

Significant function and research progress of biomarkers in gastric cancer (Review)

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Abstract. Gastric cancer is one of the most common gastrointestinal tumor types, and the incidence and mortality rates are higher in men compared with women. Various studies have revealed that gastric cancer is a spectrum of tumor types, which have biological and genetic diversity. It has proven to be difficult to improve the overall survival and disease-free survival of patients with gastric cancer through the use of traditional surgery and chemoradiation, as gastric cancer is usually identified at an advanced stage. In consequence, the outcome is frequently poor. Thus, novel biomarkers and anticancer targets are required to improve the outcome. As the identification of biomarkers has increased due to advances in research and the greater availability of bioinformatics and functional genomics, the potential therapeutic regimens available have also increased concurrently. These advances have also improved the ability to predict responses to chemotherapy, targeted therapy and immunotherapy, whilst other biomarkers predict post-treatment survival and recurrence based on their expression. This review focuses closely on the important functions of biomarkers in the timely diagnosis and treatment of gastric cancer, in addition to the advances in the study of certain novel markers in gastric cancer.

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1. Introduction

Gastric cancer is the third leading cause of cancer-associated mortality and the fifth most common malignant tumor type globally, with ~50% of all cases emerging from Eastern Asia, where China has the highest incidence rate, and where novel cases of gastric cancer and mortalities account for 42.6-45.0% of all cases globally (1,2). The majority of cases of gastric cancer are usually not diagnosed until an advanced stage, and therefore the outcome is often poor, with a 5-year survival rate of no more than 30%, including patients who have undergone surgery (3). However, the 5-year survival likelihood following early gastric cancer treatment may be >90%, and even may result in being cured (4). The rate of diagnosis and treatment of early-stage gastric cancer in China is <10%, considerably lower than Japan (70%) and South Korea (50%) (5). Therefore, it is worthwhile investigating methods for the early diagnosis and management of gastric cancer, including biomarkers associated with pathogenesis and pathological type. Understanding these biomarkers may assist in providing the ideal therapeutic option for an individuals' specific case of gastric cancer. Detecting the presence of biomarkers may have promising potential to detect cancer at earlier stages and thus improve the monitoring of the progression of a cancer. Furthermore, by obtaining information on the specific biomarker expression profile of a patient, treatment options may be better tailored for each patient, thus improving prognosis. The aim of the review was to focus on providing

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a comprehensive overview of the biomarkers in patients who suffer from stomach cancer, their diagnostic, prognostic and clinical value and therapeutic application for future prospects.

Gastric cancer is a type of epithelial malignant neoplasm. A number of various factors contribute to the pathogenesis of gastric cancer, including environmental factors and genetic factors (6). Environmental factors and lifestyle choices include obesity, smoking (7), bile reflux and chronic infections, particularly with *Helicobacter pylori* (*H. pylori*), which contribute to the development of stomach cancer (8). Globally, ~50% of all patients with gastric cancer present with evidence of a *H. pylori* infection (9), and *H. pylori* was considered to be the first carcinogen by the World Health Organization (WHO) and International Agency for Research on Cancer (IACR) in 1994 (10). There are hereditary factors, in addition to environmental factors, including a germline mutation in the cadherin-1 (CDH1) gene, which results in hereditary diffuse gastric cancer (11). Patients with inherited conditions, including Lynch syndrome, familial adenomatous polyps and Peutz-Jeghers syndrome result in a substantially higher risk of developing gastric carcinoma (12).

The treatment of gastric cancer is dependent on the morphology of the cancer tissue at the earliest stage. The pathological classification of gastric cancer is based on the histological structure and cell biological characteristics. Different classifications of gastric cancer types have different morphological structures, biological behaviors and underlying molecular mechanisms (8). At present, gastric cancer is primarily classified using the Borrmann, Lauren or WHO classification systems, although there are numerous pathological classification systems for gastric cancer (13,14). Advanced cancer types may be classified into four macroscopic types on the basis of the criteria proposed by Borrmann: Polypoid, fungating, ulcerated and infiltrative (13). The Lauren classification is the most widely used histological classification, for either early or advanced cancer types (14), which classifies gastric cancer as two major subtypes: Intestinal and diffuse. The diffuse variant may affect the majority of the stomach and is frequently called linitis plastica or leather bottle stomach. Intestinal-type gastric cancer occurs more frequently in elderly male patients and is thought to be associated with better survival rates (15). In 2010, WHO published an additional histological classification system for stomach cancer, which is divided into five categories: Tubular, papillary, mucinous, poorly cohesive (signet ring cell carcinoma belongs to this group) and mixed (8). Histological classification has no substantial impact on the treatment options available for patients with gastric cancer, therefore, novel biomarkers to aid in the early diagnosis and treatment of gastric cancer are required. In the present review, the following topics are discussed: i) Well-known and emerging biomarkers of gastric cancer; ii) the impact that high-throughput technologies have had on identifying biomarkers; and iii) biomarkers associated with the immunotherapy of gastric cancer and their value as predictors of prognosis (Fig. 1).

2. Definition of a biomarker

With the advancement of medicine, the definition of a biomarker has also changed accordingly. In 1998, the National

Institutes of Health Biomarker Definition Working Group defined biomarker as ‘a feature of objective measurement and assessment of pharmacological responses to normal biological processes, pathogenic processes or therapeutic interventions’ (16). Then, Becking and Chen (17) defined a biomarker as ‘any material structure or process that can be measured in the body or its products and effect or predict the incidence of prognosis disease’. Each of these definitions are similar. Common biomarkers include carbohydrates, proteins, nucleic acids, small metabolite lipids and cytogenetics, in addition to all tumor cells identified in body fluids that are involved in the regulation of physiological and pharmacological processes (18). However, Strimbu and Tavel (19) summarized the importance of biomarkers by stating ‘the foremost issue at present is determined by the link between any given measurable biomarker and relevant clinical endpoints’. With the development of molecular biology, an increasing number of tumor markers have been discovered. Tumor markers, not only with high sensitivity but also specificity, are still being discovered. Similarly, numerous molecular biomarkers have demonstrated their potential efficacy as diagnostic and prognostic tools in gastric cancer, yet they still require further confirmation of their use in day-to-day clinical practice (20).

3. Commonly used biomarkers

Serological biomarkers of gastric mucosa. South Korea and Japan have the most complete prevention and screening gastric cancer program globally, which result in a high detection rate of early gastric cancer in these countries (21). At present, carcinoembryonic antigens including CEA (22), CA19-9 (23) and CA72-4 (24) are the most widely used markers for detecting gastric cancer in the clinical practice. These markers lack the sensitivity and specificity required for evaluating the diagnosis and prognosis of gastric cancer, making their efficacy questionable. Therefore, the screening value for early gastric cancer is limited. However, the positive expression of monoclonal gastric cancer 7 antigen (MG7-Ag) indicates a high risk of gastric cancer (25). Nevertheless, the sensitivity and specificity of MG7-Ag as a single marker in the diagnosis of gastric cancer may not be sufficient, and further clinical research is required to evaluate its value in diagnosis for the early screening of gastric cancer.

Pepsinogen (PG) may be divided into two subtypes, PGI and PGII, according to its biochemical and immunological activity characteristics (26). PG is a good indicator of the exocrine function of the gastric antral mucosa and may be called ‘serological biopsy’ (26). When gastric mucosal atrophy occurs, serum PGI and/or the PGI/II ratio (PGR) level are decreased (27). Furthermore, *H. pylori* infection is a necessary condition for the occurrence of intestinal subtype gastric cancer which accounts for the majority of cases of gastric cancer, but it is not a sufficient condition (28,29). Thus, previously combining serum PG levels with *H. pylori* antibodies (including the ‘ABC method’) were used to assess the risk of gastric cancer and screened for patients with a high-risk of developing gastric cancer. Konturek *et al* (30) reported that elevated levels of serum gastrin-17 (G-17) may indicate the risk of gastric cancer. Additionally, Shiotani *et al* (31) reported that serum G-17 combined with PG may enhance the

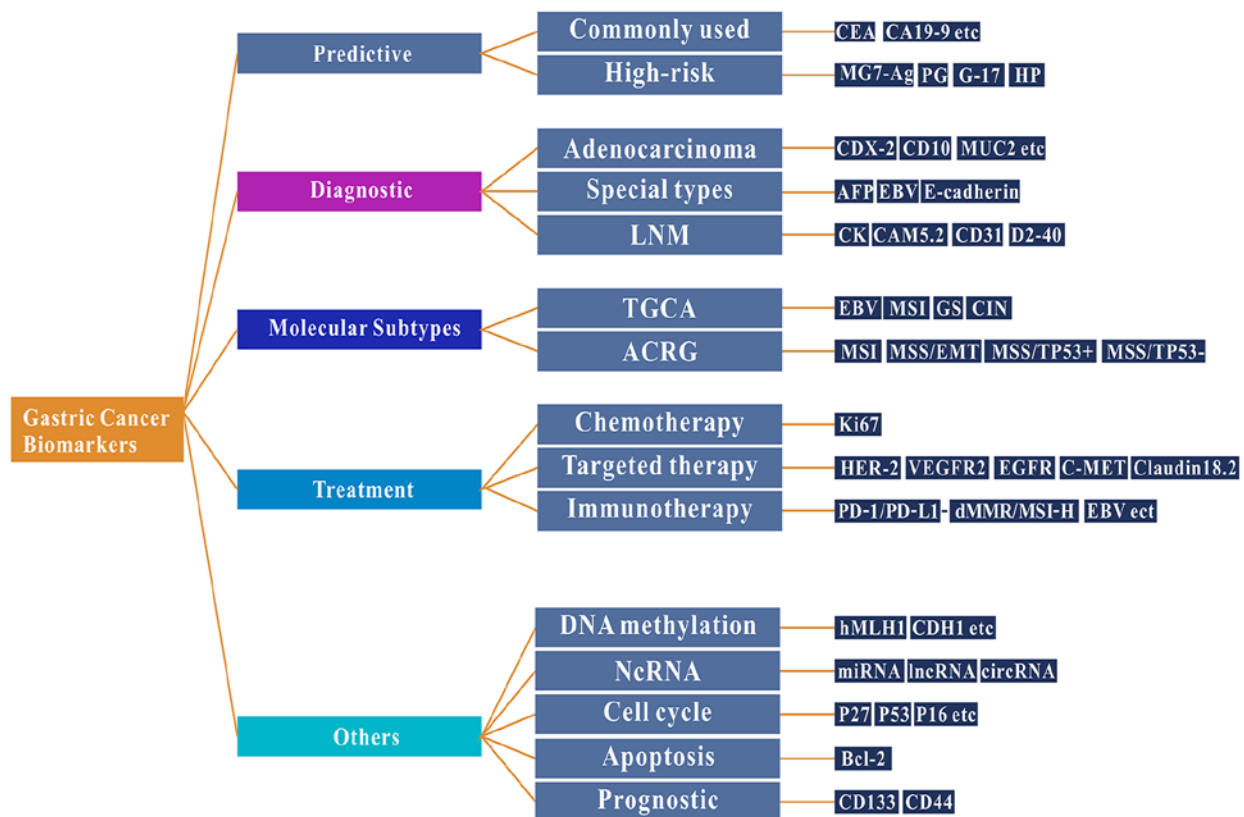


Figure 1. Function and research findings of biomarkers in gastric cancer. Common and emerging biomarkers used in gastric cancer, including biomarkers associated with the molecular subtypes, chemotherapy, targeted therapy and immunotherapy of gastric cancer in addition to their direct potential function in improving the diagnosis and treatment options in patients with gastric cancer. CEA, carcinoembryonic antigen; CA, cancer antigen; CD, cluster of differentiation; MUC2, mucin 2, oligomeric mucus/gel forming; AFP, α -fetoprotein; EBV, Epstein Barr virus; HER-2, erb-b2 receptor tyrosine kinase 2; VEGFR2, vascular endothelial growth factor receptor 2; EGFR, epidermal growth factor receptor; PD-1, programmed cell death 1; dMMR, deficient mismatch repair; MSI-H, high levels of microsatellite instability; hMLH1, human mutL homolog 1; CDH1, cadherin-1; miRNA, microRNA; lncRNA, long non-coding RNA; circRNA, circular RNA; Bcl-2, BCL2 apoptosis regulator; ncRNA, non-coding RNA; TCGA, The Cancer Genome Atlas; ACRG, Asian Gastric Cancer Research Group; MG7-Ag, monoclonal gastric cancer 7 antigen; PG, pepsinogen; G-17, gastrin-17.

diagnostic value of gastric cancer. Previously, when five serological markers were combined with PGI, PGII, PGR, (*H. pylori*) antibody and G-17 as a screening strategy for gastric cancer, it was revealed that a decrease in the levels of PGI and PGR were associated with a high risk of gastric cancer, whereas low (<0.5 pmol/l) and high (>4.7 pmol/l) G-17 levels were associated with a higher risk of suffering from gastric cancer (32). Therefore, it has been indicated that screening strategies which combine these serological markers may assist in identifying high-risk individuals, in addition to guiding targeted screening and precision prevention (32).

Diagnosis-associated tumor biomarkers of gastric cancer. Common types of adenocarcinoma, including tubular, papillary, mucinous, low adhesion carcinoma or mixed adenocarcinoma, often present with phenotypic features with intestinal epithelium [expressing mucin 2, CDX-2 and cluster of differentiation (CD)10] or gastric epithelium (expressing mucin 1, cell surface associated, mucin 5AC, oligomeric mucus/gel-forming and mucin 6, oligomeric mucus/gel forming) (33). In fact, it is frequently unnecessary to diagnose these common types of adenocarcinoma using immunohistochemistry (IHC). However, certain unique types of gastric cancer, including poorly differentiated neuroendocrine carcinoma, hepatoid adenocarcinoma, adenocarcinoma

producing α -fetoprotein, gastric cancer with lymphoid stroma [typically associated with Epstein Barr virus (EBV) infection] and choriocarcinoma require specific biomarkers to confirm the diagnosis (34). Prior to a case of hereditary diffuse gastric cancer being diagnosed, IHC detection of E-cadherin and detection of mutations in the CDH1 gene mutations are required for screening or confirmation (11).

The specific pathological features of a patients' unique case of gastric cancer will have a substantial effect on the therapeutic regimen used and the patients' outcome (15). Lymph node micrometastasis (LNM) is one of the most important prognostic factors in patients with gastric cancer, including in patients who do present with evidence of lymph node metastasis (35). Comparatively speaking, IHC may be satisfactorily accurate for the detection of LNM compared with that of haematoxylin and eosin staining. Cytokeratin AE1/AE3 and CAM5.2 are reliable biomarkers for the detection of epithelial tissues or cells in lymph nodes (36). If it is suspected that the cancer cells are present in the vasculature, the biomarkers CD31 and D2-40 are available (37). Additionally, the markers NF or S-100 may be used for the detection of nerve invasion (38).

Gastric cancer chemotherapy interrelated markers. Cell proliferation is closely associated with tumor progression, reflecting the invasiveness and final prognosis of various

malignant tumor types including gastric cancer (39). Ki67 is a nuclear antigen that may be expressed at all stages of the cell proliferation cycle except in G0 cells. Cytotoxic chemotherapeutic agents are effective against tumor cells that have entered the cell division cycle (G1, S, G2 and M phases). A high level of Ki67 indicates that a greater number of cancer cells are entering the cell division cycle and may be the most effective indicator for chemotherapeutic drug therapies. Therefore, the Ki-67 antibody is widely used to evaluate the proliferative activity of tumor cells and to identify the presence of circulating cells to measure tissue growth (40). Although there is no consensus on the prognosis and predictive value of Ki-67 in malignant tumor types, studies have revealed that the Ki-67 index has notable implications for the prognosis of cancer (41). Therefore, the high expression of Ki-67 may be used as a marker for predicting poor prognosis in patients with gastric cancer. The levels of Ki-67 expression should thus be considered when selecting a suitable treatment option and comprehensive treatment. Thus, it is recommended to perform routine Ki67 detection on gastric cancer tissues to evaluate the proliferative status of the cancer cells and provide a reference for determining the efficacy of chemotherapy.

Markers associated with gastric cancer molecular targeted therapy. Human epidermal growth factor receptor-2 (HER-2) is a proto-oncogene located on chromosome 17, encoding a 185-kDa tyrosine kinase receptor which belongs to the epidermal growth factor receptor family. The phosphorylation of HER-2 initiates a signaling pathway resulting in cell division, proliferation, differentiation and anti-apoptotic signaling (42,43). Previous research has predicted that between 7-38% of gastric cancer types exhibit the amplification and/or overexpression of HER-2 (44-46). In 2010, the trastuzumab for gastric cancer study, a phase III, open-label, randomized controlled clinical trial, revealed that patients with gastric cancer who exhibited of HER-2 upregulation and treated with trastuzumab had significantly improved overall survival (OS) and disease-free survival (DFS) times, and a significantly increased objective response proportion, compared with chemotherapy alone (cisplatin + 5-fluoropyridine or capecitabine) (44). Therefore, on October 20, 2010, the U.S. Food and Drug Administration (FDA) granted approval for trastuzumab (Herceptin) in conjunction with cisplatin and a fluoropyrimidine (cisplatin or 5-fluoropyridine), for treating patients with HER-2 amplification or upregulation in metastatic gastric or gastroesophageal junction adenocarcinoma, who were untreated for metastatic tumor (44). The National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology for gastric cancer suggest the assessment of HER-2 overexpression by IHC and/or gene amplification through fluorescence *in situ* hybridization or another *in situ* hybridization method in tumor samples for patients with an unresectable locally advanced, recurrent or metastatic stomach cancer for whom trastuzumab may be beneficial (47). A consensus for HER-2 detection in patients with gastric cancer is thus required to improve individualized treatment for patients (48). Additionally, it is recommended that there is routine detection of HER-2 expression in patients with gastric cancer, so that a greater number of patients do not forego the opportunity for targeted therapy with the aim of

improving the outcome of these patients. The vascular endothelial growth factor (VEGF) family is an important regulator of angiogenesis and lymphangiogenesis, which specifically binds to VEGFR receptor (VEGFR), promotes vascular and lymphangiogenesis and participates in the development and progression of a tumor (22). Therefore, VEGF antibody and VEGFR antagonists are used to block the blood supply to gastric cancer tissues when treating patients with gastric cancer. The anti-VEGF drug, bevacizumab, is a recombinant humanized monoclonal antibody against VEGF-A. Phase III clinical trials in the AVASTIN study revealed that bevacizumab, combined with capecitabine and cisplatin, as a first-line treatment for patients with advanced gastric cancer did not improve overall survival time. However, the results of the study revealed that there was a significant difference in survival benefits in the US, but not in Asia (49). Therefore, it is necessary to consider individual differences and other factors in future research to improved individualized treatment.

VEGFR belongs to the tyrosine kinase receptors family, which includes VEGFR-1, VEGFR-2 and VEGFR-3, of which VEGFR-2 is the primary receptor mediating increased vascular permeability (50). Ramucirumab is a completely humanized immunoglobulin G (IgG1) monoclonal antibody which targets the extracellular domain of VEGFR2, blocks the interaction with VEGFR ligands and inhibits receptor activation. It was demonstrated that in two global, randomized, double-blind phase III clinical trials, using ramucirumab combined with platinum and/or fluorouracil (REGARD trial) or ramucirumab combined with paclitaxel (RAINBOW trial), that the OS and DFS times in patients with gastric cancer whose cancer had deteriorated following the initial treatment, were significantly increased in the treatment group compared with the placebo, who were treated with the corresponding chemotherapeutics alone (51,52). In 2014, the FDA approved ramucirumab as a second-line therapy for the treatment of patients with advanced gastric or gastroesophageal junction adenocarcinoma with evidence of disease progression during or following treatment with fluoropyrimidine- or platinum-containing chemotherapeutics (53). The RAINBOW study revealed that Asian patients did not benefit significantly from anti-angiogenic therapy compared with patients treated with ramucirumab or ramucirumab combined with paclitaxel. However, there remain certain issues which need to be addressed. For example, anti-angiogenic drugs result in alterations to physiological vasoactive activity. Additionally, the mechanisms underlying the resistance to drugs targeting angiogenesis require further study which may partly be achieved through the identification of more reliable biomarkers (54). Epidermal growth factor receptor (EGFR) (also known as HER1) is a member of the human epidermal growth factor receptor family, which is a multifunctional glycoprotein on the human cell membrane with a relative molecular mass of 1.70×10^4 (55). The mutation and overexpression of EGFR is closely associated with malignant tumor types including gastric cancer (56). EGFR-targeting drugs may exhibit anti-tumor effects by inhibiting the binding of ligands to EGFR or exerting effects on the intracellular regions of EGFR and interfering with tyrosine kinase phosphorylation. Cetuximab is a human-mouse chimeric IgG1 monoclonal antibody against EGFR (57). In EXPAND, a phase III clinical trial, it was revealed that the addition of

cetuximab to capecitabine-cisplatin provided no additional benefits to progression-free survival (PFS) time compared with chemotherapy alone as a first-line treatment of advanced gastric cancer (57). Panitumumab is a humanized IgG2 monoclonal antibody against EGFR. In a large-scale clinical phase III trial (REAL-3), it was demonstrated that the addition of panitumumab to epirubicin, oxaliplatin and capecitabine (EOC) chemotherapy significantly worsened the OS time from 11.3 to 8.8 months (58). Therefore, it was not recommended for patients with advanced esophageal adenocarcinoma, who were not selected. However, a large number of studies (59-61) have revealed inconsistent results, highlighting the need for a large-scale clinical study to validate whether patients with gastric cancer with evidence of EGFR expression benefit from targeted drugs. However, at present, there are no guidelines recommending the routine use of EGFR for the detection of gastric cancer.

Mesenchymal-epithelial transition factor gene (c-MET) is located on chromosome 7q21-31, which encodes for a protein tyrosine kinase belonging to the hepatocyte growth factor receptor family, and participates in regulating important cellular processes, including proliferation, differentiation, motility, cell cycle and apoptosis (62). Additionally, numerous studies have demonstrated that c-MET overexpression is associated with a poorer survival prognosis and predicted shorter PFS time in various tumor types, including gastric cancer (63-65). Multi-center retrospective studies in Japan have revealed that there is a significant difference in the OS time of patients who had high c-Met expression compared with those with no/low levels of c-Met expression (66). Catenacci *et al* (67) observed that when the overexpression of the MET protein in patients with chemotherapy-insensitive gastric cancer was present, these patients were able to remain in remission in a two year follow-up period when treated with a monovalent MET antibody therapy. Therefore, it is expected that examining the expression of MET in gastric carcinoma tissues using IHC may have potential clinical application value for gastric cancer c-Met targeted therapy. However, a phase III, randomized, double-blind, multicenter trial (the RILOMET-1 study), 609 patients with advanced MET-positive gastric or gastroesophageal junction tumor types were divided into a rilotumumab group (rilotumumab in combination with epirubicin, cisplatin and capecitabine) or a placebo group (placebo + epirubicin, cisplatin and capecitabine). It was demonstrated that the OS time of the rilotumumab group was worse compared with the placebo group, and the incidence of negative effects was increased. The study was aborted early due to an imbalance in mortalities between the groups (62). In conclusion, the exact efficacy of a targeted MET monoclonal antibody in patients with gastric cancer requires additional study to determine the reason for the less favorable results observed in the RILOMET-1 study. Identifying suitable molecular markers and investigating the optimum combinatorial solutions may ultimately improve patient outcomes. Mammalian target of rapamycin (mTOR) is a member of the phosphoinositide-3-kinase-associated kinase family, which is highly expressed in gastric cancer tissues. Hence, blocking the mTOR signaling pathway may inhibit the proliferation and metastasis of tumor cells (68). Everolimus is an oral rapamycin derivative that blocks the phosphorylation of mTOR and

functions as an antitumor agent. A phase III clinical trial of Granite-1 revealed that everolimus did not improve the prognosis of patients with advanced gastric cancer compared with matching placebo (69). Additionally, one previous study revealed that the efficacy of everolimus was associated with the level of p-S6 (70). At present, the potential efficacy of anti-mTOR targeted therapy and biomarkers are still being determined.

The newly reported research regarding Claudin18.2 (CLDN18.2), a member of the Claudin family, has revealed that CLDN18.2 is associated with tumor development and progression and is located on the outer cell membrane. It is expressed in a variety of tumor types, in particular gastric cancer cells. These biological characteristics suggest that CLDN18.2 may be a potential therapeutic target. A monoclonal antibody against CLDN 18.2, Claudiximab (previously IMAB362), was the first targeted therapy for CLDN 18.2 expression (71). The antibody exerted an anti-tumor effect primarily through activating antibody-dependent cytotoxicity, complement-dependent cytotoxicity and regulation of the tumor microenvironment, in a recent phase II clinical study (FAST) that revealed that Claudiximab combined with EOX (epirubicin 50 mg/m², oxaliplatin 130 mg/m² d1 and capecitabine 625 mg/m² bid, d1-21, every 21 days) significantly improved the PFS and OS times compared with EOX in patients with gastric cancer (72). Treatment with IMAB362 plus EOX in patients with advanced or metastatic gastroesophageal cancer was considered safe and effective. Therefore, this may be a promising target therapeutic option for patients with typically difficult malignancies to treat. However, it is still in phase II trials and will undergo further research.

4. High-throughput technology allows for novel molecular typing of gastric cancer

As the identification of novel biomarkers has resulted in an increase in potential therapeutic regimens, it is no longer sufficient to design treatment options that are not unique for a patients unique expression profile. For example, Herceptin may be used for human epidermal growth factor receptor 2 (HER2)-positive breast cancer, but not for the negative type. However, this may not be completely accurate, as patients who are positive for this marker may exhibit additional mutations. Interestingly, high-throughput technologies have brought tremendous changes, including next generation sequencing and gene array chips, which have allowed for the detection of a large number of markers at the same time, thus making it substantially easier to tailor a specific treatment regimen to an individuals' profile (73). It is well known that there are general classifications by Borrmann and histological classifications by Lauren and WHO in gastric cancer. In previous years, developments in the field of cancer genomics have been revolutionized by the molecular characterization of the different varieties of carcinomas including gastric cancer. Initially, there were only two molecular subtypes identified (74). Subsequently, Lei *et al* (75) at the Duke National University of Singapore identified three subtypes of gastric adenocarcinoma: Proliferative, metabolic and mesenchymal. In 2014, The Cancer Genome Atlas (TCGA) integration analysis based on somatic cell copy number array analysis,

full exon sequence analysis, DNA methylation degree array analysis, mRNA sequence analysis, microRNA sequence analysis and anti-phase protein array analysis resulted in the identification of four different subtypes of gastric cancer: EBV-positive, microsatellite instability (MSI) type, stable genome (GS) and chromosomal instability (CIN) (76). Tumor types expressing EBV frequently underwent recurrent extreme DNA hypermethylation, and exhibit phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit- α mutations, Janus kinase 2 amplification, CD274 [programmed death receptor ligand-1 antibody (PD-L1)] and programmed cell death 1 ligand 2 (PD-L2). The MSI subtype exhibited mutations in its DNA sequences, including the mutations of genes which encode targetable oncogenic signaling proteins. The GS tumor types were more common in the diffuse histological variant and mutations of ras homolog family member A or fusions involving RHO-family GTPase-activating proteins. Tumor types with CIN usually exhibited substantial aneuploidy and focal amplification of receptor tyrosine kinases. Each subtype could be distinguished by different biomarkers, as different markers represent different gene mutations. Meanwhile, they are caused by different factors, which should corresponding with different treatments (77). The Asian Gastric Cancer Research Group also divided gastric cancer into four molecular subtypes, including MSI and microsatellite stable (MSS) tumor types with either MSS/epithelial-mesenchymal transition, TP53 activity (MSS/TP53⁺) or TP53 inactivity (MSS/TP53⁻) (77) based on the analysis of major components, in 2015. These subtypes are similar to the TCGA subtypes, as the two studies identified MSI immunotherapy. As gastric cancer is a multifaceted and highly heterogeneous disease, these molecular classifications allow researchers to further understand gastric cancer and provide a good basis for innovative targeted therapies for treating patients with gastric cancer.

5. Markers associated with immunotherapy in gastric cancer

Immunotherapy has received growing attention as potential mechanism for treating patients with gastric cancer, due to its favorable curative effect and improved survival time. Discussion of immunotherapy always involves a discussion of biomarkers. The programmed death receptor-1 (PD-1)/PD-L1 was considered a breakthrough for the treatment of numerous tumor types. PD-1 is a negative co-stimulatory receptor, which is primarily expressed on activated T cells (78), and suppresses an excessive immune response through binding to its ligands, PD-L1 and PD-L2 (79,80). In addition to gastric cancer, PD-L1 is expressed in a range of tissues and is also expressed in certain other malignant tumor types (78,80-84). In tumor tissues, effector T-cell function may be inhibited by the binding of PD-1 to PD-L1, which results in the inhibition of the antitumor immune response and even accelerates neoplastic growth (78,79).

Nivolumab is a human IgG4 monoclonal antibody inhibitor of PD-1. The ATTRACTION-2 (ONO-4538-12) study was a randomized, double-blind, placebo-controlled, phase III clinical trial, which compared the efficacy and safety of nivolumab and a placebo in patients with advanced gastric cancer who underwent second line systemic treatment (85).

Nivolumab treatment had previously been shown to significantly improve survival (69). In the clinical trial, nivolumab significantly reduced the risk of mortality by 37% compared with the placebo group. In addition, the 12-month OS rate was significantly higher in the nivolumab group compared with the placebo group. The OS rates were 26.2 and 10.9%, respectively (69). Nivolumab is now approved for the treatment of unresectable advanced or recurrent gastric cancer, based on the results of the ATTRACTION-2 study, and nivolumab has become an important treatment option for a variety of tumor types including gastric cancer. Pembrolizumab is an IgG4- κ humanized monoclonal antibody, which is selective and has a high-affinity, and binds to PD-1, preventing PD-1 from binding to PD-L1. Pembrolizumab is relatively safe and has exhibited potential anti-tumor activity in certain types of advanced solid tumor types and hematological malignancies (86). A number of countries have approved pembrolizumab for the treatment of advanced melanoma, and in the USA it is used for the treatment of metastatic non-small-cell lung cancer, which expresses PD-L1 and non-small-cell lung cancer with failed platinum-containing chemotherapeutics (86). Multiple studies have revealed that tumor types with an upregulated expression of PD-L1 have a higher rate of response when treated with pembrolizumab (87,88). In addition, nivolumab and atezolizumab also produced similar results (89,90). Furthermore, the higher the expression rate was of PD-L1, the higher the remission rate of the tumor was, and even resulted in complete cure in certain cases (88). Biomarkers which will help predict the response to inhibitors of PD-1 or PD-L1 are required. The detection of PD-L1 protein expression by IHC is a currently available method.

In a number of studies, PD-L1 overexpression was observed in >40% of human gastric cancer samples (81,82,91,92). The KEYNOTE-021 and KEYNOTE-059 clinical trials revealed an improved outcome in patients with advanced gastric cancer treated with pembrolizumab+5-FU+cisplatin compared with 5-FU+cisplatin and thus highlighting the potential use of immunotherapy for the treatment of patients with gastric cancer (93,94). PD-L1 Combined Positive Score (CPS) $\geq 1\%$ in tumor types or mesenchymal cells was considered to be the cut-off value for treatment with pembrolizumab. Unfortunately, in the majority of solid tumor types, the effectiveness of a PD-1 inhibitor alone is 10-30%. One exception is the classic Hodgkin's lymphoma, where the efficiency is >60%. Although PD-1 inhibitors are not so efficient, they have a long-lasting effect (94). As the immune system has a functional 'memory', once the PD-1 inhibitor has exerted its effects, patients with certain early tumor types, including patients with malignant melanoma, kidney cancer and non-small cell lung cancer, are able to achieve clinical cure, that is, no recurrence, no progression and long-term survival (95). Whether this effect may be achieved in gastric cancer requires further study. In addition, certain patients may also achieve improved results with a combination of PD-1 inhibitors with other forms of therapy. At present, PD-1 inhibitors combined with chemotherapy have been approved for treating gastric cancer (96); however, additional studies are required to determine its efficacy.

Based on these studies, in September 2017, pembrolizumab was approved by the FDA as a third-line treatment for metastatic carcinoma or patients with recurrent locally

advanced, gastric or gastroesophageal junction adenocarcinoma whose tumor types expressed PD-L1 as determined by an FDA-approved test in addition to use for any cancer with MSI-high (H) (97). A meta-analysis and systematic review of the literature have demonstrated that MSI-H and EBV-positive gastric cancer are often associated with improved prognosis and longer survival times (98). Derks *et al* (99) additionally reported that interferon-driven genes are abundant in MSI-H and EBV-positive gastric cancer types, suggesting that these patients are sensitive for PD-1 or PD-L1 inhibitors. Researchers have revealed that MSI-H and EBV positive cancer types have upregulated expression levels of PD-L1 (100). Therefore, the NCCN clinical practice guidelines for gastric cancer 2017 version 5 and 2018 version 1 have recommended that mismatch repair (MMR)/MSI-H, PD-L1 and tumor EBV status should be considered in patients with locally advanced, recurrent or distant metastases who are candidates for treatment with PD-1 inhibitors (101). The expression of the MMR proteins [mutL homolog 1 (MLH1), PMS1 homolog 2, mismatch repair system component, mutS homolog 2 and mutS homolog 6] and PD-L1 expression are assessed using IHC in clinical practice.

In addition, tumor mutation burden (TMB) is also an important biomarker for predicting the effect of PD-1 inhibitors (102). The definition of TMB is the number of somatic mutations in the whole genome subsequent to counting germline DNA variants. For efficacy analysis, patients may be divided into three groups: TMB high burden (≥ 248), medium burden (143-247) and low burden (<143) groups (103). Studies have revealed that the efficacy of PD-1/PD-L1 inhibitors in a tumor are significantly associated with TMB (102,104). Hence, patients with a high TMB may be more likely to benefit from immunotherapy. However, there are some difficulties in assessing TMB in clinical practice, including a long detection period, poor platform accessibility, high costs and inconsistent standards. TMB testing requires strict experimental conditions and has a low success rate at present. Furthermore, there is insufficient data at present to support the notion that TMB benefits OS (104). Therefore, additional research is required to fully understand the implications and clinical applications of TMB.

6. Other markers with potential clinical application value

DNA methylation. DNA methylation is a frequent epigenetic event that serves an important function in cancer development (105). Hypermethylation of gastric cancer in CPG islands are interrelated with the gene silencing of numerous tumor suppressor genes, including human MLH1, p16, CDH1 (E-cadherin) and RUNX family transcription factor 3 (RUNX3) genes. Cancer-derived DNA methylation may be detected easily in the serum of patients with gastric cancer (106). A number of studies have reported that methylation of the p16 gene and the CDH1 gene were detectable in the serum of 20-50% of patients with gastric cancer, but not in the cancer-free and control patients (107,108). Thirty percent of patients with gastric cancer had RUNX3 methylation, detected in the peripheral circulation, and it was associated with tumor stage, vascular invasion and lymph node metastasis (109). However, serum RUNX3 methylation levels were significantly reduced in patients with gastric cancer following

surgery (110). Therefore, the detection of abnormal DNA methylation in serum is an effective tool for cancer screening, disease monitoring and prognosis determination.

Among the epigenetic changes involved, DNA methylation is associated with anticancer drug resistance (111). A number of methylation alterations to apoptotic genes have been identified and used as epigenetic biomarkers for determining either chemoresistance or chemosensitivity to anticancer drugs (111). According to Choi *et al* (112), the hypermethylation of cyclin dependent kinase inhibitor 2A (p16^{INK4a}) may be a useful biomarker for 5-fluorouracil-sensitive and resistant gastric cancer, respectively. Furthermore, the hypomethylations of adhesion G protein-coupled receptor L2 and G2 and S-phase expressed 1 are potential biomarkers for cisplatin-sensitive gastric cancer. Additionally, the hypomethylation of ATP binding cassette subfamily B member 1 may be a useful biomarker, regardless of the drug type, for chemotherapy-resistant gastric cancer.

Non-coding RNA (ncRNA). In previous years, ncRNA, including microRNA (miR/miRNA), long ncRNA (lncRNA) and circular RNA (circRNA), have been identified as important regulators of protein-coding genes, and are involved in the regulation of cell development, differentiation and the occurrence of a tumor. They have also been demonstrated to serve key functions in the evolution and development of gastric cancer (113-115).

miRNAs are small ncRNA molecules that regulate gene expression at the post-transcriptional level (116). miRNAs have a large range of gene regulatory functions and are involved in a series of biological processes including regulating apoptosis, cell proliferation, cell differentiation and development, epigenetic genetic regulation and serve a notable function in the development and progression of cancer (117,118). Numerous miRNAs have been revealed to be expressed at varying degrees in gastric cancer, regulating the signaling pathways, and certain specific miRNAs are associated with the occurrence, progression and prognosis of gastric cancer (119,120). Using the microarray analysis of miRNAs, 22 types of miRNAs were demonstrated to be upregulated and 13 species were downregulated, compared with non-tumor gastric tissue (119,120). miR125b, miR199a and miR-433 are miRNAs that serve an important function in the progression of cancer. Furthermore, the low expression of miR-433 and high expression of miR214 are independent predictors of poor prognosis (121). In gastric cancer, the downregulation of miR-148a results in reduced tumor metastasis and causes lymph node metastasis and disease progression when miR-148a is downregulated (122). miR-335 inhibits metastasis by regulating BCL2 like 2 and specificity protein-1 in gastric cancer (123). Furthermore, miRNA-421 is a promising tumor biomarker with diagnostic potential to monitor a number of different types of cancer. Gastric juice levels of miR421 in patients with gastric cancer were significantly different compared with patients with a benign gastric disease (124). Therefore, miRNAs may function as potential biomarker targets for the molecular diagnosis of gastric cancer.

For the past few years, the dysregulated expression of numerous lncRNAs (ncRNAs that are >200 nucleotides long) have been demonstrated to be associated with tumorigenesis

using next-generation sequencing; and these lncRNAs perform basic regulatory functions by modulating tumor cell proliferation, apoptosis, invasion and metastasis (125). Differential expression of lncRNAs serve critical functions in the carcinogenesis of gastric cancer. A number of studies have revealed that lncRNA H19 is upregulated and highly expressed in gastric cancer and is involved in the complex molecular regulation of gastric cancer, and thus may serve as a potential diagnostic marker and molecular therapeutic target, particularly for early tumor screening (126,127). Pang *et al* (128) illuminated that the expression levels of LINC00152 in gastric cancer tissues was significantly higher compared with normal tissues and normal healthy controls, and was associated with invasion. Additionally, this previous study demonstrated that the lncRNA LINC00152 may be a novel biomarker for predicting gastric cancer (99). Furthermore, high urothelial cancer associated 1 (UCA1) expression is associated with tumor size, reduced differentiation, advanced Tumor-Node-Metastasis stages and increased invasion depth in gastric cancer. Therefore UCA1 may also serve as a potential marker for the early detection and prognostic prediction of gastric cancer (129). It was demonstrated that a three-lncRNA signature, including UCA1, long stress-induced non-coding transcript 5 and phosphatase and tensin homolog pseudogene 1, was confirmed to be a potential diagnostic biomarker for detecting gastric cancer (130). Hence, it is clear that the study of the pathophysiology of lncRNA expression in gastric cancer is a novel research trend which may improve the diagnosis and treatment of patients with gastric cancer.

CircRNA, a novel type of ncRNA which is dissimilar to the well-known linear RNA, forms a covalently closed continuous loop. In circular RNA, the 3' end and 5' end are usually joined together (131). At present, research on the function of circRNA in cancer remains in its infancy. Although a large number of circRNAs have been demonstrated to be downregulated by using next-generation RNA sequencing and bioinformatics analysis, there is emerging evidence that numerous circRNAs are abnormally expressed in various diseases, including certain types of cancer, which may function as oncogenes or tumor suppressor genes (132,133). Certain circRNAs serve a function in various aspects of biology and disease, particularly in cancer (134,135). Notable, studies have revealed that select circRNAs are upregulated or downregulated in patients with gastric cancer. Chen *et al* (136) revealed that circRNA Pvt1 oncogene was upregulated in gastric cancer and functioned as an miRNA sponge, regulating the expression of the target genes of the miRNA in gastric cancer, and ultimately demonstrated a proliferative effect and potential as a prognostic marker. In addition, three independent studies demonstrated that hsa_circ_0000190 (137), hsa_circ_0000096 (138) and hsa_circ_002059 (139) were downregulated in gastric cancer tissues compared with the adjacent noncancerous tissues. Due to the tissue, timing and disease specificity of circRNA expression, circRNAs have attracted substantial interest in cancer research. At present, circRNAs present high specificity in gastric cancer, which indicates that they may have promise as prospective novel biomarkers. However, their function and mechanism remain yet to be elucidated.

Cell cycle regulators. The normal progression of the cell cycle is regulated by positive regulators of cyclins, cyclin-dependent kinase (CDK) complexes, and negative regulators of cell cycle-dependent kinase inhibitors (CKI). The overexpression of cell cycle positive regulators, abnormal activity and lack of expression of CKI result in disorders of cell proliferation (140). Hence, any defects in cell cycle regulators affect the outcome of patients with gastric cancer, as cell-cycle regulators are involved in a number of processes, including the proliferation of cancer. An important regulator is cyclin E, which is also a useful prognostic factor in gastric cancer. The activity of CDK is inhibited by CKIs, including P16, P21 and P27 (141). The downregulation of P27 serves as a negative predictor of cyclin E-positive tumor types (142-144). In addition, the function of P53, a tumor suppressor gene, is pivotal to cell cycle regulation, DNA repair and apoptosis. Multivariate analysis has revealed that the upregulation of P53 was an independent prognostic factor in patients with advanced gastric cancer and it was associated with poor prognosis (145). However, P53 has no substantial prognostic value in the initial phase of gastric cancer in patients (146).

Apoptosis-associated factors. The growth of a tumor depends on the ratio of cell proliferation to apoptosis. In addition to the function of oncogenes and tumor suppressor genes, factors that regulate cell apoptosis also serve a crucial function in the development of a number of tumor types, including gastric cancer. Certain programmed cell death factors are good prognostic indicators for gastric cancer. Apoptosis is achieved through the interaction between pro-apoptotic and anti-apoptotic molecules (147). The BCL2 apoptosis regulator (Bcl-2) gene, which is a crucial antiapoptotic gene, influences the intrinsic apoptosis pathway (147). At the same time, Bcl-2 may facilitate tumor invasion and metastasis (148). It has been suggested that the inhibition of Bcl-2 expression is an important strategy for the treatment of various cancer types, including gastric cancer (149,150). The sensitivity of tumor cells to apoptosis may be detected and evaluated by assessing the levels of the proto-oncogene Bcl-2, which is a useful prognostic factor of survival in patients with advanced gastric cancer (151). The newest inhibitory apoptotic family, survivin, may suppress apoptosis via pathways other than those associated with the Bcl-2 family (152). One study hypothesized that survivin may be expressed in gastric cancer, although the nuclear localization of survivin is thought to delay physiological development (153). Thus, survivin may be used as a prognostic factor for poor outcome in patients with gastric cancer (154).

Prognostic markers of gastric cancer. In previous years, the association between certain potentially enriched markers of gastric cancer stem cells including CD133, CD44 and leucine rich repeat containing G protein-coupled receptor 5 and the biological behavior and prognosis of gastric cancer in patients has attracted increasing attention. If CD133 is highly expressed in gastric cancer cells, the cells display increased malignant biological behaviors, as its upregulation is associated with tumor progression, chemotherapy resistance, relapse and poor prognosis (155). Therefore, the positive expression of CD133 in gastric cancer cells may contribute to the prognosis of patients (156).

The CDH1 gene is a tumor suppressor gene which encodes E-cadherin, a transmembrane glycoprotein involved in cell adhesion and epithelial differentiation (157). Mutation of this gene are associated with the occurrence and progression of hereditary diffuse gastric cancer and sporadic gastric cancer. A lack of expression of E-cadherin is an independent prognostic factor for gastric cancer, and predicts a worse prognosis in patients (158). E-cadherin-negative and nuclear β -catenin-positive gastric cancer are often associated with the poor differentiation of gastric cancer, loss of adhesion, increased infiltration ability, increased tumor progression and a poorer prognosis (159). Consequently, the expression of CD133 or E-cadherin may be used to predict the prognosis of patients with gastric cancer.

7. Conclusion

Gastric cancer is a malignant tumor type with high rates of incidence and mortality globally (1). Gastric cancer is a group of tumor types which are diverse in their biology and genetics; and furthermore, there are various factors at work in the etiology of this cancer, including environmental and genetic factors (6). Although endoscopy and imaging technology have improved the detection of early-stage gastric carcinoma-associated lesions, gastric cancer has a wide range of morphological heterogeneity. It is easy to overlook certain cases of this disease if only the morphology is relied upon, and advances in the field of understanding biomarkers have helped to improve the early diagnosis and accuracy of diagnosis of gastric cancer early as well as the prognosis and treatment of various diseases including cancer.

The majority of cases of gastric cancer are either moderately or severely advanced at first diagnosis; however, the application of biomarkers may improve the early detection of gastric cancer screening and diagnostic accuracy. As the correct treatment may improve the prognosis of patients, it is important to tailor treatments according the unique biomarker expression profile of each patient. In particular, certain markers may guide the individual and precise therapy of gastric cancer in order to maximize a patient's survival time, instead of or in addition to relying on histological classification and chemotherapy completely. So far, targeted therapies for a small number of patients with gastric cancer have been performed instead of or in addition to using trastuzumab and ramucirumab, which are the second-line treatments for advanced gastric cancer when either HER2 or VEGFR2 expression are upregulated, respectively. The PD-1 inhibitor pembrolizumab has been approved by the FDA as a third-line (or higher) treatment for patients with recurrent locally advanced or metastatic gastric adenocarcinoma. The NCCN guidelines recommend the detection of PD-L1 or MSI/MMR, as PD-1 inhibitors are effective in patients with MSI-H/deficient MMR and a high expression of PD-L1 (76). Targeted therapy and immunotherapy have presented clinicians with a novel method for treating patients with advanced gastric cancer.

Numerous markers have now been identified for targeted therapy and immunotherapy, and each marker exhibits advantages and disadvantages (44,50,56). With the development of high-throughput technologies, various markers will be identified in the future, not only at the organ level but also at the genetic level. All tumor types that express the same markers

may be treated using similar therapeutic regimens, despite the location of the tumor. Conversely, tumor types which are located in the same organ but exhibit different biomarker profiles may be treated with using different therapeutic regimens. In summary, the identification of novel and effective biomarkers is required to improve the diagnosis of gastric cancer, in order to strengthen the accuracy of gastric cancer diagnosis, determine the prognosis and predict the pathogenesis, and establish a novel and effective treatment option for patients with gastric cancer.

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Authors' contributions

ZZ and DMY drafted the manuscript. DMY, GX and YX acquired the data. WM, YXL, WL and YL interpreted the data. DY, ZZ and YL edited the manuscript and revised it. All authors read and approved the final manuscript.

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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