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INVITED REVIEW ARTICLE

Molecular carcinogenesis of gastric cancer: Lauren classification, mucin phenotype expression, and cancer stem cells

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Abstract

Gastric cancer (GC), one of the most common human cancers, is a heterogeneous disease with different phenotypes, prognoses, and responses to treatment. Understanding the pathogenesis of GC at the molecular level is important for prognosis prediction and determining treatments. Microsatellite instability (MSI), silencing of *MLH1*, *MGMT*, and *CDKN2A* genes by DNA hypermethylation, *KRAS* mutation, *APC* mutation, and *ERBB2* amplification are frequently found in intestinal type GC. Inactivation of *CDH1* and *RARB* by DNA hypermethylation, and amplification of *FGFR* and *MET*, are frequently detected in diffuse type GC. In addition, *BST2* and *PCDHB9* genes are overexpressed in intestinal type GC. Both genes are associated with GC progression. GC can be divided into gastric/intestinal mucin phenotypes according to mucin expression. MSI, alterations of *TP73*, *CDH1* mutation, and DNA methylation of *MLH* are detected frequently in the gastric mucin phenotype. *TP53* mutation, deletion of *APC*, and DNA methylation of *MGMT* are detected frequently in the intestinal mucin phenotype. *FKTN* is overexpressed in the intestinal mucin phenotype, and *IQGAP3* is overexpressed in the gastric mucin phenotype. These genes are involved in GC progression. To characterize cancer stem cells, a useful method is spheroid colony formation. *KIFC1* and *KIF11* genes show more than twofold higher expression in spheroid-forming cells than that in parental cells. Both *KIF* genes are overexpressed in GC, and knockdown of these genes inhibits spheroid formation. Alterations of these molecules may be useful to understand gastric carcinogenesis. Specific inhibitors of these molecules may also be promising anticancer drugs.

Keywords Gastric cancer · Lauren classification · Mucin phenotype expression · Cancer stem cell

Introduction

Gastric cancer (GC), one of the most common human cancers, is a heterogeneous disease with different phenotypes, prognoses, and responses to treatment. Understanding GC pathogenesis at the molecular level is important for prognosis prediction and determining treatments. Various genetic and epigenetic alterations are associated with GC. Histologically, GC cases are classified into two major types: differentiated and undifferentiated types, as described by Nakamura et al. [1], or Lauren intestinal and diffuse types based on the glandular structure [2]. Intestinal and diffuse GC types show distinct clinical characteristics [3], and type-specific genetic and epigenetic alterations have been identified [4, 5]. Thus, the Lauren classification facilitates understanding the pathogenesis of GC. GC can also be classified into gastric or intestinal phenotypes according to mucin expression. Accumulating evidence has indicated that gastric/intestinal phenotypes of GC have distinct clinical characteristics and exhibit specific genetic and epigenetic changes [5]. Thus, mucin phenotype classification is also useful to understand GC pathogenesis. In addition to these histological classifications, studies employing next-generation sequencing (NGS) have proposed molecular classification of GC. Here, we focus on the molecular characteristics of GC according to histological and molecular classifications.

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Lauren classification

During the course of multistep carcinogenesis in the stomach, various genetic and epigenetic alterations accumulate [5]. Of these alterations, some are found in both intestinal and diffuse types of GC, while others are found in only one histological type. Microsatellite instability (MSI), silencing of *MLH1*, *MGMT*, and *CDKN2A* genes by CpG island hypermethylation, *KRAS* mutation, *APC* mutation, and *ERBB2* amplification are frequently found in intestinal type GC. In contrast, inactivation of *CDH1* and *RARB* genes by CpG island hypermethylation and amplification of *FGFR* and *MET* genes are frequently detected in diffuse type GC. Molecular alterations according to intestinal and diffuse types of GC are summarized in Fig. 1. These alterations facilitate understanding the pathogenesis of GC. In clinical practice, patients with intestinal type GC may be eligible for anti-HER2 therapy, and patients with diffuse type GC may be eligible for anti-MET therapy. Genes that encode transmembrane/secretory proteins and are expressed specifically in cancers can be ideal biomarkers for cancer diagnosis. If the gene product is involved in the neoplastic process, then the gene may be a therapeutic target. Thus, genes that encode transmembrane/secretory proteins are analyzed preferentially. Several transmembrane/secretory proteins are specifically expressed in GC.

BST2

BST2 encodes the BST-2 protein. BST-2, also known as HM1.24 or CD317, is a lipid raft-associated type II transmembrane glycoprotein that is overexpressed on multiple myeloma cells [6, 7]. A monoclonal antibody against BST-2 can induce antibody-dependent cellular cytotoxicity, suggesting that monoclonal antibodies against BST-2 may be an effective treatment for BST-2-positive malignancies. BST-2 overexpression has been reported in several human cancers

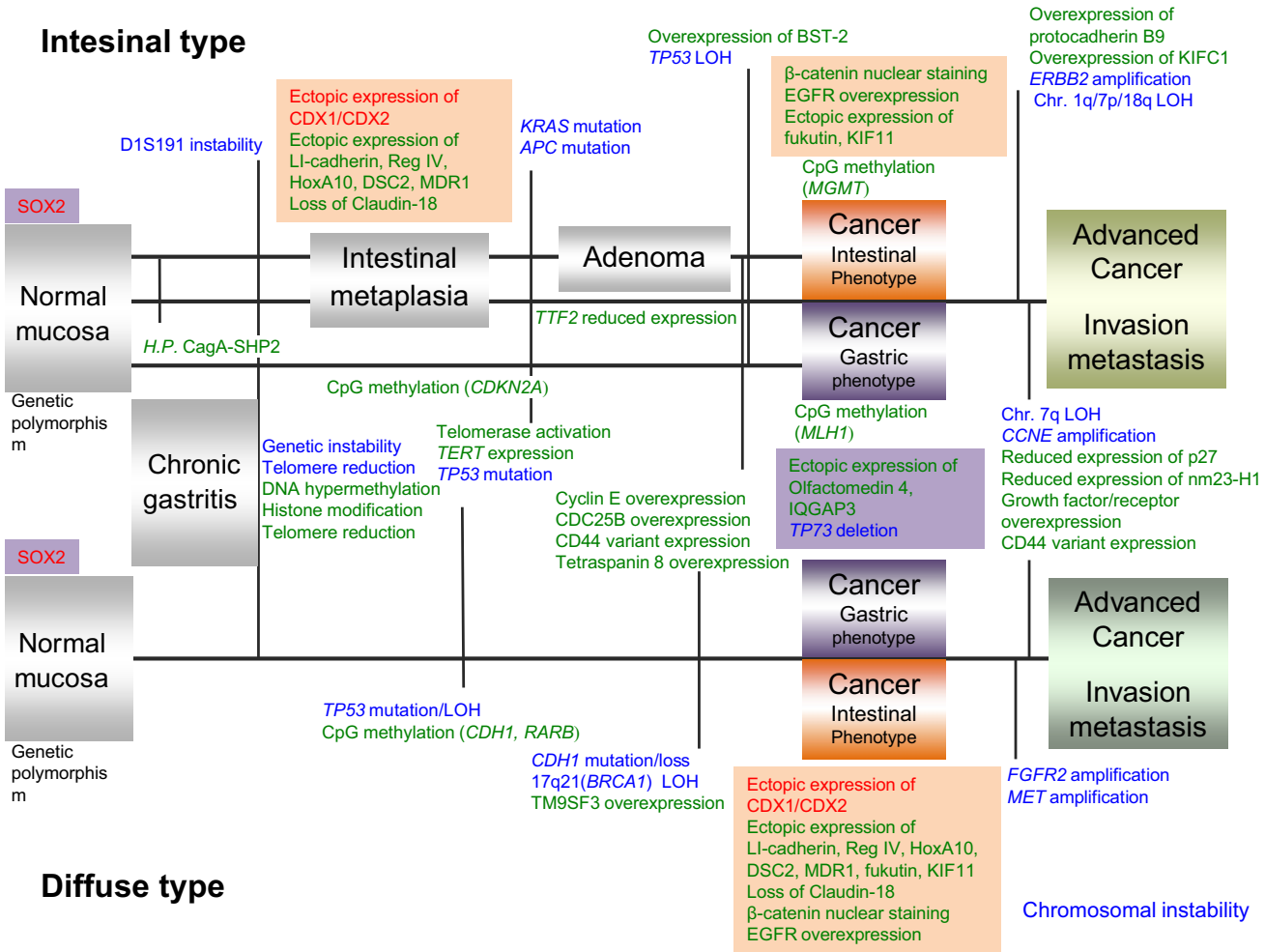

Fig. 1 Molecular alterations of GC. This graphic overview depicts the specific alterations in intestinal/diffuse type GC and the intestinal/gastric phenotypes of GC

including breast, lung, esophageal, and colorectal cancers, as well as GC [8–10]. BST-2 is overexpressed in 36% of GC tissue samples. BST-2 overexpression is preferentially found in intestinal type GC, and BST-2 expression is an independent prognostic classifier of GC patients [10]. Inhibition of *BST2* by siRNA inhibits proliferation, induces apoptosis, and represses motility of GC cells, and these effects are mediated partly through NF-kB signaling [11]. Taken together, these findings suggest that BST-2 is involved in GC progression, and that BST-2 immunostaining is a clinically useful method to predict GC patient survival. Because expression of BST-2 is found in 35% of HER2-negative GC cases, BST-2 may be a useful therapeutic target for HER2-negative GC [10].

PCDHB9

Cadherins are a family of glycosylated transmembrane proteins that mediate cell–cell adhesion. The cadherin family is classified into classical cadherins, desmosomal cadherins, and protocadherins. Protocadherins encoded by *PCDH* genes are predominantly expressed in the nervous system and comprise the largest subfamily of the cadherin superfamily of cell adhesion molecules [12]. Protocadherin B9 is overexpressed in 36% of GC tissue samples. Overexpression of protocadherin B9 is frequently found in intestinal type GC and correlated with a poor prognosis of patients with intestinal type GC [13]. The function of protocadherin B9 has also been examined [13]. *PCDHB9* knockdown or forced expression of *PCDHB9* do not change growth or invasion activities of GC cell lines. In contrast, cell adhesion to fibronectin is reduced by *PCDHB9* knockdown and enhanced by forced expression of *PCDHB9*. Expression of *ITGA3*, *ITGA4*, *ITGA5*, and *ITGB1* is reduced by knockdown of *PCDHB9* and enhanced by forced expression of *PCDHB9*. Both the number and size of spheres are decreased by *PCDHB9* knockdown and increased by forced expression of *PCDHB9*. In the peritoneal dissemination mouse model, the weight of total disseminated nodules of the MKN-74 GC cell line is increased by forced expression of *PCDHB9*. In prostate cancer cells, expression of NF-kB p65 is induced by forced expression of *PCDHB9* [14]. NF-kB p65 is a transcription factor that promotes GC cell invasion [15]. Therefore, in addition to *ITGA* genes, upregulation of NF-kB p65 is involved in the progression of protocadherin B9-positive GC. These studies indicate that protocadherin B9 plays an important role in the progression of intestinal type GC. Specific inhibitors of protocadherin B9 may also be promising anticancer drugs.

Genetic and epigenetic alterations of intestinal metaplasia (IM)

To understand gastric carcinogenesis, characterization of IM is important. IM is thought to be a premalignant condition of the gastric mucosa associated with an increased GC risk. IM shares various alterations similar to those seen in intestinal type GC [16]. Mutations of *TP53*, *APC*, or *KRAS* have been detected in IM, although at low frequencies [16]. A recent comprehensive analysis demonstrated that IMs exhibit low mutational burdens [17]. *TP53* and *ARID1A* are two of the most frequently mutated tumor-suppressor genes in GC. However, only two cases of *TP53* mutations (2%) and three cases of *ARID1A* mutations (3%) have been detected in 138 IM samples. Therefore, clonal *TP53* and *ARID1A* mutations are likely infrequent in IM. In contrast, *FBXW7* mutation is found in 4.7% of IM samples. Notably, the most frequent *FBXW7* mutations are missense point mutations, causing a dominant negative activity. Therefore, monoallelic *FBXW7* mutation is functionally active. Jiang et al. [18] have reported that the tumor incidence is obviously higher in Fbxw7+/− mice than in Fbxw7+/+ mice, and that IM and dysplasia are more severe in Fbxw7+/− mice than in Fbxw7+/+ mice. Taken together, *FBXW7* plays a crucial role in gastric carcinogenesis derived from IM.

Several epigenetic alterations are observed in IM. Some IMs show DNA hypermethylation of *MLH1*, which reduces its expression [19]. Promoter hypermethylation of *CDKN2A*, *RUNX3*, *MGMT*, and *DAPK* is found in IM [20]. These alterations are also found in GC, suggesting that DNA hypermethylation is an early event in gastric carcinogenesis. Recent comprehensive analysis has also demonstrated that 78–99% of hypermethylated regions in IM are also hypermethylated in intestinal type GC [17]. However, IMs generally lack intragenic hypomethylation signatures. Therefore, aberrant DNA hypermethylation rather than genetic alteration is associated with IM. Aberrant DNA hypermethylation is an early event in IM, whereas global intragenic hypomethylation may be a late event in gastric carcinogenesis.

Mucin phenotype classification

Gastric cancer can be divided into gastric and intestinal phenotypes. The gastric and intestinal phenotypes of GC are analyzed by immunohistochemistry of MUC5AC and MUC6 as markers for the gastric phenotype, and MUC2 and CD10 as markers for the intestinal phenotype. Based on expression of these markers, GC cases are classified into four phenotypes: gastric (G type), intestinal (I type), gastric and intestinal mixed (GI type), and neither gastric nor intestinal (N type) [21]. It is important to note that the gastric phenotype is diminishes during GC progression. GC cases at early stages, independent of the histological type, mainly consist of the gastric phenotype, and a phenotypic shift from the gastric to intestinal phenotype is clearly observed with progression of the tumor stage [22]. Therefore, GC may develop from gastric foveolar cells, but not IM.

Several genetic and epigenetic alterations are frequently detected in gastric and intestinal phenotypes of GC. Molecular alterations according to gastric and intestinal phenotypes of GC are summarized in Fig. 1. MSI is detected more frequently in the gastric phenotype of GC [23]. Alterations of *TP73*, including loss of heterozygosity and abnormal expression, play an important role in the genesis of the gastric phenotype of GC [24]. *CDH1* mutation is detected in differentiated type GC with the gastric phenotype [25]. *TP53* mutation and allelic deletion of *APC* are detected more frequently in the intestinal phenotype of GC [26, 27]. Several epigenetic alterations have also been identified. DNA methylation of *MLH1* frequently occurs in the gastric phenotype of GC, whereas *MGMT* is frequently methylated in the intestinal phenotype of GC [28].

Several transcription factors that induce the gastric/intestinal phenotypes have been identified. In the intestinal phenotype of GC, ectopic CDX2 expression has a crucial function [22]. In contrast, SOX2 may be an important transcription factor of the gastric phenotype of GC. SOX2 induces expression of MUC5AC and pepsinogen A, both of which are markers for the gastric phenotype [22]. Furthermore, SOX2 negatively regulates the CDX2 promoter by hampering the action of other transcription factors [29]. To characterize the intestinal phenotype of GC, identification of CDX2 target genes is essential. *CDH17*, *REG4*, *DSC2*, and *ABCB1* are direct targets of CDX2, which are expressed in CDX2-positive GC cells [30–33]. Notably, in patients with the gastric phenotype of GC, 5-FU-based postoperative chemotherapy is beneficial. However, in patients with the intestinal phenotype of GC, 5-FU-based postoperative chemotherapy does not improve survival [34]. *REG4* inhibits 5-FU-induced apoptosis and *ABCB1* inhibits taxane-induced apoptosis [33, 35], suggesting that chemotherapy, including 5-FU-based or taxane-based chemotherapies, is not beneficial for patients with the intestinal phenotype of GC. For these patients, molecular-targeted therapies may be suitable. In addition to these genes, altered expression of several genes is observed in gastric/intestinal phenotypes of GC.

FKTN

FKTN, which encodes fukutin protein, is responsible for Fukuyama-type congenital muscular dystrophy [36]. Fukutin is presumably involved in the glycosylation of alpha-dystroglycan, which is involved in basement membrane formation. Overexpression of fukutin is observed in 43% of GC tissue samples and correlated with the advanced T grade and N grade [37]. Fukutin expression is frequently observed in the intestinal phenotype of GC. Inhibition of *FKTN* decreases GC cell proliferation. Therefore, fukutin may be a key regulator of the progression of GC with the intestinal phenotype.

IQGAP3

The IQGAP family includes three members, *IQGAP1*, *IQGAP2*, and *IQGAP3* [38]. The name IQGAP is derived from the multiple functional domains these molecules that harbor four IQ motifs and a RasGAP-related domain (GRD) [39]. *IQGAP1* regulates the cytoskeleton and cell migration, and plays a role in cancer progression [40, 41]. In contrast, *IQGAP2* appears to act as a tumor-suppressor [42]. *IQGAP3* enhances the proliferation of epithelial cells [43]. Therefore, *IQGAP1* and *IQGAP3* are thought to have oncogenic functions. Overexpression of IQGAP3 is detected in 21% of GC tissue samples and preferentially observed in the gastric phenotype GC [44]. IQGAP3 expression is an independent prognostic predictor for survival. The growth of GC cells is inhibited by knockdown of *IQGAP3*. Both the number and size of spheres formed by GC cells are significantly reduced by knockdown of *IQGAP3*. Furthermore, phosphorylation of Akt and Erk1/2 is inhibited by knockdown of *IQGAP3*. These results suggest that IQGAP3 plays an important role in GC progression. Because IQGAP3 protein is expressed on the cell membrane, IQGAP3 protein could be a therapeutic target for GC.

Cancer stem cells (CSCs)

In the past decade, cancer has been recognized as a stem cell disease [45]. To understand the pathogenesis of GC, identification and characterization of CSCs are essential. CSCs have been described in numerous solid tumors, and have been characterized by expression of specific cell surface markers including CD44, CD133, and aldehyde dehydrogenase 1 (ALDH1) [46].

To characterize CSCs, a useful methods is spheroid colony formation [47]. To form a spheroid colony, cells are cultured in serum-free medium on low attachment culture dishes. Spheroid colonies have characteristics of the CSC phenotype. Takahashi et al. identify GC-initiating cells using cell surface marker CD44 [48]. Among six GC cell lines, MKN-45, MKN-74, and NCI-N87 have a sizeable subpopulation of CD44(+) cells, and these cells show spheroid colony formation in serum-free medium in vitro, as well as a tumorigenic ability upon injection into the stomach and skin of severe combined immunodeficient mice in vivo. Gene expression profiles of spheroid colonies derived from these GC cell lines have also been analyzed [49]. *KIF* genes, including *KIF11*, *KIF15*, *KIF2C*, *KIF20A*, *KIF20B*, *KIF22*, *KIF23*, *KIFC1*, and *KIF4A*, are upregulated in spheroid-forming cells from both MKN-45 and MKN-74 cell lines. Kinesins are a family of molecular motors, which play important roles in intracellular transport or cell division [50]. Alterations of several kinds of kinesins have been reported in

human cancers including GC. In human GC, overexpression of *KIFC1*, *KIF11*, *KIF2A*, and *KIF26B* has been reported [49, 51–53], indicating that kinesin proteins play important roles in GC pathogenesis. Inhibition of kinesin leads to cell cycle arrest at mitosis with the formation of characteristic monoaster spindles [54]. Ispinesib (SB-715992; Cytokinetics and GlaxoSmithKline) is the first potent, highly specific small molecule inhibitor of kinesin tested in clinical trials. Ispinesib achieved two complete and two partial responses among six evaluable tumors in the acute lymphoblastic leukemia xenografts panel [55]. Therefore, specific inhibitors of kinesins could be promising anticancer drugs.

KIFC1

KIFC1 (also known as HSET) is a C-type kinesin of the kinesin-14 family [56], which is assumed to be a minus end-directed motor protein [57]. Kinesins are a family of molecular motors and play important roles in intracellular transport or cell division [50]. Alteration of several kinds of kinesins has been reported in human cancers including GC. KIFC1 overexpression is found in 37% of GC tissue samples [49]. KIFC1-positive GC cases are frequently observed in the advanced stage and intestinal type of GC. Furthermore, expression of KIFC1 is detected in CD44- and ALDH1-positive GC cells. KIFC1 shows more than twofold higher expression in spheroid-forming cells than in parental cells of GC cell lines [49]. Both the number and size of spheres derived from GC cells are reduced by *KIFC1* inhibition. In prostate cancer cells, inhibition of KIFC1 resensitizes docetaxel-resistant cell lines to docetaxel treatment [58]. Docetaxel alone has little effect on the viability of docetaxel-resistant cells. However, the combination of docetaxel and CW069 (KIFC1 inhibitor) reduces the viability of docetaxel-resistant cells, indicating that CW069 resensitizes docetaxel-resistant cell lines to docetaxel treatment. These results suggest that the combination of CW069 and docetaxel could be a potential strategy to overcome docetaxel resistance. Taken together, KIFC1 likely plays an important role in CSCs.

KIF11

KIF11 encodes KIF11 protein. KIF11 protein, also known as Eg5 protein or kinesin spindle protein, is a plus-end directed heterotetrameric motor protein capable of simultaneously moving along two microtubules [59]. KIF11 is overexpressed in human cancers including breast, lung, ovarian, bladder, and pancreatic cancers, as well as GC. KIF11 is overexpressed in 72% of GC tissue samples, and KIF11 overexpression is frequently observed in the intestinal phenotype of GC [51]. Both the number and size of spheres formed by GC cells are reduced by *KIF11* inhibition. Levels of phosphorylated Erk1/2 are also reduced by *KIF11* inhibition, indicating that KIF11 protein could be a therapeutic target for GC. Because KIF11 is not expressed in the adult peripheral nervous system, KIF11 inhibitors may not cause neuropathic side effects. KIF11 inhibitors have been developed as chemotherapeutic agents for the treatment of cancer [60]. Filanesib (ARRY-520) is a highly selective, targeted inhibitor of KIF11, which induces mitotic arrest and subsequent tumor cell death. In a phase 1 clinical study, ARRY-520 had acceptable tolerability and target-specific pharmacodynamic effects [60]. There is the possibility that ARRY-520 has an activity in GC patients.

Molecular classification of GC

By comprehensive molecular analysis using NGS, two kinds of molecular classification have been proposed for GC. The Cancer Genome Atlas (TCGA) Research Network has reported that GC can be classified into four distinct molecular subtypes: GCs positive for Epstein–Barr virus (EBV type); microsatellite-unstable GCs (MSI type); genomically stable GCs (GS type); GCs with chromosomal instability (CIN type) [61]. The Asian Cancer Research Group (ACRG) has divided GCs into four groups: microsatellite instability (MSI), microsatellite-stable and epithelial-to-mesenchymal transition (MSS/EMT), and microsatellite-stable and the presence of TP53 (MSS/TP53+) or no TP53 signature (MSS/TP53−) [62]. In general, the CIN type of TCGA classification and MSS/TP53 type of ACRG classification show the intestinal type of Lauran classification. The GS type of the TCGA classification and MSS/EMT type of the ACRG classification are associated with the diffuse type of Lauran classification. The TCGA classification is not correlated with prognosis. In contrast, MSS/EMT GCs are diagnosed mainly in advanced stages and correlate with a poor prognosis. The GS type of TCGA classification often shows mutations in genes responsible for cell adhesion, such as *RHOA*, *CDH1*, and *CLDN18/ARHGAP*. The MSS/TP53 type of the ACRG classification often shows mutations in genes including *APC*, *ARID1A*, *KRAS*, *PIK3CA*, and *SMAD4*. The MSS/TP53+ type of the ACRG classification is associated with EBV infection. Most EBV-positive GCs, such as the EBV type of the TCGA classification and MSS/TP53+ of the ACRG classification, are histologically GC with lymphoid stroma or lymphoepithelioma-like, which display *PIK3CA* and *ARID1A* mutations, genome-wide hypermethylation, and amplification of *PD-L1*, an important immune checkpoint regulator [63, 64]. Although further study should be performed, these classifications may provide personalized medicine in the near future.

Conclusions

Whole genome and exon sequencing in GC has been performed by NGS. The most frequently mutated gene is *TP53* (32%) [65], and the second most frequently mutated gene is *ARID1A* (14%) [66]. The frequencies of other gene mutations are approximately 10% or below 10%. Thus, a driver gene mutation is a rare event in GC, and it is difficult to plan an effective treatment according to driver gene mutations. Therefore, alteration of gene expression is a critical event in gastric carcinogenesis. In the past few years, several targeted therapeutic agents against transmembrane proteins have been developed. For the treatment of advanced or metastatic GC, trastuzumab and ramucirumab have been approved [67, 68]. Unfortunately, the benefit from these agents is limited. Epidermal growth factor receptor (EGFR) overexpression occurs in 30–60% of GCs and is associated with a worse prognosis [69]. However, studies evaluating antibody inhibitors of EGFR have failed to demonstrate a survival advantage [70]. *MET* amplification is associated with a higher tumor stage, more aggressive phenotype, and significantly diminished survival [71]. However, monoclonal antibodies that target MET have limited activity as seen in phase III trials of rilotumumab and onartuzumab [72]. Further study is required to understand gastric carcinogenesis and treat GC.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest.

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