solid and global consensus must be taken to resolve the uncertainties present in this field.

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INTRODUCTION

Localized and locally advanced rectal adenocarcinomas below the peritoneal reflection in stages II/III present locoregional recurrence rates of approximately 6.5% following the introduction of total mesorectal excision (TME)[1], with improved outcomes seen through the introduction of multimodal treatments such as radiotherapy and chemotherapy[2-5]. However, recurrence in the lateral compartments of the pelvis is reported in 10% to 25% of patients with locally advanced rectal cancer[6,7], remaining a concern for those with rectal tumors located below the peritoneal reflection as these tend to drain along the middle and inferior rectal arteries towards the obturators, internal iliac, and external iliac, reaching the common iliac artery. These lateral nodes are precisely not encompassed in TME[8]. Some studies from Eastern countries advocate for lateral pelvic lymph node dissection (LPLND) for patients with clinical or radiological involvement and prophylactical[9-11]. Conversely, in Western countries, neoadjuvant treatment with radiotherapy (RT) with or without chemotherapy (ChT) followed by TME remains the standard treatment for these patients[2-4]. Other studies recommend selective LPLND if there are high-risk factors for nodal metastasis after neoadjuvant treatment[12-15]. In the era of total neoadjuvant therapy (TNT), a significant reduction in lateral nodal metastasis is expected, favoring selective dissection only in selected cases with limited or absent response to neoadjuvant treatment. In this sense, we discuss the article by Chua *et al*[16], evaluating the available clinical evidence from various perspectives.

DIFFERENCES IN THE MANAGEMENT OF LPLND BETWEEN EASTERN AND WESTERN VIEWS

Prophylactic management of lateral pelvic lymph nodes

The randomized controlled trial 0212 by the Japanese Clinical Oncology Group (JCOG)[9], a multicenter, non-inferiority trial, enrolled 701 patients diagnosed with lower third rectal cancer, stage II or III, without enlarged lymph nodes [short-axis diameter ≥ 10 mm on primary pelvic computed tomography (CT) or magnetic resonance imaging (MRI)]. Patients were randomized between TME with LPLND (n = 351) and TME alone (n = 350) without neoadjuvant treatment. The local recurrence rate was significantly lower in the TME plus LPLND group (7.4% vs. 12.6%; P = 0.024), with no significant differences in median follow-up of 7 years in relapse-free survival and overall survival curves between both groups. Subgroup analysis demonstrated improved relapse-free survival in clinically stage III patients undergoing TME with LPLND compared to TME alone[10]. These findings led the Japanese Society for Cancer of the Colon and Rectum (JSCCR) to recommend LPLND, even when lateral pelvic lymph nodes (LPLNs) with a short-axis diameter ≥ 10 mm are not detected by imaging[17]. However, the trial did not include patients with LPLNs ≥ 10 mm on initial radiological imaging, and only 7.3% of patients in the TME + LPLND group had pathological LPLNs[11]. Thus, these results indicate that prophylactic LPLND in patients without pathological LPLNs might be overtreatment for this patient subset. Additionally, this study demonstrates that the short-axis diameter (> 5 mm) of LPLNs is a predictive factor for positivity in pathological anatomy.

Regarding Western management in this disease scenario, neoadjuvant treatment includes radiotherapy in this area, which could effectively encompass the pelvic nodes. In this regard, the American Society for Radiation Oncology positioned itself in 2021, stating that in clinical stage II-III, there is strong evidence to recommend neoadjuvant radiotherapy[18]. Multiple clinical trials have shown that neoadjuvant radiotherapy decreases the risk of local recurrence, even in the era of TME[19-21], and the European Society for Medical Oncology guidelines[22] recommend neoadjuvant treatment with chemoradiotherapy (CRT) as superior to LPLND in terms of efficacy and morbidity. Lastly, the 2020 guideline by the American Society of Colon and Rectal Surgeons considers that in the absence of clinically positive lymph nodes in the lateral pelvic compartment, routine dissection of LPLNs is generally not required, with a strong recommendation based on low-quality evidence[23].

Selective management of LPLN and the role of imaging studies

Detecting suspicious lateral pelvic lymph nodes in rectal cancer patients using imaging studies such as CT, MRI, or positron emission tomography/computed tomography (PET/CT) with 18F-fluorodeoxyglucose poses a challenge given the heterogeneity of available studies and discrepancies between imaging diagnosis and pathological diagnosis[24].

Assessing not only the size of the nodes but also their morphological characteristics like shape, heterogeneous intensity, and borders is helpful in the initial diagnosis[25]. However, after neoadjuvant treatment with CRT/RT, it's advisable to evaluate node size in the short axis and their absence on MRI. A nodal size ≤ 2.5 mm in the short axis or a reduction of ≥ 70% in size are predictors of a good response post-surgery[26]. Nevertheless, there's no uniform international consensus on what specific sizes of lateral pelvic lymph nodes could be considered suspicious for malignancy, both at the initial diagnosis and post-neoadjuvant treatment before surgery. The presence of metastatic lateral pelvic lymph nodes in nodes ≤ 5 mm might remain hidden in up to 20% of nodes after neoadjuvant treatment[27]. A study by Ogura *et al*[28], involving 741 rectal cancer patients, revealed that lymph node size impacts locoregional recurrence rates (LRR). Nodes > 7 mm on primary MRI showed a 17.9% LRR after treatment. At 3 years, those with nodes < 4 mm had no recurrences. On the other hand, nodes > 7 mm on primary MRI and internal iliac nodes had a 52.3% LRR, considerably higher than those of similar size in the obturator compartment (9.5%). CRT with TME and LPLND in these nodes reduced LRR to 8.7% (hazard ratio, 6.2; 95%CI: 1.4-28.5; $P = 0.007$), proving significantly more effective than CRT and TME alone treatment. In this regard, the 2023 version of The Society of Abdominal Radiology's Colorectal and Anal Cancer Disease-Focused Panel [29] updated the rectal cancer lexicon, highlighting a new suggested size threshold for lateral lymph nodes. It suggests nodes with short-axis diameter (SAD) > 7 mm at the internal iliac and obturator levels as suspicious at initial staging, while post-CRT treatment considers SAD > 4 mm for internal iliac nodes and > 6 mm for obturator nodes as suspicious. However, other features should be considered, such as heterogeneity, abnormal parenchymal signal, irregular borders, and tumor deposit, with the latter being the strongest indicator of poor prognosis in lymph node involvement. Therefore, the MERCURY study considers heterogeneity and irregular borders as suspicious features of preoperative MRI[30].

ADVANTAGES OF SURGICAL TECHNIQUES AND ASSOCIATED COMORBIDITIES

LPLND is considered a relatively complex surgery in colorectal cancer, associated with longer surgical times, more significant blood loss, and a moderate risk of sexual and urinary dysfunction, although it doesn't appear to increase these risks inherent to surgery alone[7]. Studies indicate that preserving autonomic nerves during LPLND can enhance functional outcomes, especially in reducing urinary retention[31]. Comparisons among open, laparoscopic, and robotic surgery suggest the advantages of laparoscopy and robotic surgery. Robotic surgery involves less blood loss (25 mL *vs* 637 mL; $P < 0.0001$) and fewer complications, albeit with longer operating times (455 *vs* 410 min; $P < 0.007$) compared to open surgery[32]. Robotic surgery can offer improved visualization in the deep pelvis and enhanced precision in identifying vessels and nerves[32]. Despite these advancements, oncological outcomes do not differ among surgical approaches, demonstrating that both laparoscopy and robotic surgery can be equally effective in the short term for treating colorectal cancer with LPLND[33].

CURRENT CHALLENGES AND FUTURE PERSPECTIVES

The surgical approach for advanced rectal cancer with TME and LLND is common in Eastern medical societies, while the Western focus prioritizes neoadjuvant with CRT or TNT followed by TME. A Western study compared patients treated with CRT followed by TME and LPLND with those treated only with CRT and TME, reporting a local recurrence rate of 3% with LPLND *vs* 11% without LPLND ($P = 0.13$), with similar survival figures and identifying LPLND as a significant independent factor for local recurrences in multivariable analysis ($P = 0.01$). In patients with long-duration neoadjuvant and adjuvant chemotherapy, LPLND showed a lower LRR (3% *vs* 16% without LPLND; $P = 0.04$), although disease-free survival and overall survival were similar between groups ($P = 0.10$ and $P = 0.11$, respectively)[34]. These results suggest a potential shift in the therapeutic approach, assessing the role of systemic treatment in this therapeutic strategy. Indeed, the presence of mLLN should be considered locally advanced disease and treated with CRT or TNT within the Western approach. The OPRA trial[35] evaluated 324 stage II/III rectal cancer patients. After TNT treatment, those achieving complete or near-complete clinical response could adopt a wait-and-watch protocol (W&W), while others underwent TME. At 5 years, the TME-free survival was 39% *vs* 54% ($P = 0.01$), distant metastasis-free survival was 82% *vs* 79% ($P = 0.66$), and local recurrence-free survival was 94% *vs* 90% ($P = 0.27$), respectively, with similar 5-year overall survival data. These results support the safety of the W&W strategy for patients with complete or near-complete clinical responses and the use of TNT as a treatment approach in these patients. This W&W approach has gained more acceptance due, in part, to improvements/intensification in neoadjuvant treatments, where neoadjuvant systemic treatment alongside radiotherapy contributes to optimizing outcomes in these patients. Regarding radiotherapy treatment, proper coverage of the posterior compartment volume in all high-risk patients is crucial. If there are suspicions of affected lateral lymph nodes, the upper border of the mesorectal clinical target volume should be at the S1-S2 level, raising doubts about whether the radiotherapy dose coverage is adequate in routine clinical practice. In this regard, a Dutch study analyzed the coverage of internal iliac and obturator lymph nodes in standard radiotherapy treatment for rectal cancer according to volumes set by major international clinical guidelines. They observed that out of 223 patients with nodes ≥ 5 mm, 80.7% were within the treatment area, but only 33.3% were included as macroscopic tumor volume. Despite receiving adequate doses, notable local recurrence rates at 4 years were observed, especially when nodes were outside the treatment area or received lower doses. These findings suggest the need for improved techniques to locally control affected nodes[36].

For this purpose, the predictive capability of radiomic features in pre-CRT MRI images to forecast the treatment response of lymph nodes in locally advanced rectal cancer is another area of research. In a recently published study involving 78 patients who received neoadjuvant radiotherapy, five radiomic characteristics accurately discriminated

responses in the training [area under the curve (AUC) 0.908] and validation (AUC 0.865) cohorts were identified. A nomogram combining these features and morphological aspects of lymph nodes exhibited good calibration and discrimination (AUC 0.925 in training, AUC 0.918 in validation). The authors suggest that this model could personalize treatment plans and guide W&W strategies in locally advanced rectal cancer patients, offering a promising tool to enhance care and therapeutic approach[37].

Several studies explore immunotherapies such as nivolumab or toripalimab in locally advanced rectal cancer, showing high complete responses[38,39]. KRAS mutation and circulating tumor DNA (ctDNA) are biomarkers predicting recurrence and prognosis[40,41]. The GALAXY study[42] indicates that molecular residual disease detected by ctDNA is a robust indicator of recurrence. However, prospective clinical trials evaluating molecular and radiomic determinations in predicting the recurrence of LPLN are needed.

CONCLUSION

The difficulty in achieving a global consensus on the ideal treatment of LPLN in rectal cancer due to the variability of available data requires adopting an Intermediate Agreement between Western and Eastern approaches. In a context involving CRT treatment, the selective dissection of lateral pelvic lymph nodes seems to be more beneficial as part of an optimal strategy. The size of LPLN evaluated by MRI with a SAD of ≥ 7 mm, or the presence of suspicious characteristics, could be a crucial predictor of recurrence and should be considered in selective lymph node dissection. It's noteworthy that laparoscopic and robotic surgeries entail less bleeding and reduced need for transfusions, emphasizing nerve preservation to lower dysfunction risks. CRT, TNT, and surgery with selective lymph node dissection should be considered, but establishing optimal selection criteria for each therapeutic approach is necessary.

FOOTNOTES

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EDITORIAL

Tumor infiltrating lymphocytes in gastric cancer: Unraveling complex interactions for precision medicine

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Abstract

This editorial will focus on tumor immunity and the factors that alter the tumor immune micro-environment. The role of tumor infiltrating lymphocytes (TILs) will also be discussed in detail, including the types, mechanism of action, and role. Gastric cancer (GC) often presents in the advanced stage and has various factors predicting the outcomes. The interplay of these factors and their correlation with the TILs is discussed. A literature review revealed high intratumoral TILs associated with higher grade, HER2-, and *Helicobacter pylori* negativity. Moreover, stromal (ST) TILs correlated with lower grade and lesser recurrence risk in GC. High TILs in ST and invasive border also correlated with mismatch repair deficiency status. Further characterization of the CD3+, CD8+, and other cells is also warranted. In the future, this complex correlation of cancer cells with the immune system can be explored for therapeutic avenues.

Key Words: Tumor infiltrating lymphocytes; Gastric cancer; *Helicobacter pylori*; HER-2-neu

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Core Tip: Tumor infiltrating lymphocytes (TILs) are an essential component of the tumor microenvironment. The association of TIL levels with outcomes of malignancies is an upcoming field. This correlation may be utilized to explore the new immuno-oncological therapeutic avenues.

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INTRODUCTION

Gastric cancer (GC) often presents at an advanced stage, making successful treatment a daunting challenge. Immunotherapy is considered for treating GC because of the high tumor mutation burden[1]. Hence, a more in-depth understanding of tumor immunity in GC is needed. The tumor cells may be eliminated by these immune cells or escape detection. In the elimination phase, the cells, like natural killer cells, with the help of dendritic cells and CD4+ T-cells, recognize and eliminate tumor cells. However, the less immunogenic tumor cells can escape the immuno-surveillance.

Based on the presence of immune cells, tumors can be classified into inflamed and non-inflamed[2]. These inflammatory cells may contribute to pro- or anti-tumor activities. Amongst these cells, the tumor-infiltrating lymphocytes (TILs) are the significant determinants of the host immune response to tumor cells. TILs have recently gathered much attention because of their presumed role in carcinogenesis and therapeutics[3]. The "Hallmarks of Cancer" proposed by Hanahan *et al*[4] now include inflammatory infiltrates into the tumors as one of the components. This is because of their roles in tumor progression and escape from the host immunity. The new technological advancements mean improved assessment of tumor infiltrates and identification of genetic signatures expressed in the tumor micro-environment (TME). TILs and their functions have now become a leading topic of research. We can discover the prognostic relevance of TILs, which can help predict outcomes and guide therapy. The complex correlation of cancer cells with the immune system can be explored for therapeutic avenues.

TILS IN GC

The magnitude of TIL infiltration is thought to be related to the control of cancer growth, progression, and metastasis. In addition, it may be predictive of the response to cytotoxic treatment[5]. Still, various studies have shown conflicting results[6,7]. The prognostic role of TILs in GC needs further clarification. TILs, as a natural component of the immune system, can offer a tailored approach to battling GC. It is critical to understand the heterogeneity of TILs and their interaction with the tumor microenvironment. TILs differ according to their location in the tumor. These include intratumoral (IT), stromal (ST), and invasive border (IB)[8]. An analysis of the studies of IT TILs revealed robust hazard ratios (HRs) for overall cancer survival (OCS) than for other TILs. Studies of the pan-T-cell IT TILs, such as CD3/TIL, CD4, and CD8, in GC tissues revealed association with survival (CD3: HR = 0.65, 95%CI: 0.5-0.8; CD4: HR = 0.7, 95%CI: 0.55-0.9; CD8: HR = 0.65, 95%CI: 0.5-0.85). Higher CD8+ cells demonstrated the greatest overall survival (OS) improvement. In contrast, TILs with high FOXP3+ expression significantly correlated with decreased OCS (HR = 1.89, 95%CI: 1.5-2.3). The transcription factor FOXP3, presenting with the CD4+, CD25+, and FOXP3+ phenotype, is responsible for the T regulatory (Treg) cells. Treg cells promote immune tolerance in the TME by suppressing the anti-tumor T-cells. This can explain this association of decreased OCS with high FOXP3+ cells[9,10]. A meta-analysis of around 2900 cases demonstrated a significant association between higher pan T-cell marker (+ve) TILs and better survival[11]. It implies the role of adaptive immunity in the anti-tumor response. TILs have also shown apoptosis in GC models[12]. Interestingly, a higher number of TILs in patients with microsatellite instability (MSI) or Epstein Barr virus (EBV) associated GC correlated with better treatment outcomes and longer OS, prompting the association of TILs with other factors[13-15].

ASSOCIATION WITH OTHER FACTORS

Recent advances in cancer research have shed light on the intricate relationships between *Helicobacter pylori* (*H. pylori*) infection, mismatch repair (MMR) status, HER2 amplification, and TILs in the context of GC. These connections have brought a deeper understanding of this complex disease and are opening new avenues for targeted therapies and precision medicine.

H. pylori: A pervasive culprit

H. pylori is a bacterium that colonizes the stomach lining and has long been implicated as a significant risk factor for GC. Chronic *H. pylori* infection can lead to the development of chronic gastritis, which, over time, may progress to atrophic gastritis, intestinal metaplasia, dysplasia, and ultimately GC. This journey from infection to malignancy underscores the need for early detection and eradication of *H. pylori* in at-risk individuals. *H. pylori* infection triggers an inflammatory response in the stomach lining, contributing to the initiation and progression of GC. This chronic inflammation damages DNA and leads to the recruitment of TILs, which are a part of the immune system's response to the infection.

MMR status: A genetic determinant

In the realm of GC, MMR status is a crucial genetic determinant. MMR proteins are responsible for correcting DNA replication errors and ensuring genomic stability. Deficiencies in MMR (dMMR), typically characterized by MSI, can result in genetic mutations and increased susceptibility to cancer development.

The association between MMR status and GC is multifaceted. Individuals with MSI-high gastric tumors tend to have a more favorable prognosis due to the increased presence of TILs. These TILs, often enriched in MSI-high tumors, are believed to have a more potent anti-tumor effect.

HER2 amplification: A target for therapy

HER2, a member of the epidermal growth factor receptor family, is known for its role in several cancers, including breast and GC. HER2 amplification or overexpression in GC represents a specific subset of cases that can be targeted with precision therapies.

Trastuzumab, a monoclonal antibody targeting HER2, has been approved to treat HER2-positive GC. Notably, HER2-positive tumors often exhibit increased TIL infiltration, pointing to the interplay between HER2 and the immune response.

The path forward: Precision medicine and targeted therapies

Understanding the interplay between *H. pylori* infection, MMR status, HER2 amplification, and TILs in GC is vital for tailoring therapies to individual patients. Precision medicine in GC is evolving, with targeted therapies like trastuzumab for HER2-positive cases and immunotherapies that aim to enhance TIL activity showing promise. Hoilat *et al*[16] reviewed the association between *H. pylori* infection, mismatch repair, HER2, and TILs in GC. The study addresses the critical question of the TIL-associated predictive factors. They included 503 surgically treated stage I-III GC patients. Analysis of the TILs was done following standardized international TILs working group recommendations to determine IT, ST, and IB compartments. Immunohistochemistry (IHC) stained tissue tumor arrays were utilized to calculate immune cell density (CD3, CD8, and CD163). They also determined dMMR and HER2-status by IHC. *H. pylori* infection was evaluated by histology and by quantitative polymerase chain reaction in a subset. dMMR was found in 34.4%, HER2+ status in 5%, and *H. pylori* infection in 55.7%. TILs were subdivided into the IB, IT, and ST compartments. Median TIL levels were higher in IB and ST than in the IT compartment. They also found a correlation with the grade of the tumor. Grade 3 tumors were associated with high IT TIL ($P = 0.038$), whereas ST-TIL with grade 1 ($P < 0.001$). ST and IB TILs were seen to be higher in dMMR tumors. dMMR was also associated with high CD3 and CD8 densities. HER2- was associated with high IT-CD8. Also, *H. pylori* negative status correlated with higher IT-TIL ($P = 0.009$). It was also associated with high CD8 density in IT and ST compartments ($P = 0.001$). High TIL levels were associated with dMMR and *H. pylori*-negative status. Low CD8/CD3 ($P = 0.001$ in IT and $P = 0.002$ in ST compartment) and high CD3/CD163 ($P = 0.002$) predicted lower recurrence and longer survival.

These studies demonstrate that further research is required to identify *H. pylori* infection status because of the effect on the immune microenvironment, which can predict immunotherapy response. Molecular profiling and IHC can help determine the molecular subtypes of GC, guiding personalized treatment plans. The complex relationships between MMR status, HER2 amplification, and TILs in GC pave the way for more precise, effective, and individualized treatment approaches. While challenges remain in optimizing therapies for different subsets of patients, these insights represent a significant step towards conquering this relentless disease. As research progresses, we can look forward to a future where TILs may be used as prognostic and predictive factors in not only GC but also other malignancies. This warrants further studies on TILs.

CLINICAL IMPLICATIONS

TILs and their subtypes can be used in GC for predictive and prognostic purposes. The complex interplay of TILs with factors like MMR, HER2, and *H. pylori* infection demonstrates that they form an integral part of the immune response to the tumor cells. Further studies will clarify these factors' role in predicting response to therapy.

CONCLUSION

In conclusion, TILs represent a promising avenue in the battle against GC. It is incumbent upon the medical and scientific communities to come together and realize the full potential of TILs, ensuring that their immense promise becomes a reality for all those affected by this devastating disease and other malignancies.

FOOTNOTES

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REVIEW

Focus on current and emerging treatment options for glioma: A comprehensive review

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Abstract

This comprehensive review delves into the current updates and challenges associated with the management of low-grade gliomas (LGG), the predominant primary tumors in the central nervous system. With a general incidence rate of 5.81 per 100000, gliomas pose a significant global concern, necessitating advancements in treatment techniques to reduce mortality and morbidity. This review places a particular focus on immunotherapies, discussing promising agents such as Zotiraciclib and Lerapolturev. Zotiraciclib, a CDK9 inhibitor, has demonstrated efficacy in glioblastoma treatment in preclinical and clinical studies, showing its potential as a therapeutic breakthrough. Lerapolturev, a viral immunotherapy, induces inflammation in glioblastoma and displays positive outcomes in both adult and pediatric patients. Exploration of immunotherapy extends to Pembrolizumab, Nivolumab, and Entrectinib, revealing the challenges and variabilities in patient responses. Despite promising preclinical data, the monoclonal antibody Depatuxizumab has proven ineffective in glioblastoma treatment, emphasizing the critical need to understand resistance mechanisms. The review also covers the success of radiation therapy in pediatric LGG, with evolving techniques, such as proton therapy, showing potential improvements in patient quality of life. Surgical treatment is discussed in the context of achieving a balance between preserving the patient's quality of life and attaining gross total resection, with the extent of surgical resection significantly influencing the survival outcomes. In addition to advancements in cancer vaccine development, this review highlights the evolving landscape of LGG treatment, emphasizing a shift toward personalized and targeted therapies. Ongoing research is essential for refining strategies and enhancing

outcomes in the management of LGG.

Key Words: Low-grade gliomas; Monoclonal antibody; Lerapolturev; Glioblastoma; CDK9 inhibitor

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Core Tip: Our manuscript explores the dynamic landscape of glioma treatment, emphasizing the urgent need for innovative therapies to combat this prevalent central nervous system malignancy. We delve into the promising realm of immunotherapies, highlighting novel agents like zotiraciclib, pembrolizumab, and lerapolturev, offering insights into their mechanisms and clinical efficacy. Furthermore, we discuss the evolving role of radiation therapy, emphasizing recent advancements in reducing treatment-related toxicities while improving outcomes. Surgical strategies, including subtotal resection and intraoperative radiotherapy, are also explored, showcasing their potential to enhance survival while minimizing neurological morbidities.

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INTRODUCTION

Gliomas represent the most prevalent primary tumors in the central nervous system (CNS) across various age groups[1,2]. Gliomas have a general incidence rate of 5.81 per 100000 people, with older individuals having a threefold higher frequency than young children. Gliomas account for 29%-35% of all central nervous system tumors in the adolescent and young adult demographic (ages 15-39 years), with an incidence of 3.41 per 100000[3-5]. Gliomas continue to be a global concern, emphasizing the vital need to improve treatment techniques for lowering both mortality and morbidity, elevating it to the top of the neuro-oncology priority list[6,7].

Clinical care, therapeutic response, and outcomes differ significantly between pediatric and adult glioma patients. Children with high-grade gliomas (HGGs) have poor prognosis, with frequently limited long-term survival ranging from months to a few years after diagnosis[8,9]. In contrast, pediatric patients with low-grade gliomas (LGG) have good overall survival (OS)[10,11], despite significant tumor- and treatment-related morbidity[12] (Table 1). The increased likelihood of malignant transformation, which is extremely rare in children) adds to a less favorable prognosis in adults with low-grade gliomas[13,14].

IMMUNOTHERAPIES

Despite advancements in surgery, radiotherapy, and chemotherapy for LGG, the disease remains incurable and often progresses to secondary malignant transformation. Immunotherapeutic strategies have demonstrated success in various cancers, including lung, skin, colon, and blood-related cancers (Figure 1). Given that low-grade gliomas, particularly in younger patients, exhibit slower growth compared to high-grade gliomas, there is a suggestion that immunotherapies may be more effective due to the healthier immune systems of younger individuals, potentially leading to better treatment responses. Immunotherapies, including Zotiraciclib and Lerapolturev, exert their effects through distinct mechanism (Table 2).

Zotiraciclib

Zotiraciclib, a potent CDK9 inhibitor, exhibits efficacy against glioblastoma by suppressing transcription and disrupting cellular energy production. Preclinical studies, both in vitro and in vivo, have revealed its synergistic effect with temozolomide. In clinical trials, Zotiraciclib demonstrated the ability to cross the blood-brain barrier and suppress CDK9 activity in tumor tissues[15]. This promising mechanism, targeting multiple glioblastoma survival pathways, positions Zotiraciclib as a potential therapeutic breakthrough[16-19].

A two-stage, two-arm, randomized phase 1 clinical trial further investigated the potential of zotiraciclib in recurrent high-grade gliomas. This study included a comprehensive evaluation of pharmacokinetics, patient-reported outcomes, and a detailed examination of rapid-onset neutropenia. Despite this observed neutropenia, a thorough analysis concluded that it did not compromise patient safety, allowing the research and development of this novel CDK9 inhibitor to progress[19].

Pembrolizumab or nivolumab

Immunotherapy has garnered significant interest as a potential treatment for glioblastoma (GBM). Nevertheless, a recent

Table 1 World Health Organization classification of gliomas

Grade	Name	Description and characteristics
I	Pilocytic astrocytoma	Well-differentiated, often cystic, slow-growing, generally benign
II	Diffuse astrocytoma	Infiltrative, moderately cellular, tends to recur, can progress to higher grades
II	Oligodendroglioma	Composed of oligodendrocyte-like cells, often associated with 1p/19q co-deletion
II	Mixed oligoastrocytoma	Combination of features of oligodendroglioma and diffuse astrocytoma
III	Anaplastic astrocytoma	Higher grade astrocytoma with increased cellularity and mitotic activity
III	Anaplastic oligodendroglioma	Higher grade oligodendroglioma with increased cellularity and atypia
III	Anaplastic oligoastrocytoma	Higher grade mixed tumor with features of both anaplastic astrocytoma and anaplastic oligodendroglioma
IV	Glioblastoma	Highly aggressive, necrosis, endothelial proliferation, molecular heterogeneity

clinical study focusing on recurrent glioblastoma and employing PD-1 immune checkpoint inhibitors revealed that a minority of patients (8%) exhibited noticeable improvements in their condition[20]. The mechanistic underpinnings of the variability in response patterns remain unclear.

Enhanced T cell infiltration in the tumor microenvironment and elevated mutational burdens in various cancer types have been associated with improved responses to anti-PD-1 therapy[21-23]. However, GBM presents a more immunosuppressive tumor microenvironment and a lower burden of somatic mutations than melanomas or non-small cell lung cancer[24]. Immunosuppression in GBM is facilitated by the expression of PD-1 ligands (PD-L1/2) in tumor cells, leading to T cell exhaustion and apoptosis. The binding of PD-1 to the surface of cytotoxic T cells hampers their ability to mount an effective anti-tumor response. PD-1 inhibitor therapy disrupts this immune checkpoint, reinforcing the immune response against tumors[23].

PD-1 inhibitors, such as pembrolizumab and nivolumab, have gained attention for glioblastoma treatment. However, recent clinical studies have revealed variable responses, necessitating deeper understanding of the underlying mechanisms. Glioblastoma's immunosuppressive microenvironment and lower mutation burden compared to other cancers pose challenges. Molecular-tailored strategies hold promise for optimizing patient selection for immunotherapy, although further testing is required to validate their efficacy[25].

Lerapolturev

Lerapolturev, a viral immunotherapy, operates *via* a unique mechanism. As a polio-rhinovirus chimera, it induces persistent type-I interferon-dominant inflammation in glioblastoma, leading to polyfunctional antitumor CD8+ T-cell responses. Clinical trials involving Lerapolturev for recurrent adult glioblastoma demonstrated a 16% survival rate of at least 36 months, with a manageable safety profile[26-29].

In pediatric high-grade gliomas, Lerapolturev showed promise, with no grade 3 or 4 toxicity observed in early trials. The safety of treatment at this dose allows for further trials, including patients as young as 9 years of age. Ongoing research is crucial to understand the immunological factors influencing the efficacy of Lerapolturev in pediatric versus adult high-grade gliomas[30]. Our group's previous research in adults gained additional support from the inclusion of patients as young as 9 years old, including one individual with WHO grade 3 glioma[30]. Moreover, pediatric high-grade gliomas typically exhibit significantly different molecular profiles compared to adult high-grade gliomas[31]. However, whether immunological factors affecting viral immunotherapies, such as Lerapolturev, vary between pediatric and adult high-grade gliomas remains uncertain[30].

Depatuxizumab

Depatuxizumab (formerly ABT-806) is a humanized monoclonal antibody developed against epidermal growth factor receptor variant III (EGFRvIII) that also binds to wild-type EGFR at elevated levels[32,33]. The antibody-drug conjugate (ADC) Depatuxizumab mafodotin (formerly ABT-414) connects the depatux to the cytotoxic payload monomethyl auristatin F (MMAF or mafodotin). Upon binding to activated EGFR, ADC is internalized, degraded in acidic compartments, and releases the toxin, causing cell death. Unlike other treatments, this direct cytotoxic effect does not rely on inhibition of EGFR signaling and avoids typical toxicities[34]. Although unconjugated depatux is ineffective against GBMs, depatux-m demonstrates efficacy in GBM cell lines and models with EGFR amplification or EGFRvIII, showing effectiveness alone and in combination with radiotherapy and temozolomide[35]. ADCs, including depatux-m, show promise in various cancers[36], surpassing unconjugated monoclonal antibodies in efficacy, with numerous ADCs under investigation under diverse conditions[37].

Despite promising preclinical and early clinical data, depatux-m has proven ineffective in treating GBM. This disappointing outcome may result from the emergence of resistant clones over time, negating any overall survival benefit [38]. Limited penetration of depatux-m into large tumors and challenges in reaching intracranial tumors[39], especially in the non-enhancing tumor region, underscore crucial lessons for future studies involving large molecules[38]. Safety concerns with depatux-m were reversible, with adverse events, such as sensitivity to light and thrombocytopenia, being the most frequently observed.

Table 2 List of Immunotherapy

Ref.	Completion year	Demographics	Study phase	Identifier	Experimental drug	Sample size	Primary endpoint/outcomes	Results for primary outcome
BRAF/MEK inhibitors								
Nicolaides *et al* [107], 2020	2023	Pediatrics	Phase 2	NCT01748149 (Ongoing Trial)	Vemurafenib	40	Safety and pharmacokinetics	Not yet reported
Hargrave *et al* [108], 2019	2020	Pediatrics	Phase 1/2a	NCT01677741	Dabrafenib	32	Objective response rates and safety	Objective response rate was 44% and 91% experienced adverse effects
Kaley *et al*[109], 2018	2016	Adults	Phase 2	NCT01524978	Vemurafenib	24	Confirmed objective response rate, PFS, OS and safety	Confirmed objective response rate was 25% and median PFS was 5.5 months
FGFR inhibitors								
Lassman *et al*[110], 2022	2018	Adults	Phase 2	NCT01975701	Infigratinib	26	6-month PFS	6-month PFS rate was 16.0%
Bahleda *et al*[111], 2019	2017	Adults	Phase 1	NCT01703481	Erdafitinib	187	Safety	Most common treatment-related adverse events were hyperphosphatemia, dry mouth, and asthenia, generally grade 1/2 severity
HDAC inhibitors								
Wood *et al*[112], 2018	2018	Pediatrics	Phase 1	ACTRN12609000978268	Panobinostat	9	Safety and pharmacokinetics	2 patients experienced Grade 3-4 thrombocytopenia, 1 experienced Grade 3 anemia, and 2 experienced Grade 3 neutropenia
Imipridone								
Arrillaga-Romany *et al*[113], 2020	2023		Phase 2	NCT02525692 (Ongoing Trial)	ONC201	89	6-month PFS	Not yet reported
PI3K/mTOR inhibitors								
Wen *et al*[114], 2022	2023	Adults	Phase 2	NCT03522298	Paxalisib	32	Safety and pharmacokinetics	Well-tolerated with adverse events consistent with other PI3K inhibitors
Wen *et al*[115], 2020	2020	Adults	Phase 1	NCT01547546	GDC-0084	47	Safety and pharmacokinetics	Well-tolerated with adverse events consistent with other PI3K inhibitors
Franz *et al*[116], 2015	2014	Adults/Pediatrics	Phase 1/2	NCT00411619	Enviroximes	28	6-month change in the volume of sub ependymal giant-cell astrocytoma	Statistically significant reduction in the volume of the primary sub ependymal giant-cell astrocytoma at 6 months
NTRK/ALK inhibitors								
NCT02637687[117]	2026	Pediatrics	Phase	NCT02637687 (Ongoing	Larotrectinib	155	Objective response rates	Not yet reported

			1/2	Trial)			
NCT02576431[118]	2025	Adults/Pediatrics	Phase 2	NCT02576431 (Ongoing Trial)	Larotrectinib	204	Objective response rates, PFS, OS, Safety	Not yet reported
Desai *et al*[119], 2022	2025	Adults/Pediatrics	Phase 1/2	NCT02650401 (Ongoing Trial)	Entrectinib	69	Maximum Tolerated Dose and Objective response rates	Not yet reported
IDH inhibitors								
NCT05588141[120]	2029	Adults	Phase 1/2	NCT05588141 (Ongoing Trial)	Zotiraciclib	96	12-months PFS	Not yet reported
Mellinghoff *et al*[121], 2023	2027	Adults	Phase 3	NCT04164901	Vorasidenib	340	PFS	Significantly improved PFS
Mellinghoff *et al*[122], 2019	2024	Adults	Phase 1	NCT03343197	AG-120, AG881	49	2-hydroxyglutarate concentrationin resectedtumors	decreased tumorcell proliferationand immune cellactivation
EGFR inhibitors								
Weller *et al*[123], 2017	2016	Adults	Phase 3	NCT01480479	Rindopepimut/Temozolomide	745	OS	Median OS was 20.1 months in the Rindopepimut group versus 20.0 months in the control group
Lassman *et al*[124], 2023	2022	Adults	Phase 3	NCT02573324	Depatuxizumab mafodotin	691	OS	No OS benefit for depatux-m in treating EGFR-amp newly diagnosed GBM

PFS: Progression-free survival; OS: Overall survival.

Entrectinib

Entrectinib, approved by both the United States Food and Drug Administration and European Medicines Agency for tumors containing TRK or ROS1 fusions[40], encounters a challenge in treating brain neoplasms due to the blood-brain barrier (BBB)[35]. Effective targeted therapies for leptomeningeal disseminated tumors depend on their ability to penetrate this barrier. Although entrectinib, designed to cross the BBB, has demonstrated promise with a 79% objective response rate in various solid tumors, including CNS tumors, information on its cerebrospinal fluid penetrance in brain tumor patients is currently lacking[41].

The potential therapeutic efficacy of entrectinib, a selective pan TRK inhibitor, has been explored in patients with leptomeningeal disseminated pediatric high-grade gliomas (pHGG) harboring NTRK or ROS1 fusions[42,43]. The STARTRK-NG trial reported positive radiographic responses in four pHGG patients treated with entrectinib, indicating promise for CNS tumors[44,45]. This study investigated the in vitro sensitivity of pHGG cell models to entrectinib and suggested potential combination therapies[46]. The need for further studies to understand resistance mechanisms is emphasized, along with the generally well-tolerated nature of entrectinib. The observed CNS penetrance of entrectinib in a gliosarcoma patient has been discussed, highlighting its ability to cross the blood-brain barrier[47]. The text also considers the combination of entrectinib with radiotherapy and suggests the importance of intrathecal therapy in cases of leptomeningeal dissemination[41]. This conclusion underscores the need for comprehensive investigations and prospective clinical studies to establish the role of entrectinib and potential combination therapies in pHGG with ROS1/NTRK fusions.

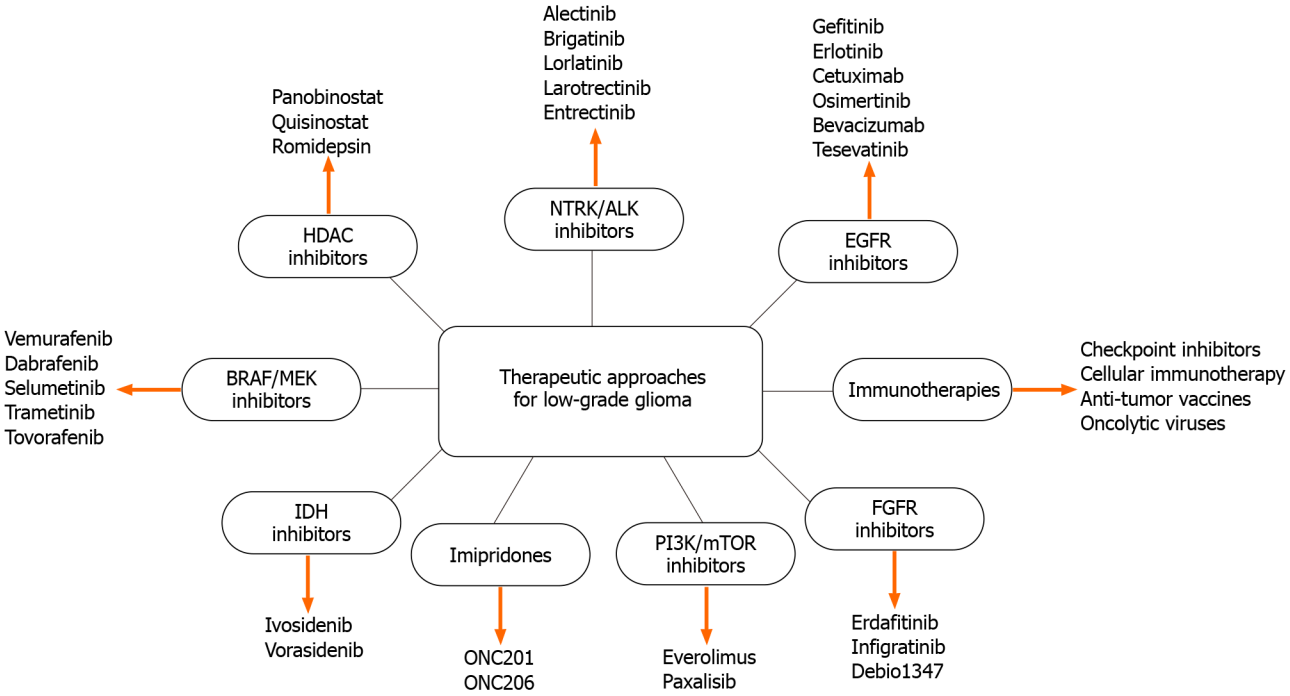

Figure 1 Illustrates a flow chart with drugs according to mutation.

ONC201 and paxalisib

ONC201, an oral small-molecule imipridone anticancer therapy, has demonstrated early clinical success in patients with diffuse intrinsic pontine glioma (DIPG)[48] and recurrent H3K27M-mutant diffuse midline gliomas[49]. Investigations across various cancer types have shown that ONC201-induced apoptosis in cancer cells, independent of p53, occurs through an atypical integrated stress response involving the expression of the antitumor protein TRAIL. This mechanism has shown promise in hematological[50], colorectal[51], breast[52], uterine cancers[53], and glioblastoma[54]. A sustained positive response was observed in a patient with secondary glioblastoma carrying an H3.3K27M mutation, prompting further exploration in patients with similar mutations, including those with DIPG[54].

Studies have discussed the therapeutic benefits of combining ONC201, a dopamine receptor D2 antagonist[55], with the blood-brain barrier-penetrant PI3K/Akt inhibitor, paxalisib, for treating DIPG. Mechanistic insights indicate that ONC201, by decreasing tyrosine hydroxylase expression, exhibits global DRD2 antagonism, with ClpP identified as a crucial target that causes mitochondrial dysfunction and oxidative stress[56]. The combination of paxalisib shows promising results in preclinical and preliminary clinical trials, leading to symptom resolution and tumor regression. Challenges related to immunologically cold tumor microenvironments in DIPG have been acknowledged, but potential changes in the epigenetic landscape and metabolic plasticity following ONC201 treatment may enhance immunogenicity [57,58]. The observed link between H3K27M mutations, metabolic changes, and the immune response highlights the complexity of DIPG treatment, presenting a potential avenue for the effective administration of therapy for glioblastoma [59].

RADIATION THERAPY

Radiation therapy (RT) is a successful management method for pediatric low-grade gliomas using both initial and salvage treatment approaches. Historically, RT was the chosen initial therapy for quickly progressing or unresectable tumors, with 10-year progression-free survival (PFS) and OS rates of 70% and 80%, respectively[60-62]. Furthermore, RT has been used as an adjuvant therapy, particularly when surgery is limited to partial resection or biopsy, particularly for tumors in the optic system, hypothalamus, deep midline tissues, and brainstem[63,64]. Adjuvant RT is suggested in cases of partial resection because PFS is greatly reduced[65,66]. However, there is a lack of agreement on its use, which is attributable in part to the paucity of randomized prospective studies[67,68].

For older children who have not responded to numerous systemic medications, RT is preferred as part of the care plan. Historically, postponing RT was motivated by concerns about RT-related toxicities such as cognitive impairment[69,70], endocrine dysfunction[71], secondary malignancies[72], vascular damage[72,73], and growth abnormalities[74]. The severity of these symptoms is directly related to the location of the tumor and the patient's age, particularly in patients under the age of 10[69,72].

An institutional evaluation covering a median follow-up of 11 years found 8-year PFS and OS rates of 83% and 100%, respectively[75]. Overall neurocognitive performance did not deteriorate in the trial; however, significant cognitive impairment was noted in young children (under 7 years old) and in patients who received high doses to the left temporal

lobe or hippocampus. Higher dosages to the hypothalamus or pituitary caused endocrine disruption, and two patients developed Moya disease. The 5-year PFS and OS rates in a recently published prospective research including 174 pediatric patients with LGG who received proton treatment were 84% and 92%, respectively, with a median follow-up of 4.4 years[76]. Four patients experienced severe late toxicities, including brainstem necrosis, symptomatic vasculopathy, radiation retinopathy, and fatal secondary cancers. While acknowledging the relevance of radiation-related damage, it is vital to emphasize that recent research has yielded promising outcomes. The extended latency of toxicity should be considered in light of the rapid developments in the field[77].

Concerns about RT-related toxicity originate mostly from long-term data collected from studies conducted during the 1970s and the 1990s using 2-dimensional RT methods that did not allow for accurate radiation dose administration. Significant technical progress has been achieved in reducing the radiation dose that reaches the normal structures surrounding the tumor. This began with the use of 3-dimensional conformal external beam RT (3D-CRT) and progressed in the 2000s with the advent of intensity-modulated RT (IMRT). Significantly, the introduction of proton therapy has reduced radiation exit dosage[78,79], contributing to its growing role in pediatric patients. Several studies have suggested that proton therapy might improve both patient quality of life and the cost-effectiveness of pediatric brain tumor treatment[80,81].

SURGICAL TREATMENT

The primary objective of glioma treatment is to strike a balance between preserving the patient's quality of life and improving PFS and OS[82,83]. The choice between oncological and surgical treatment depends on factors such as tumor size, location, and individual patient characteristics, including age and comorbidities[82,84,85]. Patients aged > 40 years at diagnosis, those with incomplete resection, and those with wild-type isocitrate dehydrogenase (IDH) status are typically considered to be at increased risk. The conventional treatment approach involves cytoreductive surgery to achieve gross total resection (GTR), followed by a combination of chemotherapy and/or radiation therapy[86,87].

The prognosis for gliomas, encompassing both LGG and HGG, is significantly influenced by the extent of surgical resection (EOSR) (Table 3). In LGG, EOSR is measured by the percentage of the FLAIR signal that is excised, whereas in HGG, it is determined by the removal of the percentage of enhancing tissue and the necrotic center. Extensive research on EOSR in LGG consistently shows that achieving GTR significantly improves survival rates, particularly among younger patients, classifying them as low-risk individuals compared to those who undergo only partial resection[82-84].

Similarly, investigations into EOSR in HGG consistently demonstrated a strong correlation between the extent of resection and survival outcomes, assuming no surgery-related neurological morbidities[88-90]. In most studies, the surgical goal is unequivocally defined as achieving GTR or complete tumor removal, typically amounting to 100% resection[91].

Recent clinical investigations have explored the concept of subtotal resection for gliomas[92]. This surgical approach aims to achieve GTR while simultaneously eliminating the FLAIR signal surrounding the necrotic and enhancing tumor mass in high-grade gliomas. Low-grade gliomas involve complete removal of the FLAIR signal along with additional radiographic extraction of the normal brain tissue adjacent to the tumor. Subtotal resection in LGG surgery was confirmed by observing that the resection cavity exceeded the initial FLAIR volume on the postoperative MRI at the three-month mark. Subtotal resection is considered justifiable when minimal neurological risks are involved, with the aim of eliminating invasive cells near the radiographic boundary[93-95]. Evidence from clinical case series of glioblastoma multiforme and HGG presents a conflicting picture, as performing supra total resection may entail an increased risk of neurological function decline, despite potential improvements in PFS and OS[92,96]. Additionally, there has been increased focus on the utilization of laser interstitial thermal (LIT) treatment for brain tumors. Recent trials investigating LIT have shown that achieving a greater level of ablation, including subtotal ablation, can lead to improved progression-free survival and overall survival outcomes in patients with HGG[97,98].

Intraoperative radiotherapy with a single high radiation dose administered following tumor resection, intraoperative radiotherapy (IORT), a novel and non-conventional form of radiotherapy, can eradicate any remaining tumor cells[99]. A wide range of cancers, including breast, pancreatic, lung, and colon cancers, have been treated with IORT[100-102]. The lack of a discernible increase in survival in IORT treatment reports for primary malignant gliomas has been ascribed to angle errors, low electrons, and small electron cones, which result in inadequate coverage of the target volume[103]. A mobile IORT unit, INTRABEAM (Zeiss, Oberkochen, Germany), can deliver an equal dose of low-energy radiation in all directions within a tumor cavity, along with spherical irradiation. According to research, IORT with low-energy X-rays increases glioblastoma patients' survival rates without causing new problems[104].

Vaccine development

Cancer vaccines targeting high-grade gliomas, predating coronavirus disease 2019, are gaining momentum. Strategies include peptide-based vaccines, dendritic cells, viral vectors, and personalized neoantigen vaccines. They are also being explored for the treatment of LGG. For IDH-mutant LGG, adjuvants such as poly (I:C) and poly-ICLC enhance immune responses, collectively reflecting a determined push for glioma immunotherapy[105]. To bolster the weak immune response in LGGs, synthetic double-stranded RNA molecules, such as polyinosinic acid homopolymers annealed to a polycytidylic acid homopolymer, have demonstrated potential[106]. They mimic viral infections and promote the release of interferon type 1 and other immune-boosting substances. Safely used as adjuvants with dendritic cells or peptide vaccines, they enhance therapeutic responses[106,107].

Table 3 Supratentorial surgical treatment options for glioma, *n* (%)

Ref.	Study origin	Study design	Total number of patients	Supratotal resection sample	Male, %	Age atresection	Permanent neurological deficits	Progression-free survival	Overall survival
Gajjar *et al* [63], 1997	United States	Cohort study	142	48 (68/142)	61	7 median (0.17-19)	Not reported	70 ± 5 at 4 years	90 ± 3 at 4 years
Fisher *et al* [67], 2008	United States	Cohort study	278	19 (52/278)	58	9.1 ± 0.3	Not reported	55 ± 3 at 5 years	87 ± 2 at 5 years
Wisoff *et al* [125], 2010	United States	Prospective trial	518	64 (332/518)	54	7.9 median (0.6-20.5)	Not reported	78 ± 2 at 8 years	96 ± 0.9 at 8 years
Yordanova *et al*[93], 2011	France	Case series	15	100.00	53.3	36.4 (24-59)	2, 13.3	73.3 at 38 months	100 at study end
Youland *et al* [11], 2013	United States	Retrospective cohort	351	67 (235/351)	55	10.9 (0.05-19.6)	Not reported	75.8 at 5 years	94.9 at 5 years
Lima *et al* [126], 2015	France	Case series	21	19.0 (4/21)	28.57	35 (18-57)	0, 0	100 at study end	100 at study end
Duffau *et al* [127], 2016	France	Cohort study	16	100.00	43.75	41.3 (26-63)	0, 0	50 relapse rate (avg 70 months)	100 at study end
Lima *et al* [92], 2017	France	Two-center prospective study	19	26.3 (5/19)	42.1	31.2 (19-51)	0, 0	100 at study end	100 at study end
Rossi *et al* [86], 2019	Italy	Case series	449	32 (145/449)	53.1	37.9 (median 36.5)	1, 0.69 (SupTR group)	Not reported	Not reported
Ng *et al* [128], 2020	France	Case series	74	28 (21/74)	41.89	35.7 (18-66)	0, 0	Not reported	100 at 5 years
Ng *et al* [129], 2020	France	Case series	47	26 (12/47)	34.04	39.2 ± 11.3	0, 0	Not reported	100 at study end
Goel *et al* [130], 2021	India	Cohort study	74	34 (25/74)	62.16	33 (21-55)	0, 0	98.7 at 2 years	100 at study end
Rossi *et al* [94], 2021	Italy	Case series	319	35 (110/319)	61.1	38.9 (18-75)	6, 1.9	94 at 92 months (SupTR group)	100 at 80 months (SupTR group)
Ius *et al* [131], 2022	United States, Canada, France, and Italy	Four center retrospective review	267	9 (24/267)	41.9	39.2 (18-71)	8, 3.1	Not reported	100 at 100 months (SupTR)

CONCLUSION

In conclusion, advancements in LGG treatment span immunotherapies, targeted therapies, radiation, surgery, and vaccine strategies. Immunotherapies like Zotiraciclib and Lerapolturev show promise, while targeted therapies such as Entrectinib and ONC201/Paxalisib combination demonstrate early success. Radiation therapy, evolving with proton therapy, remains crucial, and surgical approaches aim to achieve gross total resection. Cancer vaccines including synthetic RNA adjuvants have emerged. The evolving landscape underscores a shift toward personalized and targeted therapies, with ongoing research being essential for refining strategies and improving outcomes in LGG treatment.

FOOTNOTES

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REVIEW

Immune pathway through endometriosis to ovarian cancer

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Abstract

Endometriosis is an estrogen-dependent inflammatory disease, defined by the presence of functional endometrial tissue outside of the uterine cavity. This disease is one of the main gynecological diseases, affecting around 10%-15% women and girls of reproductive age, being a common gynecologic disorder. Although endometriosis is a benign disease, it shares several characteristics with invasive cancer. Studies support that it has been linked with an increased chance of developing endometrial ovarian cancer, representing an earlier stage of neoplastic processes. This is particularly true for women with clear cell carcinoma, low-grade serous carcinoma and endometrioid. However, the carcinogenic pathways between both pathologies remain poorly understood. Current studies suggest a connection between endometriosis and endometriosis-associated ovarian cancers (EAOCs) *via* pathways associated with oxidative stress, inflammation, and hyperestrogenism. This article aims to review current data on the molecular events linked to the development of EAOCs from endometriosis, specifically focusing on the complex relationship between the immune response to endometriosis and cancer, including the molecular mechanisms and their ramifications. Examining recent developments in immunotherapy and their potential to boost the effectiveness of future treatments.

Key Words: Ovarian neoplasms; Endometriosis; Endometriosis-associated ovarian cancers; Immune response; Immunotherapy

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Core Tip: Current investigations imply a relationship between endometriosis and endometriosis-associated ovarian cancers (EAOCs) through pathways involving oxidative stress, inflammation, and hyperestrogenism. This article endeavors to examine the current data on the molecular events associated with the development of EAOCs from endometriosis, with a particular emphasis on the intricate relationship between the immune response to endometriosis and cancer, including the molecular mechanisms and their implications.

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INTRODUCTION

Endometriosis, an estrogen-dependent inflammatory disease, is defined by the presence of functional endometrial tissue (stromal cells and gland) outside of the uterine cavity. It involves ectopic implantation of endometrial cells, marked by heightened proliferation, infiltration, and migration. This condition is one of the main gynecological diseases, affecting around 10%-15% women and girls of reproductive age. It can reach 50% of women facing infertility and often correlates with dysmenorrhea and pelvic pain[1,2]. Notably, more than 52% of women diagnosed with endometriosis are between 18-29 years[3].

Regrettably, there is a substantial delay of almost 6 years between the onset of symptoms and diagnosis in primary care, which has a detrimental impact on the quality of life of many women and the subsequent treatment of a large number of patients[4]. Currently, there is no definitive cure for endometriosis, and available treatments primarily focus on symptom management, lacking measures to prevent recurrence of the disease. Risk factors for endometriosis include early menarche, nulliparity, dysfunctional uterine bleeding, aberrant estrogen levels, and low body mass index[5-7].

The underlying mechanisms of this disease have yet to be determined, despite numerous theories attempting to clarify their nature. The most widely accepted theory posits that retrograde menstruation allows endometriotic cells to evade the apoptotic pathway, leading to a disruption of the immune balance in the surrounding endometrioid tissue and triggering an immunological cascade that produces a mixture of pro- and anti-inflammatory factors[8]. However, the precise nature of these alterations remains unclear and is the subject of ongoing research.

Although endometriosis is a benign disease, more and more studies support that it has been linked with an increased chance of developing ovarian cancer, representing an earlier stage of neoplastic processes[9-11]. The first histological correlation between endometriosis tissue and ovarian cancer specimens was first presented by Sampson (1925), proposing that endometrial ovarian cancer may develop from endometriotic tissue, creating some criteria for diagnosis: (1) Evidence of coexisting tumor and endometriosis in the same ovarian location; (2) exclusion of a second malignancy elsewhere; and (3) histological pattern that resembles endometrial origin[12]. Later on, in 1953, Scott added a fourth criteria: Histological demonstration of benign lesions of endometriosis adjacent to malignant tissue[13].

Ovarian cancer ranks as the fifth leading cause of cancer-related death among women, surpassing other female reproductive system cancers. Ovarian epithelial tumors are divided into two categories: Type I tumors, which include clear cell carcinoma, low-grade serous carcinoma, endometrioid, and mucinous carcinoma; and Type II tumors, represented by high-grade serous carcinomas[14]. Endometrioid and clear cell tumors are linked to endometriosis and are classified as endometriosis-associated ovarian cancers (EAOCs), exhibiting a crescent correlation as they progress from endometriotic cyst epithelium through various stages of tumor development[15]. Both endometriosis and cancer share certain characteristics, such as the ability to evade apoptosis, form new blood vessels, and metastasize to distant sites, as well as the capability to create a supportive microenvironment that promotes growth and immune system mobilization [11].

Our review focuses on the intricate relationships between the immune response to endometriosis and cancer, particularly on the molecular mechanisms and their consequences. We designed this article by following a chain of thought, starting with the characteristics of the diseases, the immune alterations found in both pathologies, followed by the correlations between endometriosis and ovarian cancer, and how the immune response of endometriosis can lead to the onset of ovarian cancer, or at least favor its development. We explore how the microenvironment and imbalances due to endometriosis can trigger the development of ovarian cancer. Furthermore, we provide an overview of recent advancements in immunotherapy and their potential to enhance the efficacy of future treatments of these pathologies until the new findings for treatment and the current hurdle this field faces.

OVERVIEW ON OVARIAN CANCER THAT CORRELATES WITH ENDOMETRIOSIS

Despite being a benign disorder, numerous epidemiological studies consistently identify endometriosis as a risk factor for ovarian cancer, which is the most lethal among gynecological malignancies and rank as the third most prevalent[16-18]. Several mechanistic theories are linked to endometriosis, such as the reflux of endometrial tissue through the fallopian tubes during menstruation, coelomic metaplasia, embryonic cell rests, and lymphatic and vascular dissemination[12,19,

20]. However, the etiology of endometriosis is generally considered multifactorial due to factors like genetics, hormones, and immunity. Remarkably, endometriosis can also develop in postmenopausal women, and although rare, it carries the risk of malignant transformation[21-24]. Hence, the observation of the occurrence and malignant transformation of endometriotic lesions in a hypoestrogenic environment with the absence of menstrual cycles emphasize the need for a more comprehensive understanding of the underlying mechanisms in the disease's pathogenesis, that goes beyond classical theories centered on estrogen and retrograde menstrual flow[25,26].

The invasive potential of endometriosis and the persistent maintenance of ectopic tissue are characteristics that resemble cancer[27]. Notably, a significant association has been established between a history of endometriosis and an increased risk of developing specific subtypes of epithelial ovarian carcinoma, namely, endometrioid carcinoma, clear cell carcinoma, and low-grade serous tumors[19].

It is estimated that endometriosis increases the risk by approximately 3-fold for endometrioid and clear cell carcinomas [28,29]. Through histopathological analysis, it has been observed that the carcinogenesis of these cancer subtypes can originate from cysts and other endometriotic lesions that progress to a phase of endometriosis with higher oncogenic potential, known as atypical endometriosis[30-32]. In this condition, two main histological findings are noteworthy and may be present simultaneously or independently: Cellular atypia (cytologic atypia) and architectural atypia (hyperplasia) [33]. Furthermore, a subsequent prospective histological study found that endometriosis displaying architectural atypia, and consequently exhibiting higher proliferative activity, is most strongly linked to endometriosis-associated ovarian cancer[34].

From a molecular perspective, atypical endometriosis and clear cell carcinoma share mutations in hepatocyte nuclear factor-1β and the AT-rich interactive domain-containing protein 1A gene (*ARID1A*), which encodes the tumor suppressor protein BAF250. Therefore, absence of the BAF250a protein can be a useful early biomarker indicating malignant transformation of endometriosis[35,36]. In contrast, endometrioid adenocarcinoma primarily exhibits mutations in CTNNB1 (catenin beta 1), phosphatase and tensin homolog (PTEN), and ARID1A[35].

The association between endometriosis and low-grade serous tumors is relatively recent. Previously, epidemiological studies were primarily conducted alongside the high-grade serous tumor subtype, which does not exhibit an association with endometriosis[19]. The two histological subtypes may differ in terms of etiology, with low-grade typically originating from a malignant transformation process of borderline serous tumor, while high-grade tumors often arising from intraepithelial tubal carcinoma, with rarely observed progression from low-grade to high-grade serous tumors[37, 38].

Genetically, TP53 mutations are more restricted to high-grade serous carcinomas[39], whereas low-grade serous carcinomas typically exhibit mutations in Kirsten Rat Sarcoma (KRAS) and B-type Raf kinase (BRAF), along with overexpression of hormone receptors (estrogen and/or progesterone)[38]. It's worth noting that KRAS mutations are generally associated with a worse prognosis compared to exclusive BRAF mutations[40]. This underscores the need for additional genetic research in this field.

IMMUNE DYSREGULATION IN ENDOMETRIOSIS AND ESTROGEN DEPENDENCY ON MICROENVIRONMENT DYSRUPTION

Endometriosis has garnered significant attention in the field of reproductive health due to its elusive etiology. While several theories have been proposed to explain its origin, the most widely accepted among them is Sampson's theory of retrograde menstruation[41]. According to this theory, during menstruation, endometrial cells retrograde into the fallopian tubes and subsequently into the peritoneal cavity[42,43]. In healthy females, these displaced cells typically undergo programmed cell death and are efficiently cleared by phagocytes and natural killer (NK) cells—a phenomenon known as immune surveillance[44,45]. However, in the context of endometriosis, compromised cell-mediated immune responses can disrupt this natural clearance process. Consequently, eutopic endometrial cells can adhere to the peritoneal wall, where they proliferate and eventually form endometriotic lesions[45,46].

Immune surveillance evasion: Dysregulated apoptosis signaling

The precise mechanisms responsible for the evasion of immunosurveillance by ectopic endometrial cells (EECs) remain poorly understood. Several hypotheses have been proposed to elucidate this phenomenon, including the possible dysregulation of programmed cell death pathways[47]. The FAS (CD95) and FAS ligand (FASL) extrinsic apoptosis signaling system, which is well-documented for its significant role in immune modulation[48-50], appears to hold a pivotal position in the context of endometriosis[51,52]. In response to the peritoneal microenvironment, specially elevated interleukin(IL)-8 levels, a conspicuous increase in the expression of FASL is detected within EECs[53-55]. This heightened FASL expression seems to initiate apoptotic processes through the FAS-mediated pathway in immune cells expressing FAS, including T cells and NK cells[55].

In a parallel manner, alterations in tumor necrosis factor (TNF)-α-mediated cell death signaling contribute to the advancement of endometriosis. In retrograde menstruation, the entry of menstrual tissues into the peritoneal cavity stimulates macrophages to release cytotoxic cytokines, including TNF-α, thereby initiating apoptosis signaling in the extrauterine endometrial fragments that need to be eliminated[56]. However, in individuals with endometriosis, estrogen-dependent molecular alterations in retrograde menstrual tissues enable them to evade TNF-α-mediated apoptosis[57]. In their pioneering research, Han *et al*[58] presented compelling evidence showcasing elevated expression levels of Estrogen Receptor β (ERβ) within endometriotic tissues. Erβ exerts regulatory influence over cellular apoptotic processes by impeding apoptosis initiated by TNF-α. Furthermore, ERβ engages in interactions with cytoplasmic inflammasome

constituents, consequently stimulating heightened production of interleukin-1β (IL-1β)[58]. This augmentation, in turn, amplifies cellular adhesion and proliferation characteristics.

Concurrently, within endometriotic lesions, there is a notable increase in the abundance of the nuclear receptor co-activator 1 (NCOA1) isoform[59]. NCOA1, also known as Steroid Receptor Coactivator 1, plays a pivotal role in the regulation of gene expression in response to hormonal signals[60-62] functioning as a coactivator for nuclear receptors, which are transcription factors related with physiological processes, including cell growth, differentiation, and metabolism. In endometriosis, within these specific lesions, the NCOA-1 isoform engages in an interaction with caspase 8, thereby impeding the TNF-α-triggered apoptosis process through disruption of the assembly of apoptosis complex II[59]. Altogether, it's possible to perceive that those alterations contribute to the complex mechanisms underlying the development and persistence of endometriosis. Figure 1 depicts a simplified schematic illustrating how ectopic endometrial cells evade immune surveillance.

Within the framework of endometriosis, perturbation of the intrinsic apoptotic pathway carries substantial ramifications. The B-cell lymphoma/Leukemia-2 gene (*Bcl-2*) represents a novel class of proto-oncogenes characterized by their ability to inhibit apoptosis independently of cell proliferation stimulation[63,64]. In the context of endometriosis, an upregulation of Bcl-2 protein expression was observed in the proliferative eutopic endometrial tissue of affected patients [65,66]. Conversely, Bax expression was notably absent in the proliferative endometrial phase, but displayed increased expression during the secretory phase in both patients and control subjects[66]. Accordingly, research findings demonstrate that the utilization of Gonadotropin Releasing Hormones analogs elicits an upregulation in the expression of the proapoptotic protein Bax[67], a putative antagonist protein, concomitant with a downregulation in the expression of the antiapoptotic protein Bcl-2 which can suggest potential targets for therapeutic interventions in this condition.

Dysregulation of the mitogen-activated protein kinase (MAPK) signaling pathway also seems to exert a significant influence on the advancement of the disease[68]. In tandem with the conveyance of antiapoptotic signals to endometriotic tissues, this intricate cascade promotes the recruitment of immune cells, intensifies the inflammatory response, and augments the expression of growth factors[69,70]. This coordinated synchronization of cellular processes seems to promote the initiation and progression of endometriotic lesions, while concurrently fostering a microenvironment conducive to the development of endometriosis.

Macrophages and their influence

Increasing evidence suggests that the peritoneal fluid features macrophages as the most predominant immune cells, within physiological parameters, and that they may be related to the pathogenesis of endometriosis[71]. The ectopic growth of endometriotic tissue within the peritoneal cavity leads to the onset of an inflammatory response, which, in turn, results in increased recruitment of these cells, a phenomenon mediated by colony stimulating factor-1 (CSF-1), monocyte chemoattractant protein-1 (MCP-1/CCL2), interleukin (IL)-8, and RANTES (CCL5)[72-74].

Upon recruitment, macrophages undergo activation, thus adopting specific functional profiles (M1/M2) that can either intensify inflammatory processes or contribute to tissue repair and immune regulation. In this sense, the polarization of macrophages into the M2 phenotype seems to be beneficial to the angiogenesis of endometriotic lesions, through secretion of vascular endothelial growth factor (VEGF)[75]. Additionally, the secretion of factors such as IL-10 and transforming growth factor-beta (TGF-β) by M2-polarized macrophages contributes to the growth of endometriotic lesions, since it impairs the cytotoxicity of NK cells[76-78]. Interestingly, there is also growing interest in the role of IL-17A in endometriosis, as it appears to be associated with macrophage recruitment, triggering M2 phenotype polarization, angiogenesis and maintenance of the inflammatory cascade[79,80].

In addition to the intricate interplay involving macrophages and the aforementioned mechanisms, it is essential to address the role of fibrosis in this context. In this sense, TGF-β secreted by M2 macrophages induces fibrosis, as it promotes the differentiation of fibroblasts into myofibroblasts and stimulate the synthesis of collagen and fibronectin[81-83]. On the other hand, Barcz *et al*[84] suggests that increased levels of VEGF are negatively associated to endometriosis-related pelvic fibrotic adhesions.

Macrophages also play a role in neurogenesis and, consequently, in the onset of endometriosis-associated pain[85]. Overall, nerve fibers originating from endometriotic lesions have the capacity to secrete chemokines such as CCL2 and CSF-1, which, as previously discussed, are pivotal in the recruitment of macrophages[81,86]. As a result, macrophages release neurotrophic factors, including brain-derived neurotrophic factor and neurotrophin-3, thus contributing to the heightened sensitivity and pain experienced by individuals with endometriosis[86].

Furthermore, iron accumulation into the peritoneal cavity, stemming from retrograde menstruation, holds a particular interplay with macrophages and plays a role in the pathogenesis of endometriosis[87]. Macrophages in the pelvic cavity carry out erythrocyte phagocytosis and iron metabolism, thereby resulting in elevated iron concentrations within the peritoneal fluid[88]. As a result, iron overload can trigger oxidative stress and contribute to chronic inflammation, thus leading to increased proliferative capacity of endometriotic lesions[89].

Finally, it is crucial to acknowledge that the phenotypic distinction between M1 and M2 macrophages is currently viewed as oversimplified[87]. Indeed, emerging evidence underscores the plasticity of macrophages, suggesting the potential for a hybrid M1/M2 profile or a dynamic switch between these phenotypes[87,88]. This adaptability appears to be influenced by the specific microenvironment to which these cells are exposed[88]. Such complexity in the interplay between macrophages and the pathogenesis of endometriosis emphasizes the need for further studies to thoroughly elucidate these intricate mechanisms.

Natural Killer cells dysfunction

Natural Killer cells play a crucial role in the immune system's surveillance and defense against endometriosis. These immune cells are capable of identifying and eliminating abnormal endometrial cells, thereby contributing to the body's

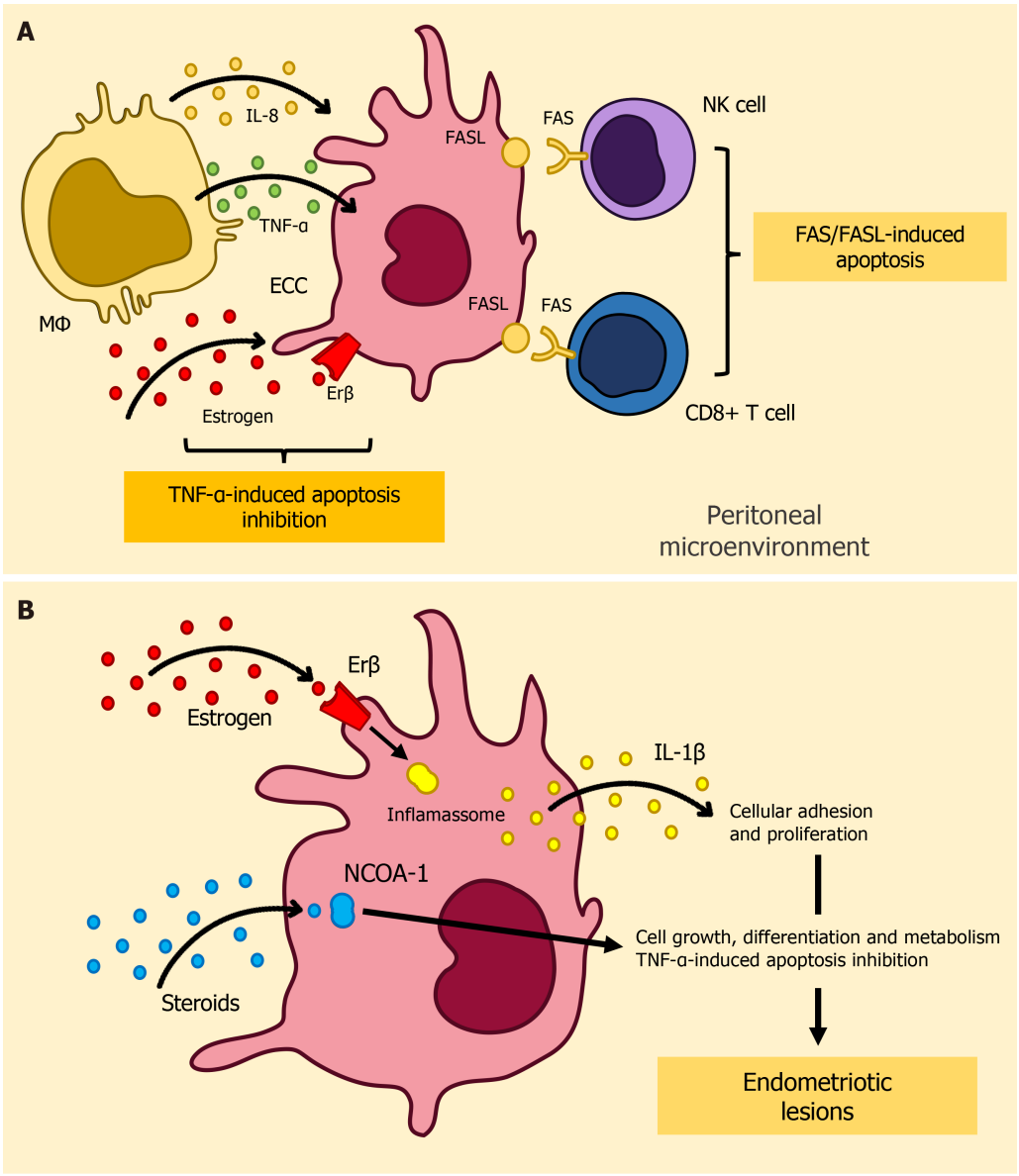

Figure 1 Depiction of immune surveillance evasion mechanisms in endometriosis. A: Illustration of FAS/FASL-mediated apoptosis in cytotoxic lymphocytes and TNF-α-induced ectopic endometrial cell apoptosis resistance; B: Key molecular factors contributing to dysregulated apoptosis signaling in endometriosis. CD8+ T cell: Cytotoxic T-cells; ECC: Ectopic Endometrial Cells; Erβ: Estrogen receptor β; FAS (CD95): Cluster of Differentiation 95; FASL: FAS Ligand; IL-1β: Interleukin-1β; IL-8: Interleukin-8; MΦ: Macrophage; NCOA-1: Nuclear receptor coactivator 1; NK cell: Natural killer cell; TNF-α: Tumor necrosis factor-alpha.

efforts to combat this condition[90,91]. Additionally, NK cells help regulate inflammation, modulate angiogenesis, and assist in maintaining immune tolerance within the endometrial environment[92,93].

In individuals with endometriosis, the peripheral circulation is characterized by a predominance of $CD16^{+}/CD56^{dim}$ NK cells, which are well-known for their heightened cytotoxic capabilities. In contrast, the endometrium and peritoneal fluid (PF) predominantly harbor $CD16^{-}/CD56^{bright}$ NK cells, renowned for their robust production of cytokines[94]. Accordingly, among females diagnosed with endometriosis, there is a noteworthy decrease in cytotoxicity observed in NK cells present within the peritoneum and PF[95]. Reduced cytotoxicity in the context of endometriosis may result from a complex interplay of cytokines within the intricate microenvironment of endometriotic lesions.

For instance, Yang *et al*[76] recently proposed that the interaction between macrophages and endometrial stromal cells (ESCs) could downregulate NK cell cytotoxicity. This downregulation might occur through the induction of cytokine secretion, including IL-10 and TGF-β, by the interacting cells. Such an interaction could potentially facilitate immune evasion by ectopic fragments and contribute to the development of endometriosis. Building on this idea, Kang *et al*[96] demonstrated that an increased level of IL-6 in the PF of patients with endometriosis might also suppress NK cell activity *via* regulation of SHP-2 expression. Likewise, elevated IL-15 levels were demonstrated to foster the proliferation and invasive behavior of ESCs while concurrently suppressing the cytotoxic capabilities of NK cells in individuals with endometriosis[97]. These cytokines, each wielding a distinct mechanism, collectively contribute to the precise regulation of NK cell responses in various immunological contexts.

The NK cell detection system employs a set of receptors on the surface of NK cells, including activating receptors like NKG2D and CD16 (FcgRIIIa), to regulate NK cell activities. This system is crucial for the immune system's ability to identify and eliminate abnormal cells—such as ECCs. In comparison to healthy women, the PF of individuals with endometriosis exhibited decreased levels of various markers associated with NK cell cytotoxicity. These markers include the natural receptors NKp46, NKp44, and NKG2D, as well as CD16 and CD107a, which are indicative of NK cell activation, and CD69[98].

Conversely, González-Foruria *et al*[99] demonstrated that there is a notable rise in soluble NKG2D ligands in the PF of endometriosis patients, indicating reduced expression of these ligands on the surface of ectopic endometrial cells[99]. These soluble NKG2D ligands serve as decoy receptors, contributing to increased evasion from NK cell recognition[100]. Notably, several studies have also reported elevated levels of Inhibitory Receptor Tyrosine-based Inhibition Motif-Killer Immunoglobulin-like Receptors, including KIR2DL1, Natural Killer Cell Inhibitory Receptor NKB1, EB6, soluble intracellular adhesion molecule-1, and Human Leukocyte Antigen class I in the PF of endometriosis patients[101-104].

Thus, the immune dysregulation associated with endometriosis involves intricate interactions between NK cells, various immune cells, and cytokines, ultimately impacting NK cell function and contributing to the development and persistence of this condition.

Altered T-cell-mediated cytokine profiling and cytotoxicity in endometriosis

In the context of endometriosis, there is a notable reduction in the Th1/Th2 cell ratio within the peritoneal fluid (PF) when compared to women with a healthy condition[105]. This shift is accompanied by increased concentrations of IFN-γ and IL-10, resulting in elevated IL-4/IFN-γ, IL-4/IL-2, IL-10/IFN-γ, and IL-10/IL-2 ratios within endometriotic lesions [106]. Furthermore, individuals with endometriosis show a substantial reduction in the T-bet/GATA-3 protein ratio compared to their healthy counterparts[107].

To our current understanding, T-bet regulates the expression of the Th1-specific cytokine IFN-γ while inhibiting the production of the Th2-specific cytokine IL-4[108-110]. Conversely, GATA-3, a transcription factor specific to Th2 differentiation, orchestrates the differentiation of Th2 cells and promotes the production of Th2 cytokines, including IL-4, IL-6, and IL-10[111-113]. Within endometriotic lesions, there is a significant upregulation of GATA-3 protein mRNA levels influenced by estrogen, a hormone central to GATA-3 regulation[107]. Consequently, the interplay between GATA-3 and estrogen signaling governs the production of Th2-type cytokines in affected endometrial cells[114]. This dynamic contributes to the elevated levels of Th2-type cytokines in endometriotic lesions—despite the increase in IFN-γ concentrations—ultimately promoting the progression of endometriosis.

Recent research conducted by Xia *et al*[115] underscores the diagnostic significance of serum cytokine concentrations in the context of endometriosis-associated pelvic pain (EAPP). Specifically, the study identifies IFN-γ and IL-2 as independent protective factors against EAPP, while recognizing IL-4 and IL-10 as independent risk factors for the condition[115]. Notably, IL-4, a hallmark cytokine associated with the Th2 immune response, is shown to elevate localized estrogen levels, thereby facilitating the estrogen-dependent progression of endometriosis[116]. Furthermore, this cytokine enhances the proliferation of endometriotic stromal cells through the activation of pathways such as p38 MAPK, stress-activated protein kinase/c-Jun kinase, and p42/44 MAPK, thereby leading to the advancement of the disease[117].

Apart from Th2 cells, T-helper-17 (Th17) cells and Regulatory T (Treg) cells may also be involved in endometriosis [118]. Khan *et al*[118] recently demonstrated that $CD4^+IL\text{-}17A^+$ Th17 cell percentage was consistently reduced in both peripheral blood and PF of individuals with early and advanced endometriosis. In contrast, Gogacz and colleagues reported an elevated proportion of Th17 cells in PF when compared to peripheral blood in individuals with endometriosis[119]. Their findings further indicated that the percentage of Th17 cells in PF was associated with the severity of endometriosis[119].

Zhang *et al*[120] pioneered the empirical validation of elevated IL-17 levels in the PF of individuals with endometriosis. Their research provided substantial evidence of statistically significant increases in IL-17 concentrations in individuals with minimal/mild endometriosis compared to those with moderate/severe disease and healthy individuals[120]. Subsequent to their groundbreaking work, multiple other authors have also confirmed elevated IL-17 levels in the PF of women diagnosed with this condition[121].

Interleukin-17A exhibits the capability to induce the secretion of IL-8 and the upregulation of cyclooxygenase-2 (COX-2) expression, thereby instigating inflammatory reactions and fostering the proliferation of stromal cells associated with endometriosis[122]. In a similar vein, the research conducted by Ahn *et al*[79] has provided evidence that IL-17A also contributes to the pathogenesis of endometriosis by triggering the expression of angiogenic factors such as VEGF and IL-8, as well as proinflammatory cytokines including IL-6 and IL-1β, along with chemotactic cytokines such as granulocyte colony-stimulating factor, C-X-C motif chemokine ligand 12 (CXCL12), C-X-C motif chemokine ligand 1 (CXCL1), and C-X3-C motif chemokine ligand 1[79].

Subsequently, it was also observed that the presence of $IL\text{-}10^+Th17$ cells significantly rises in the PF of females suffering from endometriosis[123]. Additionally, there is an upregulation of IL-27, IL-6, and TGF-β in this context. In comparison to peripheral CD4+ T cells, endometrial $CD4^+$ T cells exhibit a pronounced expression of IL-27 receptors, particularly in the ectopic endometrium. Apparently, in later stages endometriosis, IL-27 seems to plays a role in suppressing the development of Th17 cells while stimulating the production of IL-10 within these cells through the c-Maf/RORC/Blimp-1 complex—thereby contributing to the establishment of an immune tolerance pattern[123]. Consequently, these Th17 cells, which produce IL-10, enhance the growth, adhesion, invasion, and deep infiltration of endometrial stromal cells, thereby hastening the progression of endometriosis[123,124].

In contrast to the CD4+IL-17A+Th17 cell subset, there is a substantial increase in the proportions of CD25+FOXP3+ Treg cells within the CD4+ T-cell population among patients with advanced endometriosis, as opposed to those with early-stage endometriosis or control subjects ($P < 0.05$ in both instances)[118]. The induction of Treg cells, characterized by the expression of the transcription factor FOXP3, may be facilitated by specific cytokines, notably TGF-β and IL-10 [125]. Consistent with these findings, heightened levels of TGF-β and IL-10 have been consistently documented in the PF of individuals afflicted with endometriosis[90,126]. Notwithstanding this, endometriosis is correlated with elevated PF concentrations of numerous cytokines, encompassing various chemotactic and activatory factors, such as RANTES and MCP-1, known as robust chemoattractants for Treg cells[127,128]. Hence, the heightened prevalence of the CD4+ T cell phenotype may arise from either local stimulation or represent a secondary occurrence associated with their chemotactic response due to the sustained presence of a local inflammatory response[118].

It is hypothesized that the abundance of Treg cells within the peritoneal cavity hinders the recognition and selective targeting of ectopic endometrial tissues—thereby contributing to the persistence of ectopic lesions.

B cell dysregulation in endometriosis

Notably, the role of B cells in endometriosis is an area of ongoing research, and the exact mechanisms underlying their dysregulation in the disease are not fully understood. One intriguing finding is the decreased B-cell leukemia lymphoma (Bcl)-6 and increased B lymphocyte inducer of maturation program (Blimp)-1—transcription factors that regulate B-cell function—in the peritoneal cavity of patients with endometriosis[129].

Blimp-1 serves as a pivotal regulator of plasma cell differentiation[130,131]. The pronounced elevation of Blimp-1 in individuals suffering from endometriosis implies a heightened commitment to the differentiation of B cells into plasma cells. This observation raises the intriguing possibility of an intensified antibody response occurring within the peritoneal cavities of these patients, potentially bearing significance for their immune function. Conversely, Bcl-6 functions as an antagonist to Blimp-1, primarily inhibiting the process of plasma cell differentiation[132]. The diminished levels of Bcl-6 in endometriosis patients suggest a compromised ability to regulate the differentiation of B cells into plasma cells. This imbalance may contribute to an exaggerated antibody response or potentially exert influence over other facets of immune function.

The PF of individuals with endometriosis also seems to express high levels of the B lymphocyte stimulator (BLys)[133]—a protein that plays a critical role in the development of B cells and their differentiation into plasma cells[134]. Increased BLys levels in endometriotic lesions, in turn, suggest that the local microenvironment within the peritoneal cavity of endometriosis patients may be conducive to enhanced B-cell activation and maturation.

Accordingly, the presence of autoantibody responses targeting endometrial antigens represents a prevalent characteristic in endometriosis. In 1980, Startseva[135] was the first to report an elevated responsiveness of B cells in individuals with endometriosis. Since then, antibody responses directed against a range of both serum and tissue antigens, including alpha(2)-Heremans Schmidt glycoprotein (alpha(2)-HSG), transferrin, and carbonic anhydrase, have been discerned in this condition[136]. Nevertheless, additional research is required to gain a more comprehensive understanding of the connection between autoantibodies and the disease's onset and progression.

Estrogen and the immune microenvironment in endometriosis

The development of endometriotic lesions relies on estradiol—an estrogenic steroid hormone[137]. The heightened activity within the 17β-estradiol axis serves as a pivotal trigger for the activation of macrophages intricately associated with endometriosis pathogenesis[138-141]. In response to the escalated signaling of estradiol, the ectopic endometrial tissue, a hallmark feature of endometriosis, undergoes a noteworthy upregulation in the expression of ERβ. Indeed, higher ERβ levels, as opposed to ERα, have been observed in endometriotic tissues when compared to normal endometrial tissues[142]. An elevated ERβ-to-ERα ratio within endometriotic stromal cells is linked to the downregulation of progesterone receptors and an upsurge in cyclo-oxygenase-2 Levels, thereby playing a role in the development of progesterone resistance and inflammation[143,144].

Moreover, elevated prostaglandin levels hinder the immune system, enabling ectopic endometrial cells to evade immune surveillance and form endometriotic lesions. Additionally, ERβ engages in interactions with cytoplasmic inflammasome components and TNF-α-mediated programmed cell death pathways, resulting in increased production of IL-1β and enhanced cellular adhesion and proliferation[58]. This intricate modulation ultimately creates a cellular environment favoring enhanced cell survival and the sustained orchestration of the inflammatory response, both of which are pivotal factors in the perpetuation of endometriosis.

IMMUNE FACTORS DRIVING OVARIAN CANCER:

The correlation between chronic inflammation and development of tumors is not a unique feature of ovarian cancer. It has been described for many years, as various risk factors of cancer development are linked to inflammatory processes, such as viral infections, smoking and UV exposure[145]. The process of ovarian carcinogenesis is attributed to multiple factors, and while inflammation does not account for all of them, it serves as a pivotal element in the development of this particular disease[146]. Firstly, despite ovulation being a physiological process, multiple factors that alter the ovulation cycle, such as contraceptive pills, parity and age of menarche and menopause are related to a reduced risk of ovarian cancer development[147]. Fathalla proposed, in 1971, the theory of incessant ovulation, suggesting that the repetitive damage and subsequent repair of the ovarian epithelium may elevate the risk of neoplastic development and be the reason for the above-mentioned risk factors[147].

Presently, it is well-established that ovulation is closely connected to the inflammatory cascade, as the ovarian population of immune cells play critical roles in various processes within the menstrual cycle. As an example, ovarian macrophages contribute to tissue repair and proliferation through the secretion of several growth factors, TGF-β and IL-10, as well as apoptosis *via* the secretion of Reactive Oxygen Species (ROS), and IL-1β during physiological destruction, resulting in the necessary rupture of the follicle wall for ovum liberation and remodeling processes in the ovarian epithelium associated with the menstrual cycle phases[148].

The result of this is a chronic and periodic exposition of ovarian epithelial cells to a complex and dysregulated interplay of molecular events, involving both inflammation and tissue proliferation stimuli. These events encompass the nuclear factor-kappa B (NF-κB) activation, which has been reported to be an important element in tumorigenesis and further fueling the inflammatory milieu, as it enhances cytokine and growth factors production, induces cell proliferation and impedes cell apoptosis[149-151]. It is also important to note that the high levels of ROS may induce DNA damage that can facilitate the development of mutations that could induce the ovarian carcinogenesis process[152]. Ultimately, the complicated network of interacting events offers insights into its plausible involvement in instigating the mechanisms underpinning ovarian carcinogenesis.

Furthermore, various inflammatory conditions, including infections and reproductive system disorders, have been identified as risk factors for the development of ovarian cancer. Lin and colleagues, in 2011, found that women with Pelvic Inflammatory Disease exhibited an adjusted Hazard Ratio for ovarian tumor development almost twice as high as non-affected women[145]. Additionally, in 2012, a combination of results from 13 case-control studies demonstrated a significant association between the presence of clear-cell, low-grade serous, and endometrioid invasive ovarian cancers and a history of endometriosis among patients[19].

A determining factor in how ovarian cancer will progress, is the individual aspects of the tumor microenvironment. Increased number of Tumor infiltrating lymphocytes (TILs) with active CD3+ T cells have been associated with increased survival rate in patients with ovarian cancer[153,154]. On the other hand, the anti-tumor action of these cells can be rendered less effective by Tumor-infiltrating immune cells with immunosuppressive activity, such as M2 macrophages, regulatory T cells and Myeloid-derived Suppressive Cells, whose increased presence have been consistently related with poor prognosis of the disease[155,156].

Previous studies have evaluated the specific action of live Treg cells in this scenario. Initially, production of CCL22 chemokine by cancerous cells and macrophages attracts CCR4 expressing Treg cells to tumor site. After reaching the tumor microenvironment, these cells highly express several immunosuppressive cytokines, such as IL-10, IL-35, and TGF-β[157,158] and immune checkpoint inhibitors (CPI), such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), that binds to CD80/CD86 in Antigen presenting cells (APC), decreasing co-stimulation of T cells and inhibiting their activity [157]. In summary, the interplay among all of these factors underscores a multifaceted tumor microenvironment that hinders immune surveillance and the fight against malignant cells.

Another important molecule for the immunomodulator properties of ovarian cancer microenvironment is T cell immunoglobulin and mucin domain-containing protein 3 (TIM3), associated with higher IL-10 production and inhibition of T cell action in multiple tumors[158]. Currently, it is being tested in preclinical settings as a possible target for monoclonal antibodies in cancer treatment in association with PD-1/PD-L1 inhibitors[159,160].

It is important to note that proinflammatory cytokines, such as IL-6 and TNF-α, can also exhibit high expression levels within the context of ovarian cancer[161]. The correlation between IL-6 and the tumor's progression is well-established, and it is believed to contribute to various functions, primarily through the JAK/STAT pathway, including angiogenesis, cell proliferation, differentiation, and resistance to chemotherapy[162]. This could explain why the overexpression of the IL-6 receptor (IL-6R) in ovarian tissue has been linked to a poor prognosis for the disease[163]. Regarding M2 macrophages, in addition to playing an immunosuppressive role, they can induce angiogenesis and fibroblast proliferation by secreting various growth factors that promote the replication of cancer cells[164]. This is particularly significant in the clinical context of ovarian cancer, as vasculogenesis driven by VEGF secretion is the primary target of bevacizumab, one of the immunotherapy drugs currently used in the treatment of this condition[165].

Another type of CPI is PD-1, whose ligands PD-L1 and PD-L2 (both members of B7 superfamily) can be expressed by both immune and cancerous cells in the tumor microenvironment. When the PD-1 receptor of T cells binds to its ligands, it causes a reduction in proliferation, cell activity, IFN-γ and IL-2 production, and may even induce apoptosis of these cells[157,166,167]. Given the pressing need for additional therapeutic targets to enhance outcomes in combined ovarian cancer treatment, current research focuses on investigating the potential roles of other B7 family members, specifically B7-H3 (CD276) and B7-H4 (B7x) in TME immune suppression, as their expression relate to poor survival rates and treatment resistance[168-170].

Research suggests that B7-H3 and B7-H4 exhibit distinct expression patterns within the ovarian cancer microenvironment, as B7-H3 has been shown to be present both in stromal and tumor cells in Epithelial Ovarian Cancer TME, whereas B7-H4 seems to be primarily restricted to tumor cells within the ovarian cancer[171]. Furthermore, the expression of B7-H4 by Tumor-Associated Macrophages is induced by IL-6 and IL-10 and B7-H4 expressing TAMs in the ovarian cancer microenvironment exhibit an enhanced suppressive effect on T cell responses[172]. B7-H4 is believed to trigger cell cycle arrest in T lymphocytes, leading to the inhibition of cell division and proliferation.

Additionally, it has been linked to reduced cytokine production and a decrease in the cytotoxic capabilities of these immune cells[173]. On the other hand, B7-H3 has a complex and conflicting role in the immune response, as it may function as both a co-stimulatory and immunoregulatory molecule. This dual role could explain why a study discovered a positive correlation between B7-H3 expression, CD8+ cell infiltration in the TME, and improved prognosis for patients with pancreatic cancer[174]. However, in most neoplasms, including ovarian cancer, high B7-H3 expression doesn't appear to be associated with a favorable prognosis. Instead, it is correlated with increased therapy resistance and enhanced proliferation of cancerous cells, both *in vitro* and *in vivo*[170].

B7-H3 may be expressed in Antigen-Presenting Cells, and the knockout of its expression has been shown to induce enhanced cytolytic activity in tumor antigen-specific CD8+ cells among Tumor-Infiltrating Lymphocytes of mice, resulting from a higher production of IFN-γ and Granzyme B enzymes[175]. It is important to note, however, that the precise mechanisms and functions of B7-H3 and B7-H4 in immune cell activity are still areas of active research. Despite this, these proteins offer potential avenues for future tailored immunotherapies that can improve outcomes in ovarian cancer treatment.

ENDOMETRIOSIS AND OVARIAN CANCER: WHAT IS KNOW UNTIL NOW?

The presence of endometriosis leads to an increased risk of malignant tumors, and it is a well-documented finding that both pathologies appear together[176]. Suggesting some kind of transformation from endometriosis constituents into tumor cells[177]. Current molecular studies aim to establish links between endometriosis and EAOCs through pathways related to oxidative stress, inflammation, and hyperestrogenism[178]. Several researches have indicated that atypical endometriosis precedes clear cell or endometrioid ovarian cancers[31,32,179-181], suggesting a precancerous behavior [182]. A study conducted by Kato *et al*[183] concluded that certain epithelial cells in ovarian endometriosis, including atypical endometriosis and endometriosis - with many inflammatory and regenerative changes - have already acquired a clear cell phenotype.

Thinking about the pathway through endometriosis to derived ovarian cancer, the first thing to point out is the common component of both pathologies, the inflammatory pattern and immune system mobilization[11]. Those alterations can lead to a disruption and tumor formation. Endometriosis patients may have an inflammation profile similar to those with EAOC, even present in patients with benign lesions[184], suggesting that tumor-like immune signatures may develop even earlier than imagined. Deep infiltrating endometriosis with infrequent occurrences exhibits a tumor-like behavior, and may even resemble a metastatic disease[185,186].

Suryawanshi *et al*[184], in 2014, demonstrated a correlation between upregulation of the complement pathway and the KRAS and PTEN-regulated pathways, those two frequently related in oncogenesis and maintenance of the cancer phenotype in vitro. Also, this study showed that 33% of the patients with endometriosis revealed a tumor-like inflammation. Complement was previously linked with the support of tumor growth, being engaged in both chronic and acute inflammation[187,188].

It is crucial to comprehend the onset of the lesion and its connection to the inflammatory component. The development of endometriosis initiates with the implantation of ectopic tissue, which leads to bleeding. This is followed by inflammation, which triggers fibrin deposition and adhesion formation, eventually leading to scarring and distortion of the affected surfaces[189]. It has been reported that eutopic endometrium has a significant decrease in apoptosis compared to women without endometriosis[190]. The inflammatory process of endometriosis is strongly correlated with the peritoneal space experiencing high levels of oxidative stress, this leads to the proliferation of endometriosis as well as increased angiogenesis[191].

Increased proliferation of endometrial tissue and the occurrence of retrograde menstruation result in elevated levels of hemoglobin, heme, and iron[192,193]. Intense hemolysis observed in endometriosis results in high levels of free heme and iron; these molecules have a prominent effect as proinflammatory factors[193]. Excessive exposure to iron in the context of endometriosis sustains a state of chronic inflammation, modulates several mechanisms for the progression of endometrial lesions, and generates intracellular reactive oxygen species, as well as activating neutrophil responses[194, 195].

These substances modify crucial structures, enhance adhesion of refluxed endometrial cells, result in cell damage and DNA methylation, and consequently lead to the development of fibrosis and progression of endometriosis[189,193,196]. Subsequent transcription activation occurs (NF-kB, AP-1, and SP-1), along with oxidative burst, production of ROS, and IL-8[197-199]. Iron overload worsens the activation of peritoneal macrophages. And help to maintain a state of chronic inflammation. The inflammatory is further accentuated by the increased expression and activity of COX- 2, interleukins, and oxidative stress that act through the MAPK pathways[190].

The peritoneal fluid in cases of endometriosis typically has elevated levels of activated cytokines and macrophages[200, 201]. Macrophage activation is implicated in the pathogenesis of endometriosis and its association with ovarian cancer. Primarily due to the trophic factors secreted by macrophages that promote the growth of neoplastic lesions while at the same time increasing the conditions of oxidative stress due to the production of lipid peroxides[202,203]. Macrophage proliferation can alter the immune response at the site of inflammation, M2 phenotype molecules attract additional proinflammatory mediators to the lesion site, amplifying the inflammatory microenvironment[204].

Tumor-associated macrophages (TAMS) are a key component of the tumor stroma, essential for angiogenesis and matrix remodeling[189], they spontaneously release large amounts of IL-10 to TGF47, and some chemokines induce IL-10 in macrophages and the monocyte chemotactic protein-1 polarizes immunity in the Th2 direction[205,206].

Other than that, macrophages secrete many products such as TGF-beta, VEGF, IL-1, Prostaglandin E2 (PGE2) and macrophage migration inhibitory factor (MIF)[35]. Importantly, MIF sustains macrophage viability, which sustains inflammation through TAMS activation, and leads to tumor progression and the development of metastases[207]. Also, MIF upregulates COX-2 synthesis and PGE2 secretion in ectopic endometrial cells[208].

In a synergistic manner, IL-1 is associated with the induction of COX-2 and IL-8 expression, which facilitate migration, proliferation, and angiogenesis in endometriotic tissue[209]. And as a cytokine that promotes tumor growth, IL-1 triggers an ongoing chemical dialogue between the progressing tumor and its supportive stroma[210]. In the ovary, COX-2 is involved in the early events of neoplastic transformation; it is rarely found in normal ovarian epithelium, but is present in

endometriosis and in ovarian cysts considered to be premalignant[211].

In addition, other cytokines and chemokines are present in increased concentrations, on both ovarian CA and endometriosis. These include TNF-α, IL-1β, IL-6, IL-8 and RANTES. The proinflammatory cytokines TNF-α and IL-1β are elevated in the peritoneal fluid of women with endometriosis[201]. IL-1β induces expression of RANTES more in endometriotic stromal cells than in normal endometrial stromal cells, functioning as a chemoattractant for monocytes, memory T cells, and eosinophils[193,201]. TNF also can be found in malignant and/or stromal cells in human ovarian and the expression of TNF-α is increased on clear cell ovarian carcinoma, when compared with normal ovarian tissue[205,211]. Elevated TNF network gene expression resulted in increased signaling related to angiogenesis, cell adhesion, cell cycle and inflammation[211]. IL-8 increases ERα activity to induce ovarian cancer cell proliferation, it acts as an autocrine growth factor to promote proliferation of endometrial stromal cells in normal endometrioma and endometriotic cells.

Endometrial fragments are able to adhere to surfaces due to the presence of molecules that regulate cell-matrix and cell-cell interactions expressed by endometrial cells. These molecules include cadherins, integrins, proteoglycans such as the immunoglobulin superfamily, and CD44[212]. Cell-cell and cell-matrix adhesion molecules are engaged both in cancer tumors and endometriosis[213]. Endometriosis is reported to have an overall highly variable and aberrant integrin expression as compared with eutopic endometrium[190,214-216]. Endometrioid tissue may share molecular mechanisms of invasion and metastasis with carcinoma cells that are related to the level of E-cadherin expression[213,216].

The failure to remove fragments of menstrual effluent from the abdominal cavity induces excessive local inflammation [217,218]. The chronic aberrant expression of proinflammatory cytokines alters regulatory signaling pathways, which may facilitate cancer growth, invasion and metastasis through DNA damage and inhibition of DNA repair *via* reactive oxygen, autocrine/paracrine growth, survival factors for malignant cells, induction of vascular permeability and extravasation of fibrin/fibronectin[205]. And, result in accumulation of genetic mutations in endometriotic cells, through the changing of physiological homeostasis and progressive transcriptional changes can drive sustained proliferation and increase the rate of DNA repair[189].

Another factor that has an imperative role on disruption of homeostasis, and inflammation, is the hormonal component. As previously mentioned, hormonal management, upregulation of estrogen and intolerance to progesterone, plays a fundamental role in the maintenance and development of endometriosis, as well as on the tumor development. This said, an important alteration is the estrogen role on endometriosis, and its intense presence on peritoneum of afflicted women, and the consequently exacerbation of the immune inflammatory pathway.

Endometriotic stromal cells contain numerous specific epigenetic defects that favor overproduction of E2 and overexpression of the steroid receptor ER-beta that mediates an intense and E2-induced inflammatory process involving overproduction of cytokines and prostaglandins[219-221]. Being a major regulator of all key pathological processes in endometriosis and enhances lesion survival and inflammation leading to pain[220]. And excess E2 can result in cellular proliferation through the stimulation of cytokine production, specifically IL-8 and RANTES[222]. In addition, E2 stimulates the production of PGE2, the micro-environment in endometriotic tissue is marked by proliferative pressure with an enhanced level of reparative activity and thus, a higher chance for DNA damage and mutations[178]. Thus, PGE2 is a central mediator of the inflammatory response on endometriosis, but it has also been shown to regulate vital processes related to tumor growth, including angiogenesis, proliferation and inhibition of apoptosis[223].

Moreover, massive concentrations of estrogen in the ovary may also stimulate the inflammatory process *via* ER-beta in endometriotic stromal cells, which may contribute to the carcinogenic process in neighboring epithelial cells[58,224]. Is speculated that intense inflammation, progesterone resistance, and high levels of E2 (unopposed by progesterone action) in the stromal component led to a high proliferative activity and enrichment of driver mutations (*e.g.*, PIK3CA, KRAS, ARID1A) in attached endometriotic epithelial cells[68]. And it is associated with pro-inflammatory cytokines, which leads to (VEGF) expression, cell cycle activation, and activation of the anti-apoptotic gene Bcl-2[225,226]. Figure 2 depicts a simplified schematic illustrating of general immune response on endometriosis and ovarian cancer, and their similarities.

The dysregulation of apoptotic pathways and subsequent resistance to apoptosis contribute to the failure of immune clearance[45,227]. Accumulation of mutations in tumor suppressor genes and oncogenes is a crucial step during tumor development[228]. It is known that hormonal dysregulation in endometriotic implants, along with Inflammatory responses, may drive carcinogenesis[71]. Mutations secondary to endometriosis, is a fair finding. A study conducted by Koppolu *et al*[185], in 2021, showed that all the patients with endometriosis recruited on the study had no history or features of neoplastic disease, however the results revealed mutations in known cancer driver genes, especially in ectopic lesions. Some cancer-related mutations are found in endometriosis without cancer, in particular recurrent KRAS mutations[229]. Otherwise, some studies have shown confirmatory evidence that mutations found in endometriosis-associated cancers are found in adjacent endometriosis[36,230-232], and has been reported to exhibit a high percentage of PIK3CA and KRAS activating mutations and ARID1A and PTEN inactivating mutations[232,233]. A study conducted in 2023, with mice, was capable of successfully developed carcinoma by inducing the knockout (KO) of ARID1A and PTEN in the epithelium of endometriotic cysts, which were formed by the transplantation of small uterine pieces onto the peritoneum or ovarian surface, having a EAOC developed as early as 4 wk after the KO[232]. Helping to consolidate and bring up more data surrounding the intrinsic correlation between the dysfunctions caused by endometriosis and the onset of ovarian cancer.

IMPLICATIONS FOR CLINICAL MANAGEMENT

Immunotherapy in ovarian cancer and endometriosis

Unlike the traditional strategies of killing tumor cells, immunotherapy is a treatment approach that utilizes cells, viruses,

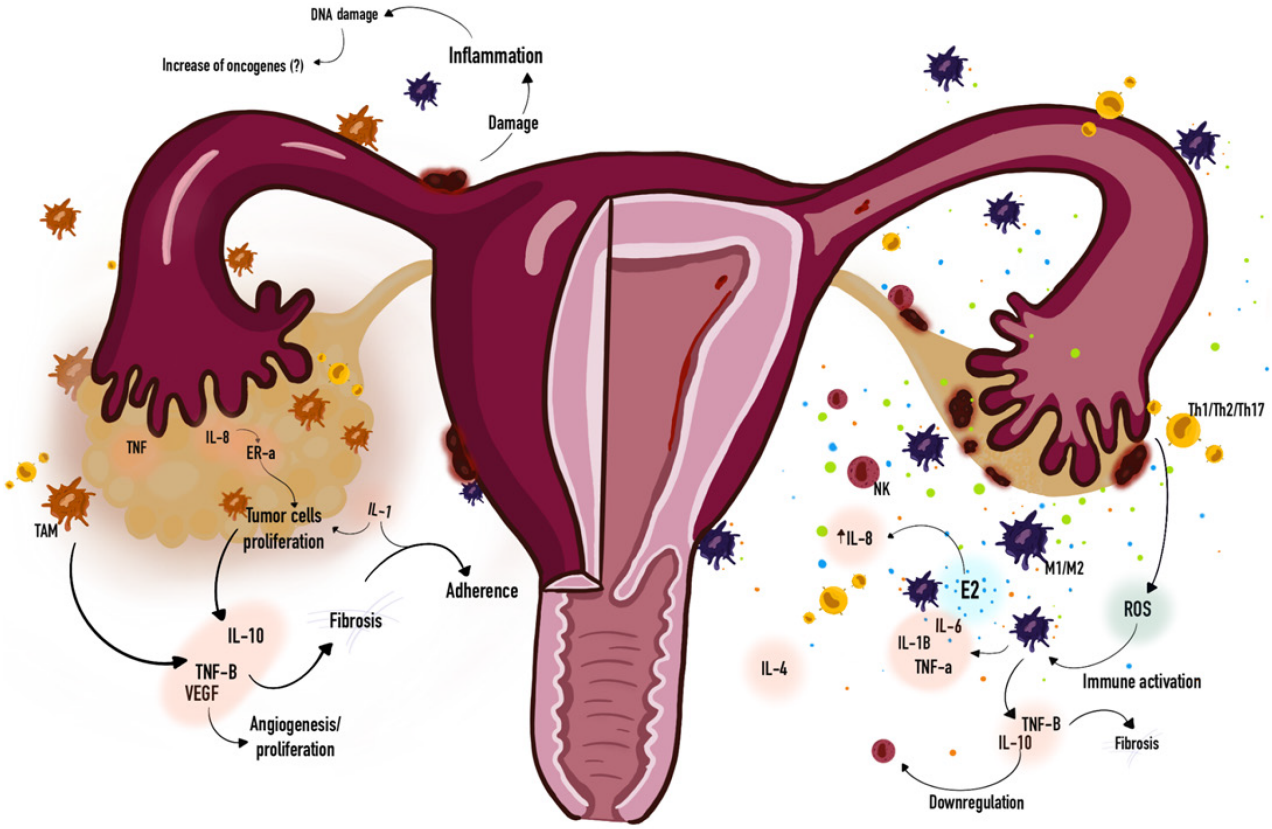

Figure 2 Overview of immune dysregulation similarities between on endometriosis and ovarian cancer. IL-1: Interleukin-1; IL-1B: Interleukin-1β; IL-4: Interleukin-4; IL-6: Interleukin-6; IL-8: Interleukin-8; IL-10: Interleukin-10; E2: Prostaglandin E2; ER-a: Estrogen Receptor Alpha; M1/M2: Macrophages; NK: Natural killer cell; TAM: Tumor-associated macrophages; Th1/Th2/Th17: T helper cells; TNF: Tumor Necrosis Factor; TNF-a: Tumor Necrosis Factor Alpha; TNF-B: Tumor Necrosis Factor Beta; ROS: Reactive oxygen species; VEGF: Vascular endothelial growth factor.

peptides, small molecules, or antibodies to activate or modulate the immune system to attack cancer cells[234]. This treatment has brought about a significant transformation in the approach of various solid tumors, including malignant melanoma, non-small-cell lung cancer, and renal cell carcinoma. It has become the leading choice for managing recurrent or metastatic solid tumors, surpassing conventional chemotherapy and targeted therapy[235].

Over the past few decades, immunotherapy has surfaced as a hopeful treatment alternative for gynecologic malignancies, including ovarian cancer. The first line of treatment for ovarian cancer is usually cytoreductive surgery combined with chemotherapy[236]. However, chemoresistance is one of the most prevalent factors for the poor prognosis of this pathology, especially when associated with metastatic capacity and clinical course of the disease[237]. As ovarian cancer is a tumor with an extremely immunosuppressive microenvironment, the immunological approach shows great promise [238]. Currently, immune strategies for the treatment of ovarian cancer are being tested in clinical trials, and include checkpoint inhibition, cancer vaccines, oncolytic virotherapy and adoptive cell therapy. Figure 3 depicts a simplified schematic illustrating of current possible immunotherapy approaches to ovarian cancer.

Immune checkpoint inhibitors

Effective immunotherapy for ovarian cancer hinges on activating antigen-presenting cells, reducing the immunosuppressive microenvironment, and enhancing the performance of effector T cells. The T cell-mediated immune response is controlled through both inhibitory and activating signals, and immune checkpoint receptors play a crucial role in restraining T cell activation to prevent excessive stimulation. Nonetheless, numerous types of tumors exhibit immune checkpoint expression, which results in immune evasion. Consequently, inhibitors targeting immune checkpoints play a significant role in immunotherapy[238]. Up until now, the most promising immune checkpoint inhibitors for solid tumors have been antibodies that hinder CTLA4, PD-1 and PD-L1, which are presented in some drugs approved by The Food and Drug Administration, such as CTLA4 antibodies (Ipilimumab), PD-1 antibodies (Pembrolizumab and Nivolumab), and PD-L1 antibodies (Avelumab, Atezolizumab and Durvalumab)[239,240]. However, the clinical application of checkpoint inhibitors in ovarian cancer has yielded limited success, with single-agent response rates in clinical trials typically hovering around 6%-15%[241-243]. As single agents, the results in ongoing clinical trials showed modest effects of immune checkpoint inhibitors (ICI) in ovarian cancer, limiting its approval for use in patients with ovarian cancer[244].

Another potential target of immune checkpoint blockade is B7-H3, which is an immunosuppressive molecule present on tumor cells but absent in host cells, and researchers have explored the therapeutic impacts of blocking B7-H3 and PD-1 in the context of cancer[238]. The results indicate that, in ID8 tumor-bearing mice with ovarian cancer, it is B7-H3 inhibition, not PD-1 blockade, that prolongs the median survival time[245].

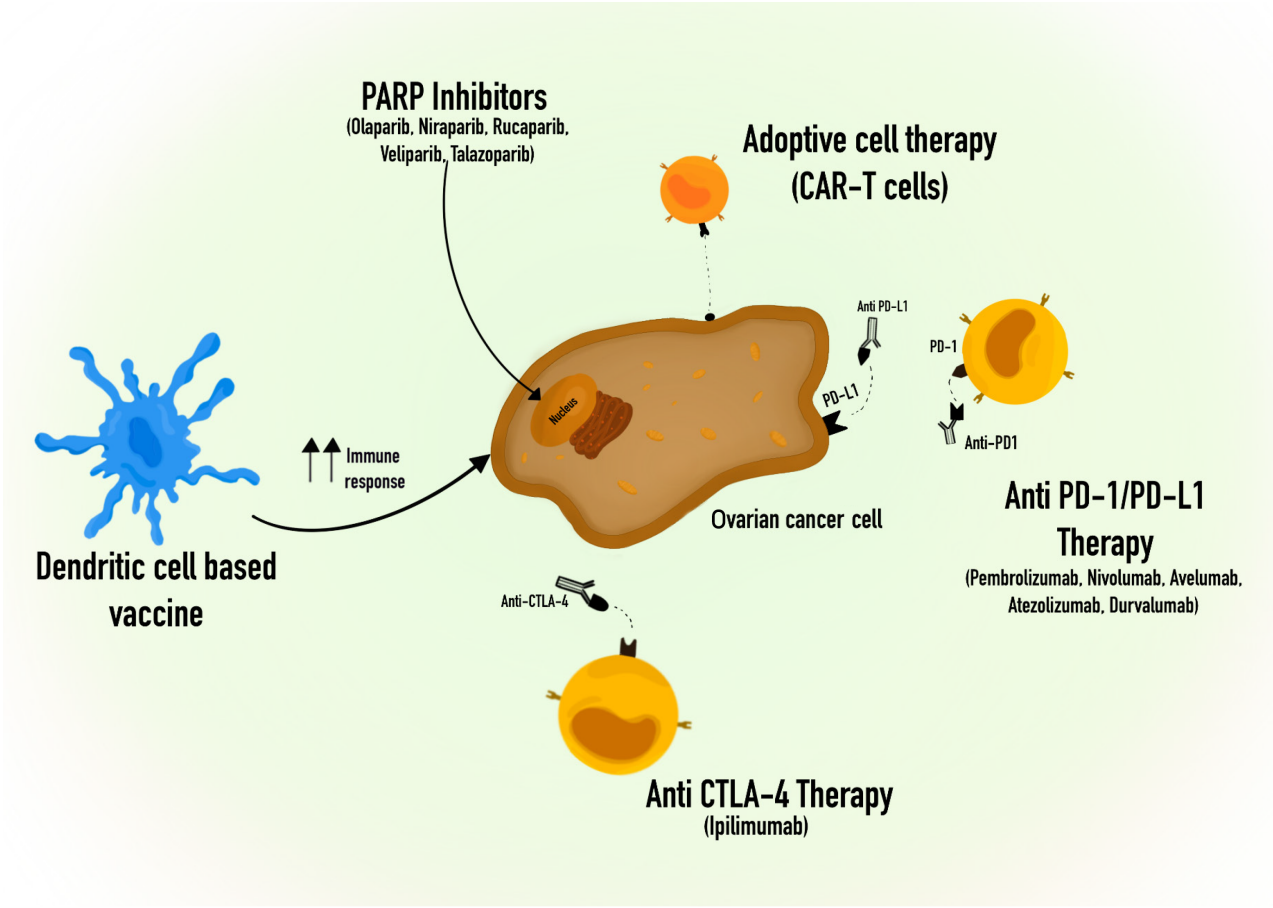

Figure 3 Some of the current immunotherapy approaches of treatment to ovarian cancer. Anti-CTLA-4: Cytotoxic T-Lymphocyte Associated Protein 4 Antibody; Anti-PD1: Programmed Cell death Protein 1 Antibody; Anti PD-L1: Programmed Death-ligand 1 Antibody; CAR-T cells: Chimeric Antigen Receptor-T cells; PARP: Poly Adenosine Diphosphate-Ribose Polymerase; PD-1: Programmed Cell death Protein 1; PD-L1: Programmed Death-ligand 1.

Immune checkpoint inhibitors monotherapy has limited anti-tumor effects in ovarian cancer, and the efficacy of ICI depends on the condition of TILs and the expression of specific molecules. Hence, ideal candidates for this strategy should be well-chosen. To surpass these barriers, combining therapies are being tested to improve the anti-tumor activity in ovarian cancer[246].

PARP inhibitors

The poly (ADP-ribose) polymerase (PARP) is a well-acknowledged detector of DNA damage, renowned for its involvement in repairing DNA base excision and single-strand breaks. PARP inhibitors have emerged as a novel targeted therapy for ovarian cancer, especially for women carrying BRCA1 and BRCA2 mutations or individuals lacking a functional homologous recombination repair pathway[247]. Cells with impaired homologous recombination are vulnerable to PARP inhibitors. BRCA1 and BRCA2 are tumor suppressor genes known for their essential involvement in DNA repair, as they create a complex responsible for homologous recombination repair[248]. The FDA has sanctioned various PARP inhibitors for use, and some of them are currently under investigation in clinical trials. These include olaparib, niraparib, rucaparib, veliparib, and talazoparib[249]. Despite the encouraging advantages offered by PARP inhibitors, numerous limitations persist, as shown in some studies[250-253]. Future research should prioritize exploring and developing combinations that can amplify the impact of PARP inhibitors, such as antiangiogenic agents and ICIs combining therapies[254].

Adoptive cell therapy

Adoptive cell therapy (ACT) primarily relates to the utilization of chimeric antigen receptor (CAR)-modified T cells, T-cell receptor (TCR)-engineered T cells, natural tumor-infiltrating lymphocytes (TILs), CAR-NK cells, and CAR-macrophages. ACT has brought about a significant breakthrough in treating blood-related tumors. However, when it comes to solid tumors like ovarian cancer, ACT appears to be inadequate in inducing substantial anti-tumor responses [255]. Up until now, there has not been a notable therapeutic effectiveness. The primary challenges lie in the weak binding affinity and inconsistent presence of targetable surface antigens, as well as obstacles related to the infiltration and viability of CAR-T cells[256]. Therefore, additional clinical data is necessary to verify their effectiveness in individuals with ovarian cancer.

Cancer vaccine

Vaccination plays a crucial role in immunotherapy, offering advantages to individuals affected by diverse forms of cancer, and there is extensive research into therapeutic vaccines in the context of ovarian cancer. The investigation into the potential of single application of a cancer vaccine for ovarian cancer is ongoing, and this includes the examination of various types of vaccines such as peptide vaccines, whole tumor cell vaccines, cancer stem cell vaccines, APC vaccines, DNA/RNA vaccines, bacterial vaccines, and more[255]. Many of these vaccines enhance the body's immune response against ovarian cancer, but clinical evidence has only demonstrated modest effectiveness in the majority of patients[257-259]. It is feasible to assess the therapeutic potential in a broader group of patients. The use of dendritic cell-based vaccines is another treatment approach, which is also under investigation in patients with ovarian cancer, being shown that it can produce improved outcomes[236,246]. However, the evaluation of the clinical use of vaccines in cancer patients has certain limitations, such as the heterogeneity of antigen expression within a tumor[260,261].

Immunotherapy in endometriosis

The immune system has a significant role in endometriosis. Therefore, immune therapy holds promise as a potential treatment approach for this condition. The reduced macrophagic phagocytosis observed in the serum and peritoneal fluid of individuals with endometriosis is a significant contributor to the disruption of immune balance[262]. In this sense, the expression of CD47 is used by macrophages to distinguish "self" or "non-self" cells. The CD47 site inhibitor disrupts this signal, enabling macrophages to carry out regular phagocytosis[263]. Clinical observations have revealed a substantial elevation in CD47 Levels within the ectopic endometrial tissue of individuals with endometriosis[264]. Immunotherapy targeting the CD47-SIRPa signaling pathway appears to show promise in the management of endometriosis[265].

Furthermore, it has been reported that exosomes originating from endometriosis can induce a shift in macrophage phenotype toward M2 polarization, leading to a reduction in macrophage phagocytic activity both in laboratory settings (*in vitro*) and within the body (*in vivo*)[262]. As a result, employing anti-exocrine therapy for individuals with endometriosis appears to have a notable effect on attracting macrophages to ectopic lesions[266]. This therapy can decrease the presence of M2-type macrophages, leading to an overall enhancement in macrophage-mediated phagocytosis of ectopic endometrial cells[98].

Moreover, encouraging NK cell cytotoxic activity is a potential novel therapeutic approach for managing endometriosis[267]. The treatment is based on NK inhibitory receptors that dampen their response to ectopic or malignant cells. The PD1, one such receptor that interacts with the PDL1 Ligand has already shown promise in cancer immunotherapy [268]. This approach aims to potentially enhance the rescue of endometrial cells, counteract the suppression of NK cells' regulatory function, and enable the elimination of misplaced endometrial cells. The proposed immunotherapy strategy suggests utilizing existing medications, commonly employed for conditions like cancer, to aid in identifying and prompting the removal of endometrial cells through apoptosis[269].

Besides this, vitamin D has been noted for its immunomodulatory properties in the medical management of endometriosis[270]. In a recent animal model study focused on endometriosis, it was observed that the use of synthetic vitamin D derivatives significantly curbed the progression of both endometriosis and peritonitis[271]. Considering the role of inflammatory cytokines in the development of endometriosis, recent studies have suggested that the impact of 1,25(OH)2D3 on the cytokine production within human endometrial stromal cells could be a contributing factor to the therapeutic benefits of this compound in treating endometriosis. Additionally, another investigation has shown that 1,25(OH)2D suppresses the immune response of Th1 cells while promoting the response of Th2 cells. It achieves this by inhibiting the release of IL-12, IL-2, and TNF from T cells, macrophages, and DCs, respectively[272].

Targeting macrophages and inflammatory mediators

The direct involvement of tumor-associated macrophages (TAMs) in the oncogenesis and prognosis of ovarian cancer based on the imbalance in polarization between M1 (pro-inflammatory) and M2 (anti-inflammatory) macrophages has been previously established[273]. Thus, it was discovered that the presence of a majority of M2 (anti-inflammatory) macrophages in the ovarian environment is a determining factor for a worse cancer prognosis, through the interaction of specific receptors of these cells with the immune system and also through the stimulation of various cytokines, generating an environment more conducive to the establishment of ovarian cancer of all types, including EAOC[274]. In this way, investigation of new ways of interfering in this process using the specific receptors expressed on M2 macrophages and the cytokines produced can be a new approach in order to achieve better therapeutic results and greater survival[275].

CD47- SIRPα: CD47 is a glycoprotein that is very present in the tumor environment and exerts its inhibitory activity by binding to its counter-receptor, the signal regulatory protein-α (SIRPα), expressed in macrophages[276,277]. Liu *et al*[277] conclude that the impact of this inhibitory process is a reduction in phagocytosis by these macrophages (known as the "don't eat me signal"), which culminates in the progression of the tumor microenvironment, thus serving as one of the strategies for inhibiting immune activity by tumor cells[277].

Based on this principle, therapies based on blocking CD47 (anti-CD47) and SIRPα have emerged, which aim to interfere with the inhibitory process[278]. Son *et al*[278] demonstrated that these two therapies are promising in the treatment of solid cancers, such as ovarian cancer, and have shown good progress in terms of improved anti-tumor activity of macrophages based on the established blockade of inhibitory receptors.

Kaur *et al*[279] reported a series of preclinical studies in mice and humanized clinical studies of anti-CD47 therapy, bringing a positive result, since both the preclinical study and the humanized clinical studies showed promising data in relation to limiting tumor cell growth.

Sikic *et al*[280] also demonstrated a promising result when they carried out a phase I study in humans using the anti-CD47 antibody Hu5F9-G4, which showed safety, tolerability and also tumor regression in some patients undergoing therapy. It is also important to note that Tian *et al*[281] were able to demonstrate that anti-CD47 therapy has a promising future in improving the activity of innate immunity and the oncolytic process.

In addition, Li *et al*[264] also reported a study showing high CD47 expression in patients with endometriosis, in which CD47 blocking treatment resulted in an increase in the phagocytic process by macrophages and also in an increase in apoptosis of endometrial stromal cells, representing a protective effect against the development of EAOC. Figure 4 depicts a simplified schematic illustrating of CD47- SIRP-based immunotherapy.

CSF-1/CSF-1R: The colony-stimulating factor-1 receptor (CSF-1R) is a receptor that exists in several human cells during homeostasis, but is overexpressed in tumor-associated macrophages in several types of cancer, including ovarian cancer [282]. The specific ligand of this glycoprotein is CSF-1, which is found in high levels in tumor cells and the binding of these two receptors culminates in an oncogenic role through the release of growth factors and substances that stimulate the cellular differentiation of macrophages into M2[282]. In this way, the relationship between both glycoproteins has become the object of study for the development of new therapeutic techniques, based on the inhibition of these receptors, with the aim of reducing TAMs and tumor growth[283].

Based on this premise, Ries *et al*[284] used a monoclonal antibody directed at blocking CSF-1R in cancer patients, with the aim of manipulating the activity of TAMs, and the result was promising, since a reduction in tumor-associated macrophages was achieved in patients as well as an increase in the levels of TCD8/CD4 cells in animal models.

Lu *et al*[285] conducted a study in murine models of ovarian cancer and associated a CSF-1R inhibitor with docetaxel and concluded that treatment with the inhibitor alone resulted in increased cell apoptosis of tumor-associated macrophages due to the repolarization effect, transforming them into M1. Combined treatment resulted in a significant reduction in tumor growth, a reduction in TAMs and increased levels of TCD8+ cells[285].

Finally, Xiaocui *et al*[286] identified a significant presence of the glycoprotein CSF-1 in patients with endometriosis, which resulted in an elevated appearance of macrophages with an anti-inflammatory effect and, consequently, a depletion of the activity of the immune system in this environment, leaving it vulnerable to the appearance of possible ovarian cancer. This study also found that the use of an anti-CSF-1 antibody reduced these negative effects, demonstrating the promising activity of this immunotherapeutic field[286]. Figure 5 depicts a simplified schematic illustrating of CSF-1/CSF-1R-based immunotherapy.

CCL2/C-C motif chemokine receptor 2: The CCL2/C-C motif chemokine receptor 2 (CCR2) axis is involved in the recruitment of monocytes from the bloodstream to the tumor environment, since CCL2 is a chemokine that attracts CCR2+ monocytes, which will become M2 macrophages when they arrive in the tumor environment[287].

This axis has also become a therapeutic target, as demonstrated by Miyamoto *et al*[288] who based their study on blocking the CCL2/CCR2 interaction. The study achieved a positive result in mice by reducing M2 macrophages and increasing TCD8+ cells and IFNγ by inhibiting the CCL2/CCR2 relationship[288].

In addition, it is also necessary to highlight the relationship between CCL2 and endometriosis, as reported by Hogg *et al*[289], who reported that high levels of CCL2 are present in endometriosis, thus resulting in greater recruitment of macrophages that may impact on the inflammatory environment and contribute to the process of malignization of the lesion, generating ovarian cancer.

CXCR4-CXCL12: C-X-C receptor 4 (CXCR4) is a chemokine that functions through its binding to the CXCL12, derived from stromal cells, and the activation of this axis is closely linked to the initiation and progression of ovarian tumors from the invasion of ovarian cancer cells[290,291].

With this in mind, Xue *et al*[292] used an antagonist of the CXCR4 receptor in order to reduce the impacts derived from its activity and achieved promising results, which are the blocking of the activation of the NF-κB signaling pathway and, consequently, a reduction in tumor growth and a reduction in the possibility of metastasis.

Pharmacological modulation of TAMs: The polarization of macrophages and their influence on the oncogenesis and progression of ovarian cancer has also extended the discussion into the field of pharmacological modulation, with the clear aim of finding a way to revert M2 macrophages into M1, promoting anti-tumour activity and, consequently, regression of the already established tumour[293,294].

In their study, Bolli *et al*[295] used imidazoquinoline (IMDQ), an agonist of the Toll 7/8 receptor, which was coupled to an antibody and infused intravenously into patients with ovarian cancer with the aim of blocking the macrophage mannose receptor (MMR) and causing a repolarization of macrophages from M2 to M1. The results of the study were promising and showed a reduction in tumor growth, associated with an increase in the pro-inflammatory process derived from the change in the types of macrophages employed in the environment[295].

On the other hand, the study by Xiao *et al*[296] addressed the development of a nanodrug specific for tumor-associated M2 macrophages, from siRNA IKKβ (an activator of NF-kβ and STAT6, participants in the polarization process), with the clear objective of inhibiting STAT6, thus resulting in a safe conversion of macrophages and the reduction of tumor growth with the activation of the antitumor axis in an *in vivo* experiment.

Finally, Hsieh *et al*[297] presented a study based on the use of vorinostat in mice with EAOC, in which the result was a reduction in the tumor from the inhibition of the polarization of M2 macrophages.

Anti-inflammatory agents in the context of endometriosis and ovarian cancer

COX-2 is an enzyme directly linked to inflammatory processes from arachidonic acid and the consequent production of prostaglandins[298]. COX-2 has been described as an overexpressed marker in ovarian cancer, playing a direct role in the

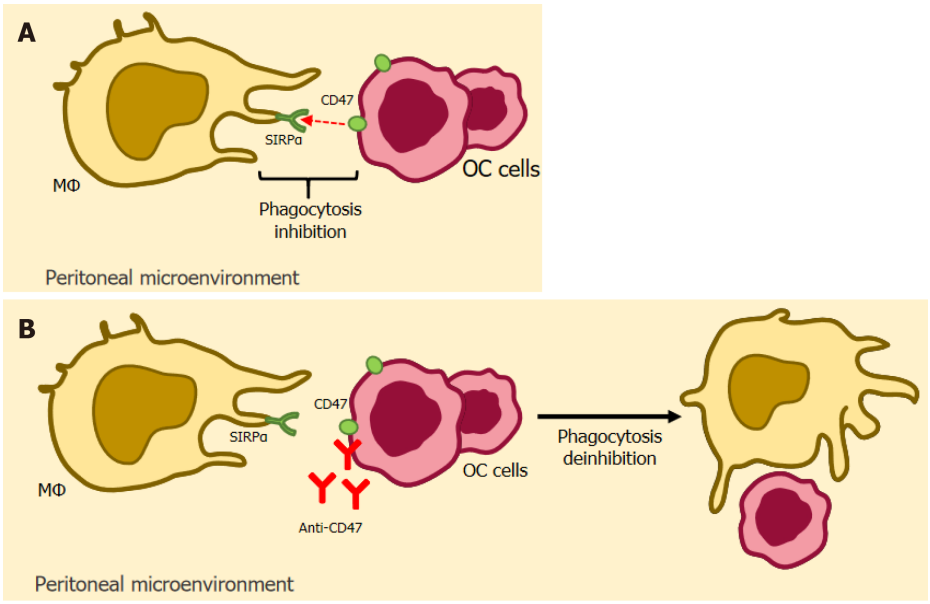

Figure 4 Illustration of anti-CD47-based immunotherapy. CD47 is a glycoprotein that is very present in the tumor environment and exerts its inhibitory activity by binding to its counter-receptor, the signal regulatory protein-α (SIRPα), expressed in macrophages. It reduces phagocytosis by these, which culminates in the progression of the tumor microenvironment. It is highly expressed patients with endometriosis. A: CD47 binding to SIRPα inhibits phagocytosis; B: Anti-CD47 blocks the binding between CD47 and SIRPα, allowing phagocytosis to occur. Anti-CD47: Integrin-associated protein Antibody; CD47: Integrin-associated protein; MΦ: Macrophage; OC cells: Ovarian Cancer cells; SIRPα: signal regulatory protein alpha.

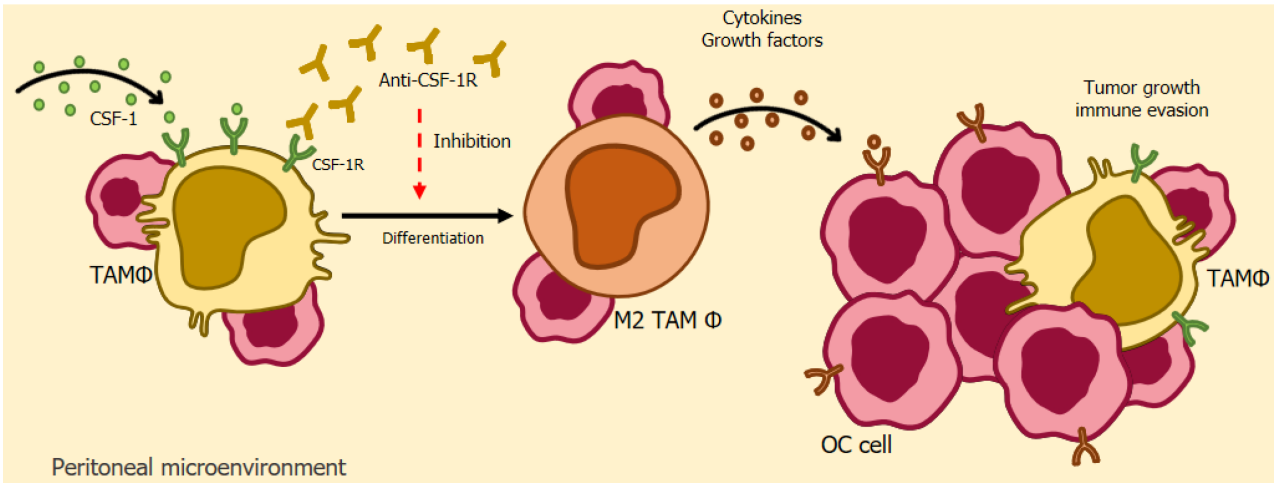

Figure 5 Illustration of anti-CSF-1R-based immunotherapy. The colony-stimulating factor-1 receptor (CSF-1R) is a receptor that exists in several human cells during homeostasis, but is overexpressed in tumor-associated macrophages in ovarian cancer. The use of a monoclonal antibody directed at blocking CSF-1R in cancer patients, aims to manipulate the activity of TAMs, reducing tumor-associated macrophages in patients, as well as an increase in the levels of TCD8/CD4 cells in animal models. Anti-CSF-1R: Colony-Stimulating Factor 1 receptor Antibody CSF-1: Colony-Stimulating Factor 1; CSF-1R: Colony-Stimulating Factor 1 receptor; M2 TAM Φ: Tumor-associated macrophages M2; OC cell: Ovarian Cancer cells; TAM Φ: Tumor-associated macrophages.

poor prognosis of patients with this type of cancer, since it participates in the migration and invasion of tumor cells[299]. In addition, Zhang *et al*[300] and Lai *et al*[301] bring up the direct involvement of COX-2 in endometriosis, since, as found in the study, cyclooxygenase-2 promotes increased cell proliferation, reduced apoptosis and increased angiogenesis, factors that are also linked to oncogenesis.

Thus, studies such as that by Li *et al*[302] show the importance of investigating the impact of anti-inflammatory drugs on ovarian cancer, since, as reported in this analysis, a positive effect was found when associating a COX-1 inhibitor with Cisplatin or Taxol in order to reduce angiogenesis in ovarian cancer in murine models.

Therefore, the direct involvement of COX-1 and COX-2 in the oncogenesis and progression of ovarian cancer, as well as in the progression of endometriosis, has already been established. Therefore, new clinical studies with a large number of patients using COX inhibitors are still needed in order to establish an even more efficient treatment for ovarian cancer and endometriosis.

CONCLUSION

Endometriosis and ovarian cancer are still underreported diseases. This means that much involved in their pathophysiology and correlations still remains hazy. This is also due to the lack of feasible and efficient diagnostic resources in clinical practice. Especially with regard to the applicability of biomolecular resources to support the diagnosis and monitoring of those pathologies.

Future studies should aim to better elucidate the roles of oxidative stress, inflammation, and estrogen in EAOC development. Existing evidence suggests a shared microenvironment between endometriosis and EAOC in terms of cytokines and mediators, but further research is necessary to confirm a direct link between the two. Additionally, further research should focus on discovering biomarkers capable of identifying endometriosis cases with oncogenic potential, with the aim of detecting premalignant lesions, and consequently develop better interventions in order to decrease the incidence of EAOCs.

Research efforts should prioritize establishing model systems for endometriosis. These investigations could yield valuable knowledge about risk factors, molecular traits specific to subtypes, novel therapeutic testing, and the factors that contribute to the development of EAOCs. Additionally, it is crucial to emphasize the necessity of current interventions that lower the risk of ovarian cancer, including endometrioid carcinoma and clear cell carcinoma.

Moreover, non-invasive methods to help diagnose the disease should be a priority, as well as clarification of the genetics and genomics that control disease development, environmental contributions, and the involvement of the immune system. Other than that, well-designed clinical trials are essential to determine which therapies are safe and effective, and which markers and targets on the immune system may be useful in treatment and management. And confirmation of the veracity of these biomarkers can help in the development of true research, with larger populations, to understand endometriosis in particular.

FOOTNOTES

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MINIREVIEWS

Britanin – a beacon of hope against gastrointestinal tumors?

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Abstract

Britanin is a bioactive sesquiterpene lactone known for its potent anti-inflammatory and anti-oxidant properties. It also exhibits significant anti-tumor activity, suppressing tumor growth *in vitro* and *in vivo*. The current body of research on Britanin includes thirty papers predominantly related to neoplasms, the majority of which are gastrointestinal tumors that have not been summarized before. To drive academic debate, the present paper reviews the available research on Britanin in gastrointestinal tumors. It also outlines novel research directions using data not directly concerned with the digestive system, but which could be adopted in future gastrointestinal research. Britanin was found to counteract liver, colorectal, pancreatic, and gastric tumors, by regulating proliferation, apoptosis, autophagy, immune response, migration, and angiogenesis. As confirmed in pancreatic, gastric, and liver cancer, its most commonly noted molecular effects include nuclear factor kappa B and B-cell lymphoma 2 downregulation, as well as Bcl-2-associated X protein upregulation. Moreover, it has been found to induce the Akt kinase and Forkhead box O1 axis, activate the AMP-activated protein kinase pathway, elevate interleukin-2 and peroxisome proliferator-activated receptor-γ levels, reduce interleukin-10, as well as downregulate matrix metalloproteinase-9, Twist family bHLH transcription factor 1, and cyclooxygenase-2. It

also inhibits Myc–HIF1α interaction and programmed death ligand 1 transcription by interrupting the Ras/RAF/MEK/ERK pathway and mTOR/P70S6K/4EBP1 signaling. Future research should aim to unravel the link between Britanin and acetylcholinesterase, mast cells, osteolysis, and ischemia, as compelling data have been provided by studies outside the gastrointestinal context. Since the cytotoxicity of Britanin on noncancerous cells is significantly lower than that on tumor cells, while still being effective against the latter, further in-depth studies with the use of animal models are merited. The compound exhibits pleiotropic biological activity and offers considerable promise as an anti-cancer agent, which may address the current paucity of treatment options and high mortality rate among patients with gastrointestinal tumors.

Key Words: Britanin; Sesquiterpene lactones; Chemotherapeutics; Gastrointestinal tumors; *In vitro*; *In vivo*

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Core Tip: Natural compounds have settled in the development of novel drugs. Britanin is a sesquiterpene lactone whose effect on gastrointestinal tumors has not been summarized before. Our paper reviews the current state of knowledge and proposes novel research directions. Britanin was found to counteract liver, colorectal, pancreatic, and gastric tumors *via* the regulation of proliferation, apoptosis, autophagy, immune response, migration, and angiogenesis. Future research should examine the link between Britanin and acetylcholinesterase, mast cells, osteolysis, and ischemia. The compound holds promise as an anti-cancer agent and may overcome the paucity of treatment options or high mortality rate in gastrointestinal tumors.

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INTRODUCTION

Natural compounds have long been established in the development of novel drugs. One such group, the sesquiterpene lactones, are organic terpenoids that exhibit a broad spectrum of biological activities, with their anti-cancer, anti-parasitic, and anti-inflammatory properties being the most prominent[1-3]. One of the representatives of this group is a compound termed Britanin (C19H26O7), a pseudoguaianolide-type sesquiterpene lactone present in various Inula species. It has been found to demonstrate anti-cancer agent activities by affecting tumor cell survival[3,4]. Although Britanin has been present in the PubChem database since 2005, the current body of research is limited to about thirty papers in total, mostly related to cancer. The vast majority of the literature concerns gastrointestinal tumors that have not been summarized before. Britanin has also been evaluated in leukemia[5-8] and tumors of the breast[9-12], head and neck[13], kidney[14], prostate[15], or lung[14]; however, insufficient data exists on each disease type to draw firm conclusions. Given its promising implications in oncology, Britanin is likely to be the subject of considerable research in the upcoming years. To drive academic debate, the present paper reviews and discusses available research on Britanin in gastrointestinal tumors. A literature search was performed *via* PubMed using the "britanin" and "britannin" terms, focusing on gastrointestinal tumors. Moreover, the present paper outlines novel research directions using data outside the scope of the digestive system, which could be adopted in future gastrointestinal research.

RESEARCH ON BRITANIN IS FOCUSED ON LIVER, COLORECTAL, PANCREATIC, AND GASTRIC TUMORS

The first report on the anti-proliferative properties of Britanin was published in 2012 by Moghadam *et al*[14] who extracted a compound from Inula aucheriana. A strong cytotoxic effect was noted on the liver cancer cell line HepG2 based on MTT assay, *i.e.*, utilizing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, with the half-maximal inhibitory concentration (IC_{50}) of 2.2 μg/mL[14]. In the following year, Fischedick *et al*[16] found that 10 μmol/L of Inula britannica-derived Britanin inhibited cell growth by ~80%, as estimated on colorectal cancer cell line DLD1 and its multidrug resistant counterpart with P-glycoprotein overexpression.

In 2016, Piao *et al*[17] evaluated the activity of fourteen Inula japonica-derived compounds that inhibit DNA topoisomerases. Among them, Britanin exhibited better inhibitory activity against topoisomerase II (IC_{50} = 6.9 μmol/L) than against topoisomerase I (IC_{50} > 80 μmol/L). Interestingly, the inhibitory capabilities of Britanin directed at topoisomerase II were found to surpass those of Etoposide (IC_{50} = 26.9 μmol/L), a commonly used inhibitor. Moreover, Britanin showed low toxicity against liver hepatoblastoma (HepG2 cell line) and colon adenocarcinoma (HT-29 cell line), with IC_{50} values

of 35.5 μmol/L and 3.9 μmol/L, respectively[17].

In 2017, Moeinifard *et al*[18] assessed the chemotherapeutic potential of Britanin derived from Inula aucheriana in pancreatic cancer therapy. The results indicated that the compound induces apoptosis in human pancreatic cancer cell lines AsPC-1 and PANC-1 by simultaneously decreasing B-cell lymphoma 2 (BCL-2) expression and increasing that of Bcl-2-associated X protein (BAX). Additionally, Britanin increased the generation of reactive oxygen species (ROS) and activated the axis of Akt kinase and Forkhead box O1 (AKT-FOXO1), inducing the mitochondrial apoptotic pathway in both cell lines[18]. IC_{50} values for AsPC-1 and PANC-1 cell lines equaled 30 ± 4.61 μmol/L and 40 ± 5.63 μmol/L, respectively.

In the following year, Cui *et al*[19] reported that Britanin extracted from Inula aucheriana could induce apoptosis and autophagy *via* ROS-driven activation of the AMP-activated protein kinase (AMPK) pathway in the liver cancer cell lines HuH-7, SMMC-7721, and HepG2. Britanin reduced the survival rate of the cells in a dose- and time-dependent manner, with respective IC_{50} values of 27.86 ± 1.35 μmol/L, 28.92 ± 1.09 μmol/L and 15.69 ± 1.58 μmol/L after 24-h treatment (8.81 ± 0.95 μmol/L, 8.12 ± 1.15 μmol/L, and 6.86 ±1.05 μmol/L after 48 h). Furthermore, the compound exhibited no cytotoxicity against normal human liver cells. Further *in vivo* tests on the most susceptible cell line (HepG2) found Britanin to suppress liver cancer proliferation in a dose-dependent manner[19].

In 2020, Shi *et al*[20] found Inula japonica-derived Britanin to inhibit the growth and progression of gastric cancer cells using *in vitro* and *in vivo* models. The *in vitro* study examined the influence of Britanin on the proliferation and migration of BGC-832 and SGC-7901 gastric cell lines, while the mouse xenograft model involving the BGC-823 allowed for real-time tracking of tumor growth through bioluminescent imaging. Cytotoxicity testing indicated IC_{50} values of 4.999 μmol/L for BGC-823 and 2.243 μmol/L for SGC-7901. Treatment with Britanin was associated with alterations in the nuclear factor kappa B (NF-κB) pathway which reduced the proliferation of gastric cancer cells. It also resulted in elevated interleukin-2 levels (activator of Natural Killer cells, B-cells, CD4+ and CD8+ T-cells) and decreased interleukin-10 levels (CD4+ T-cell inactivator), thus promoting the immune response and inhibiting cancer cell development[20].

A study by Li *et al*[21] found Britanin to have similar effects on hepatocellular carcinoma. The cytotoxicity and anti-tumor effects were studied on HepG2 and BEL-7402 cell lines *in vitro* and a subcutaneous BEL-7402 tumor model in mice *in vivo*. The IC_{50} values were found to be 2.702 μmol/L in the BEL-7402 and 6.006 μmol/L in the HepG2 cells. Colony formation assay, transwell migration, and tumor size measurements showed that Britanin possesses a reliable anti-tumor effect. Additionally, Western Blotting indicated that Britanin inhibited p65 protein and modulated the BCL-2/BAX ratio [21].

The effect of Britanin from Inula linearifolia on pancreatic cancer was examined by Li *et al*[22]. The anti-tumor effects were determined *in vitro* on three pancreatic cancer cell lines: PANC-1, MIA CaPa-2, and BxPC-3. Respective IC_{50} values equaled 1.348, 3.104, and 3.367 μmol/L. PANC-1 was utilized to establish a murine xenograft model. Britanin exhibited very low toxicity *in vivo* and excellent inhibitory effects against pancreatic cancer *in vivo* and *in vitro*. The compound diminished cell proliferation and migration by inhibiting the p50-p65/NF-κB pathway. The authors suggest that, due to its very low toxicity, Britanin could be safer for use than small molecule inhibitors[22].

In 2021, Zhang *et al*[23] investigated the potential of Britanin in cancer immunotherapy, specifically its impact on the Programmed death receptor 1 and ligand 1 (PD-1/PD-L1) immune pathway. The study used Hep3B liver cancer cells and HCT116 colorectal cancer cells, with the latter utilized to establish a mouse xenograft model. It was found that Britanin maintains the activity of T-cells and reduces proliferation and angiogenesis by inhibiting PD-L1 transcription; this was achieved by interrupting the Ras/RAF/MEK/ERK pathway and mTOR/P70S6K/4EBP1 signaling, ultimately affecting communication between myelocytomatosis oncogene (Myc) and hypoxia-inducible factor 1α (HIF-1α). Moreover, molecular docking data revealed that Britanin interacts with PD-L1, HIF-1α, and Myc[23]. A later docking analysis of Britanin, and fifteen of its analogues, to the PD-L1 protein was used in the design of novel molecules based on the structure of pseudoguaianolide-type sesquiterpene lactones[4].

The most recent gastrointestinal study was conducted by Abdolmohammadi *et al*[24] who evaluated the mode of action of Britanin from Inula aucheriana in gastric cancer. Growth inhibition and apoptosis induction were noticed in AGS and MKN45 cell lines, where Britanin suppressed the NF-κB pathway by increasing the mRNA and protein levels of peroxisome proliferator-activated receptor-γ (PPARγ). Upregulation of BAX and downregulation of BCL-2, matrix metalloproteinase-9 (MMP-9), Twist family bHLH transcription factor 1 (TWIST-1), and cyclooxygenase-2 (COX-2) were also noted. The authors concluded that Britanin is an encouraging anti-cancer agent that still requires further examination [24].

The main biological and molecular findings from the above studies are briefly summarized in Figure 1, whereas available IC_{50} values are collected in Table 1. It is worth recapitulating a few aspects that make Britanin a promising anti-cancer agent. Above data certify that the compound exhibits pleiotropic biological activity, providing a multimodal approach against gastrointestinal tumors. Combining these properties with the impact of Britanin on the PD-1/PD-L1 pathway[4,23], it seems that the compound might be valuable for both chemotherapeutic and immunotherapeutic settings. Moreover, available studies report that the cytotoxic effect of Britanin on noncancerous cells is significantly lower than that on tumor cells, while still being effective against the latter[18,19,22]. Ultimately, it has been suggested that Britanin could be safer than small molecule inhibitors[22], which are currently used for targeting gastrointestinal tumors [25,26].

FUTURE PROSPECTS

A wealth of data on the effect of Britanin has been obtained from studies other than those associated with liver, colorectal, pancreatic, and gastric cancer. Such information may suggest the direction of further research on gastrointestinal tumors.

Table 1 Efficacy of Britanin from various plant sources in inhibiting gastrointestinal cancer cell lines

Gastrointestinal tumor	Cell line	Source of Britanin	IC_{50} (μmol/L)	Ref.
Liver cancer	HuH-7	*Inula aucheriana*	27.86 ± 1.35[3]	Cui *et al*[19]
Liver cancer	SMMC-7721	*Inula aucheriana*	28.92 ± 1.09[3]	Cui *et al*[19]
Liver cancer	HepG2	*Inula aucheriana*	15.69 ± 1.58[3]	Cui *et al*[19]
Liver cancer	HepG2	*Inula aucheriana*	6.004[1,4]	Moghadam *et al*[14]
Liver cancer	HepG2	*Inula japonica*	35.5[5]	Piao *et al*[17]
Liver cancer	HepG2	*Unspecified*[2]	6.006[5]	Li *et al*[21]
Liver cancer	BEL-7402	*Unspecified*[2]	2.702[5]	Li *et al*[21]
Colorectal cancer	HT-29	*Inula japonica*	3.9[5]	Piao *et al*[17]
Pancreatic cancer	MIA CaPa-2	*Inula linearifolia*	3.104[4]	Li *et al*[22]
Pancreatic cancer	BxPC-3	*Inula linearifolia*	3.367[4]	Li *et al*[22]
Pancreatic cancer	PANC-1	*Inula linearifolia*	1.348[4]	Li *et al*[22]
Pancreatic cancer	PANC-1	*Inula aucheriana*	40 ± 5.63[3]	Moeinifard *et al*[18]
Pancreatic cancer	AsPC-1	*Inula aucheriana*	30 ± 4.61[3]	Moeinifard *et al*[18]
Gastric cancer	BGC-832	*Inula japonica*	4.999[4]	Shi *et al*[20]
Gastric cancer	SGC-7901	*Inula japonica*	2.243[4]	Shi *et al*[20]

[1]Recalculated from μg/mL to μmol/L to standardize the unit (molecular weight of Britanin, *i.e.*, 366.4 g/mol, was acquired from PubChem 2.1).
[2]Unspecified Britanin source (non-open access paper with no data in abstract).
[3]24-h incubation time with Britanin.
[4]72-h incubation time with Britanin.
[5]Unspecified incubation time with Britanin (non-open access paper or no data).
IC_{50}: Half-maximal inhibitory concentration.

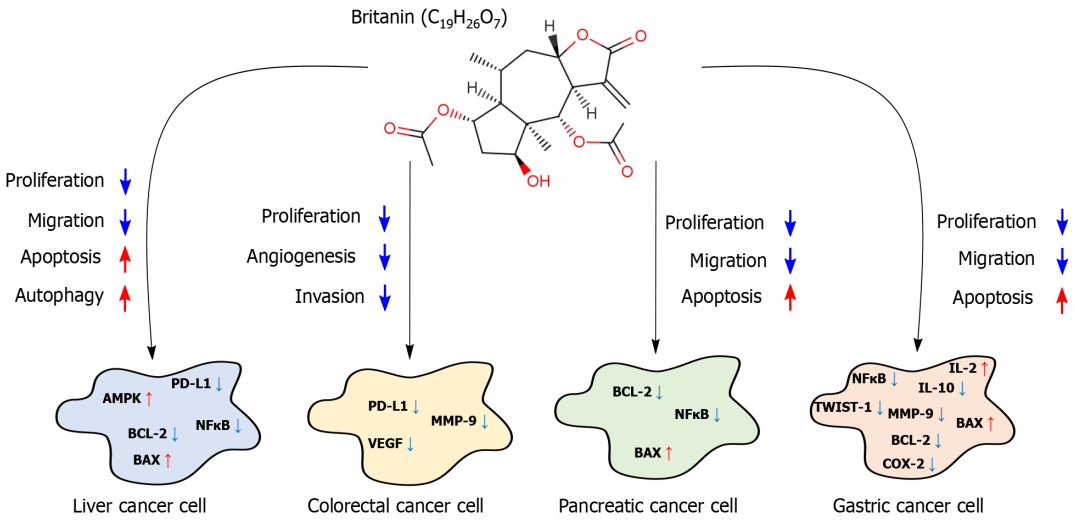

Figure 1 Influence of Britanin on biological processes and related proteins in gastrointestinal tumors. A red upward pointing arrow ("↑") indicates biological process activation by Britanin, whereas a blue downward pointing arrow ("↓") signifies biological process inhibition by the same compound. Similar applies to the level of proteins, the symbols of which are located in four multicolored areas representing liver, colorectal, pancreatic, and gastric cancer cells.

Firstly, Hajimehdipoor *et al*[27] discovered that three sesquiterpene lactones extracted from Inula aucheriana hold promise as inhibitors of acetylcholinesterase (AChE). While the research was primarily focused on Alzheimer's disease, Britanin emerged as the second most potent inhibitor of AChE, exhibiting 25.2% inhibitory activity at a concentration of 300 μg/mL. The researchers suggest that altering the structure of Britanin could enhance its AChE inhibitory potential and reduce its cytotoxicity[27]. This could be of value in cancer treatment, as the cholinergic system and AChE activity are known to play important roles in tumor development and microenvironmental alterations[28]. Modifying the structure of Britanin to reduce cytotoxicity is noteworthy since gastrointestinal toxicity remains a common complication

of cytotoxic anti-cancer chemotherapy[29].

Secondly, gastrointestinal research on Britanin should be directed at mast cells, which appear to play pro-tumorigenic and anti-tumorigenic roles[30]. Lu *et al*[31] assessed the anti-allergic activity of an Inula japonica extract *in vivo* and investigated its mode of action on mast cells *in vitro*. Britanin was found to be one of the most abundant sesquiterpenes. The extract attenuated the mast cell-mediated passive cutaneous anaphylaxis reaction and exhibited an anti-allergic effect by modulating eicosanoid generation and degranulation *in vitro*[31]. Park *et al*[32] found Inula japonica-derived Britanin to ameliorate mast cell-mediated pro-inflammatory responses, which they attributed to NF-κB activation. Similarly, Lu *et al*[33] found the mast cell-suppressing ability of Britanin to be associated with the inhibition of the spleen tyrosine kinase (Syk) pathway *via* Syk protein dephosphorylation, as well as deactivation of NF-κB and mitogen-activated protein kinases.

It has been observed that mast cell density appears to correlate with angiogenesis and progression in patients with gastric carcinoma[34]. Moreover, mast cells were found to be abundant in gastric cancer, which shorten patient survival [35]. The latter study also revealed that cancer-derived tumor necrosis factor alpha induces PD-L1 overexpression in mast cells *via* activation of the NF-κB signaling pathway. PD-L1+ mast cells suppressed T-cell growth and function in a PD-L1-dependent manner. Given that Britanin is associated with NF-κB, PD-L1, and T-cells, future gastrointestinal research should include Britanin and mast cells.

Thirdly, Britanin has been found to inhibit osteoclastogenesis and osteolysis. The compound inhibited osteoclast differentiation by downregulation of B lymphocyte-induced maturation protein 1 and nuclear factor of activated T cells 1 *in vitro*, as well as protected bone from titanium-induced calvarial osteolysis *in vivo*[36]. Although osteolysis is a complication among patients carrying titanium-based implants after long-term usage[37], it also occurs as an outcome of bone metastasis in colorectal cancer. The mechanism by which colorectal cancer cells influence the differentiation of bone marrow-derived monocytes into osteoclasts has been described previously[38]. However, further studies are needed to confirm whether Britanin can prevent metastasis of colorectal cancer while also counteracting the tumor itself.

Lastly, Britanin was found to relieve ischemic injury, a phenomenon characterized by tissue damage due to the lack of perfusion and oxygenation. Although a higher risk of hypoxia is typically associated with organ transplantation, the tumor microenvironment is similar to ischemic tissue in this regard[39]. Outside the gastrointestinal context, Britanin was found to ameliorate cerebral and myocardial ischemia *via* pathways incorporating the nuclear factor erythroid 2-related factor 2, which is one of the most important defenders against oxidative stress[40,41]. Thus, Britanin might be an important protector against negative outcomes of oxidative stress, to which rapidly dividing cells of colonic mucosa are steadily exposed[42]. Moreover, subsequent research on gastrointestinal tumors is necessary, since ischemia mediates metastasis in liver, pancreatic, and colon cancer[43-45].

The novel research directions which could be adopted in future gastrointestinal research on Britanin are recapitulated in Figure 2. Regardless of the topic, any studies of the relationship between Britanin and its influence on signaling pathways or the proteome should be supported by molecular docking. Existing data indicates that Britanin interacts with such essential proteins as NF-κB, PD-L1, Myc, and HIF-1α[4,12,23,46], and it may also influence other important proteins and pathways, such as BCL-2, BAX, AMPK, MMP-9, TWIST-1, COX-2, or PPARγ.

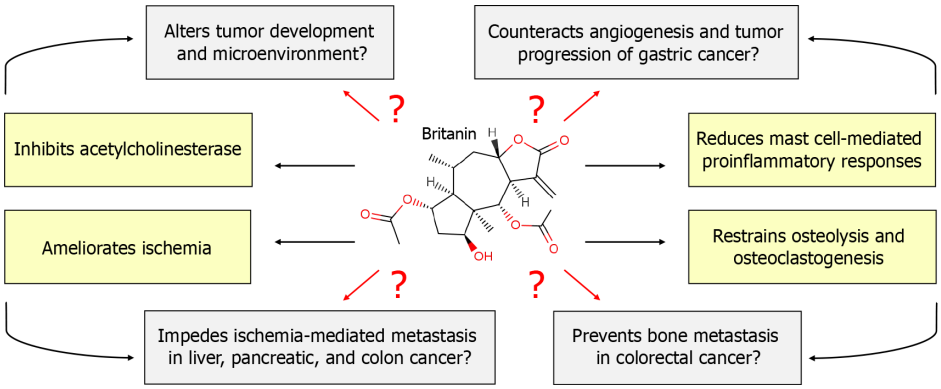

Figure 2 Novel research directions which could be adopted in future gastrointestinal research on Britanin. The light-yellow rectangles represent data on Britanin obtained from studies other than those associated with liver, colorectal, pancreatic, and gastric cancer. Processes included therein are linked to various tumor-related phenomena, which are depicted in gray rectangles. Britanin was not yet investigated in these tumor-related phenomena, which was marked with solid red arrows and question marks ("?"). Such information may suggest the direction of further research on gastrointestinal tumors.

CONCLUSION

Britanin is a natural compound that counteracts liver, colorectal, pancreatic, and gastric tumors by regulating proliferation, apoptosis, autophagy, immune response, migration, and angiogenesis. Its cytotoxicity on noncancerous cells is significantly lower than that on tumor cells, while still being effective against the latter, warranting further in-depth studies based on animal models. The ability to reduce the cytotoxicity of Britanin *via* structural modification may be useful in limiting gastrointestinal toxicity after cytotoxic anti-cancer chemotherapy. Outside the chemotherapeutic context, Britanin might also be valuable in an immunotherapeutic setting since it affects the PD-1/PD-L1 pathway. The

compound acts against negative outcomes of oxidative stress, to which rapidly dividing cells of colonic mucosa are steadily exposed. Given the pleiotropic biological activity, Britanin ensures a multimodal approach against gastrointestinal tumors, which may provide additional treatment options or reduce the high mortality rate. However, it has yet to be included in clinical trials as no data on its use exists in the National Institutes of Health. Future research should incorporate molecular docking simulations and focus on the link between Britanin and acetylcholinesterase, mast cells, osteolysis, and ischemia, as considerable data on its potential already exists outside the gastrointestinal context.

FOOTNOTES

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MINIREVIEWS

Molecular targets and mechanisms of different aberrant alternative splicing in metastatic liver cancer

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Abstract

Metastasis remains a major challenge in the successful management of malignant diseases. The liver is a major site of metastatic disease and a leading cause of death from gastrointestinal malignancies such as colon, stomach, and pancreatic cancers, as well as melanoma, breast cancer, and sarcoma. As an important factor that influences the development of metastatic liver cancer, alternative splicing drives the diversity of RNA transcripts and protein subtypes, which may provide potential to broaden the target space. In particular, the dysfunction of splicing factors and abnormal expression of splicing variants are associated with the occurrence, progression, aggressiveness, and drug resistance of cancers caused by the selective splicing of specific genes. This review is the first to provide a detailed summary of the normal splicing process and alterations that occur during metastatic liver cancer. It will cover the role of alternative splicing in the mechanisms of metastatic liver cancer by examining splicing factor changes, abnormal splicing, and the contribution of hypoxia to these changes during metastasis.

Key Words: Alternative splicing; Carcinoma; Hepatocellular; Metastasic; Liver neoplasms; Prognosis

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Core Tip: Metastatic liver cancer refers to tumors formed outside the liver that metastasize to the liver and colonize it. Abnormal alternative splicing is a molecular characteristic unique to almost all tumor types. Most tumors exhibit a wide range of splicing abnormalities compared to the surrounding healthy tissues. This review is the first to provide a detailed summary of the normal splicing process and alterations that occur during metastatic liver cancer by examining splicing factor changes, abnormal splicing, and the contribution of hypoxia to cellular changes.

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INTRODUCTION

Primary liver cancer, also known as hepatocellular carcinoma (HCC), originates in the liver and is often associated with chronic liver diseases such as cirrhosis, infections with hepatitis B or C viruses, or alcohol-related liver disease[1]. Conversely, metastatic liver cancer refers to tumors formed outside the liver that metastasize to the liver and colonize it. Owing to the dual blood supply from the hepatic artery and portal vein, the liver has become the most common parenchymal organ to which most malignant tumors metastasize[2]. Metastatic cancer has become a major clinical challenge because of its high incidence and poor prognosis. Metastatic liver cancer, or liver metastasis, is caused by the spread of cancer cells from other primary sites (such as the colon, rectum, stomach, and breast) to the live[3-6]. The prognosis of patients with liver metastasis varies depending on the type of primary cancer. Liver metastasis in some cancers, such as lung cancer, is associated with a poor prognosis[7].

Currently, the treatment of metastatic liver cancer is completely different from that of the primary cancer. Although tumors grow in the liver, the biological activity of metastatic liver cancer is different from that of tumors at the primary site, and liver metastasis has the characteristics of multifocal and late-stage diseases[7]. The treatment of metastatic liver cancer usually involves systemic treatments such as chemotherapy and targeted therapy[3-6]. Therefore, initially determining the organ or tissue source of the primary cancer is necessary (to obtain pathology findings) and then use systemic treatment (choosing a plan based on the pathology of the primary cancer) and local liver resection, including surgical, ablation, and systemic treatment methods[7-10]. The combination of minimally invasive image-guided therapies, such as radioembolization and percutaneous liver-guided therapy, has expanded the treatment options for patients with obvious metastatic liver disease. However, further research is required to optimize the timing and safety of combining systemic and local regional therapies. Metastatic liver cancer presents a complex clinical environment with different primary cancer origins, prognostic impacts, and challenges in accurate diagnosis and management. Understanding the metastatic patterns, prognostic factors, and immune microenvironments of liver metastases is crucial for developing effective treatment strategies and improving patient prognosis[11-13].

Abnormal alternative splicing (AS) is a molecular characteristic unique to almost all tumor types[14]. Most tumors exhibit a wide range of splicing abnormalities compared to the surrounding healthy tissues, including frequent retention of normally excised introns, inappropriate expression of isoforms that are typically limited to other cell types or developmental stages, splicing errors that damage tumor suppressor genes or promote oncogenic gene expression, and promotion of tumor development through various mechanisms, including increased cell proliferation, reduced apoptosis, enhanced migration and metastasis, drug-resistant chemotherapy, and evasion of immune monitoring[15,16]. Metastatic liver cancer undergoes significant changes over time. In cancer cells derived from the liver and bile ducts, abnormal proteins are synthesized due to abnormal splicing associated with cancer. This leads to the dysproliferation of these cells, ultimately transforming them into invasive, migratory, and multidrug-resistant phenotypes, resulting in a poor prognosis for these liver cancers[8,17,18].

In this review, we highlight the recent developments in AS events. We will also describe the regulation of AS in primary and metastatic liver cancers. In addition, this review integrates the biological functions of AS and splicing products as well as current efforts to develop their potential for clinical application in the diagnosis or treatment of cancer.

ALTERNATE SPLICING

Some genes have one mRNA precursor that produces different mRNA splicing isomers using different splicing methods (choosing different splicing sites) in a process known as variable splicing (or AS)[14,15,18-20]. Variable splicing is the most common and widespread type of splicing[14]. Variable splicing is an important mechanism for regulating gene expression and generating proteomic diversity and is an important reason for the large differences in the number of genes and proteins in eukaryotes[21]. In vivo, there are seven types of variable splicing: (1) Exon skip; (2) Retained intron; (3) Alternate Donor site; (4) Alternate acceptor site; (5) Alternate promoter; (6) Alternate terminator; and (7) Mutually exclusive exons[14,15,17] (Figure 1). During variable splicing, the different exons of a gene sequence are selectively linked to form multiple transcripts. Consequently, the same gene can encode many different proteins, thereby increasing the

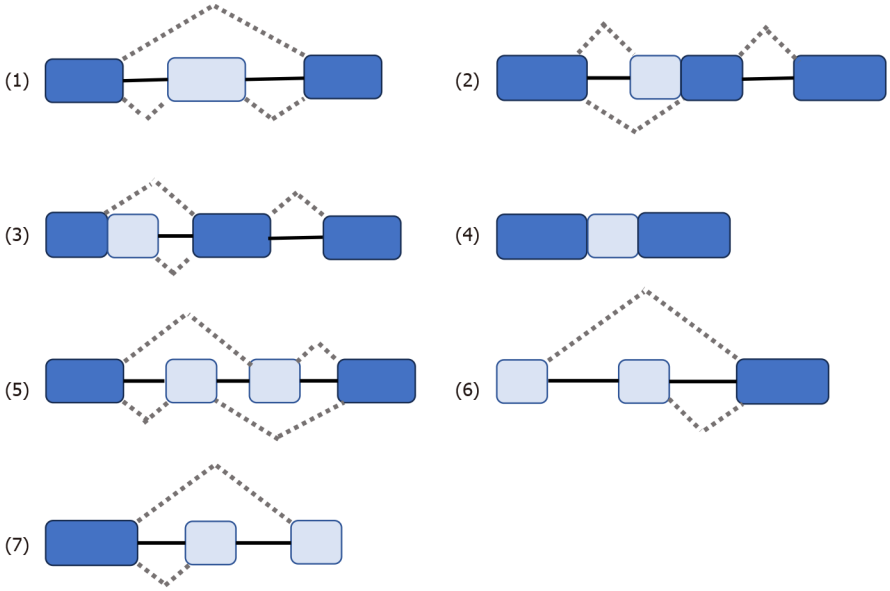

Figure 1 Seven types of variable splicing *in vivo*. (1) ES: Exon skip; (2) RI: Retained intron; (3) AD: Alternate Donor site; (4) AA: Alternate acceptor site; (5) AP: Alternate promoter; (6) AT: Alternate terminator; (7) ME: Mutually exclusive exons.

functional diversity of the gene. This type of splicing is very common in mammals and is found in more than 90% of genes[22]. The development of metastatic cancer is influenced by multifactorial conditions, possibly due to: (1) Altered expression of the spliceosome; (2) mutations affecting genes encoding spliceosome components and related regulatory proteins; (3) disruption of splice site or splicing regulatory sites (enhancers or silencers); and (4) impaired signaling pathways involved in the regulation of splicing mechanisms[17,23,24].

ALTERNATE SPLICING IN PRIMARY LIVER CANCER

Primary HCC tumor tissue exhibits a high degree of differential splicing compared to normal liver tissue. A growing body of research has shown that alterations in the splicing program in HCC tumor cells generate novel protein subtypes that often have different and sometimes opposite functions to their classical counterparts[25]. These changes were significantly associated with patient survival[12]. These findings suggest that AS plays a crucial role in HCC progression and prognosis. Primary liver cancer includes various tumors such as HCC and intrahepatic cholangiocarcinoma (iCCA) [26]. One study showed that differences between HCC and iCCA AS affected hundreds of genes[19]. Thus, alternative and tumor-specific subtypes caused by abnormal splicing are common during liver tumorigenesis[21,27].

AS disorder is also associated with the pathogenesis of liver cancer. For example: Loss of SRSF3 induces *IGF2* expression and altering *INSR* splicing to allow insulin-like growth factor II (IGF2) signaling to be conducted through insulin receptor (IR)-A in hepatocytes[28]. Hepatic *IGF2* expression is a carcinogenic driver in aging-related HCC mouse models, causing DNA damage and supporting hepatocyte proliferation. This allowed for the accumulation of somatic mutations. EGFR regulates the selective splicing of IR pre-mRNA in HCC cells. After ligand binding, EGFR activation triggers an intracellular signaling cascade, which implies MEK activation. This stimulates the transcription of genes encoding different splicing factors, namely *CUGBP1*, *hnRNPH*, *hnRNPA1*, *hnRNPA2B1*, and *SF2/ASF*. *hnRNPF* expression is not regulated by the EGFR-dependent pathway. Interaction between splicing factors and IR pre-mRNA promotes the selective splicing of IR exon 11. Consequently, the expression of the IR-A subtype increases to the detriment of IR-B, which allows for the transmission of proliferative signals in response to insulin and IGF2, leading to HCC development [29].

In summary, the dysregulation of AS in liver cancer has been shown to affect various molecular pathways, underscoring the influence of AS dysregulation on the molecular mechanisms of liver cancer development and its extensive involvement in the pathogenesis, progression, and prognosis of HCC. The replacement of gene products produced by abnormal splicing has been linked to positive effects in cancer, making AS a potential target for gene therapy[30]. These findings suggest that understanding AS in liver cancer may lead to the development of novel therapeutic interventions.

METASTASIS MECHANISMS IN METASTATIC LIVER CANCER

Pathogenesis of metastatic liver cancer involves a complex interplay of molecular mechanisms, including the role of splicing factors in cancer progression, AS, and hypoxia-induced splicing changes[31].

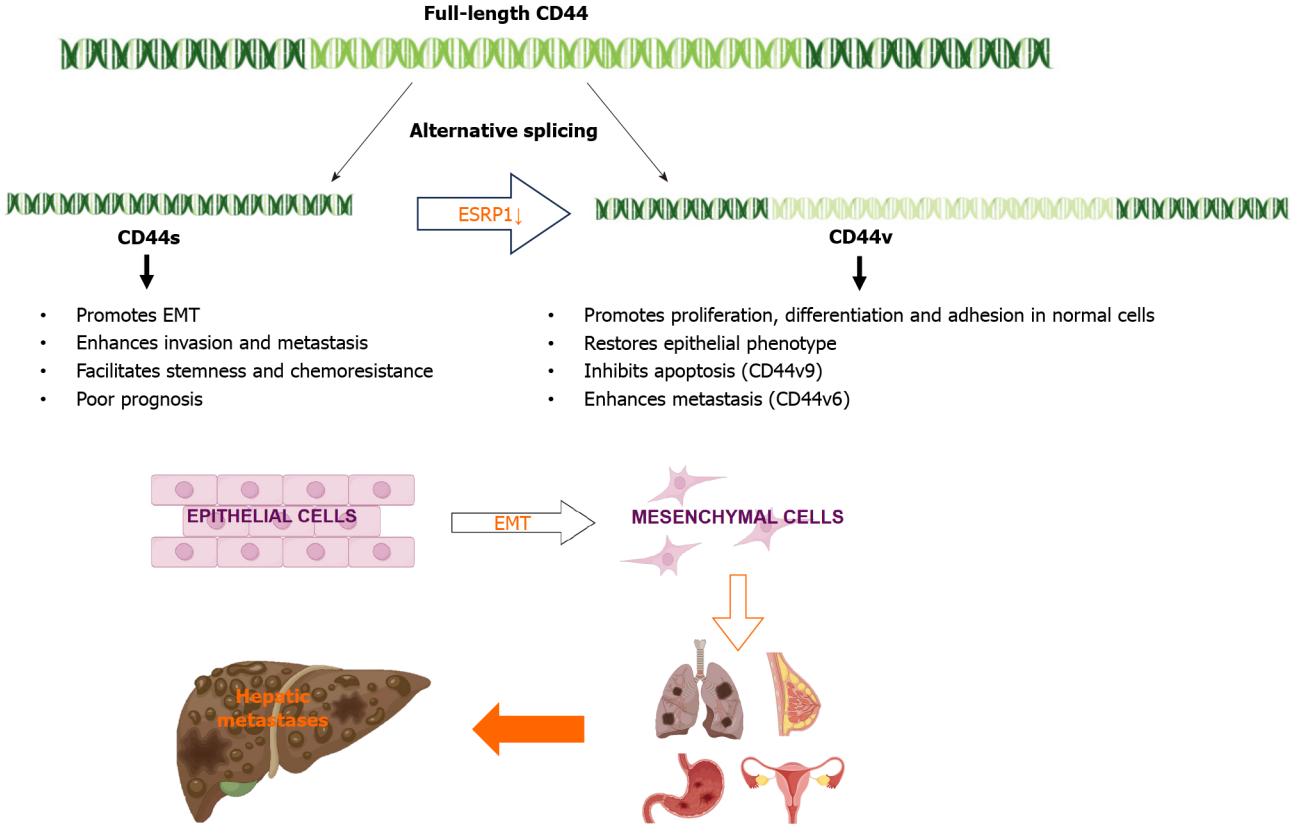

Figure 2 During Epithelial-mesenchymal transition, epithelial splicing regulatory protein 1 expression is reduced, promoting the transition from variant CD44 to standard CD44, and can promote liver metastasis of lung, breast, stomach, and ovarian cancers. CD44s: Standard CD44; CD44v: Variant CD44; ESRP1: Epithelial splicing regulatory protein 1; EMT: Epithelial-mesenchymal transition.

Splicing factor changes in metastasis liver cancer

The role of splicing factors in metastatic liver cancer has attracted increasing interest in cancer research. Splicing factors, including those in metastatic liver cancer, play a direct role in cancer development[32]. Abnormal RNA splicing has been recognized as a driver of cancer development and changes in AS of RNA have been associated with liver cancer progression[26]. In addition, alterations in splicing are associated with liver cancer markers, including de-differentiation and genomic instability, which are the core processes of tumor transformation[16].

In liver cancer, the AS of specific genes has been shown to contribute to cancer progression and metastasis. For example, epithelial splicing regulatory protein 1 (ESRP1) plays a key role in the regulation of *CD44* AS[33]. ESRP1 is an epithelium-specific splicing factor that regulates the AS of several genes, including fibroblast growth factor receptor 2 and *CD44*[34]. Epithelial-mesenchymal transition (EMT) is a specific biological process in which epithelial cells are transformed into stromal cells. It is important for epithelial cell-derived malignant tumor cells to acquire migration and invasion abilities. EMT plays a crucial part in embryonic development, chronic inflammation, tissue reconstruction, cancer metastasis, and various fibrotic diseases[35]. The main characteristics of EMT include reduced expression of cell adhesion molecules (such as E-cadherin), transformation of the cytoskeleton from keratin to vimentin, and altered morphological characteristics of mesenchymal cells. Through EMT, epithelial cells lose cell polarity, their connection to the basement membrane, and other epithelial phenotypes, while gaining higher interstitial phenotypes, such as migration and invasion, apoptosis inhibition, and degradation of the extracellular matrix[36]. During EMT, *ESRP1* expression is sharply reduced, facilitating the transition from variant CD44 (CD44v) to standard CD44 (CD44s), mediating the expression of isoforms required for EMT. ESRP1 promotes liver metastasis in breast cancer cells by enhancing EMT[34]. ESRP1 regulates subtype conversion and determines gastric cancer metastasis[37]. ESRP1 drives AS of *CD44*, thereby enhancing invasion and migration of epithelial ovarian cancer cells[38]. ESRP1 has been identified as a favorable prognostic factor for pancreatic cancer[39], alleviating pancreatic metastasis. In contrast, the silencing of *ESRP1* has been shown to drive the malignant transformation of human lung epithelial cells[40], suggesting that cancer progression is strongly influenced by splicing factors (Figure 2).

Abnormal splicing in metastatic liver cancer

AS events have been identified as prognostic factors for HCC, highlighting their potential impact on the development and prognosis of liver cancer[41]. Various AS events may also influence the development of metastatic liver cancer. Studies have shown that abnormal AS events promote malignant cancer progression[42].

In 2015, a team found that PC-3 and its derived cell lines crossed the transfer barrier *in vitro* and in vivo, providing an excellent, unbiased system for comprehensively characterizing AS events and identifying the key splicing factors that

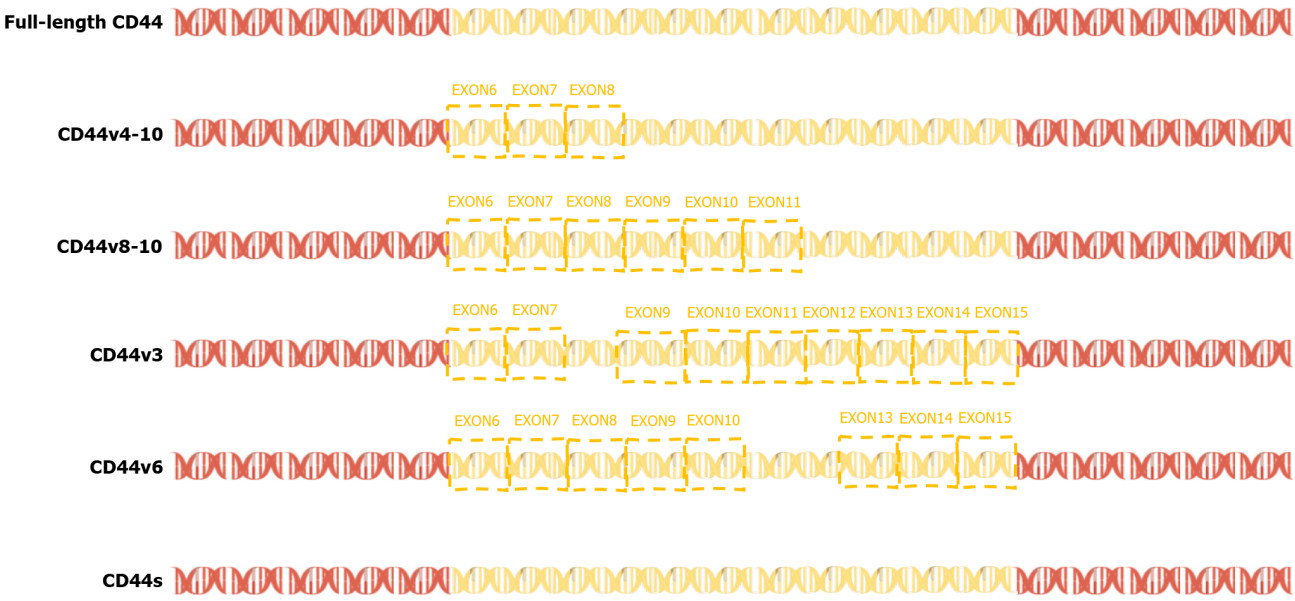

Figure 3 Exons 6-14 of CD44 gene undergo alternative splicing in the membrane-proximal stem region, resulting in a variety of variable splicing variants (CD44 variant isoform, variant CD44; Including CD44v2-v10). CD44s: Standard CD44; CD44v: Variant CD44.

influence the splicing regulation of transfer. This suggests that partially selective splicing events are associated with metastatic colonization of cancer cells, suggesting a potential role in promoting metastasis[43]. Liver metastasis may occur in different tumors, and different splicing events may promote liver metastasis. For example, the splicing mediated by RBFOX2 shifts from an epithelial-specific event to a mesenchymal-specific event, leading to a higher degree of tissue invasion, which in turn leads to liver metastasis[44]. Different splicing subtypes of the same spliceosome, such as CD44, promote liver metastasis. CD44 is a cell surface glycoprotein involved in cancer progression and metastasis. AS of CD44 mRNA produces various subtypes, including CD44s and CD44v (Figure 3), which are associated with cancer metastasis. The CD44-ZEB1-ESRP1 feedback loop can control the cell phenotype and prognosis of patients with cancer by determining the CD44 subtype expression[45]. Some splicing events that lead to liver cancer metastasis, such as the overexpression of IGF2 and decreased splicing activity of SRSF3, are considered major causes of DNA damage and drivers of liver cancer, indicating the importance of specific splicing factors in liver cancer development. While affecting the process of liver metastasis, it also affects changes in tumor drug resistance. The FUS/circEZH2/KLF5 feedback loop promotes liver metastasis of breast cancer by FUS promoting the reverse splicing process of circEZH2 by binding to the 3′-lateral intron portion of pre-EZH2 to enhance the EMT, and may also influence drug resistance of liver metastases through this mechanism[46]. In summary, AS events are involved in the occurrence and development of metastatic liver cancer, highlighting the importance of splicing regulation in cancer progression and metastasis.

Hypoxia-induced splicing changes in metastasis liver cancer

Hypoxia is associated with changes in EMT, angiogenesis, local tissue invasion, endothelium, exocytosis, and pre-metastatic niche formation[47]. Hypoxia, a hallmark of the tumor microenvironment, induces AS, thereby promoting the invasive behavior of cancer cells[31]. Hypoxia inhibits cancer cell differentiation and promotes cancer cell invasion and metastasis, emphasizing its role in promoting cancer cell metastasis[48]. Hypoxia-induced splicing changes play a crucial role in the occurrence and progression of cancer metastasis. Hypoxia-induced selective splicing is cell type-specific and has highly conserved universal target genes, indicating that hypoxia has a broad impact on splicing[49]. In the DNA damage response, hypoxia drives the selective splicing of genes towards non-coding subtypes by increasing intron retention[50]. Similarly, hypoxia promotes the expression of splicing subtypes of Myc-related factor X in endothelial cells, mediated by nonsense decay degradation, and another splicing subtype that encodes unstable proteins[51].

Hypoxia leads to significant changes in the selective splicing of prostate cancer cells and increased expression of CLK splicing factor kinase, leading to liver metastasis[52]. In addition, hypoxia regulates *CD44* and its variant subtypes through HIF-1α in triple-negative breast cancer, highlighting the role of hypoxia in regulating various splicing events associated with cancer progression[48].

The effect of hypoxia on AS has been recognized as a powerful driving force for tumor pathogenesis and progression, and various studies have emphasized the important influence of hypoxia-induced splicing changes on the pathogenesis of metastatic liver cancer. Understanding the molecular mechanisms underlying hypoxia-induced splicing changes is essential for developing targeted therapeutic strategies to mitigate the invasive behavior of metastatic liver cancer cells and improve patient outcomes.

DISCUSSION

The liver has a rich blood supply, so it provides fertile "soil" for metastasis to spread[53]. The liver is one of the largest blood vessel networks in the body. It receives blood from the gut, which contains a lot of nutrients. The blood vessels at the end of the liver also have high pressure, so it is easier to accommodate and colonize metastasized cancer cells[54]. The most common source of metastatic liver cancer is colorectal cancer, followed by pancreatic, breast, melanoma and lung cancer. The common ways of metastasis include direct invasion, lymphatic metastasis and blood-derived metastasis. Malignant tumors that directly invade the organs and tissues around the liver, such as gastric cancer, gallbladder cancer, pancreatic cancer, colon cancer and duodenal cancer. Lymphatic metastasis is more common in digestive system malignancies, pelvic or retroperitoneal malignancies, breast cancer, lung cancer and gallbladder cancer. Hematogenous metastasis can also be further subdivided into hepatic artery and portal vein metastasis. Any tumor cells entering the liver through these vessels can cause liver metastasis, such as esophageal and gastrointestinal tumors and some sarcomatoid tumors with higher malignant degree.

Metastatic liver cancer presents significant clinical challenges owing to its aggressiveness, poor prognosis, and limited treatment options. Studies based on the SEER database emphasize that patients with primary extrahepatic metastases have poor prognosis[55]. In 2020, a practical study of high-intensity focused ultrasound ablation in 250 patients, including a primary liver cancer cohort (n = 80) and metastatic liver cancer cohort (n = 195), yielded 1-year survival rates of 70.69% and 48.00%, respectively[56]. These findings highlight the need for innovative therapeutic modalities to address the adverse effects of metastatic liver cancer. Metastatic liver cancer is a serious detrimental condition. Addressing the challenges associated with metastatic liver cancer requires a comprehensive understanding of its harmful nature and development of targeted treatment strategies.

Mechanisms underlying liver cancer metastasis are complex. In 2012, Biamonti *et al*[57] explored the role of AS in EMT, elucidating the link between AS and the invasive abilities of cancer cells. Understanding the effects of AS on EMT is critical for elucidating the underlying mechanisms of cancer metastasis and drug resistance. Next, a systematic review of liver transplantation in patients with liver metastases from neuroendocrine tumors highlighted the challenges posed by high recurrence rates, underscoring the need for precise patient selection and new treatment strategies[58]. Breakthroughs in the understanding of variable splicing in metastatic liver cancer have the potential to revolutionize cancer treatment. A comprehensive analysis of tumor AS in 8705 patients showed that tumors had 30% more AS events than normal samples[59]. This highlights the importance of AS in cancer, including metastatic cancer, and illustrates the potential of targeting splicing events for therapeutic interventions. In addition, the 2021 study by Fish *et al*[60] identified a previously unknown structural splicing enhancer rich in near-box exons with increased inclusions in highly metastatic cells. These findings provide valuable insights into the molecular mechanisms of metastasis and offer potential targets for the suppression of cancer metastasis. Subsequently, 2022 revealed an FUS/circEZH2/KLF5 feedback loop that promoted liver metastasis of cancer by enhancing EMT[46]. These findings provide insights into the molecular pathways of liver metastasis and a potential target for therapeutic intervention.

Metastatic liver cancer is a complex and multifaceted disease, and AS has been identified as a key factor in its progression. Several themes regarding the role of AS in metastatic liver cancer have emerged in the literature. AS is associated with EMT, a key process in cancer metastasis[57]. In addition, the splicing of specific genes such as *CD44* has been shown to enhance the metastatic potential of cancer cells[61-63]. In addition, regulatory strategies to control AS in cancers, including metastatic liver cancer, remain largely unknown, suggesting gaps in our understanding of the underlying mechanisms[37,60]. In addition, associations between AS and metastatic phenotypes have been studied in various types of cancers, including colorectal and prostate cancers, suggesting that AS has a broader relevance in cancer metastasis[64,65]. Despite these insights, the existing studies of AS for metastatic liver cancer have some shortcomings. The functional mechanisms of AS in cancer, particularly liver metastasis, remain unclear[46,66]. Although a link between AS and cancer metastasis has been established, the specific regulatory procedures governing this process remain unclear [60]. In addition, the literature highlights the disappointing outcomes of liver transplantation for both primary and metastatic liver cancers, suggesting a lack of effective treatment strategies for metastatic liver disease[67]. This finding suggests that further research is needed to develop new treatment options for metastatic liver cancer. However, significant gaps exist in our understanding of the functional mechanisms and regulatory processes involved in AS in metastatic liver cancer. Addressing these gaps is critical for developing effective interventions for this challenging disease. This article is the first review of variable splicing in metastatic liver cancer, with the hope of providing new directions for future research.

CONCLUSION

In recent years, the importance of variable splicing in the development of liver metastases has been increasingly recognized. These breakthroughs underscore the potential of targeting AS events and related molecular pathways to inhibit the development and progression of metastatic cancers. Further research and clinical studies are essential to translate these findings into effective treatments for patients with metastatic liver cancer.

FOOTNOTES

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ORIGINAL ARTICLE

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Chen-Gu Niu, Jing Zhang, Aniket-Vijay Rao, Utsav Joshi, Patrick Okolo

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Abstract

BACKGROUND

Immunotherapy have demonstrated promising outcomes in patients with high microsatellite instability (MSI) (MSI-H) metastatic colorectal cancer. However, the comparative effectiveness of Immunotherapy and chemotherapy for patients with low MSI (MSI-L), and microsatellite stable (MSS) metastatic colorectal cancer remains unclear.

AIM

To investigate immunotherapy *vs* chemotherapy for treatment of MSI-L/MSS metastatic colorectal cancer, and to evaluate the success of immunotherapy against chemotherapy in managing MSI-H metastatic colorectal cancer during a follow-up of 50 months.

METHODS

We conducted a retrospective cohort study using the National Cancer Database (NCDB) to evaluate the overall survival (OS) of patients with metastatic colorectal cancer treated with immunotherapy or chemotherapy. The study population was stratified by MSI status (MSI-H, MSI-L, and MSS). Multivariable Cox proportional hazard models were used to assess the association between treatment modality and OS, adjusting for potential confounders.

RESULTS
A total of 21951 patients with metastatic colorectal cancer were included in the analysis, of which 2358 were MSI-H, and 19593 were MSI-L/MSS. In the MSI-H cohort, immunotherapy treatment (n = 142) was associated with a significantly improved median OS compared to chemotherapy (n = 860). After adjusting for potential confounders, immunotherapy treatment remained significantly associated with better OS in the MSI-H cohort [adjusted hazard ratio (aHR): 0.57, 95% confidence interval (95%CI): 0.43-0.77, P < 0.001]. In the MSS cohort, no significant difference in median OS was observed between immunotherapy treatment and chemotherapy (aHR: 0.94, 95%CI: 0.69-1.29, P = 0.715).

CONCLUSION
In this population-based study using the NCDB, immunotherapy treatment was associated with significantly improved OS compared to chemotherapy in patients with MSI-H metastatic colorectal cancer, but not in those with MSI-L/MSS metastatic colorectal cancer. Further studies are warranted to determine the optimal therapeutic approach for patients with MSI-L/MSS metastatic colorectal cancer.

Key Words: Immunotherapy; Chemotherapy; Metastatic colorectal cancer; Microsatellite instability; National cancer database

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Core Tip: Our population-based study demonstrates that immunotherapy treatment is associated with significantly improved overall survival in patients with high microsatellite instability (MSI-H) metastatic colorectal cancer. However, immunotherapy does not significantly benefit patients with microsatellite stable (MSS) metastatic colorectal cancer. The lower response rates to immunotherapy in MSS tumors can be attributed to the lower tumor mutational burden and reduced immunogenicity compared to MSI-H tumors. These findings indicate that while immunotherapy is a promising treatment for MSI-H colorectal cancer, its efficacy in MSS cases remains uncertain, warranting further investigation to develop targeted therapies for these patients.

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INTRODUCTION

Colorectal cancer is globally recognized as the third most widespread form of cancer and the second leading cause of death due to cancer[1,2]. The 2023 statistics from the American Cancer Society predict that there will be 153020 new cases of colorectal cancer in the United States, with an estimated death count of 52550[3]. The treatment of metastatic colorectal cancer poses a significant difficulty in clinical practice, with an overall 5-year survival rate of just 14%[4]. Conventional frontline therapies for this condition often consist of Fluoropyrimidine-based chemotherapy, complemented by targeted treatments including anti-vascular endothelial growth factor and anti-epidermal growth factor receptor agents[5-8]. A mounting body of evidence suggests that tumors with high microsatellite instability (MSI) (MSI-H) may not be ideally suited to standard chemotherapy treatments[9-11]. MSI-H colorectal cancers, known for their high mutation rate, generate neoantigens that activate the immune system[11]. The KEYNOTE-177 and CheckMate-142 trials have demonstrated that immunotherapy offers significant clinical benefit in the treatment of MSI-H/dMMR metastatic colorectal cancer[12,13]. While immunotherapy has shown enhanced effectiveness in treating metastatic colorectal cancers characterized by MSI-H, it demonstrates limited success in microsatellite stable (MSS) variants, which account for the majority (95%) of these cases[14].

A thorough literature review highlights a significant data gap in immunotherapy application for MSS patients. Consequently, the majority of those with MSS metastatic colorectal cancer have yet to see the benefits of current immunotherapy methods[14]. Meanwhile, large-scale data evaluating the relationship between MSI-H metastatic colorectal cancer and immunotherapy is scarce. Hence, leveraging the National Cancer Data Base (NCDB)—which captures over 70% of new cancer diagnoses in the United States [15]—this research intends to: (1) Investigate immunotherapy *vs* chemotherapy for treatment of MSS colorectal cancer; and (2) Evaluate the success of immunotherapy against chemotherapy in managing MSI-H metastatic colorectal cancer during a follow-up of 50 months.

MATERIALS AND METHODS

Data source and study population

Our research involved a retrospective cohort analysis utilizing the NCDB, a collaborative initiative between the American College of Surgeons and the American Cancer Society, encompassing over 70% of new cancer diagnoses in the United States[16]. Our research entailed a detailed retrospective analysis utilizing the NCDB, focusing on a cohort of adult patients diagnosed with stage IV colorectal adenocarcinoma on 2020. This study encompassed patients identified by primary tumor site codes C18 (malignant neoplasm of the colon) and C20 (malignant neoplasm of the rectum), which are ICD-10 codes. The analysis concentrated on key variables, including gender, age at diagnosis, and tumor size. Tumor size was categorized into two clinically relevant groups: ≤ 20 mm and > 20 mm. Furthermore, patient MSI status was a crucial variable, alongside the initial treatment strategy, categorized into immunotherapy and chemotherapy. Vital status was utilized to determine whether each patient in the study was deceased or alive. The present study was a database analysis using de-identified data; therefore, institutional review board approval was not required for this type of study.

Study population characteristics

In profiling the study population, we gathered demographic information and clinical characteristics. This included age at diagnosis, gender, race, socioeconomic background, and types of healthcare facilities where treatment was administered. The Charlson-Deyo Comorbidity score was employed to evaluate comorbid conditions, with scores truncated to 0, 1, 2, or 3 (for scores ≥ 3). Data regarding treatment modalities, immunotherapy, chemotherapy, and additional supportive treatments, were analyzed with a primary focus on the initial course of therapy.

Outcome of interest

The focus of our research was on the initial systemic therapy administered to patients, divided into two categories: Immunotherapy and chemotherapy, including both single-agent and combination therapies. The primary outcome for evaluation was overall survival (OS), which we defined as the period from the diagnosis of metastatic colorectal cancer until death from any cause or the most recent follow-up. We tracked OS from the point of cancer diagnosis, monitoring up to the occurrence of death or the last recorded follow-up, and calculated both one-year, three-year, and 50 months survival rates. Our methodology and data analysis conformed to the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.

Statistical analysis

All analyses were conducted using Stata version 17.0 (StataCorp, College Station, Texas 77845, United States). We calculated the median follow-up duration, with survival time measured from the date of diagnosis to either death or the last known contact. Descriptive statistics were employed to summarize the baseline characteristics of the patient cohort. The Kaplan-Meier method was used to estimate survival probabilities, and the log-rank test was applied to compare differences between prognostic factors. To assess the impact of various factors on five-year OS, Cox proportional hazards models were utilized. These models generated hazard ratios (HR) along with their 95%CI. Additionally, multivariate analysis was conducted to calculate the adjusted HR (aHR), accounting for variables like race, gender, and age. The proportional hazards assumptions of our models were graphically verified. Furthermore, the accuracy of the American Joint Committee on Cancer sixth edition staging system was evaluated by calculating a concordance index, complete with 95%CIs. All statistical tests were two-sided, with a significance threshold set at $P < 0.05$.

RESULTS

Baseline characteristics of the study cohort

Our comprehensive study analyzed 21951 patients diagnosed with stage IV colorectal cancer, categorized based on MSI status. Within this cohort, 2358 patients were identified as MSI-H, and 19593 as MSS. The treatment breakdown revealed that in the MSI-H group, 142 patients opted for the novel approach of immunotherapy, while a significant portion, 860 patients, underwent conventional chemotherapy. Similarly, in the MSS group, 88 patients received immunotherapy, compared to 8085 who chose chemotherapy. This distinction in treatment choices underscores the evolving landscape of cancer therapeutics. The average follow-up duration for patients receiving immunotherapy in the MSI-H group was 21.91 ± 12.23 months, and 19.83 ± 12.89 months for those receiving chemotherapy. The MSS group had a slightly longer mean follow-up of 18.48 ± 11.37 months for immunotherapy and 20.61 ± 11.71 months for chemotherapy. The median ages in these groups varied, with 77 years and 63 years for MSI-H patients on immunotherapy and chemotherapy, respectively, and 67.5 and 62 years for the MSS cohort, reflecting the demographic diversity of the study population (Table 1).

Survival outcomes based on MSI status

Analyzing the survival outcomes, MSI-H patients who received immunotherapy experienced a pronounced survival benefit with an aHR of 0.57 (95%CI: 0.43-0.77), suggesting a robust response to this treatment modality. This benefit contrasts with the MSS group, where immunotherapy did not provide a significant survival advantage (aHR = 0.94; 95%CI: 0.69-1.29). The one-year survival rates further illustrate this difference: 71.96% for MSS patients on immunotherapy and 76.78% for those on chemotherapy, compared to 76.55% and 69.91% for MSI-H patients, respectively. A similar pattern was observed at the three-year follow-up, with survival rates of 48.06% for immunotherapy and 40.38%

Table 1 Basic characteristics

	Microsatellite instability-high, *n* = 2358		Microsatellite stable, *n* = 19593	
	Immunotherapy, *n* = 142	Chemotherapy, *n* = 860	Immunotherapy, *n* = 88	Chemotherapy, *n* = 8085
Follow up duration (month)				
mean ± SD	21.91 ± 12.23	19.83 ± 12.89	18.48 ± 11.37	20.61 ± 11.71
Median (Range)	22.46 (0.53-48.76)	18.58 (0.26-48.69)	18.88 (0.79-47.31)	30.52 (0-49.97)
Age (yr)				
mean ± SD	72.32 ± 14.70	62.43 ± 14.42	66.10 ± 15.41	61.53 ± 13.38
Median (Range)	77 (27-90)	63 (21-90)	67.5 (27-90)	62 (19-90)
< 65, *n* (%)	34 (23.94)	465 (54.07)	39 (44.32)	4661 (57.65)
≥ 65, *n* (%)	108 (76.06)	395 (45.93)	49 (55.68)	3424 (42.35)
Sex, ***n*** (%)				
Male	52 (36.62)	437 (50.81)	48 (54.55)	4466 (55.24)
Female	90 (63.38)	423 (49.19)	40 (45.45)	3619 (44.76)
Race, *n* (%)				
White	9 (6.34)	47 (5.47)	76 (6.36)	6356 (78.61)
Black	123 (86.62)	679 (78.95)	8 (9.09)	1123 (13.89)
Other	9 (6.34)	125 (14.53)	0	8 (0.10)
Unknown	1 (0.70)	9 (1.05)	4 (4.41)	598 (7.4)
Charlson-Deyo Score, *n* (%)				
0	9 (6.34)	644 (74.88)	65 (73.86)	6039 (74.69)
1	123 (86.62)	132 (15.35)	16 (18.18)	1260 (15.58)
2	9 (6.34)	43 (5.00)	6 (6.82)	400 (4.95)
≥ 3	1 (0.70)	41 (4.77)	1 (1.14)	386 (4.77)
Tumor size, *n* (%)				
≤ 20 mm	107 (75.35)	623 (72.44)	61 (69.32)	5621 (69.52)
> 20 mm	35 (24.65)	237 (27.56)	27 (30.68)	2464 (30.48)
Tumor grade, *n* (%)				
Well differentiated	0	0	0	0
Moderate differentiated	0	0	0	0
Poorly differentiated	0	0	0	0
Unknown	142 (100.00)	860 (100.00)	88 (100.00)	8085 (100.00)

SD: Standard deviation.

for chemotherapy in the MSS group, and 50.96% and 44.35% in the MSI-H group, indicating a more pronounced long-term benefit for immunotherapy in the MSI-H category (Tables 2 and 3). The Kaplan-Meier survival curves for these groups are depicted in Figure 1A (MSS) and Figure 1B (MSI-H).

KRAS mutation and survival

The study also delved into the impact of KRAS mutation status on treatment outcomes. For KRAS wild-type patients, no significant difference in survival was observed between immunotherapy and chemotherapy (HR = 1.16; 95%CI: 0.86-1.56). However, in KRAS mutated patients, a trend toward improved survival was noted with immunotherapy (HR = 0.67; 95%CI: 0.42-1.07), hinting at the potential effectiveness of personalized treatment based on genetic profiles. This trend, though not statistically significant, signals a possible avenue for enhancing patient-specific treatment strategies in the future (Table 4). The corresponding survival curves are shown in Figure 1C (KRAS wild type) and Figure 1D (KRAS mutated type).

Table 2 Comparative analysis of survival outcomes

Survival analysis	Microsatellite instability-high		Microsatellite stable	
Immunotherapy *vs* chemotherapy	Hazard ratio (95%CI)	Adjusted hazard ratio (95%CI)	Hazard ratio (95%CI)	Adjusted hazard ratio (95%CI)
Overall	0.75 (0.57-0.99)	0.57 (0.43-0.77)	1.05 (0.77-1.43)	0.94 (0.69-1.29)
One year	1.32 (0.92-1.92)	1.23 (0.84-1.81)	1.43 (0.95-2.14)	1.37 (0.91-2.06)
Three year	0.74 (0.56-0.98)	0.62 (0.46-0.82)	0.98 (0.72-1.34)	0.88 (0.65-1.21)

Table 3 Comparative analysis of survival rates

Survival rate	Microsatellite stable		Microsatellite instability-high	
	Immunotherapy	Chemotherapy	Immunotherapy	Chemotherapy
1 yr (%)	71.96 (61.14-80.25)	76.78 (75.83-77.70)	76.55 (68.64-82.72)	69.91 (66.65-72.91)
3 yr (%)	48.06 (35.30-58.70)	40.38 (39.01-41.74)	50.96 (39.83-61.04)	44.35 (40.38-48.24)

Table 4 Comparative analysis of survival analysis by KRAS status

Survival analysis	KRAS wild type		KRAS mutated type	
Immunotherapy *vs* chemotherapy	Hazard ratio (95%CI)	Adjusted hazard ratio (95%CI)	Hazard ratio (95%CI)	Adjusted hazard ratio (95%CI)
Overall	1.16 (0.86-1.56)	1.01 (0.75-1.37)	0.67 (0.42-1.07)	0.70 (0.44-1.12)
One year	1.28 (0.88-1.87)	1.14 (0.78-1.68)	1.33 (0.71-2.49)	1.33 (0.71-2.50)
Three year	1.17 (0.87-1.58)	1.02 (0.76-1.37)	0.66 (0.41-1.07)	0.68 (0.42-1.09)

DISCUSSION

In our study, utilizing data from the NCDB, we observed that in patients with MSI-H metastatic colorectal cancer, immunotherapy significantly improved OS in long-term follow-up, aligning with some previous studies[12,13]. However, our results reveal no significant survival benefit with immunotherapy in MSI-L/MSS patients. These findings suggest that immunotherapy treatment should be considered for patients with MSI-H metastatic colorectal cancer, while further studies are warranted to determine the optimal therapeutic approach for patients with MSS metastatic colorectal cancer.

Our findings echo those of Le *et al*[12] and Overman *et al*[13], underscoring the divergent responses to immunotherapy in MSI-H *vs* MSI-L/MSS metastatic colorectal cancers. Le *et al*'s research delves into the efficacy of programmed death-1 (PD-1) blockade in mismatch repair-deficient tumors, showing significant positive responses in colorectal and other cancers with MSI-H – a notable advancement in immunotherapy for these patients[12]. Similarly, Overman *et al*'s study focuses on the use of Nivolumab, a PD-1 inhibitor, in treating metastatic colorectal cancer patients with mismatch repair deficiencies or MSI-H, adding to the growing body of evidence in this field[13]. Boland and colleagues highlighted the significant influence of MSI on colorectal cancer, particularly emphasizing the unique tumor characteristics and varied treatment responses associated with it[17]. These findings collectively underline the intricacies of tumor biology and the critical need to incorporate MSI status in devising treatment strategies.

Our research indicates that immunotherapy does not significantly benefit patients with MSI-L/MSS metastatic colorectal cancer, a finding that contrasts sharply with the substantial efficacy observed in MSI-H metastatic colorectal cancer. This notable difference may imply a potential resistance to immunotherapeutic strategies within the MSI-L/MSS subtype, hinting at a complex, yet unexplored aspect of its molecular profile. The lower response rates to immunotherapy in MSI-L/MSS tumors can be attributed to the lower tumor mutational burden and reduced immunogenicity compared to MSI-H tumors[17]. Nonetheless, several ongoing clinical trials are investigating combination strategies, such as the use of immunotherapy with chemotherapy, targeted therapies, to enhance the efficacy of immunotherapy in MSI-L/MSS metastatic colorectal cancer[18-21].

While at first glance these results in MSI-L/MSS metastatic colorectal cancer patients may seem like a setback, they actually represent a significant advancement in our understanding of metastatic colorectal cancer. They highlight the necessity of re-evaluating our current therapeutic approaches and underscore the importance of further investigation into the distinct molecular features of the MSI-L/MSS subtype. Our findings serve as a catalyst for this critical research, driving the development of more targeted and effective treatment strategies for metastatic colorectal cancer. Echoing the sentiments of Mármol *et al*[22], our study supports the push towards personalized medicine in the treatment of metastatic colorectal cancer. Tailoring treatments based on genetic markers such as MSI can potentially lead to more effective and

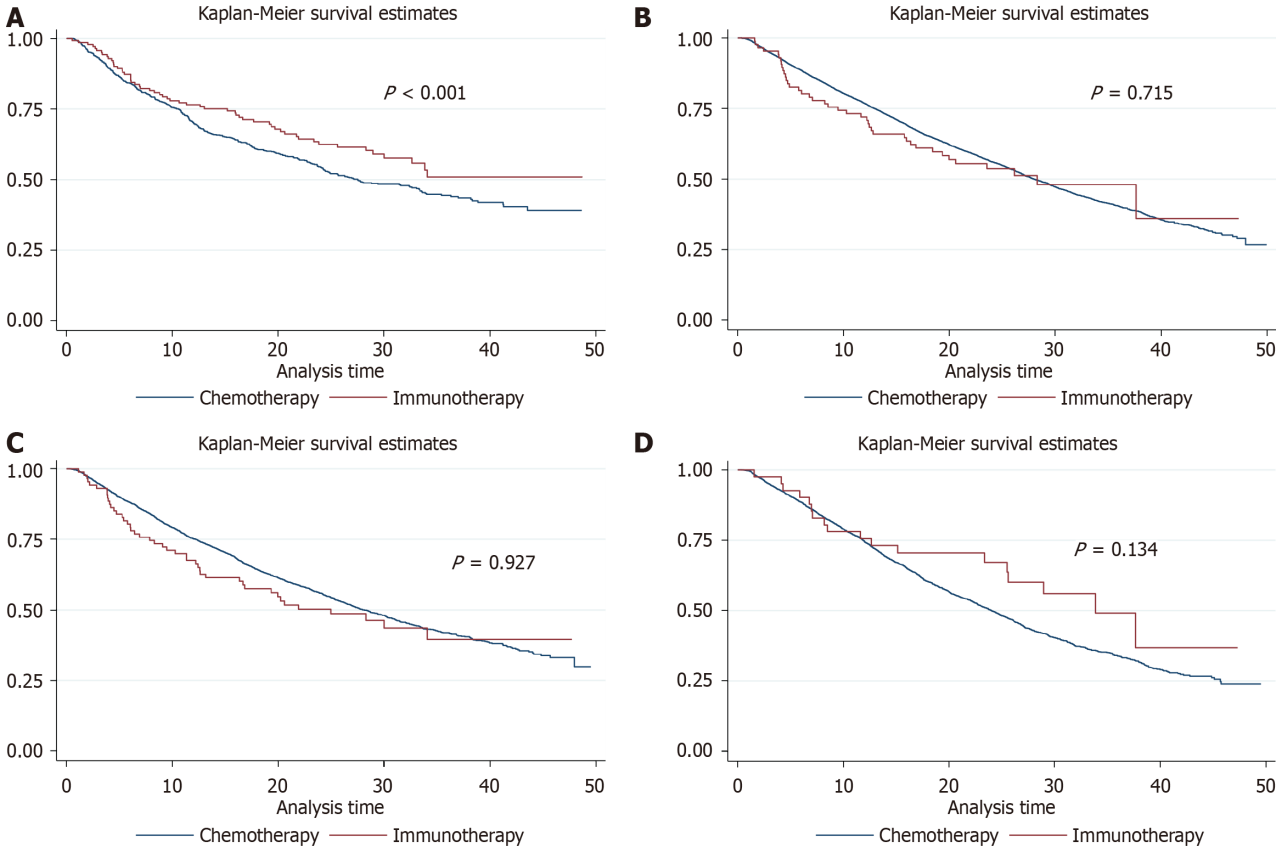

Figure 1 Survival analysis. A: Survival analysis among microsatellite instability-high population; B: Survival analysis among microsatellite stable population; C: Survival analysis among KRAS wild type population; D: Survival analysis among KRAS mutated type population.

targeted therapies.

In our study, the evaluation of OS benefits associated with immunotherapy, in comparison to chemotherapy, revealed no significant differences in both KRAS mutated and wild-type colorectal cancer populations. This outcome highlights the complex interplay between genetic profiles and tumor response to immunotherapeutic agents. Existing literature has consistently shown that KRAS mutations are a common feature in colorectal cancers, often correlating with a challenging prognosis and reduced responsiveness to certain treatments, such as anti-EGFR therapies. The lack of a distinct OS advantage in either KRAS cohort within our study may suggest a broader pattern of resistance or insensitivity to immunotherapy across these genetic variations. This observation emphasizes the critical need for developing more refined and individualized treatment strategies, especially for KRAS-mutated colorectal cancer, a substantial subset of the patient population.

Our study underscores the necessity of integrating genetic profiling into therapeutic decision-making, potentially improving patient outcomes in metastatic colorectal cancer. Such an approach aligns with the evolving paradigm of personalized medicine. However, this endeavor requires careful consideration of the metastatic colorectal cancer's genetic heterogeneity, the development of sophisticated genomic analysis techniques, and a thorough understanding of the practicalities and challenges in implementing personalized treatment regimens, including economic and logistical factors.

Limits of the study

This study encountered several limitations that are important to acknowledge. Firstly, the retrospective nature of the study may have introduced selection bias, as the choice of treatment might have been influenced by unmeasured factors. Additionally, the NCDB lacks detailed information on treatment regimens, duration, and response to therapy, which precludes further exploration of the impact of different agents, combinations, or lines of therapy. Information on potential predictive biomarkers, such as tumor mutational burden and PD-L1 expression, was not available. Another significant limitation is the variability in data due to incomplete information on specific molecular characteristics of the colorectal tumors in some patients, which may impact the study's conclusions. Lastly, our study population included patients diagnosed till 2020, which may not reflect the most recent advances in metastatic colorectal cancer treatment. Given these limitations, it is crucial to undertake further research in this field to enhance our understanding of MSS metastatic colorectal cancer and to develop more effective treatment strategies.

CONCLUSION

Our population-based study demonstrates that immunotherapy treatment is associated with significantly improved OS in patients with MSI-H metastatic colorectal cancer, but not in those with MSI-L/MSS metastatic colorectal cancer. These findings suggest that immunotherapy treatment should be considered for patients with MSI-H metastatic colorectal cancer, while further studies are warranted to determine the optimal therapeutic approach for patients with MSI-L/MSS metastatic colorectal cancer.

FOOTNOTES

Author contributions: All authors jointly conceptualized the article. Okolo P critically revised the manuscript and provided substantial feedback; Okolo P and Zhang J were responsible for the statistical analysis; Niu CG, Zhang J, Rao AV and Joshi U conducted the literature review and drafted the manuscript. All authors approved the final version of the manuscript.

Institutional review board statement: The present study was a database analysis using de-identified data; therefore, institutional review board approval was not required for this type of study.

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STROBE statement: The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

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ORIGINAL ARTICLE

Retrospective Study

Elevated cardiovascular risk and acute events in hospitalized colon cancer survivors: A decade-apart study of two nationwide cohorts

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Abstract

BACKGROUND

Over the years, strides in colon cancer detection and treatment have boosted survival rates; yet, post-colon cancer survival entails cardiovascular disease (CVD) risks. Research on CVD risks and acute cardiovascular events in colorectal cancer survivors has been limited.

AIM

To compare the CVD risk and adverse cardiovascular outcomes in current colon cancer survivors compared to a decade ago.

METHODS

We analyzed 2007 and 2017 hospitalization data from the National Inpatient Sample, studying two colon cancer survivor groups for CVD risk factors, mortality rates, and major adverse events like pulmonary embolism, arrhythmia, cardiac arrest, and stroke, adjusting for confounders *via* multivariable regression analysis.

RESULTS

Of total colon cancer survivors hospitalized in 2007 (n = 177542) and 2017 (n = 178325), the 2017 cohort often consisted of younger (76 *vs* 77 years), male, African-

American, and Hispanic patients admitted non-electively *vs* the 2007 cohort. Furthermore, the 2017 cohort had higher rates of smoking, alcohol abuse, drug abuse, coagulopathy, liver disease, weight loss, and renal failure. Patients in the 2017 cohort also had higher rates of cardiovascular comorbidities, including hypertension, hyperlipidemia, diabetes, obesity, peripheral vascular disease, congestive heart failure, and at least one traditional CVD ($P < 0.001$) *vs* the 2007 cohort. On adjusted multivariable analysis, the 2017 cohort had a significantly higher risk of pulmonary embolism (PE) (OR: 1.47, 95%CI: 1.37-1.48), arrhythmia (OR: 1.41, 95%CI: 1.38-1.43), atrial fibrillation/flutter (OR: 1.61, 95%CI: 1.58-1.64), cardiac arrest including ventricular tachyarrhythmia (OR: 1.63, 95%CI: 1.46-1.82), and stroke (OR: 1.28, 95%CI: 1.22-1.34) with comparable all-cause mortality and fewer routine discharges (48.4% *vs* 55.0%) ($P < 0.001$) *vs* the 2007 cohort.

CONCLUSION

Colon cancer survivors hospitalized 10 years apart in the United States showed an increased CVD risk with an increased risk of acute cardiovascular events (stroke 28%, PE 47%, arrhythmia 41%, and cardiac arrest 63%). It is vital to regularly screen colon cancer survivors with concomitant CVD risk factors to curtail long-term cardiovascular complications.

Key Words: Colon cancer; Colorectal cancer; Cardiovascular diseases; Cardiovascular disease risk; Cardiac events; Stroke

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Core Tip: Colon cancer survivors hospitalized 10 years apart in the United States showed an increased cardiovascular disease risk with an increased risk of acute cardiovascular events (stroke 28%, pulmonary embolism 47%, arrhythmia 41%, and cardiac arrest 63%). Increased screening in this cohort is important.

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INTRODUCTION

Cardiovascular disease (CVD) and cancer remain the leading causes of death in the United States, with colon cancer being the third leading cause of all cancer-related deaths in both men and women. According to 2017 Global Burden of Disease data, there were 1.8 million incident colon cancer cases with an age-standardized incidence rate of 23.2 per 100000 person-years[1]. However, with improvements in screening strategies, early detection and treatment, and better lifestyle modifications, the survival rates have improved significantly[2].

Studies have shown increased CVD risk in cancer survivors which includes heart failure, stroke and coronary artery disease[3]. This is explained by the fact that CVD and colon cancer survivors both share risk factors such as age, obesity, a sedentary lifestyle, and smoking. Patients after cancer chemo and radiotherapy enter a chronic inflammatory state secondary to the cancer burden and the treatment effects. These lead to the development of new chronic conditions such as diabetes, hypertension, and hyperlipidemia, which in themselves increase adverse cardiovascular event risk[4-6]. There is also increased cardiotoxicity from these treatments, which is understudied in colon cancer survivors. The risk of CVD has been well described for breast[7], lung[8,9], lymphoma/leukemias[10] and prostate cancers[11] amongst various population groups however for colon cancer, it is understudied. There has been a paucity of data regarding the CVD burden and trend in colon cancer in the last decade. Hence, it is imperative to understand the CVD risk and how it has varied over time. We therefore performed a retrospective analysis of colon cancer survivors and compared the CVD risk and adverse cardiovascular outcomes in current colon cancer survivors compared to a decade ago.

MATERIALS AND METHODS

We conducted a retrospective analysis of hospitalizations among colon cancer survivors in the years 2007 and 2017 using the National Inpatient Sample (NIS) from the Agency for Healthcare Research & Quality-supported Healthcare Cost Utilization Project[12]. The records of NIS comprise demographics of patients, hospital characteristics, several diagnoses, procedures, and comorbidities with pertinent International Classification of Diseases Clinical Modification, Ninth Revision (ICD-9-CM), or Tenth Revision (ICD-10-CM) codes. As the datasets are publicly available and de-identified, they were exempt from institutional review board approval.

The study included patients from January 1st to December 31st in 2007 and 2017. Using the ICD-9-CM and ICD-10-CM code V10.05 and Z85.038 respectively, we identified patients aged 18 or older who were admitted to the hospital with a prior history of colon cancer. Hospitalization with information missing on age, race, gender, length of stay, cost of a stay, or in-hospital death were excluded. The primary outcomes were major adverse cardiovascular and cerebrovascular events and healthcare resource utilization. Secondary outcomes included the prevalence of CVD risk factors. The ICD-9 and ICD-10 codes for complications are listed in Supplementary Table 1, and the comorbidities were determined using the Elixhauser software.

We performed multivariable regression analysis, adjusting for sociodemographic confounders such as age, sex, median household income, type of admission, teaching facility, and comorbid conditions, to assess the risk of cardiovascular events across these two cohorts a decade apart. We also compared the CVD risk factors and in-hospital outcomes, including all-cause mortality, PE, arrhythmia, atrial fibrillation/flutter, cardiac arrest, including ventricular tachyarrhythmias, stroke, and patient disposition (routine, short-term rehabilitation, including skilled nursing facilities, intermediate care facility, home health, and leaving against medical advice). Categorical and continuous data were assessed using Pearson's chi-square test and the Mann-Whitney U test for non-normally distributed continuous data. Statistical significance was measured at a two-sided *P* value of 0.05. All analyses were conducted using weighted data and complex survey modules in IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, United States).

RESULTS

Of the total hospital admissions among colon cancer survivors in 2007 (n = 177542) and 2017 (n = 178325), the 2017 cohort often consisted of younger [median age: 76 (65-84) *vs* 77 (67-84) years], black (12.2% *vs* 9.6%), Asian or Pacific Islander (2.9% *vs* 2.2%), and Hispanic (7.3% *vs* 5.4%), males (50.2% *vs* 48.9%) ($P < 0.001$) and a lower median household income quartile (26.4% *vs* 25.6%). There were also more non-elective admissions (82.9% *vs* 76.9%) from urban teaching facilities (53.2%) *vs* 50.9% ($P < 0.001$) (Table 1).

Furthermore, the 2017 cohort had higher rates of smoking (40.9% *vs* 17.6%), alcohol abuse (2.2% *vs* 1.7%), drug abuse (1.5% *vs* 0.7%), coagulopathy (6.5% *vs* 3.2%), liver disease (3.8% *vs* 1.9%), weight loss (8.6% *vs* 3.4%), and renal failure (19.7% *vs* 10.9%). The 2017 cohort of colon cancer survivors also had higher rates of cardiovascular comorbidities, including hypertension (73.9% *vs* 61.8%), hyperlipidemia (43.5% *vs* 26.4%), diabetes (29.7% *vs* 25.0%), obesity (11.1% *vs* 4.5%), peripheral vascular disease (6.7% *vs* 6.4%), congestive heart failure (14.3% *vs* 10.3%), and at least one traditional CVD (89.5% *vs* 77.9%) ($P < 0.001$).

Comparing colon cancer survivors from 2007 and 2017, the 2017 cohort had a significantly higher risk of PE (1.4% *vs* 1.3%, OR: 1.47, 95%CI: 1.37-1.48), arrhythmia (30.6% *vs* 23.6%, OR: 1.41, 95%CI: 1.38-1.43), atrial fibrillation/flutter (25.2% *vs* 17.6%, OR: 1.61, 95%CI: 1.58-1.64), cardiac arrest, However, there was no significant difference in all-cause mortality (2.9% *vs* 3.0%, OR: 0.99, 95%CI: 0.95-1.04, $P = 0.77$) (Table 2).

DISCUSSION

In this nationwide study, we compare cardiovascular risk factors and outcomes among colon cancer survivors in 2017 with those in 2007. Cardiovascular risk has been shown to be elevated in patients diagnosed with colon cancer in several studies[13-15]. However, CVD risk in survivors hasn't been extensively studied[16]. In an era with an increasing prevalence of both colon cancer survivors and cardiovascular disease, it is paramount to explore cardiovascular morbidity and mortality. The key findings from our study were: (1) The number of colon cancer survivors has almost remained the same, but they are younger; (2) CVD risk factors were significantly higher in the 2017 cohort; (3) The 2017 cohort also had higher rates of in-hospital complications such as PE, atrial and ventricular tachyarrhythmias, cardiac arrest, and stroke; and (4) Despite increased complication rates and overall CVD morbidity, all-cause mortality was not significant in the 2017 cohort.

With improvements in screening criteria and advancements in treatment modalities, colon cancer is being diagnosed earlier. In one of the studies from the National Cancer Database (2004-2015), it was found that cancer is being diagnosed at a much younger age compared to 2005[17]. This is also concerning, as there has been an increase in colon cancer incidence in the younger population (50 years old)[18]. This warrants further exploration to see if this is due to early diagnosis and effective therapeutics that has developed in the past decade[19], or if it is due to rising sedentary lifestyles, obesity, and alcohol use, which are co-existent with cardiovascular diseases[20]. It is already established that cardiovascular risk is high[13,14], and with the increased pool of colon cancer survivors cardiovascular disease risk factors would be expected to be high. Our study supported this by demonstrating that the 2017 cohort of colon cancer survivors had a higher prevalence of the current increase in CVD risk factors, such as obesity, hypertension, diabetes, and hyperlipidemia.

The rise in the prevalence of cardiovascular risk factors over time may help to explain why we are seeing an increase in complication rates for cardiovascular end-points like PE, cardiac arrhythmia, stroke, and cardiac arrests in our study. Colon cancer itself is a risk factor for the development of these complications, and it has been studied for other cancers as well. Hence, it is particularly important to identify at-risk population groups and control these risks to prevent worse outcomes.

Table 1 Demographics and comorbidities of hospitalizations among colon cancer survivors a decade apart: Propensity matched analysis

Variable	2007 (*n* = 177542)	2017 (*n* = 178325)	*P* value
Age (yr) at admission, median (IQR)	77 (67-84)	76 (65-84)	< 0.001
Sex, *n* (%)			
Male	86792 (48.9)	89485 (50.2)	< 0.001
Female	90750 (51.1)	88840 (49.8)	
Race			
White	142763 (80.4)	132770 (74.5)	< 0.001
Black	16975 (9.6)	21750 (12.2)	
Hispanic	9506 (5.4)	13045 (7.3)	
Asian or Pacific Islander	3962 (2.2)	5140 (2.9)	
Native American	836 (0.5)	830 (0.5)	
Others	3499 (2.0)	4790 (2.7)	
Median household income quartile, *n* (%)			< 0.001
0^{th}-25^{th}	45378 (25.6)	47100 (26.4)	
76^{th}-100^{th}	44838 (25.3)	41700 (23.4)	
Urban teaching facility, *n* (%)	90450 (50.9)	94790 (53.2)	< 0.001
Non-elective admission, *n* (%)	136359 (76.9)	147545 (82.9)	< 0.001
Comorbidities, *n* (%)			
Alcohol abuse	3069 (1.7)	3835 (2.2)	< 0.001
Congestive heart failure	18256 (10.3)	25510 (14.3)	< 0.001
Coagulopathy	5738 (3.2)	11535 (6.5)	< 0.001
Hypertension	109779 (61.8)	131870 (73.9)	< 0.001
Hyperlipidemia	46873 (26.4)	77505 (43.5)	< 0.001
Diabetes	44331 (25.0)	52910 (29.7)	< 0.001
Smoking	31260 (17.6)	72955 (40.9)	< 0.001
Obesity	8031 (4.5)	19750 (11.1)	< 0.001
At least 1 Traditional CVD risk factor	138285 (77.9)	159640 (89.5)	< 0.001
Peripheral vascular diseases	11370 (6.4)	11890 (6.7)	0.001
Renal failure	19316 (10.9)	35075 (19.7)	< 0.001
Liver disease	3369 (1.9)	6760 (3.8)	< 0.001
Weight loss	5993 (3.4)	15405 (8.6)	< 0.001
Drug abuse	1165 (0.7)	2650 (1.5)	< 0.001

IQR: Interquartile range; CVD: Cardiovascular disease.

Despite increasing cardiovascular morbidity and complication rates, overall mortality was not found to be significantly higher in the 2017 cohort compared to 2007. This provides an opportunity to shed more light on the fact that in the past decade, the intensive management of cardiovascular issues has changed[23,24]. With improved cardiac critical care management, including the implementation of evidence-based protocols[25], rapid recognition of life-threatening conditions, and attention to patient safety, we have been able to reduce cardiovascular mortality in the past decade[24].

We used the data from a publicly accessible database, which has limited applicability since cancer-related information like the stage of colon cancer, any second incident malignancies, the exact type of chemotherapy, and the history of past treatment are not specified. Additionally, there was conflicting information regarding the number of years that patients survive after receiving a cancer diagnosis and whether they are still battling the disease or have it in remission. The cohorts were sampled from patients all over the United States, and our analysis requires external validation from other regions. Also, there is unclear data on whether these patients had any previous cardiovascular diseases before a diagnosis

Table 2 Hospitalization outcomes among colon cancer survivors a decade apart: Propensity matched analysis

Variable	2007 (*n* = 177542)	2017 (*n* = 178325)	OR	CI (UL-LL)	Adjusted P value
All-cause mortality	5245 (3.0)	5165 (2.9)	0.32	0.99 (0.95-1.04)	0.77
Pulmonary embolism	2290 (1.3)	2470 (1.4)	0.013	1.47 (1.37-1.58)	< 0.001
Arrhythmia	41948 (23.6)	54595 (30.6)	< 0.001	1.41 (1.38-1.43)	< 0.001
Atrial fibrillation/flutter	31280 (17.6)	44875 (25.2)	< 0.001	1.61 (1.58-1.64)	< 0.001
Cardiac arrest including ventricular tachyarrhythmias	609 (0.3)	1065 (0.6)	< 0.001	1.63 (1.46-1.82)	< 0.001
Stroke	4409 (2.5)	5675 (3.2)	< 0.001	1.28 (1.22-1.34)	< 0.001
Routine discharge	97712 (55.0)	86785 (48.7)	< 0.001		

Multivariable analysis was adjusted for demographics, hospital characteristics and all relevant comorbidities. IQR: Interquartile range; CVD: Cardiovascular disease.

of colon cancer. Apart from that, there might be inherent errors in coding. And lastly, no associations can be made between cardio-cerebrovascular outcomes and a previous history of colon cancer.

CONCLUSION

With increasing cardiovascular risk factors in the general population and increasing cancer survivorship, we have found that the prevalence of CVD and its complications is higher than ever. With improvements in acute cardiovascular treatment, we haven't seen an improvement in mortality, which we would expect. Hence, we need better control of the cardiovascular risk factor from a primary care standpoint as well to prevent worse outcomes in colon cancer survivors. We need further studies comparing cardiovascular morbidity and outcomes in colon cancer survivors with other cancer survivors, which are more extensively studied, and how they have evolved in the past years.

FOOTNOTES

Author contributions: Desai R, Singh S and Chauhan S conceptualized the methodology of manuscript; Desai R and Mondal A collected resources to write the manuscript; Desai R, Patel V, Singh S, Chauhan S and Jain A reviewed and edited the manuscript; Desai R analyzed the manuscript with software; Desai R, Mondal A, Singh S, Chauhan S and Jain A visualized the results; Singh S, Chauhan S and Jain A supervised the manuscripts. All authors have read and approved the final manuscript.

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ORIGINAL ARTICLE

Basic Study

Regulation of *TMEM100* expression by epigenetic modification, effects on proliferation and invasion of esophageal squamous carcinoma

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Abstract

BACKGROUND

Esophageal squamous cell carcinoma (ESCC) is a prevalent malignancy with a high morbidity and mortality rate. *TMEM100* has been shown to be suppressor gene in a variety of tumors, but there are no reports on the role of *TMEM100* in esophageal cancer (EC).

AIM

To investigate epigenetic regulation of TMEM100 expression in ESCC and the effect of TMEM100 on ESCC proliferation and invasion.

METHODS

Firstly, we found the expression of TMEM100 in EC through The Cancer Genome Atlas database. The correlation between *TMEM100* gene expression and the survival of patients with EC was further confirmed through Kaplan-Meier analysis. We then added the demethylating agent 5-AZA to ESCC cell lines to explore the regulation of TMEM100 expression by epigenetic modification. To observe the effect of TMEM100 expression on tumor proliferation and invasion by overexpressing TMEM100. Finally, we performed gene set enrichment analysis using the Kyoto Encyclopaedia of Genes and Genomes Orthology-Based Annotation System database to look for pathways that might be affected by *TMEM100* and verified the effect of *TMEM100* expression on the mitogen-activated protein kinases (MAPK) pathway.

RESULTS

In the present study, by bioinformatic analysis we found that TMEM100 was lowly expressed in EC patients compared to normal subjects. Kaplan-meier survival analysis showed that low expression of TMEM100 was associated with

poor prognosis in patients with EC. Then, we found that the demethylating agent 5-AZA resulted in increased expression of TMEM100 in ESCC cells [quantitative real-time PCR (qRT-PCR) and western blotting]. Subsequently, we confirmed that overexpression of TMEM100 leads to its increased expression in ESCC cells (qRT-PCR and western blotting). Overexpression of TMEM100 also inhibited proliferation, invasion and migration of ESCC cells (cell counting kit-8 and clone formation assays). Next, by enrichment analysis, we found that the gene set was significantly enriched in the MAPK signaling pathway. The involvement of TMEM100 in the regulation of MAPK signaling pathway in ESCC cell was subsequently verified by western blotting.

CONCLUSION

TMEM100 is a suppressor gene in ESCC, and its low expression may lead to aberrant activation of the MAPK pathway. Promoter methylation may play a key role in regulating TMEM100 expression.

Key Words: Esophageal squamous cell carcinoma; TMEM100; Invasion; Mitogen-activated protein kinases pathway; Epigenetic

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Core Tip: *TMEM100* has been shown to be an oncogene in a variety of tumors, but there are no reports on the role of *TMEM100* in esophageal cancer. In the present study, we found that TMEM100 was lowly expressed in esophageal squamous cell carcinoma (ESCC). Methylation may play a key role in regulating TMEM100 protein low expression. Overexpression of TMEM100 resulted in its increased expression in ESCC cells. Overexpression of TMEM100 also inhibited proliferation, invasion and migration of ESCC cells. Low expression of TMEM100 in ESCC may lead to aberrant activation of the mitogen-activated protein kinases pathway.

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INTRODUCTION

Esophageal cancer (EC) is a common malignant tumour of the digestive tract and is recognised for its high incidence and mortality rate[1,2]. The disease primarily manifests in two forms, namely squamous carcinoma and adenocarcinoma[2]. Esophageal squamous cell carcinoma (ESCC) represents the predominant subtype of EC and is particularly prevalent in Asia, while esophageal adenocarcinoma is more commonly observed in Europe[3]. China bears a significant burden, accounting for nearly 50% of ESCC cases worldwide and over 90% within Asia[4]. The predominant treatment approach for ESCC primarily involves surgical procedures. While outcomes are relatively favourable for early-stage patients with EC, those with intermediate to advanced disease face a more challenging prognosis, with a 5-year overall survival rate ranging from 10%–30%[5]. The emergence of immunotherapy brings a promising dimension to EC treatment[6]. However, the efficacy and safety of immunotherapy for patients with tumours require further validation. Anticipated advancements in identifying more clinical targets hold the potential to improve the effectiveness of immunotherapy.

TMEM100 is a gene that encodes a 134-amino-acid protein located at locus 17q32. This gene possesses two hypothetical transmembrane structural domains (amino acids 53–75 and 85–107)[7]. Initially identified as a transcription factor in the murine gene, *TMEM100* is highly conserved and exhibits a structure dissimilar to any known protein family across various species[8]. In the context of TMEM100's involvement with tumours, research findings indicate its association with a variety of malignancies. A study by Han *et al*[9] revealed a correlation between TMEM100 and the proliferation of lung cancer cells. Similarly, a study by Ou *et al*[10] suggested that TMEM100 exhibits low expression in hepatocellular carcinoma and is closely related to both its proliferation and invasion. A study by Ye *et al*[11] revealed that TMEM100 exhibits low expression in patients with prostate cancer and is associated with tumour stage and metastasis. In a study conducted by Li *et al*[12], TMEM100 demonstrated significantly low expression in colorectal cancer, and the overexpression of TMEM100 inhibited the malignant progression of tumours through the regulation of the transforming growth factor β pathway.

Epigenetic modifications are heritable alterations in gene expression that do not stem from primary DNA sequence changes, playing a pivotal role in the development of tumours such as leukaemia. These modifications primarily encompass three regulatory mechanisms: DNA methylation, non-coding RNA regulation, and histone modification[13]. DNA methylation involves the transfer of a methyl to the 5' position of cytosine through the action of DNA methyltransferase. This process utilises S-adenosylmethionine as the methyl donor, resulting in the formation of 5'-methylcytosine [14]. In the context of EC, multiple oncogenes, including EPB41L3/GPX3/TMEM176A, exhibit methylation in their

promoter regions[15-17]. Despite the critical role of epigenetics in gene regulation, the literature on the mechanisms governing the expression of *TMEM100* in EC is limited. Nevertheless, the significance of epigenetic regulation cannot be overlooked. The impact of DNA methylation on TMEM100 expression in tumours remains unexplored.

In this study, our objective was to elucidate the function of TMEM100 in malignant growth and invasion *in vitro* within ESCC cells. We sought to investigate the expression of TMEM100 and its impact on the activation of the mitogen-activated protein kinases (MAPK) signalling pathway in ESCC cells. Additionally, we aimed to explore the epigenetic regulation of TMEM100 expression in ESCC to provide a theoretical foundation for considering TMEM100 as a potential new therapeutic target for ESCC.

MATERIALS AND METHODS

Materials and reagents

Hieff Trans Liposomal Transfection Reagent and PAGE Gel Quick Preparation Kit (12.5%) were purchased from Yeasen (Shanghai, China). Penicillin-streptomycin solution (100 ×), RIPA lysis buffer, and crystal violet were sourced from Beyotime (Shanghai, China). Fetal bovine serum (FBS) and RPMI-1640 medium were obtained from Bio-Channel (Nanjing, China). TRIzol reagent and dimethyl sulfoxide were purchased from Biosharp (Hefei, China). 5-Azacytidine was acquired from Selleck (Houston, United States of America). Paraformaldehyde was obtained from Servicebio (Wuhan, China). Cell counting kit-8 (CCK-8) was sourced from topscience (Shanghai, China). Nitrocellulose filter (NC) membranes were purchased from PALL (New York, United States of America). *TMEM100* and *β-actin* primers were procured from Tsingke (Beijing, China). TMEM100 monoclonal antibodies were purchased from Proteintech (Wuhan, China). Human monoclonal antibodies against extracellular regulated kinase 1/2 (ERK1/2), phosphorylated (p-) ERK1/2, the c-Jun N-terminal kinase (JNK), phosphorylated (p-)JNK, p38, phosphorylated (p-) p38, goat anti-rabbit horse radish peroxidase (HRP) IgG, goat anti-mouse HRP IgG, and GAPDH were purchased from Zen Bioscience (Chengdu, China).

Cell culture

Human ESCC cell lines KYSE-450 (Cobioer Biosciences, Nanjing, China) and KYSE-150 (Typical Culture Preservation Committee Cell Bank, Chinese Academy of Sciences, Shanghai, China) were used in this study. Both cell lines were cultured in RPMI-1640 medium supplemented with 10% FBS and 1% penicillin-streptomycin solution (100 ×). The culture conditions were maintained at 37 °C with 5% CO_2.

Gene overexpression and transient transfection

The recombinant plasmid overexpressing TMEM100 was designed by General Biol (Chuzhou, China). Cells cultured at 70% density in 6-well plates were transfected with recombinant plasmids using Hieff Trans Liposomal Transfection Reagent, following the manufacturer's protocol. After 24 h, cells were collected for quantitative real-time PCR (qRT-PCR), CCK-8 assay, colony formation assay, and western blotting.

qRT-PCR

Total RNA was isolated from K-150 and K-450 cells using TRIzol reagent, following the manufacturer's instructions. Subsequently, the RNA was reverse transcribed using a cDNA synthesis kit (Promega, Fitchburg, United States of America). The resulting cDNA was amplified through 42 cycles, and the initial reaction volume was 20 μL, comprising 1 μL of reverse transcription product and 0.8 μL of primers. The housekeeping gene *β-actin* was used as a standardized internal control. Table 1 provides details on the gene-specific primers utilised in PCR amplification.

Western blotting

ESCC cells were lysed using RIPA lysis buffer. The resulting total cell lysates were then separated on a 12.5% sodium dodecyl sulfate polyacrylamide gel and transferred to NC membranes. After blocking in phosphate buffered saline with tween-20 containing 5% non-fat milk, membranes were incubated overnight at 4 °C with specific primary antibodies, followed by a 2 h incubation at 27 °C with HRP-conjugated specific secondary antibodies. Detection was achieved using the enhanced chemiluminescence western blotting detection system (Tanon, Shanghai, China). GAPDH was utilized to ensure equal protein loading on the gel.

Colony formation assay

For colony formation studies, ESCC cells were harvested following a 24-h treatment with transient transfection. These cells were then seeded at a density of 300 cells per 35 mm plate in RPMI-1640 medium with 10% FBS and cultured at 37 °C for two weeks. Thereafter, the cells were treated with 4% paraformaldehyde for 20 min and dyed with 1 mL of 0.1% crystal violet for 30 min. Photographs were captured after the stain was removed.

CCK-8 assay

During the exponential growth phase, three thousand cells treated with transient transfection were seeded into each well of a 96-well plate (100 μL/well). At specified time points (day 1, day 2, day 3), 10 μL of CCK-8 solution was added to each well, and the optical density (450 nm) values were measured using a microplate reader after 1 h of incubation.

Table 1 Primer sequences for quantitative real-time reverse transcription polymerase chain reaction

Gene	Primer pair
TMEM100	F: 5-ACAGTCCCTCTGGTCAGTGAGA-3 R: 5-GGCGATGAAGACAACCACAGCA-3
β-actin	F: 5-CACCATTGGCAATGAGCGGTTC-3 R: 5-AGGTCTTTGCGGATGTCCACGT-3

Bioinformatic analysis

The efficient channel attention transcriptional data, sourced from The Cancer Genome Atlas (TCGA) database, encompasses data from 161 patients and 11 normal subjects[18]. Differential expression analysis was conducted using the R package "Limma" applying the filtering criteria of $|\log \text{FoldChange}| \geq 1$, P value < 0.00001, and adjusted P value < 0.0001 to identify differentially expressed genes (DEGs). Visualisation of DEG expression was accomplished through the generation of a volcano plot and heatmap using the R packages "ggplot2" and "pheatmap". For a deeper insight into the functional implications of DEGs containing *TMEM100*, gene set enrichment analysis was performed using the Kyoto Encyclopaedia of Genes and Genomes (KEGG) Orthology-Based Annotation System database[19]. The top 69 enriched terms or pathways were selected and visualised using the R packages "gridExtra", "grid", and "ggplot2". Additionally, boxplots were constructed using the gene expression profiling interactive analysis (GEPIA) tool, and Kaplan-meier survival analysis was performed using the online analysis tool[20,21].

Statistical analysis

Statistical analysis and data visualization were performed using R software and GraphPad Prism 9.0. A P value < 0.05 was considered statistically significant unless otherwise specified. R software, comprising several packages, was employed for various analyses. When assessing differences between groups, statistical comparisons were conducted in GraphPad Prism 9.0 using the Student's *t*-test.

RESULTS

Low TMEM100 expression is associated with reduced overall survival in patients with EC

Analysis of TCGA data extracted from GEPIA revealed that the *TMEM100* gene exhibited underexpression in EC specimens compared to adjacent normal tissue (Figure 1A). The correlation between *TMEM100* gene expression and the survival of patients with EC was further confirmed through Kaplan-Meier analysis. Patients with high *TMEM100* expression demonstrated a significantly higher overall survival rate compared to those with low expression of this gene (Figure 1B).

Elevated expression levels of TMEM100 in ESCC cell lines treated with 5-AZA

To validate the impact of decreased DNA methylation on TMEM100 expression, ESCC cell lines were treated with 5-AZA. Both qRT-PCR and western blotting analyses revealed upregulation of TMEM100 at both mRNA and protein levels (Figure 1C). These findings suggest that changes in DNA methylation levels affect the expression levels of TMEM100.

Overexpression effect of TMEM100 in ESCC

To ascertain the impact of TMEM100 overexpression, recombinant plasmids were transfected into K-150 and K-450 cell lines using Hieff Trans Liposomal Transfection Reagent. Examination of TMEM100 expression through qRT-PCR and western blotting analyses revealed a significant increase in both mRNA and protein levels upon transfection with the recombinant plasmid (Figure 2A and B).

Effect of TMEM100 overexpression on the proliferation and invasion ability of ESCC

In order to explore the long-term effects of TMEM100 on cancer cell growth, the colony-forming capacity was evaluated. TMEM100 overexpression was observed to significantly inhibit the colony-forming ability of both K-150 and K-450 cells (Figure 2C). Additionally, the impact of altered TMEM100 expression on the proliferation of K-150 and K-450 cells was examined using the CCK-8 assay (Figure 2D). These results indicate that the overexpression of TMEM100 exerts inhibitory effects on the proliferation and invasive ability of ESCC.

Identification and enrichment analysis of DEGs containing TMEM100

An analysis of the TCGA database resulted in the identification of a total of 50940 differential genes between EC tissue and normal tissue. Further screening narrowed down the list to 3720 differential genes containing *TMEM100* (Figure 3A and B). Subsequently, the KEGG pathway enrichment analyses were conducted (Figure 3C and D), revealing a significant enrichment in the MAPK signalling pathway (P < 0.0005).

Effect of TMEM100 on the activity of the MAPK signalling pathway in ESCC

The MAPK signalling pathway plays a pivotal role in various cellular physiological activities, including cell growth, development, differentiation, and apoptosis. Given its significant involvement in tumourigenesis, we investigated

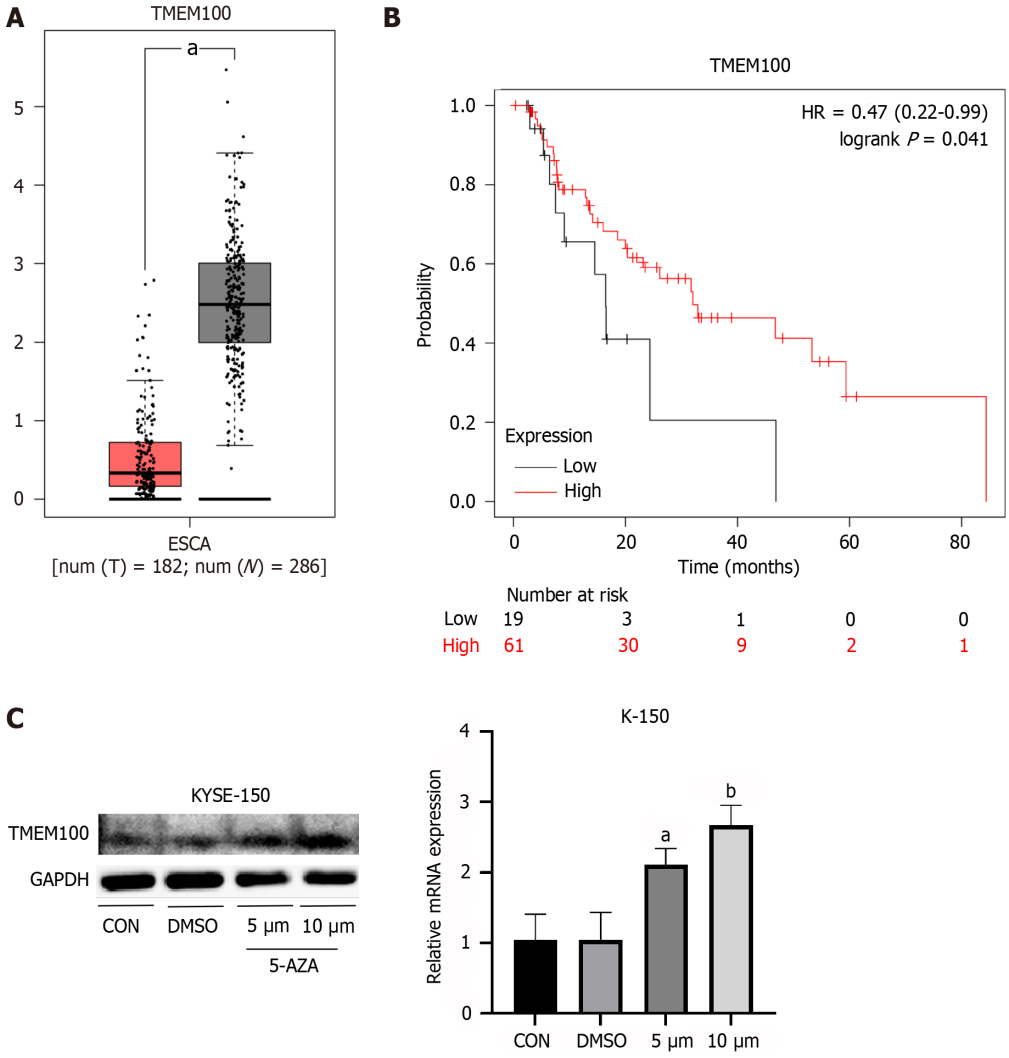


Figure 1 Relationship between low TMEM100 expression in esophageal cancer and patient survival and the effect of 5-AZA on *TMEM100* expression in esophageal squamous cell carcinoma lines. A: Expression profile of TMEM100 in EC samples compared with normal samples, showing reduced expression of TMEM100 in EC tissues; B: Overall survival of patients with high *vs* low TMEM100 expression levels. Survival was poorer for those with low TMEM100 expression ($P = 0.041$); C: 5-AZA induced a dose-dependent expression of TMEM100 in K-150 cells. Real-time PCR and western blotting results showed that after 24 h of treatment, TMEM100 expression increased with increasing 5-AZA concentration. $^{a}P < 0.05$, $^{b}P < 0.01$. DMSO: Dimethyl sulfoxide; EC: Esophageal cancer; ESCA: Esophageal cancer; HR: Hazard Ratio.

whether TMEM100 mediated the cascade of the classical MAPK pathway. Western blotting results demonstrated a significant reduction in the expression of phosphorylated ERK, phosphorylated JNK, and phosphorylated p38 following transfection with the TMEM100 overexpression plasmid (Figure 4). These findings suggest that the impact of TMEM100 on ESCC cell proliferation may be regulated through the ERK/MAPK, JNK/MAPK, and p38/MAPK signalling pathways.

DISCUSSION

The prognosis for ESCC remains challenging, partially due to the absence of prognostic biomarkers capable of identifying high-risk patients and facilitating the assignment of risk-appropriate monitoring and treatment regimens. TMEM100 is well established as an oncogene, as demonstrated by its inhibitory role in colorectal cancer progression through the promotion of ubiquitin/proteasome degradation of hypoxia-inducible factor-1 alpha[22]. The downregulation of TMEM100, mediated by histone deacetylase 6, expedites the development and progression of non-small cell lung cancer [23]. However, the expression and function of TMEM100 in ESCC have yet to be elucidated.

In our study, we initially identified *TMEM100* as a DEG between patients with EC and individuals without the condition by analysing gene expression data obtained from the TCGA database. Using online bioinformatics tools, we observed that TMEM100 exhibited low expression in patients with EC and that individuals with higher expression levels demonstrated a better prognosis. This suggests that TMEM100 may serve as a novel biomarker for EC. Given that over 70% of EC cases occur in China, with ESCC being the predominant subtype (80%)[24,25], we hypothesised that TMEM100

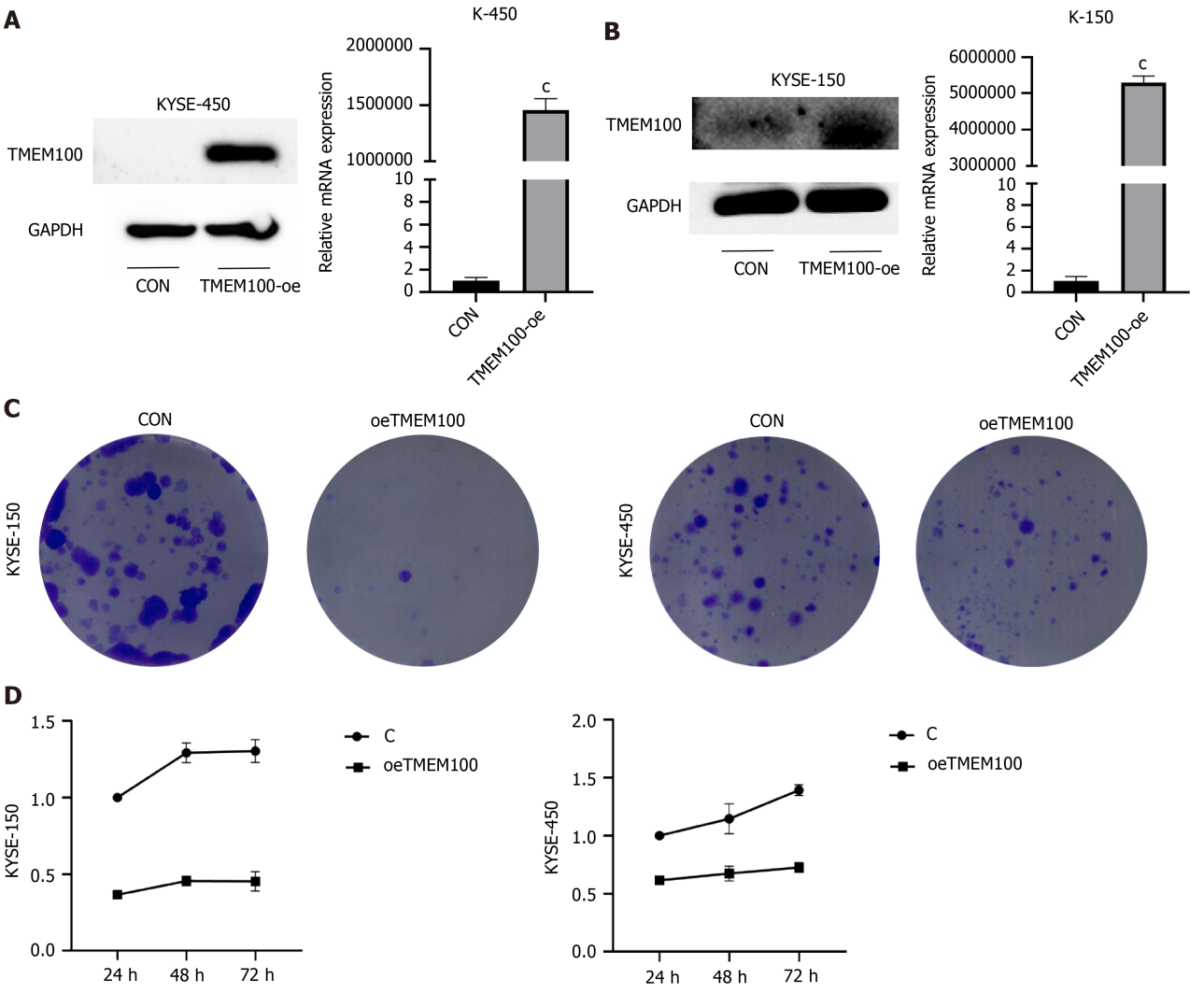
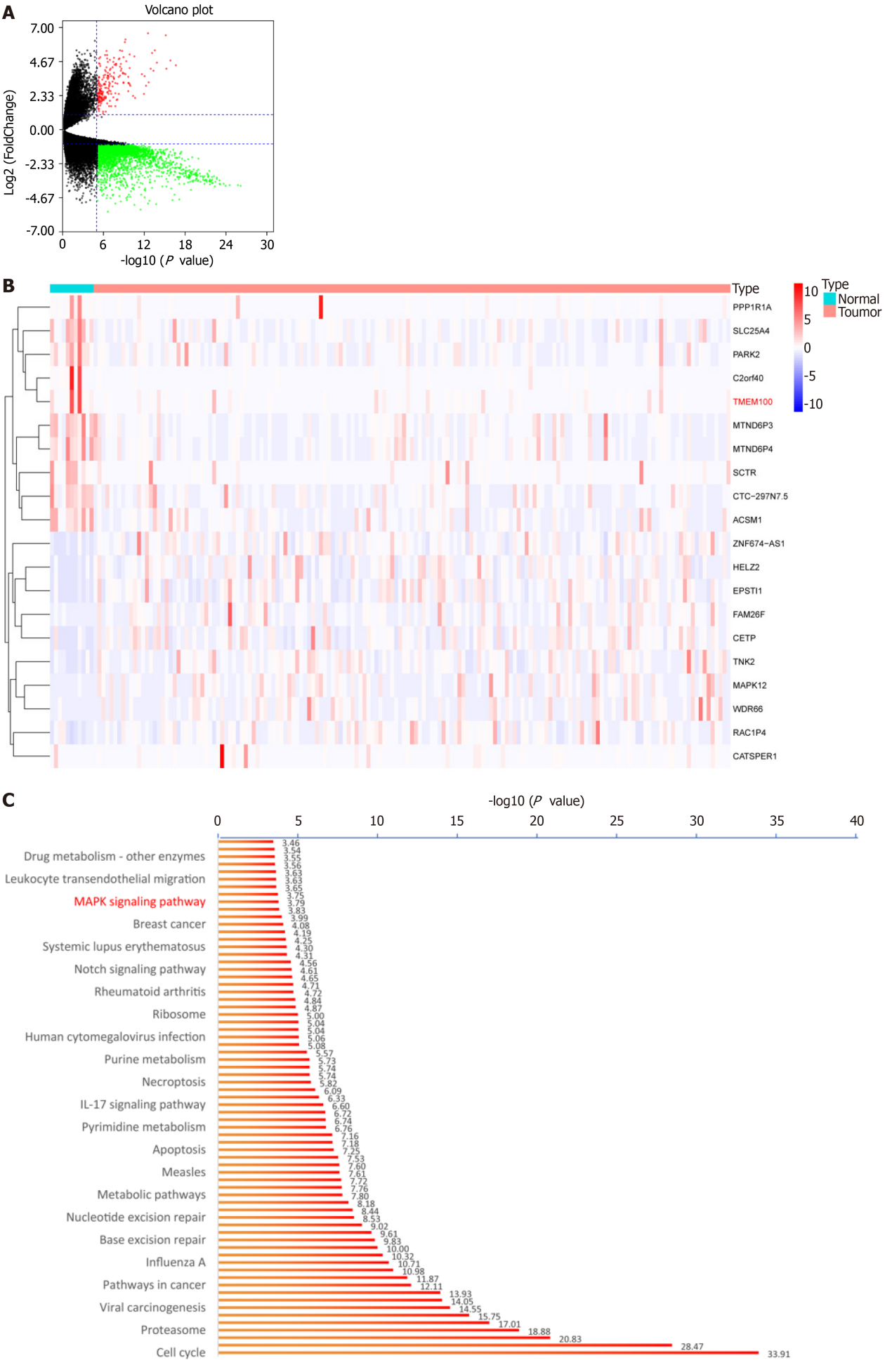


Figure 2 Overexpression effect of TMEM100 in esophageal squamous cell carcinoma lines and the inhibitory effect of TMEM100 overexpression on proliferation, migration, and invasion of esophageal squamous cell carcinoma cells *in vitro*. A and B: K-150/K-450 cells transfected with TMEM100-oe were assayed using real-time PCR and western blotting, and the results showed that the expression of TMEM100 was significantly upregulated in the transfected cells compared to that in the control group; C: Colony formation viability of K-150/K-450 cells after transient transfection treatment for 14 d was analysed by staining with 1% crystal violet; D: Cell counting kit-8 assay results show that overexpression of TMEM100 inhibits the proliferation of K-150/K-450 cells. $^{c}P < 0.0001$.

functions as an oncogene suppressor in ESCC. In further experiments, we observed that the overexpression of TMEM100 inhibited the proliferation and invasion of ESCC cells, supporting our conjecture. Additionally, we conducted a preliminary investigation into the mechanisms regulating TMEM100 expression in ECSS and observed that TMEM100 expression was significantly higher in ESCC cells treated with methylation inhibitors compared to that in normal ESCC cells. This suggests that DNA methylation in epigenetics may be involved in the regulation of TMEM100 expression in ESCC.

To explore the underlying mechanisms of ESCC, we performed a KEGG enrichment analysis to identify potential pathways. The analysis revealed that TMEM100 may be involved in signalling pathways, including p53, interleukin-17, and MAPK. We chose to focus on the MAPK signalling pathway in our research, as it has been extensively shown to be associated with tumour cell proliferation, differentiation, apoptosis, and stress response compared to other pathways[26-29]. This choice aligns with the results of our CCK-8 and clone formation experiments. Subsequent investigations revealed that the phosphorylation levels of ERK, p38, and JNK were significantly inhibited in ESCC cells overexpressing TMEM100. These results suggest that TMEM100 exerts an inhibitory effect on ESCC proliferation and invasion by negatively regulating the ERK, p38, and JNK pathways.

This study has several limitations. First, the robustness of TMEM100 as a prognostic indicator for ESCC requires further validation in large or prospective cohort studies. Second, the *in vivo* effects of TMEM100 overexpression on ESCC proliferation need additional clarification. Third, the regulation of DNA methylation for TMEM100 expression in ESCC requires further investigation. Nevertheless, this study provides initial insights into the role of TMEM100 in the development of ESCC and its specific mechanism of action. These findings lay the foundation for further understanding the mechanism of action of TMEM100 in other malignant tumours, carrying important theoretical and clinical significance.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ACSM1
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Leukocyte transendothelial migration
MAPK signaling pathway
Breast cancer
Systemic lupus erythematosus
Notch signaling pathway
Rheumatoid arthritis
Ribosome
Human cytomegalovirus infection
Purine metabolism
Necroptosis
IL-17 signaling pathway
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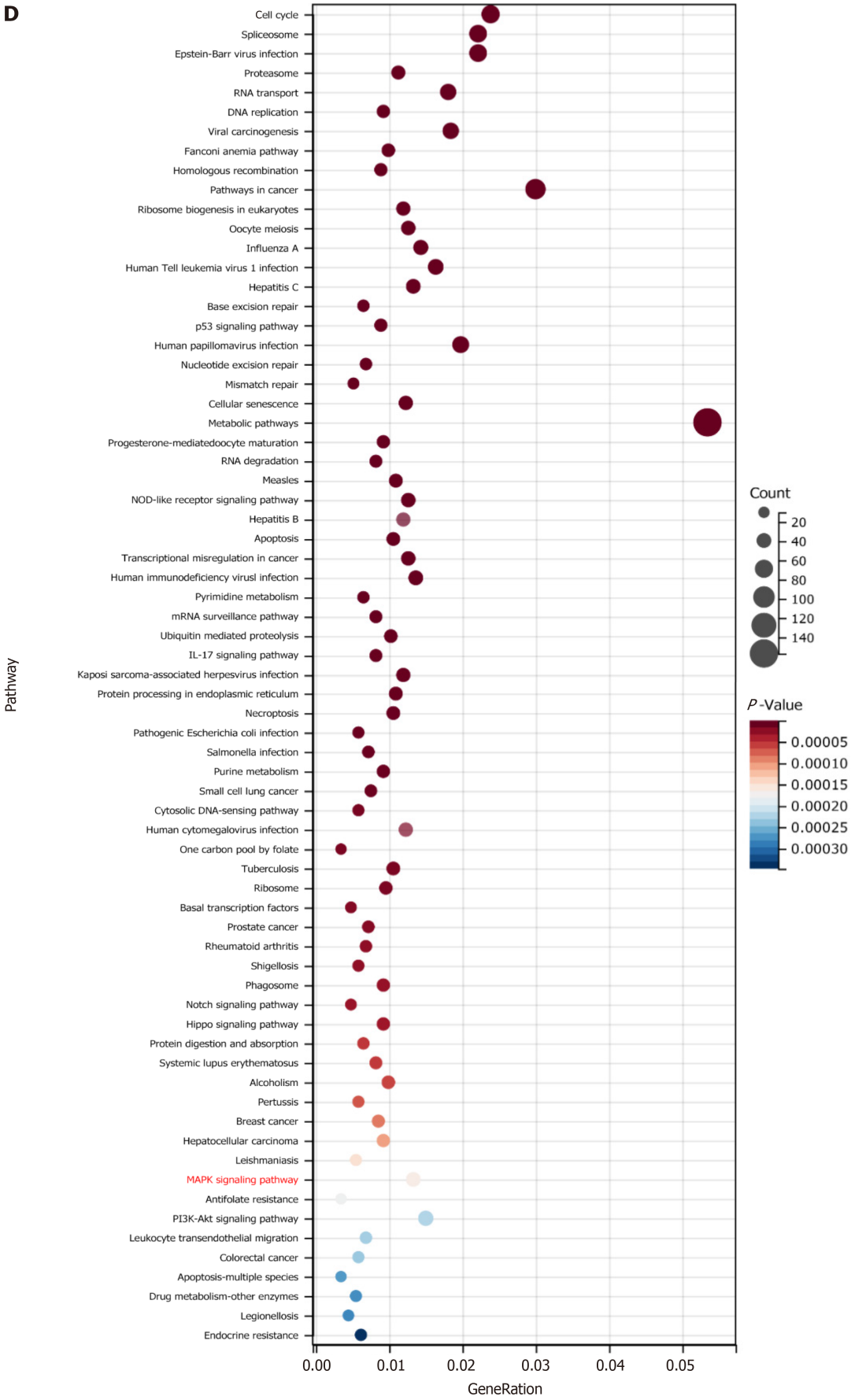

Figure 3 Identification of differentially expressed genes and functional enrichment analysis. A: In the volcano plot, upregulated genes are indicated by red dots, and downregulated genes are indicated by green dots; B: The heatmap represents the expression levels of the genes, with the blue to red spectrum indicating low to high expression; C and D: The top 69 enriched Kyoto Encyclopedia of Genes and Genomes pathways.

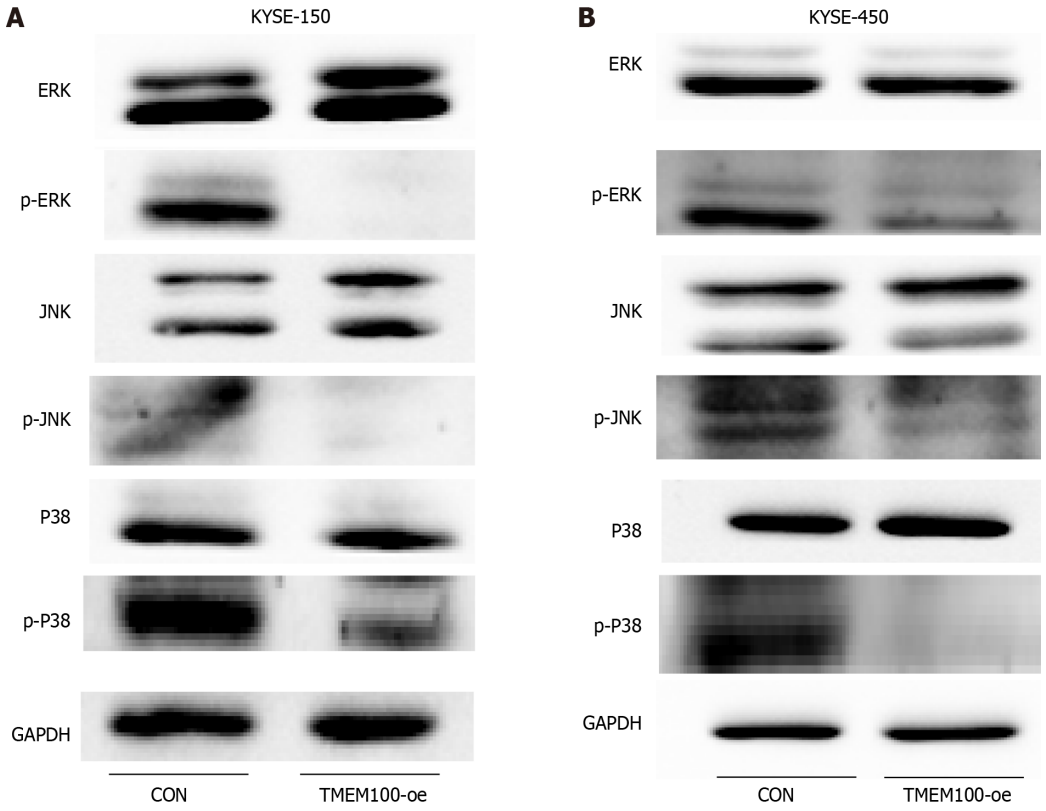

Figure 4 Effect of TMEM100 overexpression on mitogen-activated protein kinase pathway activation in KYSE-150/KYSE-450 cells. A: K-150 cells were harvested 24 h after transfection with TMEM100-oe, and total proteins were extracted for western blotting analysis. Phosphorylated-extracellular regulated kinase (p-ERK) and ERK, phosphorylated-c-Jun N-terminal kinase (p-JNK) and JNK, and p-p38 and p38 were analysed. The result demonstrated a reduction in the expression of p-ERK, p-p38, and p-JNK in K-150/K-450 cells transfected with TMEM100-oe; B: The experiment was repeated again with K-450 cells. p-ERK: Phosphorylated-extracellular regulated kinase; p-JNK: Phosphorylated-c-Jun N-terminal kinase.

CONCLUSION

TMEM100 functions as a suppressor gene in ESCC cells, and its low expression in ESCC may contribute to aberrant activation of the MAPK pathway. Promoter methylation likely plays a crucial role in regulating the low expression of TMEM100.

ARTICLE HIGHLIGHTS

Research background

TMEM100 is associated with multiple malignancies but its role in esophageal squamous cell carcinoma (ESCC) remains unknown.

Research motivation

This study aimed to investigate the regulatory mechanism of TMEM100 expression in ESCC and its effect on ESCC cell growth and proliferation.

Research objectives

This study hopes to clarify the role of TMEM100 in ESCC as well as to preliminarily investigate the epigenetic regulation of TMEM100 expression.

Research methods

We used R software and online analysis databases to analyze the expression, prognosis and pathway of TMEM100 in esophageal cancer (EC). Utilization of real-time PCR and western blotting to probe the expression of TMEM100 and pathway proteins in ESCC. In addition, the effects of TMEM100 overexpression on the proliferation, invasion and migration of ESCC cells were assessed by CCK-8 and clone formation assays.

Research results

Kaplan-meier survival analysis revealed that low expression of TMEM100 correlated with poor prognosis in patients with EC. Further, treatment with the demethylating agent 5-AZA resulted in increased TMEM100 expression in ESCC cells. Additionally, TMEM100 overexpression exhibited inhibitory effects on the proliferation, invasion, and migration of ESCC cells. Enrichment analysis highlighted significant enrichment in the mitogen-activated protein kinases (MAPK) signalling pathway, which was validated using western blotting, confirming TMEM100's involvement in the regulation of the MAPK signalling pathway in ESCC cells.

Research conclusions

TMEM100 is highly expressed in normal subjects and lowly expressed in EC patients, and patients with high TMEM100 expression in EC patients have a better prognosis. The expression of TMEM100 was increased in ESCC cells treated with the methylation inhibitor 5-AZA. Overexpression of *TMEM100* gene inhibited the growth and proliferation of ESCC cells and negatively regulated the MAPK signaling pathway.

Research perspectives

The robustness of TMEM100 as a prognostic indicator for ESCC needs to be further validated. Further clarification of the *in vivo* effects of overexpression of TMEM100 on the proliferation of esophageal squamous carcinoma is needed.

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FOOTNOTES

Author contributions: Xu YF and Dang Y are responsible for data curation and writing (original draft preparation); Kong WB is responsible for visualisation; Wang HL and Chen X are responsible for software and validation; Zhao Y is responsible for methodology; Yao L is responsible for writing (reviewing and editing); Zhang RQ is responsible for conceptualization and resources.

Institutional review board statement: This study does not involve human subjects.

Institutional animal care and use committee statement: This study does not involve animal subjects.

Conflict-of-interest statement: No conflicts of interest are associated with any of the senior authors or other co-authors who contributed their efforts to this manuscript.

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CASE REPORT

Low-grade myofibrosarcoma of the maxillary sinus: Two case reports

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Abstract

BACKGROUND

Low-grade myofibroblastic sarcoma (LGMS) is an extremely rare tumor characterized by the malignant proliferation of myofibroblasts. LGMS most commonly develops in adults, predominantly in males, in the head and neck region, oral cavity, especially on the tongue, mandible, and larynx. This article presents 2 cases of LGMS localized to the maxillary sinus and provides an overview of the available literature.

CASE SUMMARY

Two patients with LGMS located in the maxillary sinus underwent surgery at the Department of Head and Neck Surgery. Case 1: A 46-year-old patient was admitted to the clinic with suspected LGMS recurrence in the right maxillary sinus (rT4aN0M0), with symptoms of pain in the suborbital area, watering of the right eye, thick discharge from the right nostril, and augmented facial asymmetry. After open biopsy-confirmed LGMS, the patient underwent expanded maxillectomy of the right side with immediate palate reconstruction using a microvascular skin flap harvested surgically from the middle arm. The patient qualified for adjuvant radiotherapy for the postoperative bed, with an additional margin. Currently, the patient is under 1.5 years of observation with no evidence of disease. Case 2: A 45-year-old man was admitted to our clinic with facial

asymmetry, strabismus, exophthalmos, and visual impairment in the right eye. Six months earlier, the patient had undergone partial jaw resection at another hospital for fibromatosis. A contrast-enhanced computed tomography scan revealed a tumor mass in the postoperative log after an earlier procedure. An open biopsy confirmed low-grade fibrosarcoma (rT4aN0M0). The patient qualified for an extended total right maxillectomy with orbital excision and right hemimandibulectomy with immediate microvascular reconstruction using an anterolateral thigh flap. The patient subsequently underwent adjuvant radiotherapy to the postoperative area. After 9 months, recurrence occurred in the right mandibular arch below the irradiated area. The lesion infiltrated the base of the skull, which warranted the withdrawal of radiotherapy and salvage surgery. The patient qualified for palliative chemotherapy with a regimen of doxorubicin + dacarbazine + cyclophosphamide and palliative radiotherapy for bone metastases. The patient died 26 months after surgical treatment. The cases have been assessed and compared with cases in the literature.

CONCLUSION

No specific diagnostic criteria or treatment strategies have been developed for LGMS. The treatment used for LGMS is the same as that used for sinonasal cancer radical tumor excision; adjuvant radiotherapy or chemoradiotherapy should also be considered. They have low malignant potential but are highly invasive, tend to recur, and metastasize to distant sites. Patients should undergo regular follow-up examinations to detect recurrence or metastasis at an early stage. Patients should be treated and observed at the highest referral centers.

Key Words: Head and neck cancer; Paranasal sinuses; Maxillary sinus; Sarcoma; Low-grade myofibroblastic sarcoma; Case report

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Core Tip: Low-grade myofibroblastic sarcomas are tumors of low malignant potential; however, they are highly invasive and a high tendency to recur and metastasize to distant sites. Since only 55 cases of low-grade myofibroblastic sarcoma have been described, it is impossible to establish guidelines. As there are no specific diagnostic criteria, it is necessary to consider the occurrence of myofibroblastic sarcoma more often than reported in the literature.

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INTRODUCTION

Low-grade myofibroblastic sarcoma (LGMS) is characterized by malignant proliferation of myofibroblasts. LGMS is extremely rare and most commonly presents on the tongue in the head and neck region. According to the literature, LGMS may also be present in the limbs, abdominal cavity, pelvis, and long bones and pelvis. Sarcomas are histologically atypical with infiltrating myoepithelial cells and morphological, immunochemical, and ultrastructural features of myofibroblast origin.

Myofibroblasts, also called modified fibroblasts, are myoepithelial cells or stellate cells of mesenchymal origin, discovered in 1971 during the healing of granulation tissue[1]. These cells have contractile properties, have characteristics of both fibroblasts and smooth muscle cells, and are present in almost every tissue[2]. In adults, myofibroblasts have also been discovered in the periodontium and around the seminiferous tubules in the testicle[3].

Myofibroblasts have an irregular, hyperchromatic, enlarged nucleus with moderate atypia in amphophilic cytoplasm [4]. They are characterized by the expression of α-Smooth muscle actin (SMA), vimentin and extra domain A of the fibronectin domain; however, they do not express smooth muscle markers Desmin and Smooth muscle myosin, differentiating them from other cells. These cells play a crucial role in physiological and pathological processes such as fibrotic diseases (lungs, kidney, intestine, and liver) and the etiopathogenesis of bronchial asthma. Myofibroblasts are particularly important during wound healing[5]. It is suspected that the transformation of fibroblasts to myofibroblasts occurs under the influence of transforming growth factor-B and extra domain A of fibronectin or the mesenchymal transformation of fibrocytes from bone marrow[1,6].

LGMS most frequently occurs in men and is extremely rare in children. It is highly malignant and characterized by metastasis to distant sites. To the best of our knowledge only 5 cases of maxillary sinus LGMS are available[2,7,8]. Patients rarely report symptoms, and the primary complaint is painless edema. Radiologically, LGMS can present a destructive growth pattern.

CASE PRESENTATION

Chief complaints

Case 1: A 46-year-old male previously treated at another hospital was admitted to the outpatient clinic of the Maria Sklodowska-Curie National Research Institute of the Oncology Department of Head and Neck Oncology. The patient presented with right-sided pain in the suborbital area, watering of the right eye, and thick discharge from the right nostril with augmented facial asymmetry.

Case 2: A 45-year-old male was admitted to Maria Sklodowska-Curie National Research Institute of Oncology presenting with strabismus, exophthalmos, and visual impairment.

History of present illness

Case 1: The patient had previously undergone surgery at another hospital for LGMS. The patient underwent resection of the maxilla using a lateral rhinotomy. A second operation was performed because of the positive surgical margins. Histopathological examination confirmed radical resection and the patient qualified for observation. Thirty months after the surgery, clinical examination confirmed an advanced tumor infiltrating the right nasal cavity, hard palate, and soft palate.

Case 2: Six months earlier, the patient underwent partial resection of the maxilla because of fibromatosis.

History of past illness

Case 1: Generally healthy, did not report chronic diseases, allergies, or medications taken regularly. At the age of 4 years, there was an electric burn on the index finger of the left hand and subsequent amputation.

Case 2: Overall healthy. He does not take medications regularly. Allergy to penicillin.

Personal and family history

Case 1: Professional driver by profession. No family history of malignancy.

Case 2: No family history of malignancy.

Imaging examinations

Case 1: Computed tomography (CT) (Figure 1) and magnetic resonance imaging (MRI) (Figure 2) of the head and neck region revealed extensive soft tissue masses in the right maxillary sinus, nasal cavity, nasopharynx, ethmoid cells, and frontal sinus. Infiltration and partial osteolysis were observed in the bone structures on the right side, including the sinus walls, hard palate, medial and suborbital bones, and pterygoid plates.

Case 2: CT, with contrast scan (Figure 3), revealed a tumor mass in the postoperative lobe after the first surgery.

Tumor infiltration was observed in the pterygopalatine and right temporal fossa. Infiltration also involved the lateral pterygoid and masseter muscle, the lateral wall of the nasal cavity and the oral cavity.

Soft tissue mass protruding from the tumor into the posterior orbit through the superior orbital fossa.

Tumor progression and rapid recurrence after primary surgery. The histopathological examination results were verified at the Maria Sklodowska-Curie National Research Institute of Oncology. After additional examinations and multispecialty consultation, the primary diagnosis was changed from fibromatosis to inflammatory myofibroblastic tumor.

Laboratory examinations

Case 1: Laboratory tests without deviations.

Case 2: Laboratory tests without any significant deviations.

Physical examination

Case 1: Facial asymmetry, highlighting of the right cheek. Eyeball movement was preserved, and the patient denied diplopia or any other deviation from the norm. On intraoral examination, an exophytic tumor of the hard palate reached the midline. Lymphadenopathy was not present during the physical examination.

Case 2: Facial asymmetry, swelling of the right cheek. Scars on the right cheek from previous surgery. Strabismus and exophthalmos of the right eye, significant visual impairment, preserved response to light.

During intraoral examination, a palpable tumor on the palate on the right side was observed. Palpable cervical bulb on the right in group 2.

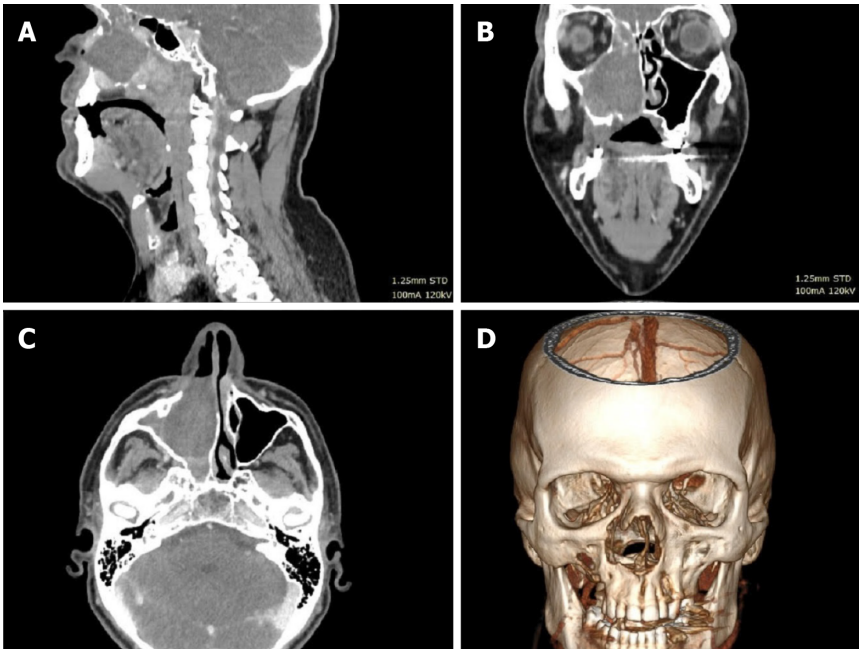

Figure 1 Case 1 computed tomography scan with contrast and 3D reconstruction. Solid contrast-enhancing tumor filling the maxillary sinus and eroding the bony plate is shown. A: Sagittal section; B: Coronal section; C: Axial section; D: 3D reconstruction.

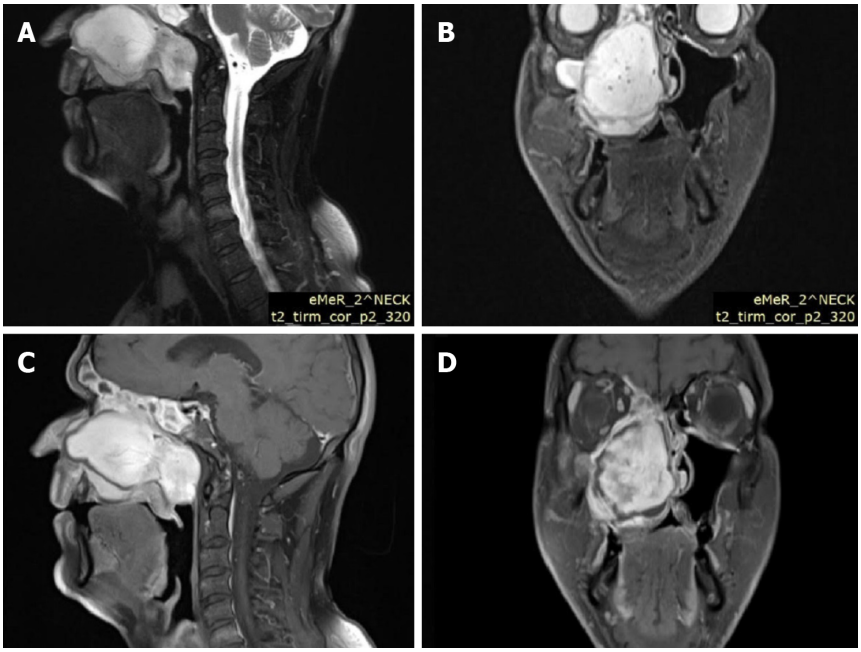

Figure 2 Case 1 T2 magnetic resonance imaging illustrating the extent of the tumor to the right maxilla sinus. A and C: Sagittal sections; B and D: Coronal sections.

FINAL DIAGNOSIS

Case 1

Histological examination confirmed the recurrence of LGMS (rpT4aN0M0) (8th Edition, American Joint Committee on Cancer) of the right maxilla, 8 cm in size. Neoplasms with spindle-cell proliferation and moderate cellular atypia.

Mitotic activity was low [four mitoses per 10 high power field (HPF)], without atypical mitosis. The collagenous stroma was partially myxoid and contained an increased number of thick-walled capillaries; no necrosis was observed. Bone destruction was also observed.

Immunohistochemistry staining performed: SMA (+, in parts of cell population), reaction type "tram truck", cytokeratin AE1 and AE3 (CKAE1/3) (-/+, insufficient focal reaction), Mucin 4 (MUC4) (-), CD34 (-), Desmin (-), SOX10 (-), S100 protein (-), Ki-67 protein (5%), hHf35 (-), Epithelial membrane antigen (EMA) (-) (-/+), Caldesmon (-/+, trace), H3K27me3

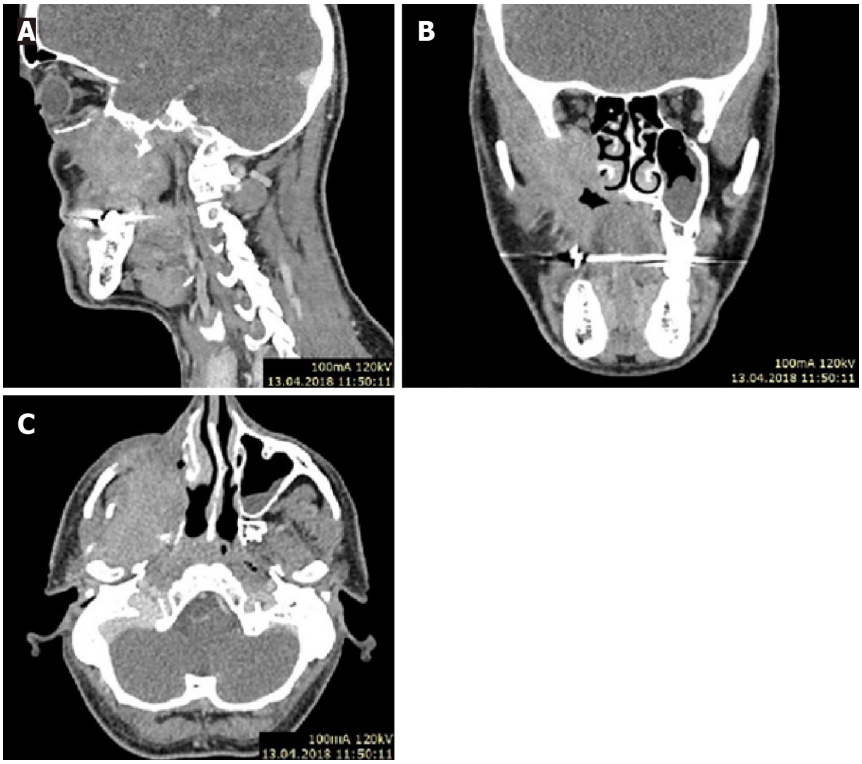

Figure 3 Case 2 computed tomography with contrast scan. Solid contrast-enhancing tumor filling the maxillary sinus is shown. A: Sagittal section; B: Coronal section; C: Axial section.

(+, expression prohibited), ALK (-), ROS1 (-), HMB45 (-), Melan-A (-), Myogenin (-), MyoD1 (-).

Case 2

Histopathological examination confirmed LGMS. The tumor was poorly demarcated, cream-gray in color, macroscopically without necrosis, and 8 cm in diameter with endophytic growth (rpT4aN0M0) (8th Edition, American Joint Committee on Cancer).

Microscopic examination revealed proliferation of spindle cells with moderate cellularity and focal moderate cellular atypia; mitotic activity was low (four mitoses per 10-HPF) without atypical mitosis. The collagen stroma was partially edematous without necrosis. Natural invasion was also observed.

Immunohistochemical staining performed: SMA (+), Desmin (-), CD34) (-), EMA (-), CKAE1/3 (-), Caldesmon (-), MUC1 (-), S100 protein (-), ALK1 (-), Signal transducer and activator of transcription 6 (+/-), B-creatinin (-).

TREATMENT

Case 1

Based on the physical, histopathological, and radiological examinations, the patient qualified for an expanded maxillectomy of the right side with immediate palate reconstruction using a microvascular skin flap harvested surgically from the middle arm. An intraoperative photograph was captured (Figure 4A) after buccal flap creation. The right lymph nodes were selectively resected for vascular anastomosis. Because infiltration was present within the tissues, exenteration was performed on the right side with a partial right-sided sphenoethmoidectomy (Figure 4B). Figure 5 shows the post-resection lodges. Fastened preparation of blocks. During the procedure, leakage of cerebrospinal fluid from the olfactory filament area and right sphenoid sinus was observed. Duraplasty was performed using the latae of the tensor fascia, a mucoperiosteal flap, and a topical fibrin sealant patch. The patient did not experience any complications peri- or postoperatively.

The patient qualified for adjuvant radiotherapy radiation therapy (IMRT) and cone beam CT for postoperative treatment, with additional margins. The patient received a fractional dose of 200 centiGray (cGy) for a total dose of 6600 cGy.

Case 2

Open biopsy confirmed recurrence of low-grade myofibrosarcoma. Based on clinical, histopathological, and radiological results, the patient qualified for expanded complete right-sided maxillectomy and right-sided hemimandibulectomy with immediate microvessel reconstruction using an anterolateral thigh flap and selective resection of the lymph nodes on the right side. Because of infiltration of the orbital tissues, right-sided exenteration was performed.

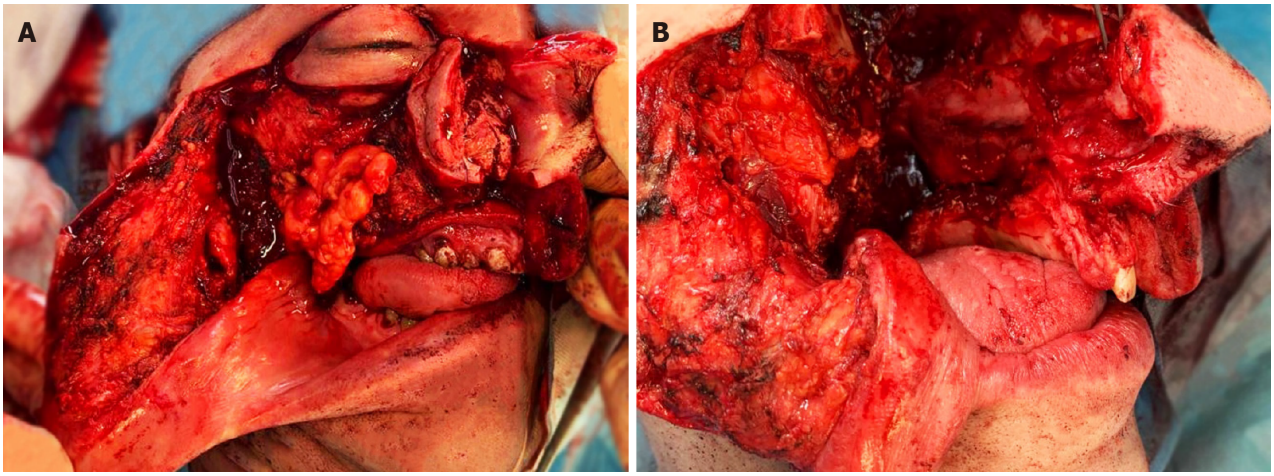

Figure 4 Case 1 intraoperative photo and post-resection lodge. A: Intraoperative photo; B: Post-resection lodge.

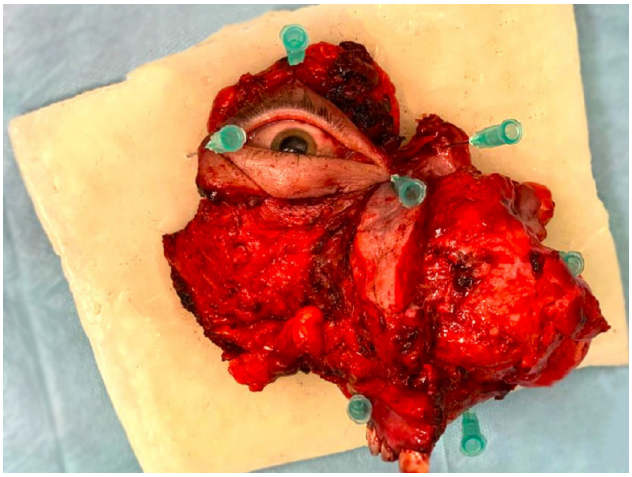

Figure 5 Case 1 fastened preparation in block.

The patient qualified for adjuvant radiation therapy (IMRT) of the postsurgical bed with a fraction dosage of 200 cGy to a total dose of 6600 cGy.

OUTCOME AND FOLLOW-UP

Case 1

Currently, the patient is under observation with no evidence of disease.

Case 2

After 9 months of observation, recurrence appeared in the right mandibular arch below the irradiated area. CT confirmed the progression in both the irradiated and the previously irradiated areas. The lesion is located at the base of the skull.

There were increasing postoperative risks, which justified refrainment from radiotherapy and salvage surgery.

The patient qualified for palliative chemotherapy with doxorubicin + dacarbazine + cyclophosphamide regimen. Due to pathological L1 and L5 fractures, metastases to L1 and L2, and metastasis to the right hip bone, the patient was eligible for Radiation Therapy [fractions of 3 Gray (Gy) to a total dose of 36 Gy] and second-line chemotherapy (gemcitabine + docetaxel). The patient died 26 months after surgical treatment.

A comparison of the immunohistochemical studies of Case 1 and Case 2 is shown in Table 1.

Table 1 Comparison of immunohistochemical results in Cases 1 and 2

Case	Vimentin	SMA	CK AE 1/3	Desmin	h-Caldesmon	CD 34	S100 protein	EMA	ALK 1
1	+	+	-/+	-	-/+ trace	-	-	-/+	-
2	+	+	-	-	-	-	-	-	-

+: Positive reaction; -: Negative reaction. ALK 1: Anaplastic lymphoma kinase 1; CD 34: Cluster of differentiation 34; CKAE 1/3: Cytokeratin AE1 and AE3; EMA: Epithelial membrane antigen; SMA: Smooth muscle actin.

DISCUSSION

LGMS

LGMS is a recently discovered and extremely rare malignant tumor. The first such case was diagnosed in the 1998s. In 2002, the World Health Organization made the LGMS a separate unit in the pathology and genetics of soft tissue and bone tumors[9]. Clinically, it manifests as a slow-growing and infiltrating tumor. LGMS is a low-grade malignant tumor with a high tendency for recurrence and distant metastases, even after several years[10,11].

LGMS most commonly develops in adults, predominantly in males, and in the head and neck. The tumor most often appears in the oral cavity, especially in the tongue, mandible, and larynx[6]. Other localizations include the limbs, abdominal cavity, pelvis, and long bones[12].

LGMS of the maxillary sinus is extremely rare, and only five cases have been described so far. Here, we present two more cases (Table 2)[2,7,8,13]. In cases of soft tissue sarcomas of the head and neck, MRI with contrast and/or CT with contrast should be performed (NCCN Guidelines version 2. 2022)[14]. Radiologic imaging typically shows a well-limited tumor with visible margins of destructive growth[6,10].

Histology

Histologically, the tumor is composed of spindle and stellate cells collected in clusters of different lengths, with a focal herringbone, spiral, or no pattern[13]. Cancerous cells are composed of a mild to moderate amount of pale eosinophilic cytoplasm and a spindle nucleus, which can be spiral or circular, and vesicles with cavities.

In most cases, focal atypia of the nucleus is observed; however, this is usually benign with enlarged hyperchromatic nuclei. Additionally, larger atypical cells can sometimes be observed[11].

Microscopic Figure 6A shows spindle cell infiltration, hypocellularity with mild atypia, and stromal collagen. Hypercellular proliferation and bundles of spindle cells are observed with hematoxylin and eosin staining (Figure 6B). Figure 7A shows focal expression of SMA and Figure 7B shows no expression of ALK.

Immunophenotype

Neoplastic cells in LGMS have a variable immunophenotype: Actin positive (+)/Desmin negative (-), Actin negative (-)/Desmin positive (+), and Actin positive (+)/Desmin (+) positive. In addition, tumor cells may stain positively for fibronectin. Focal expression of CD34 and Cluster of differentiation 99 has been reported, while S100 protein, epithelial markers, laminin, and h-Caldesmon are negative (-)[2,15].

Differential diagnosis

The differential diagnosis of LGMS includes both malignant and benign tumors such as nodular fasciitis, myofibroblastic tumors, fibromatosis, myofibroma, myopericytoma, monophasic synovial sarcoma, malignant peripheral nerve sheath tumors, spindle cell rhabdomyosarcoma, fibrosarcoma, leiomyosarcoma, and melanoma[5,6,16].

Procedure

The gold standard procedure in cases of sarcoma infiltrating bones is radical excision of the tumor[12,17]. In cases of positive margins, the radicalization procedure should be primarily considered. When radicalization is impossible, soft tissue margins are narrow and large. If tumors infiltrate the blood vessels or nerves, radiotherapy or chemoradiotherapy should be considered[12,18,19]. The LGMS head and neck recurrence rate is 25%-40% and is the highest when the tumor is in the nasal cavity or paranasal sinuses. A higher frequency of recurrence was observed in patients who underwent adjuvant radiation therapy. This is probably a result of the qualification of patients with unfavorable prognostic factors. The most important prognostic factor was the resection state. Positive margins, regional lymph node involvement, and age > 60 years[12].

The clinical cases presented above were characterized by characteristics specific to the described type of sarcoma, which enabled the identification of certain groups of tumors. As shown in Table 2, males are mostly affected (57%), which is also indicated in previous literature. The average age of the patients is 41 ± 17.2 years (females 45 ± 25.8 years, males 38 ± 10.6 years). The most common symptoms are nasal congestion, rhinorrhea, edema, and pain. Exophthalmos was present in two patients; however, visual impairment was present in one patient.

LGMSs are tumors of low malignancy; however, they are highly invasive, with a high tendency for recurrence and a high risk of distant metastases[6]. Several factors may contribute to this paradox of LGMS. Tumors with a low grade of malignancy may have a lower mitotic index, but this does not necessarily reflect their invasive potential or likelihood of

Table 2 Clinical features of reported cases of low-grade myofibrosarcomas of the maxillary

Ref.	Age	Sex	Size in cm	Side	Main symptoms	Necrosis	Mitotic rate, 10/10 HPF scale	Follow up in months	IHC	Results
Meng *et al*[7], 2007	33	F	6.5	Left	Nasal obstruction and leakage	Yes	10/10/HPF	12	Vimentin (+); SMA (+); Fibronectin (+); Calponin (+); Desmin (-); h-Caldesmon (-); Laminin (-); Type IV collagen (-); CD34 (-); CD68 (-); ALK1 (-)	Diagnostic testing of 1 yr
Meng *et al*[7], 2007	28	F	3	Left	Nasal obstruction and leakage	Yes	8/10 HPF	21	Vimentin (+); SMA (+); Fibronectin (+); Calponin (+); Desmin (-); h-Caldesmon (-); Laminin (-); Type IV collagen (-); CD34 (-); CD68 (-); ALK 1 (-)	Recurrent at 0.5 yr
Ghosh *et al*[2], 2019	35	M	No data	Left	Exophthalmos	No data	< 2/10 HPF	72	α-SMA (+); MIC-2 (+); Desmin (-); CD 34 (-); S100 protein (-); Cytokeratin (-); EMA (-); Calponin (-); Bcl-2 (-)	Recurrent at 6 yr
Bisceglia *et al* [13], 2001	24	M	4	Left	Pain, swelling of the midface	No data	1/10HPF	40	Vimentin (+); α-SMA (+); MSA (+); CD34 (-); Desmin (-); S100 protein (-); Cytokeratin (-)	Observation 3 yr
Gómez-Oliveira *et al*[8], 2015	75	F	No data	Left	Pain, swelling	Yes	No data	12	Vimentin (+); SMA (+); CD10 (+); Cytokeratin (+); h-Caldesmon (+); Desmin (-); CD34 (-); ALK (-); EMA (-); S100 protein (-)	After 1 yr metastasis-left femur
Current article	46	M	8	Right	Pain, swelling of the midface, nasal obstruction and leakage	No	4/10HPF	15	SMA (+) in some cells; tram truck type reaction; CK AE1/3 (-/+) focal weak reaction; MUC4 (-); CD34 (-); Desmin (-); SOX10 (-); S100 (-); Ki-67 5%; hHf35 (-); EMA (-/+) trace; Caldesmon (-/+) trace; H3K27me3 (+) preserved expression; ALK (-); ROS1 (-); HMB45 (-) Melan-A (-); Myogenin (-); MyoD1 (-)	Observation 1.5 yr
Current article	45	M	8	Right	Exophthalmos, strabismus, visual impairment	No	4/10HPF	26	SMA (+); Desmin (-); CD34 (-); EMA (-); CKAE1/3 -; Caldesmon (-); MUC1 (-); S100 (-); ALK1 (-); STAT6 (+/-); B-catenin (-)	Recurrent

α-SMA: α-Smooth muscle actin; ALK: Anaplastic lymphoma kinase; ALK1: Anaplastic lymphoma kinase 1; Bcl-2: B-cell lymphoma 2; CD34: Cluster of differentiation 34; CD68: Cluster of differentiation 68; CKAE1/3: Cytokeratin AE1 and AE3; EMA: Epithelial membrane antigen; F: Female; H3K27me3: Trimethylation of lysine 27 on histone H3; HMB45: Human melanoma black-45; HPF: High power field; M: Male; MIC-2: Monoclonal intestinal cancer-2; MSA: Muscle specific actin; MUC1: Mucin 1; MUC4: Mucin 4; MyoD1: Myogenic differentiation 1; ROS1: Receptor tyrosine kinase 1; SMA: Smooth muscle actin; SOX10: Sex determining region Y-box 10; STAT6: Signal transducer and activator of transcription 6.

metastasis. It is suspected that these tumors may show infiltrative growth patterns, making complete surgical removal difficult, and allowing residual microscopic disease left after surgery to cause recurrence.

Even within a specific histological subtype, tumors can be significantly heterogeneous in terms of biological behavior. Some cells may have more aggressive features. Tumor behavior is also influenced by genetic and molecular characteristics. Some low-grade tumors may contain genetic changes or mutations that contribute to their ability to recur or metastasize.

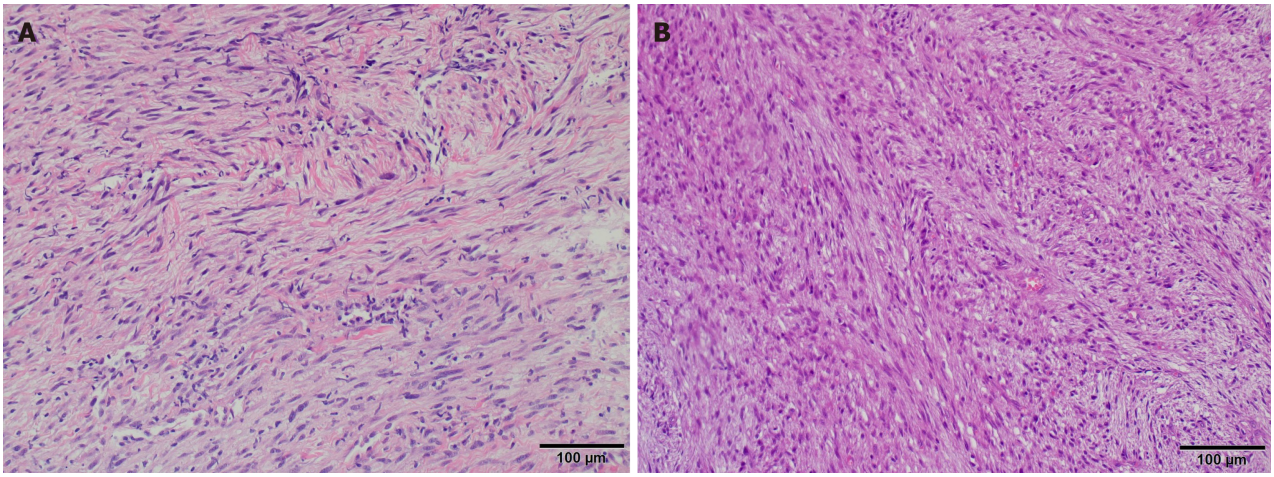

Figure 6 Hematoxylin and eosin staining. A: Spindle cell infiltration, hypocellular with mild atypia, stromal collagen; hematoxylin and eosin (H&E) 20 ×; B: Hypercellular proliferation, fascicles of spindle cells; H&E 20 ×.

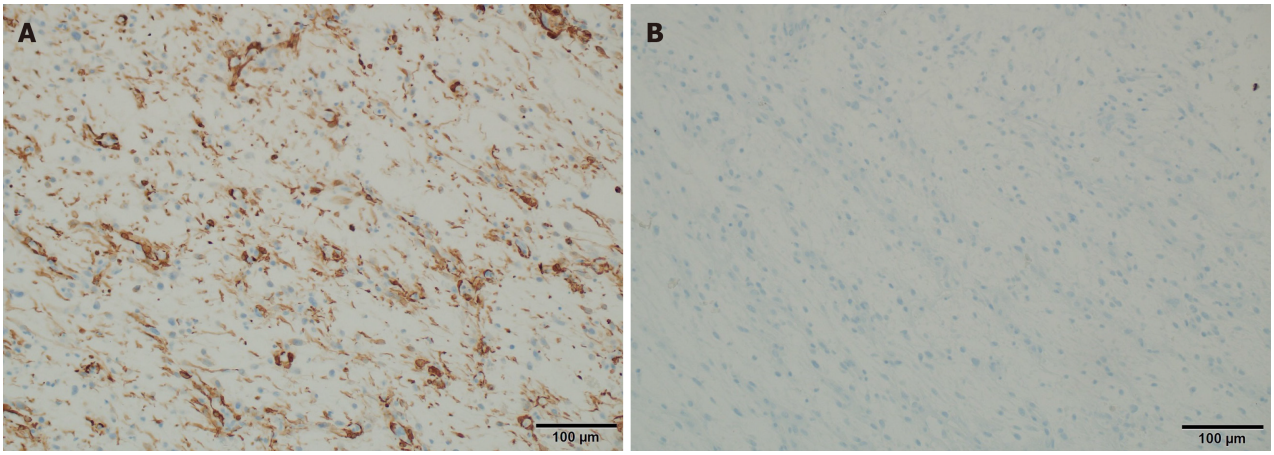

Figure 7 Focal expression of smooth muscle actin and no expression of anaplastic lymphoma kinase (magnification 20 ×). A: Focal expression of smooth muscle actin (magnification 20 ×); B: No expression of anaplastic lymphoma kinase (magnification 20 ×).

CONCLUSION

LGMSs are tumors of low malignant potential; however, they are highly invasive and have a high tendency to recur and metastasize to distant sites. A standard treatment strategy has not been developed yet for LGMS patients. Because of its low frequency of occurrence, it is impossible to establish guidelines. Therefore, the treatment used for LGMS is the same as that used for sino-nasal carcinoma.

It is important that LGMS patients be closely monitored by a multidisciplinary healthcare team to determine the most appropriate treatment plan and follow-up. Regular follow-up examinations are crucial to detect recurrence or metastasis at an early stage. Considering the lack of precise diagnostic criteria, LGMS occurs more often than the literature indicates and may include various clinicopathological forms.

FOOTNOTES

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