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Bao Jun, M.D., Chief Physician. He is mainly engaged in clinical and basic research on the prevention and treatment of gastrointestinal tumours, studying the pathogenesis, metastasis and prognostic factors of gastrointestinal tumours as well as the complications of hereditary gastrointestinal tumours. He has co-chaired one project of Jiangsu Provincial Science and Technology Department and two projects of Jiangsu Provincial Health Planning Commission. He has published more than 20 academic papers in domestic and international academic journals, and participated in the preparation of 2 monographs. He was awarded the third prize of Jiangsu Provincial Health Department for the introduction of new medical technology in TCM and the second prize of Jiangsu Provincial Medical New Technology Introduction Award.

Claudin18.2: an emerging target for the treatment of gastric cancer

WANG Qiangcheng, BAO Jun

Department of Medical Oncology, The Affiliated Cancer Hospital of Nanjing Medical University, Nanjing, Jiangsu 210009, China

Corresponding author: BAO Jun, E-mail: junbao@jszlyy.com.cn

Abstract: Gastric cancer is a malignant tumor that seriously endangers human health. Claudin-18.2 (CLDN18.2) is a transmembrane protein and a main component of tight junctions that plays an important role in maintaining barrier function. Under normal physiological conditions, CLDN18.2 is not expressed in healthy tissues, but it is expressed in differentiated epithelial cells of the gastric mucosa. With the progress of research, it has been found that CLDN18.2 is highly expressed or ectopically activated in primary malignant tumors such as gastric cancer, esophageal cancer, ovarian cancer, pancreatic cancer, lung cancer, and breast cancer. Due to its potential for specific expression, CLDN18.2 has become a new emerging target in anti-tumor drug development. This article reviews the expression characteristics, regulation, and clinical pathological features of CLDN18.2 in gastric cancer, and the progress of clinical drug research targeting CLDN18.2.

Keywords: Claudin18.2; Gastric cancer; Targeted therapy; Monoclonal antibodies; Bispecific antibodies; Chimeric antigen receptor T cells; Antibody-drug conjugates
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Introduction

Gastric cancer accounts for 7.7% of all tumors and ranks fourth among all malignancies globally [1]. Despite significant progress in early screening and treatment in recent years, the prognosis for patients with advanced gastric cancer remains poor, with a median overall survival (mOS) of only 14.2 months [2]. Surgical resection is the preferred treatment for early gastric cancer, while chemotherapy-based systemic therapy , is often used for patients with inoperable, recurrent, or advanced gastric cancer [3]. However, the improvement in prognosis with chemotherapy is limited, and it is associated with significant toxic and side effects. In recent years, with the continuous advancement of oncology research, cancer treatment has shifted from traditional "one-size-fits-all" cytotoxic chemotherapy to personalized therapy targeting patient molecular characteristics [4]. The progress in immunotherapy represented by immune checkpoint inhibitors and molecular targeted therapy has led to some improvement in the prognosis of patients with advanced gastric cancer. Pembrolizumab, nivolumab, and the HER2-targeted drug trastuzumab have been recommended as standard first-line treatments for advanced gastric cancer by guidelines both domestically and internationally [5]. However, patients with gastric cancer who are HER2-negative and have a combined positive score (CPS) of <5 for programmed death-ligand 1 (PD-L1) show limited benefits and lower 5-year survival rates from the aforementioned treatment. Therefore, it is urgent to develop drugs against new therapeutic targets to enhance the prognosis of patients with gastric cancer.

In recent years, with the development of oncology and computational biology, the significance of Claudin18.2 (*CLDN18.2*) in the advancement of gastric cancer has attracted considerable interest among researchers. *CLDN18.2* is a tight junction protein expressed only minimally in normal gastric tissue. However, the occurrence of gastric cancer disrupts the

tight junctions on the surface of tumor cells, exposing the *CLDN18.2* antigenic epitope, making it a potential specific therapeutic target for gastric cancer [6]. Various therapies targeting *CLDN18.2* for gastric cancer have been developed, including monoclonal antibodies (mAb), chimeric antigen receptor T cell (CAR-T) therapy, bispecific antibodies (BsAb), and antibody-drug conjugates (ADC), and corresponding clinical trials have been conducted. This article provides an overview of the expression characteristics, regulatory mechanisms, biological functions, clinical pathological features, and research progress of clinical drugs targeting *CLDN18.2* in gastric cancer, aiming to better assist clinicians and researchers in developing and applying drugs targeting *CLDN18.2*.

1. Expression characteristics and regulatory mechanism of *CLDN18.2* gene

Claudins (CLDNs) are a class of membrane proteins with a molecular weight of approximately 20-27 kDa, consisting of four transmembrane domains (TMDs), an N-terminus, and a C-terminus, as well as two extracellular loops spanning the TMDs. There are 27 different subtypes of CLDNs in various human organs, and abnormalities in the function of these subtypes may have different effects on the occurrence and progression of cancer in corresponding tissues, involving various stages from dysplasia to cancer progression and metastasis [7]. Among them, Claudin-18 (CLDN18) plays an important role in maintaining cell polarity and barrier function, as well as promoting acid resistance [8]. The *CLDN18* gene locus is located on chromosome 3q22, with a molecular weight of approximately 35 kb, containing six exons and five introns. The first exon of CLDN18 can undergo selective splicing to form two different splice variants (*CLDN18.1* and *CLDN18.2*) with highly homologous amino acid sequences [9].

1.1 Expression characteristics of CLDN18.2 gene

CLDN18.2 is a highly and selectively expressed molecule, typically expressed only in differentiated epithelial cells of normal gastric mucosa [6], and is present at the tight junctions of gastric mucosal cells [9]. Therefore, it cannot be recognized and bound by antibodies in the bloodstream. However, the occurrence of gastric cancer leads to the disruption of tight junctions and disturbances in cell polarity, exposing *CLDN18.2* antigenic epitopes on the cell surface. Antibodies targeting *CLDN18.2* can bind highly and specifically to the surface of tumor cells without damaging normal cells, making *CLDN18.2* an ideal molecular target for anti-gastric cancer drugs [10]. In addition, *CLDN18.2* is highly expressed or ectopically activated in malignant tumors such as pancreatic cancer, esophageal cancer, ovarian cancer, and lung cancer [11], indicating its potential in the treatment of other tumors.

1.2 Functions of CLDN18.2 gene

The exact mechanism through which *CLDN* expression promotes tumor development is unclear yet. The main view at the moment is that *CLDNs* activate various signaling pathways or proteases, directly or indirectly promoting tumor development. One direct pathway involves association with other molecules, such as EpCAM, MT-MMPs, ADAM10, and integrins, which activate *CLDNs* to participate in signal transduction, extracellular matrix degradation, and receptor cleavage. Another direct pathway is associated with transcription factors (TFs), such as YAP/TAZ and β-catenin, to induce nuclear accumulation of TFs. Indirect pathways involve two types of molecules: proteinases such as MMPs, which degrade the extracellular matrix (ECM) to release growth factors (GFs) to activate RTK/PI3K, MAPK, TGF-β/SMAD, and JAK/STAT signaling pathways; and protein kinases such as SFK, ABL, and Tyk2, which phosphorylate downstream molecules. The interconnection between related signaling pathways integrates signals from *CLDNs* to promote tumor development [12].

Methylation of the CpG island-associated gene promoters is considered one of the key mechanisms regulating the expression of CLDN18 [13]. Methylation in the CpG island is associated with the expression of the CLDN18 gene. Sahin *et al.* [14] demonstrated that methylation in the CpG island can completely block the necessary TFs for the activation of *CLDN18.2* transcription. CREB binds to the promoter of *CLDN18.2*. Activator protein-1 (AP-1) is an intracellular transcription activator, one of the most significant TFs which is critical for regulating cell growth and differentiation. Studies have found that AP-1 can bind to the cis-regulatory elements of the *CLDN18.2* promoter, promoting the transcription of CLDN18.2. Furthermore, activation of the PKC and ERK-MAPK pathways enhances the phosphorylation of AP-1, thereby increasing the transcription of *CLDN18.2* mRNA [15].

2. Clinicopathologic features of *CLDN 18.2*

The expression level of *CLDN18.2* in metastatic tumor lesions is comparable to that in primary lesions, suggesting that patients with metastatic tumors may also benefit from *CLDN18.2*-targeted therapy [16-17]. The expression level of *CLDN18.2* is also associated with the histological subtypes of gastric cancer, with a significant increase in *CLDN18.2* expression in diffuse-type gastric cancer. Additionally, CLDN18-ARHGAP26/6 fusion genes are specifically present in diffuse-type gastric cancer [18]. Shu *et al.* found that the proportion of CLDN18-ARHGAP26/6 fusion genes in diffuse-type gastric cancer (signet ring cell carcinoma) is 25%, and it is related to the content of mucin cells, age, gender, and TNM staging. Patients

with CLDN18-ARHGAP26/6 fusion genes have a poorer prognosis and lower response rates to chemotherapy drugs such as oxaliplatin/fluoropyrimidine [19]. Nakayama *et al.* [20]found that among 146 gastric cancer patients under 40 years old, the proportion of CLDN18-ARHGAP26/6 fusion genes was 15.1%, and gene fusion-positive patients had larger tumors, higher rates of lymph node metastasis, and later TNM staging. These results suggest that the proportion of CLDN18-ARHGAP fusions is higher in young gastric cancer patients and may be associated with the highly invasive nature of tumors.

3. Targeted therapies for *CLDN18.2*-positive gastric cancer

Currently, targeted therapies for *CLDN18.2*-positive gastric cancer mainly include monoclonal antibodies (mAb), CAR-T cell therapy, bispecific antibodies (BsAb), and antibody-drug conjugates (ADC)., and so on.

3.1 CLDN18.2-mAb

3.1.1 Zolbetuximab

Zolbetuximab is a chimeric IgG1 mAb targeting *CLDN18.2*, which mediates immunolytic effects through complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity [21]. The effectiveness of zolbetuximab with chemotherapy in the first-line treatment of advanced gastric or esophagogastric junction cancer was examined in the recent phase III SPOTLIGHT study [22]. The combination of Zolbetuximab with mFOLFOX6 (oxaliplatin + 5-fluorouracil/ leucovorin) significantly improved the median progression-free survival (mPFS) from 8.67 months in the placebo group to 10.61 months. The mOS was 18.23 months in the Zolbetuximab group and 15.54 months in the placebo group. The objective response rate (ORR) and duration of response (DOR) were similar between the two groups. In terms of safety, 14% of patients in the Zolbetuximab group discontinued treatment due to grade 3 or higher adverse events, compared to 6% in the placebo group.

In March 2023, the American Society of Clinical Oncology (ASCO) presented preliminary results of another Phase III trial, the GLOW study, which investigated the addition of Zolbetuximab to CAPOX regimen chemotherapy as first-line treatment [23]. Zolbetuximab combined with chemotherapy significantly increased PFS, confirming the results of the SPOTLIGHT study. Compared to the placebo group, the mPFS increased from 6.80 months to 8.21 months in the Zolbetuximab group. Similarly, the mOS was significantly prolonged in the Zolbetuximab group (14.39 months *vs.* 12.16 months). The ORR was 53.8% in the Zolbetuximab group and 48.8% in the placebo group. Treatment-related adverse events led to discontinuation in 7.1% of patients in the Zolbetuximab group and 4.4% in the placebo group.

In summary, these two Phase III studies demonstrate significant clinical benefits of the first anti-*CLDN18.2* antibody, Zolbetuximab, in gastric or gastroesophageal junction cancer. However, the enrolled patients in both studies were *CLDN18.2*-positive (≥75% of tumor cells showing moderate-to-strong membranous *CLDN18.2* staining), and further exploration is needed for the therapeutic effects in patients with low *CLDN18.2* expression.

3.1.2 TST001

TST001 is a *CLDN18.2*-mAb with high affinity. The drug reduces the fucose content of the Fc terminus and further enhances its ability to bind to the Fc receptor on natural killer (NK) cells. This enhances the binding of TST001 to NK cells and further strengthens the activity of antibody-dependent cell-mediated cytotoxicity (ADCC). Preclinical studies have shown that TST001 exhibits a stronger tumor-reducing effect compared to Zolbetuximab at equivalent doses. Additionally, the anti-tumor effect of TST001 is independent of the expression level of *CLDN18.2*, meaning that even in gastric cancer cells with low-to-moderate levels of *CLDN18.2* expression, TST001 can still exhibit anti-tumor activity [24].

Cohort C from the TranStar102/TST001-1002 study (NCT04495296) evaluated the efficacy and safety of TST001 in combination with CAPOX regimen chemotherapy as first-line treatment for gastric cancer. In terms of efficacy, the mPFS was 9.5 months, and ORR was 66.7%. The median DOR was 9.9 months. There were no dose-limiting toxicities in the overall population, and most treatment-related adverse events (TRAEs) during treatment were grade 1-2. It is worth noting that, compared to the GLOW study, this study relaxed the criteria for *CLDN18.2* expression in enrolled patients (≥10% tumor cells with *CLDN18.2* membrane staining intensity ≥1+), and also showed a prolonged mPFS [25]. Furthermore, a phase III clinical trial for TST001 has recently been initiated, selecting TST001 in combination with nivolumab and chemotherapy as first-line treatment for gastric cancer (NCT06093425), and its efficacy and safety need further exploration. Other *CLDN18.2* antibodies are mostly in phase I or preclinical studies.

3.2 Bispecific antibody (BsAb) targeting CLDN18.2

BsAb is another important direction for drug development targeting *CLDN18.2*, with three major mechanisms of action, including recruiting and activating immune cells to kill tumor cells; inhibiting or stimulating multiple signaling pathways to exert coordinated effects; and mediating the formation of protein complexes through the bivalent structure of antibodies to exert biological effects, etc. [26]. BsAbs that have been tested by clinical trials include *anti-CLDN18.2/CD3*, *anti-CLDN18.2/4-1BB*,

anti-CLDN18.2/PD-L1, and *anti-CLDN18.2/CD47*. Compared with mAbs, these BsAb drugs have an additional specific antigen-binding site, making them more specific and able to target tumor cells more accurately while reducing off-target toxicity, showing great potential in the treatment of gastric and pancreatic cancer. Most BsAbs are currently in phase I or preclinical stages, and here we summarize several representative BsAbs.

3.2.1 AMG-910

Based on the findings of Zhu *et al.* [27], it appears that BsAb targeting *CD3*/*CLDN18.2* may be an effective treatment for gastric and pancreatic cancer, and *in vivo* models have validated its efficacy and safety. AMG-910 is a half-life extended (HLE) bispecific T cell engager (BiTE) antibody targeting CD3/*CLDN18.2*, which guides the lysis of tumor cells and kills tumor cells by binding to *CLDN18.2* on tumor cells and CD3 on T cells. A phase I clinical study (NCT04260191) is currently underway to evaluate the safety, tolerability, pharmacokinetics, and efficacy of AMG-910 in treating patients with *CLDN18.2*-positive gastric cancer and gastroesophageal junction adenocarcinoma.

3.2.2 Q-1802

Q-1802 can bind to PD-L1 and *CLDN18.2* simultaneously, inducing antibody-dependent cell-mediated cytotoxicity and blocking PD-1 signal transduction, activating innate and adaptive immunity. Preclinical studies have shown that Q-1802 can accurately target tumor tissue and has a strong therapeutic effect in killing tumor cells, providing a new treatment option for advanced solid tumors expressing *CLDN18.2* [28]. The antibody has been in a phase I clinical trial in patients with advanced solid tumors, and interim data showed that, in the dose-expansion stage, among 9 patients with *CLDN18.2*-positive gastrointestinal tumors, 2 patients achieved partial response (PR) and 4 achieved stable disease (SD). In terms of safety, most TRAEs were grade 1-2, with a 24.1% (7/29) incidence of grade 3 TRAEs, and one case of grade 4 TRAE, hyponatremia, occurred in a patient with a history of long-term diuretic use [29].

In addition, BsAbs also face challenges, such as the need for precise engineering design to avoid stability, solubility, and aggregation issues, as well as how to avoid potential non-specific effects and toxicity. Additionally, the production cost of bispecific antibodies is high, limiting their use in patients. Continuous research and development are needed to improve the efficacy and safety of BsAbs in cancer treatment [29].

3.3 CAR T-cell therapy targeting CLDN18.2

CAR-T cell therapy is an immunotherapy approach that involves genetically engineering a patient's own immune cells and using gene editing to recognize and attack specific targets [30].

Jiang *et al.* [31] successfully developed humanized *CLDN18.2*-specific mAbs, hu8E5 and hu8E5-2i single-chain fragments, and prepared *CLDN18.2*-specific CAR-T cells. These CAR-T cells effectively suppressed tumor growth in xenograft mouse models without causing significant damage to other normal tissues. Luo *et al.* [32] further enhanced the anti-tumor activity of *CLDN18.2*-specific CAR-T cells by introducing cytokines such as IL-7 and CCL-21. Studies found that *CLDN18.2*-specific CAR-T cells expressing IL-15 exhibited better amplification *in vitro* and stronger anti-tumor activity both *in vitro* and *in vivo*, with a higher proportion of central memory T cells [33].

Currently, domestically developed CAR-T cell therapy CT-041 has been tested by phase Ⅰ/Ⅱ clinical trials. A interim analysis of a phase I trial (NCT03874897) targeting advanced gastric/esophagogastric junction adenocarcinoma showed a mPFS of 4.2 months, with a 6-month overall survival rate of 81.2% [34]. Additionally, an analysis of 18 patients with gastric/esophagogastric junction adenocarcinoma who had previously failed at least two lines of treatment showed an ORR of 61.1% and an mPFS of 5.6 months after receiving treatment with 2.5×10^8 CAR-T cells. Safety analysis showed no dose-limiting toxicities within 28 days after the first infusion of CT-041. Common treatment-related adverse events (TRAEs) of grade ≥3 included pre-treatment hematologic toxicities (100%), leukopenia (83.8%), neutropenia (67.6%), anemia (40.5%), and thrombocytopenia (16.2%). Moreover, there were no immune effector cell-associated neurotoxicity syndromes (ICANS) reported. Overall, CT-041 demonstrated acceptable safety and promising anti-tumor activity in *CLDN18.2*-positive gastric cancer patients, positioning it as a leading entity in the global therapy field of solid tumor CAR-T cells. Other CAR-T cell therapies targeting *CLDN18.2,* such as LY-011, KD-498, and LB-1908, are in phase I clinical trials.

3.4 ADC targeting CLDN18.2

ADCs are potent anticancer therapeutics that deliver cytotoxic molecules directly to cancer cells. ADCs have been widely used in many solid tumors and hematologic malignancies [35]. Zhu *et al.*[27] conjugated anti-*CLDN18.2* antibodies with cleavable auristatin to generate ADCs. Their research showed effective inhibition of the growth of gastric cancer cells (KATO-III/h*CLDN18.2*) and pancreatic cancer cells (BxPC3/h*CLDN18.2*) *in vitro*, and efficacy in high *CLDN18.2*-expressing PDX models. This preclinical study suggests that anti-*CLDN18.2* ADCs may be effective in the treatment of gastric and pancreatic cancers.

CMG901 is an ADC composed of humanized mAb CM311 targeting *CLDN*18.2 and the inhibitor of microtubule polymerization monomethylauristatin E (MMAE) linked by a cleavable connecting arm. Upon binding to *CLDN18.2*-positive cells, CMG901 is

endocytosed into lysosomes within tumor cells, where it releases cytotoxins, leading to apoptosis of tumor cells. Mechanistic studies demonstrate that CMG901 can stimulate cell and soluble immune effector activation, including ADCC and complement-dependent cytotoxicity (CDC), to kill *CLDN*18.2-positive tumor cells. Preclinical studies also indicate that CMG901 effectively kills gastric cancer cells, with greater antitumor efficacy than zolbetuximab [36]. Phase Ⅰ clinical trials (NCT04805307) show good safety and tolerability of CMG901. In terms of efficacy, for 8 patients with *CLDN18.2*-positive gastric or esophagogastric junction adenocarcinoma treated with CMG901, the ORR reached 75% [37].

4. Challenges and perspectives of *CLDN18.2* in tumor-targeted therapy

Due to the use of different detection methods and positive thresholds in various studies, as well as differences in the populations studied, the positivity rates of *CLDN18.2* vary. Highly standardized and specific reagents must harmonize detection methods and distinguish *CLDN18.2* from *CLDN18.1* isoforms. A molecular beacon-based ultra-sensitive detection for *CLDN18.2* in circulating tumor cells has shown promising potential [38]. Further research is needed to determine the optimal cutoff value for *CLDN18.2*-positivity to identify patients who should receive targeted therapy. Whether different patient populations require different cutoff expression levels remains further investigation. Another controversy is the varying significance of *CLDN18.2* for the prognosis of gastric cancer in different studies, lacking consensus. Arnold *et al.* [39] reported that immunohistochemistry results from a cohort of Caucasian gastric cancer suggested that high expression of *CLDN18.2* was not associated with OS. Dottermusch *et al.* [40] reported no significant correlation between tumor-specific survival and *CLDN18.2* expression in tumor cells among 430 advanced gastric cancer patients. However, Jun *et al.* [41] found through immunohistochemistry that gastric cancer patients with high *CLDN18.2* expression had longer OS compared to patients without *CLDN18.2* expression. Furthermore, clinical applications are mainly focused on the combination of targeted drugs with chemotherapy, with no reports yet of combination with immune checkpoint inhibitors. Gastric cancers positive for *CLDN18.2* exhibit an immunosuppressive microenvironment characterized by high infiltration of CD4+ FoxP3+ Treg cells and CD4+PD-L1+ T cells, while infiltration of CD8+PD1+ T cells is reduced, suggesting limited benefit from conventional PD-1 monoclonal antibody therapy for *CLDN18.2*-positive gastric cancer patients [42]. Combination therapy with novel immune checkpoint inhibitors such as anti-LAG-3 and anti-TIM-3 monoclonal antibodies may benefit *CLDN18.2*-positive gastric cancer patients, but their effectiveness and safety require further research [43-44].

In conclusion, as a molecule with relatively high specificity in tumor tissue expression, *CLDN18.2*-targeted drugs may reshape the current treatment approach for gastric cancer and have broad clinical application prospects. Molecular subtyping studies of gastric cancer patients based on multi-omics data contribute to exploring patients who would benefit most from targeted *CLDN18.2* therapy. Additionally, more precise clinical studies are still needed to determine the safe dosage and effective combination strategies for targeted *CLDN18.2* therapy.

Conflict of interest None

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Claudin18.2——胃癌治疗的新兴靶点

王羌成，　鲍军
南京医科大学附属肿瘤医院肿瘤内科，江苏 南京 210009

鲍军，医学博士，主任医师。主要从事消化道肿瘤防治的临床与基础研究，研究消化道肿瘤的发病机制、转移及预后因子以及遗传性消化道肿瘤的并发症。曾共同主持承担江苏省科学技术厅课题 1 项，江苏省卫健委课题 2 项。在国内外专业学术期刊发表学术论文共 20 余篇，参与编写专著 2 部。荣获江苏省卫生厅中医药医学新技术引进三等奖 1 项、江苏省医学新技术引进奖二等奖 1 项。

摘要：胃癌是一种严重危害人类健康的恶性肿瘤。Claudin18.2（CLDN18.2）是一种跨膜蛋白，作为紧密连接的主要成分，在维持屏障功能中发挥重要作用。在正常生理条件下，CLDN18.2 在健康组织中不表达，在胃黏膜的分化上皮细胞中特异性表达。近年来研究发现，CLDN18.2 也可在胃癌、食管癌、卵巢癌、胰腺癌、肺癌、乳腺癌等恶性肿瘤中高表达或异位激活。基于其特异性表达的特点，CLDN18.2 已经成为抗胃癌药物研发中的明星靶点。本文对 CLDN18.2 在胃癌中表达特点、调控方式、临床病理特征以及靶向 CLDN18.2 的临床药物研究进展作一综述。

关键词：Claudin18.2；胃癌；靶向治疗；单克隆抗体；双特异性抗体；嵌合抗原受体 T 细胞；抗体药物偶联物
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Claudin18.2: an emerging target for the treatment of gastric cancer

WANG Qiangcheng, BAO Jun
Department of Medical Oncology, The Affiliated Cancer Hospital of Nanjing Medical University, Nanjing, Jiangsu 210009, China
Corresponding author: BAO Jun, E-mail: junbao@jszlyy.com.cn

Abstract: Gastric cancer is a malignant tumor that seriously endangers human health. Claudin18.2 (CLDN18.2) is a transmembrane protein and a main component of tight junctions that plays an important role in maintaining barrier function. Under normal physiological conditions, CLDN18.2 is not expressed in healthy tissues, but it is expressed in differentiated epithelial cells of the gastric mucosa. With the progress of research, it has been found that CLDN18.2 is highly expressed or ectopically activated in primary malignant tumors such as gastric cancer, esophageal cancer, ovarian cancer, pancreatic cancer, lung cancer, and breast cancer. Due to its potential for specific expression, CLDN18.2 has become a new emerging target in anti-tumor drug development. This article reviews the expression characteristics, regulation and clinical pathological features, of CLDN18.2 in gastric cancer, and the progress of clinical drug research targeting CLDN18.2.

Keywords: Claudin18.2; Gastric cancer; Targeted therapy; Monoclonal antibodies; Bispecific antibodies; Chimeric antigen receptor T cells; Antibody-drug conjugates
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通信作者：鲍军，E-mail：junbao@jszlyy.com.cn
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胃癌占所有肿瘤的 7.7%，位居第四位[1]。尽管近年来在早期筛查和治疗方面取得了重大进展，但晚期胃癌患者的预后仍然较差，中位总生存期仅为 14.2 个月[2]。早期胃癌最好的治疗方式是手术切除，而对于不可切除、复发或晚期的胃癌患者，常采用以化疗为主的全身性治疗[3]。然而，化疗对患者的预后改善有限，且毒副作用较大。近年来，随着肿瘤学研究的不断深入，癌症治疗已经从传统的“一刀切”细胞毒性化疗转变为针对患者分子特征的个性化治疗[4]。以免疫检查点抑制剂为代表的免疫治疗和分子靶向治疗的进展使得晚期胃癌患者的预后得到了一定的改善，帕博利珠单抗（pembrolizumab）、尼伯替尼（nivolumab）和人类表皮生长因子受体 2（HER2）靶向治疗药物曲妥珠单抗（trastuzumab）已被国内外指南推荐为晚期胃癌的标准一线治疗方案[5]。然而，HER2 阴性、程序性死亡配体 1（PD-L1）联合阳性分数<5 的胃癌患者从以上的治疗方式中获益有限，5 年生存率较低，亟需开发针对新治疗靶点的药物以改善这些胃癌患者的预后。

近年来，随着肿瘤学和计算生物学的发展，Claudin18.2（CLDN18.2）在胃癌发生发展中的关键作用受到研究者的广泛关注。CLDN18.2 是一种紧密连接蛋白，在正常胃组织中表达有限，胃癌的发生使得肿瘤细胞表面的紧密连接破坏，CLDN18.2 抗原表位得以暴露，使之成为潜在的胃癌治疗特异性靶点[6]。针对胃癌，目前已有多种靶向 CLDN18.2 的疗法，包括单克隆抗体（monoclonal antibody，mAb）、嵌合抗原受体 T 细胞（chimeric antigen receptor T cells，CAR-T）疗法、双特异性抗体（bispecific antibodies，BsAb）和抗体药物偶联物（antibody-drug conjugates，ADC），并且已经进行了相应的临床试验。本文对 CLDN18.2 在胃癌中的表达特点、调控方式、生物学功能、临床病理特征以及靶向 CLDN18.2 的临床药物研究进展作一综述，以期更好地帮助临床医生和科研工作者研发并应用靶向 CLDN18.2 的药物。

1　CLDN18.2 的表达特点及调控方式

Claudins（CLDNs）是一类膜蛋白，分子量为 20～27 kDa，由四个跨膜结构域（transmembrane domain，TMD）、一个 N 末端和一个 C 末端组成。此外还包含两个横跨 TMD 的细胞外环。CLDNs 在不同的人体器官中有 27 种不同的亚型，而这些亚型的功能异常可能会对相应组织的癌症发生和发展产生不同的影响，从而涉及从异型增生到癌症进展和转移的各个阶段[7]。其中，Claudin18（CLDN18）在维持细胞极性和屏障功能，以及促进耐酸性方面发挥着重要作用[8]。CLDN18 基因位点位于染色体 3q22，分子量约为 35 kb，包含 6 个外显子和 5 个内含子。CLDN18 的第一个外显子可以发生选择性剪接形成两个具有高度同源氨基酸序列的不同剪接突变体（CLDN18.1 和 CLDN18.2）[9]。

1.1　CLDN18.2 的表达特点　CLDN18.2 是一种高度选择性表达的分子，通常只在正常胃黏膜的分化上皮细胞内表达[6]，存在于胃黏膜细胞的紧密连接处[9]，因此无法被血液中的抗体识别并结合。然而，胃癌的发生会导致紧密连接的破坏和细胞极性的紊乱，使得 CLDN18.2 抗原表位暴露于细胞表面。靶向 CLDN18.2 的抗体可以高度特异性地结合在肿瘤细胞表面，而不损伤正常细胞，因此 CLDN18.2 是一种理想的抗胃癌药物分子靶点[10]。此外，CLDN18.2 在胰腺癌、食管癌、卵巢癌和肺癌等恶性肿瘤中高表达或异位激活[11]，这也表明 CLDN18.2 在其他肿瘤治疗中的潜力。

1.2　CLDN18.2 基因的功能　CLDNs 表达促进肿瘤发生的相关机制尚不明确。目前的主要观点是 CLDNs 激活各种信号通路或蛋白酶，直接或间接地促进肿瘤的发生。一种直接途径是与其他分子联合，如 EpCAM、MT-MMPs、ADAM10 和整合素，这些分子激活 CLDNs 参与信号传导、胞外基质降解和受体裂解。另一种直接方式与转录因子（transcription factors，TFs）有关联，如 YAP/TAZ 和 β-catenin，以诱导 TFs 的核积累。间接方式涉及两种分子，一种是如 MMPs 的蛋白酶，它裂解细胞外基质（ECM）以释放生长因子来激活 RTK/PI3K、MAPK、TGF-β/SMAD 和 JAK/STAT 途径；另一种是蛋白激酶，如 SFK、ABL 和 Tyk2，它们可以使下游分子磷酸化。相关信号通路之间相互联系，整合了来自 CLDNs 的信号，促进肿瘤的产生[12]。

启动子区 CpG 岛的甲基化被认为是调节 CLDN18 表达的关键机制之一。CpG 岛的甲基化与 CLDN18 基因表达相关[13]。Sahin 等[14]研究表明，CpG 岛的甲基化可以完全阻止激活 CLDN18.2 转录所必需的 TFs。CREB 与 CLDN18.2 的启动子区结合。激活蛋白-1（activator protein-1，AP-1）为细胞内转录激活因子，是调节细胞生长和分化最为关键的 TFs 之一。研究发现，AP-1 能够结合 CLDN18.2 启动子的顺式调控元件，促进 CLDN18.2 的转录[15]。此外，PKC 和 ERK-MAPK 途径的活化，促进了 AP-1 的磷酸化，从而增强 CLDN18.2 mRNA 的转录[15]。

2　CLDN18.2 的临床病理特征

CLDN18.2 在肿瘤转移病灶的表达水平与原发病灶相当[16-17]，提示转移性肿瘤患者也可能是 CLDN18.2 靶向治疗的获益人群。CLDN18.2 的表达水平还与胃癌的组织学亚型相关，弥漫型胃癌中 CLDN18.2 显著增高。此外，在弥漫型胃癌中还特异性存在 CLDN18-ARHGAP26/6 融合基因[18]。Shu 等[19]的研究表明，CLDN18-ARHGAP26/6 融合在弥漫型胃癌（印戒细胞癌）中占比 25%，并且与黏液细胞含量、年龄、性别和 TNM 分期有关。存在 CLDN18-ARHGAP26/6 融合基因的患者预后较差，其对奥沙利铂/氟嘧啶类等化疗药物应答率较低[19]。Nakayama 等[20]的研究发现 146 名 40 岁以下的胃癌患者中，CLDN18-ARHGAP26/6 融合占比为 15.1%，并且融合基因阳性患者拥有较大的肿瘤、更高的淋巴结转移比例和更晚的 TNM 分期。这些结果表明，在青年胃癌患者发生 CLDN18-ARHGAP 融合的比例较高，并可能与肿瘤高度的侵袭性相关[20]。

3　CLDN18.2 阳性胃癌的靶向治疗方式

目前，针对 CLDN18.2 阳性胃癌的靶向治疗主要包括单克隆抗体、CAR-T 细胞治疗、BsAb 和 ADC 等。

3.1　CLDN18.2 单克隆抗体

3.1.1　Zolbetuximab　Zolbetuximab 是一种嵌合的 IgG1 单克隆抗体，靶向 CLDN18.2，通过补体依赖性毒性和抗体依赖性细胞毒性介导免疫裂解作用[21]。最近的Ⅲ期临床试验 SPOTLIGHT 研究了一线治疗中将 Zolbetuximab 与化疗联合应用于晚期胃癌或食管胃交界处癌的作用[22]。Zolbetuximab 与 mFOLFOX6（奥沙利铂+5-氟尿嘧啶/亚叶酸钙）的联合应用使安慰剂组的中位无进展生存期（median progression-free survival, mPFS）从 8.67 个月显著改善到 10.61 个月。Zolbetuximab 组的中位总生存期（median Overall Survival, mOS）为 18.23 个月，安慰剂组为 15.54 个月。两组的客观缓解率（objective response rate, ORR）和缓解持续时间（duration of response, DOR）均相似。在安全性方面，Zolbetuximab 组有 14%的患者因为 3 级或更严重的不良事件而中止治疗，安慰剂组则为 6%。

2023 年 3 月，美国临床肿瘤学学会主题报告系列介绍了另一项Ⅲ期试验 GLOW 研究的初步结果，该研究将 Zolbetuximab 作为一线治疗加入到 CAPOX 化疗中，并与单独化疗相比[23]。Zolbetuximab 与化疗的联合应用使 PFS 显著增加，验证了前述 SPOTLIGHT 研究的结果。与安慰剂组相比，Zolbetuximab 组的 mPFS 从 6.80 个月改善到 8.21 个月。同样，Zolbetuximab 组的 mOS 也显著延长（14.39 个月 *vs* 12.16 个月）。Zolbetuximab 组的 ORR 为 53.8%，安慰剂组为 48.8%。与治疗相关的不良事件导致 7.1%的患者停用 Zolbetuximab，安慰剂组为 4.4%。

综上所述，这两项Ⅲ期研究表明了首个抗 CLDN18.2 抗体 Zolbetuximab 在胃或食管胃交界处癌中具有显著的临床益处。但两项研究的入组患者均为 CLDN18.2 阳性（≥75%的肿瘤细胞显示中度至强膜性 CLDN18.2 染色）的患者，仍需进一步探索 CLDN18.2 低表达患者的治疗效果。

3.1.2　TST001　TST001 是具有高亲和力的 CLDN18.2 单克隆抗体。该药物降低了 Fc 末端的岩藻糖含量，并进一步增强了该药物与 NK 细胞上 Fc 受体的结合能力。这使得 TST001 与 NK 细胞的结合以及抗体依赖性细胞毒性反应（ADCC）活性进一步增强。临床前研究发现，同等剂量下，相较于 Zolbetuximab，TST001 展示了更强的缩瘤作用。而且，TST001 的抗肿瘤效应与 CLDN18.2 的表达水平无关，意味着即使在 CLDN18.2 表达较低至中等水平的胃癌细胞中，TST001 也能够产生抗肿瘤活性[24]。

TranStar102/TST001-1002 研究（NCT04495296）的队列 C 评估了 TST001 联合 CAPOX 化疗方案一线治疗胃癌的疗效和安全性，中位 mPFS 为 9.5 个月，ORR 为 66.7%，中位 DOR 为 9.9 个月。总人群中未出现剂量限制性毒性，治疗期间出现的不良事件（TRAE）大多为 1~2 级。值得一提的是，相比 GLOW 研究，该研究放宽了入组患者的 CLDN18.2 表达水平（≥10%肿瘤细胞的 CLDN18.2 膜染色强度≥1+），同时表现出了 mPFS 的延长[25]。此外，最近也开启了 TST001 的临床Ⅲ期试验，选择了 TST001 联合纳武利尤单抗和化疗作为胃癌的一线治疗（NCT06093425），其有效性及安全性仍需进一步探索。其余的 CLDN18.2 单抗多处于临床Ⅰ期或临床前研究。

3.2　靶向 CLDN18.2 的双特异性抗体

双特异性抗体是靶向 CLDN18.2 药物研发的另一个重要方向，双抗药物主要存在 3 种作用机制，包括招募和激活免疫细胞杀伤肿瘤细胞；抑制或激发多个信号通路，发挥协调效应；借助抗体双价结构，介导蛋白复合物形成，发挥生物学效应等[26]。目前进入临床试验的双抗包括 CLDN18.2/CD3 双抗、CLDN18.2/4-1BB 双抗、CLDN18.2/PD-L1 双抗、Claudin18.2/CD47 双抗。与

单克隆抗体相比，这些双抗药物增加了一个特异性抗原结合位点，因而特异性更强、可较准确靶向肿瘤细胞并降低脱靶毒性，在胃癌和胰腺癌的治疗中具有很大潜力。大多数的双抗目前皆处于临床Ⅰ期或临床前阶段，在这里对几种代表性双抗进行总结。

3.2.1　AMG-910　Zhu 等[27]的研究数据表明，靶向 CD3/Claudin18.2 双特异性抗体可能是治疗胃癌和胰腺癌的有效方法，其有效性和安全性已经在动物活体模型中得到了验证。AMG-910 是一种半衰期延长（HLE）的 CD3/CLDN18.2 双特异性 T 细胞连接子（BiTE）抗体，通过与肿瘤细胞上的 CLDN18.2 和 T 细胞上的 CD3 结合，引导肿瘤细胞裂解以杀死肿瘤细胞。目前正在开展Ⅰ期临床研究（NCT04260191），以评估 AMG-910 治疗 CLDN18.2 阳性的胃癌和胃食管交界腺癌患者的安全性、耐受性、药代动力学和疗效。

3.2.2　Q-1802　Q-1802 可以同时结合程序性死亡配体 1（PD-L1）和 CLDN18.2，从而诱导抗体依赖性细胞介导的细胞毒性作用，并且阻断 PD-1 信号传导，激活固有免疫和适应性免疫。临床前研究显示，Q-1802 能够准确靶向肿瘤组织，并且对于杀死肿瘤细胞具有强大的疗效，为具有 CLDN18.2 表达的晚期实体肿瘤提供了新的治疗候选药物[28]。该抗体已在患有晚期实体肿瘤的患者中展开了一项Ⅰ期临床试验，中期数据表明，在剂量扩展阶段，9 例 CLDN18.2 阳性胃肠道肿瘤患者中，2 例患者达到部分缓解，4 例达到疾病稳定。安全性方面，TRAE 大多为 1～2 级，3 级 TRAE 发生率为24.1%（7/29），出现 1 例 4 级 TRAE，为低钠血症，该患者有长期服用利尿剂的病史[29]。

同时双抗也面临着一些挑战，如需要精确的工程设计以避免稳定性、溶解度和聚集等问题，以及如何避免可能发生的非特异性效应和毒性。此外，双特异性抗体的生产成本较高，限制了患者的使用。需要进行持续的研究和开发工作，以改进双特异性抗体在癌症治疗中的疗效和安全性[29]。

3.3　*靶向 CLDN18.2 的 CAR-T 细胞治疗*　CAR-T 细胞治疗是一种经过基因工程改造的免疫疗法，其通过提取患者自身的免疫细胞，并对其进行基因编辑，使其具有识别和攻击特定靶标的能力[30]。

Jiang 等[31]成功开发了人源化的 CLDN18.2 特异性单克隆抗体 hu8E5 和 hu8E5-2i 单链片段，并制备了 CLDN18.2 特异性 CAR-T 细胞。该 CAR-T 细胞能够有效地抑制异种移植瘤小鼠模型的肿瘤生长，而且对其他正常组织没有明显的损伤。Luo 等[32]还将 IL-7 和 CCL-21 等细胞因子引入到 CLDN18.2 特异性 CAR-T 细胞中，以提高 CAR-T 细胞的抗肿瘤活性。通过体外和体内实验，研究发现表达 IL-15 的 CLDN18.2 CAR-T 细胞在体外更好地扩增，在体内外均具有更强的抗肿瘤活力，其中央记忆型 T 细胞的比例也更高[33]。

目前我国自主研发的 CAR-T 细胞药物 CT-041 已进入Ⅰ/Ⅱ期临床试验。一项针对晚期胃癌/食管胃结合部腺癌的Ⅰ期试验（NCT03874897）的中期分析表明：mPFS 为 4.2 个月，6 个月时的总生存率达到了 81.2%[34]。另外，该研究还对 18 例既往接受至少两线治疗失败的胃癌/食管胃结合部腺癌患者进行了分析。这些患者接受了 2.5×10^8 CAR-T 细胞的治疗。结果显示，ORR 为 61.1%，mPFS 为 5.6 个月。在安全性方面，首次输注 CT041 后 28 d 内未观察到剂量限制性毒性。常见的≥3 级 TRAE 包括预处理相关的血液学毒性（100%）、白细胞减少症（83.8%）、中性粒细胞减少症（67.6%）、贫血（40.5%）和血小板减少症（16.2%）。未观察到 3 级及以上的细胞因子释放综合征（cytokine release syndrome，CRS），CRS 通常是由 CAR-T 细胞治疗引起的免疫活化反应所致。此外，这项研究未出现免疫效应细胞相关的神经毒性综合征。总体而言，CT-041 在 CLDN18.2 阳性胃癌患者中显示出了可接受的安全性和有前景的抗肿瘤活性，在全球实体肿瘤 CAR-T 细胞治疗领域中处于领先地位。其他靶向 CLAN18.2 的 CAR-T 细胞药物如 LY-011、KD-498、LB-1908 等均处于临床Ⅰ期阶段。

3.4　*靶向 CLDN18.2 的 ADC*　ADC 是一类强大的抗癌治疗药物，可以直接将细胞毒性的分子直接送达癌细胞。ADC 已被广泛应用于许多实体肿瘤和血液恶性肿瘤患者[35]。

Zhu 等[27]将抗 CLDN18.2 抗体与可分解的奥瑞他汀结合生成 ADC。研究结果表示其能够有效抑制体外胃癌细胞（KATO-Ⅲ/hCLDN18.2）和胰腺癌细胞（BxPC3/hCLDN18.2）的生长，并且在高表达 CLDN18.2 的 PDX 模型中也能产生效果。这项临床前研究表明了抗 CLDN18.2 ADC 在胃癌和胰腺癌治疗中可能是有效的。

CMG901 是由靶向 ClDN18.2 的人源化单抗 CM311 和微管聚合蛋白抑制剂 MMAE 通过可裂解连接臂偶联而成的 ADC，通过其单克隆抗体部分与 ClDN18.2 阳性细胞结合。结合后，CMG901 被肿瘤细胞内吞到溶酶体中，并释放细胞毒素，导致肿瘤细胞凋亡。一项作用机制研究显示，CMG901 可以刺激细胞和可溶性免疫效应器激活 ADCC 和补体依赖性细

胞毒性 CDC,以杀伤 CLDN18.2 阳性肿瘤细胞。临床前研究也表明,CMG901 可以有效杀死胃癌细胞,其抗肿瘤效力比 zolbetuximab 更强[36]。Ⅰ期临床试验(NCT04805307)表明 CMG901 在安全性和耐受性方面表现良好。在疗效方面,对于接受 CMG901 治疗的 8 例 CLDN18.2 阳性胃癌或胃食管结合部腺癌患者,ORR 达到了 75%[37]。

4　CLDN18.2 在肿瘤靶向治疗中的挑战与展望

由于不同研究中使用了不同的检测方法和阳性阈值,并且人群不同,CLDN18.2 的阳性率有所不同。必须使用标准化的高度特异的试剂来统一检测方法,以区分 CLDN18.2 与 CLDN18.1 剪切体。一种基于分子信标的超灵敏检测循环肿瘤细胞中 CLDN18.2 的方法显示出良好的潜力[38]。需要进一步的研究确定 CLDN18.2 阳性的最佳截断值,以明确应接受靶向治疗的患者。不同的患者群体是否需要不同的截断表达水平仍需要进一步探究。另一个争议是 CLDN18.2 对胃癌预后的意义在不同的研究中各不相同,缺乏共识。Arnold 等[39]报道,白种人胃癌队列的免疫组化结果提示 CLDN18.2 高表达与 OS 无关。Dottermusch 等[40]报道,在 430 例晚期胃癌患者中,肿瘤特异性生存率与肿瘤细胞中 CLDN18.2 表达之间无显著相关性。然而,Jun 等[41]通过免疫组化发现,CLDN18.2 高表达的胃癌患者的 OS 较无 CLDN18.2 表达的患者 OS 长。此外,临床应用主要集中在靶向药物与化疗的结合上,尚无免疫检查点抑制剂与其结合的报道。CLDN18.2 阳性的胃癌局部呈现免疫抑制微环境,表征为 $CD4^+$ $FoxP3^+$Treg 细胞和 $CD4^+$ $PD\text{-}L1^+$T 细胞的高度浸润,而 $CD8^+PD1^+$T 细胞的浸润减少,因此接受传统的 PD-1 单抗治疗对 CLDN18.2 阳性的胃癌患者可能受益有限[42]。联用新型免疫检查点抑制剂如抗 LAG-3、抗 TIM-3 单抗可能对 CLDN18.2 阳性的胃癌患者具有获益,但其有效性和安全性仍有待进一步的研究[43-44]。总的来说,CLDN18.2 作为一种在肿瘤组织表达特异性程度较高的分子,其靶向药物可能重塑当前的胃癌的治疗方式,具有广阔的临床应用前景。基于多组学数据的胃癌患者分子分型研究有助于探索靶向 CLDN18.2 治疗具有较好获益的患者。此外,仍需要开展更多的精密的临床研究以明确靶向 CLDN18.2 治疗的安全剂量及有效联合方式。

利益冲突　无

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