Review Article

Prognostic biomarker in advanced gastric cancer

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Abstract: Gastric cancer is considered one of the most lethal tumors. Even with the decline in its incidence, the mortality rate of this disease has remained high-gastric cancer ranks third in terms of cancer-related death worldwide. Patient survival is highly dependent on the tumor stage at the time of diagnosis. Yet, gastric cancer is often either asymptomatic or causing only nonspecific symptoms in its early stages. By the time the symptoms occur, the cancer has usually reached an advanced stage. The current management for advanced gastric cancer (AGC) is a multidisciplinary approach; nevertheless, the prognosis of AGC is poor. The primary tumor (T), regional nodes (N), and metastasis (M) (TNM) classification, which was validated as the best predictor of patient survival, has limited power to fully reflect the prognosis. In the last decade, several biomarkers are identified to ameliorate the accuracy of patient prognosis and subsequent treatment decision-making. Undoubtedly, the discovery of novel molecular biomarkers and their establishment in clinical practice make sense in the era of "personalized" oncologic practice. The purpose of this review is to discuss the prognostic significance of the currently well-known biomarkers as well as to highlight some of the new candidate prognostic molecular markers. These prognostic markers include conventional tissue-based genetic and epigenetic alterations, noncoding RNAs, and proteins. We will also discuss the non-invasive biomarker with the ability to monitor real-time tumor dynamics, such as circulating tumor cells (CTCs) and cell-free nucleic acids (cfNAs): DNA, microRNA and long ncRNAs (lncRNAs).

Keywords: Gastric cancer (GC); prognostic; biomarkers

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Introduction

Gastric cancer (GC) still remains one of the leading challenges in the oncologic research area due to its frequent occurrence as well as its poor prognosis. The incidence of GC has decreased in most parts of the world, with estimated 952,000 new cases in 2012 (1). However, the drop in the number of cases of GC is not coupled with a decrease in the mortality rate. GC ranks third in terms of cancer-related death worldwide (2,3). Substantial geographic variation exists in incidence and mortality, as more than 50% of GCs arise in Japan and other East Asian countries, including Korea and China (4,5). Japan owned superior 5-year survival rates of approximately 60% and the percentage of early gastric cancer (EGC) cases reported in Japan is higher (more than 50%) compared with other areas, where screening programs have not been implemented because of cost ineffectiveness (6-8). In Western countries and China, the majority of patients is always diagnosed at an advanced, unresectable stage and tends to have disease relapse within 5 years after initial curative-intent surgery. As a result, the 5-year survival rate of locally advanced disease in these areas is only about 25% (1,9). The average time between EGC diagnosis and progression is about 37 months (10),

and it commonly takes 8 months for EGC to progress into advanced gastric cancer (AGC). Once a patient presented classic symptoms of weight loss, consistent and dull pain in the epigastrium, loss of appetite, nausea, vomiting and chronic bleeding, he or she might already be at a later stage rather than the first stage of disease progression (11). The median survival of patients with late stage GC who do not receive chemotherapy is less than 6 months (12).

To achieve better survival results, currently recommended management of AGC is a standardized multidisciplinary approach, which involves surgery, neoadjuvant and adjuvant chemotherapies, chemoradiation as well as targeted therapy. Depending on the site and extent of cancer, surgery is still the unique potentially curative treatment for AGC. Tumor staging has been conceived and validated as the best predictor of patient survival. The TNM classification is the most important tool for making therapeutic plan in oncology and for predicting the patient's prognosis. Nevertheless, even the latest edition of the TNM classification has limited power to fully reflect the complicated progression events because of the heterogeneous clinical behavior of GC (13). Prognosis varies from individual to individual even though the two patient stay in a similar tumor stage, therefore disease staging alone is not able to accurately predict the outcome for individual patients. Another well-recognized classification system is Lauren's classification, subdividing GC as two histomorphologic subtypes—"intestinal-differentiated" and "diffuse-undifferentiated" (14,15). Case comes the same; Lauren's classification also fails to accurately guide patient therapy, especially when dealing with the molecular heterogeneity of GC (16,17).

Figuring out the molecular biology of the individual tumor might be the key point to better understand the nature of GC and to improve the prognosis of GC patients. The introduction of targeted therapies in molecularly selected populations offers a real opportunity for better outcomes. The identification of various tumor biomarkers has added to our basic knowledge of molecular and cellular mechanisms of GC tumorigenesis and progression. The National Cancer Institute defines a biomarker as "a biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease" (18).

Throughout the whole process of carcinogenesis, there are several opportunities to identify cancer biomarkers. These include assessment of genetic mutations and epigenetic modifications, altered gene expression, protein production, and metabolites; and changes in molecular pathways that control the hallmarks of cancer (19,20). With the advent of complete human genome sequence era, and advancement in key technologies such as high-throughput DNA sequencing, microarrays, and mass spectrometry, the potential tumor biomarkers has expanded dramatically to include the sequence and expression levels of DNA, RNA, and protein as well as metabolites (21,22). The majority of tumor markers are effective prognostic tools that might be used to identify groups of patients at risk of relapse or metastasis or to monitor cancer survivors following treatment. In this review, we will outline the currently available and developing tumor markers associating with the advancement of GC, focusing on markers with potential prognostic significance.

Tissue-based prognostic biomarkers

Currently well-known tissue-based biomarkers

Vascular endothelial growth factor (VEGF)

The recruitment of new blood vessels for the supply of the growing tumor with nutrients and oxygen is essential for tumor growth, development, and distal metastasis (23). Angiogenesis is the process by which blood vessels sprout in an uncontrolled manner from pre-existing vasculature and the VEGF family participated in continually encouraging angiogenesis in neoplasms included five members VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor (PGF) (24). In addition, VEGF and its downstream effectors are ubiquitously over-expressed in various types of tumors including GC (25,26). It has been reported that VEGF is more frequently dysregulated in intestinal type than in diffuse type GC (36% and 16%, respectively) (25). A prospective biomarker analysis showed that higher levels of VEGF-A are associated with worse survival in GC (27,28). Furthermore, low expression of a co-receptor for VEGF, neuropilin-1 is found to be associated with poor prognosis (28,29). Meanwhile, VEGF and its receptors (VEGFRs) acted as predictive biomarkers in GC as well. Bevacizumab, a humanized monoclonal antibody against VEGF-A, has been shown promising results on GC progression in some phase II studies (30,31). However, the results of phase III studies were disappointing. The phase III randomized controlled Avastin in Gastric Cancer (AVAGAST) trial studied the effect on overall survival (OS) when bevacizumab was added to capecitabine and cisplatin in the first-line setting for patients with AGC. Although the overall median survival was slightly longer in patients given

bevacizumab plus standard chemotherapy, these results did not reach a statistically significant level (12.1 and 10.1 mo, P=0.1002) (27,28). Therefore, bevacizumab currently is not recommended in the treatment of AGC at present.

Epidermal growth factor receptor (EGFR)

EGFR is over-expressed in 60% to 70% of GC cases, and there is evidence that EGFR overexpression in GC is associated with poor survival and poor response to chemotherapy (32,33). Contrarily, there are also studies demonstrating that EGFR expression may promise good prognosis or have no prognostic significance at all (34). Considering its central role in signaling pathways and its biological functions, EGFR has also been investigated as a predictive biomarker in GC. cetuximab, an EGFR-targeted monoclonal antibody, functioned well in stage 4 colorectal cancer (with k-ras wild type) and in several head and neck malignancies (35,36). Nevertheless, the outcome of the phase III Erbitux (cetuximab) in Combination With Xeloda (capecitabine) and Cisplatin in Advanced Esophagogastric Cancer (EXPAND) trial was disappointing, because the addition of cetuximab to chemotherapy did not significantly improve the primary endpoint of progression-free survival (PFS). The explorers also made retrospective assessment of EGFR immunohistochemistry (IHC) score, coming to a conclusion that there was no relationship between EGFR IHC score and PFS or OS in either treatment group (37). According to these results, the prognostic role of EGFR is considered unclear and that EGFR seems not to be a predictive biomarker for GC.

Human epidermal growth factor receptor 2 protein (HER2)

The HER2 protein is a member of the EGFR family and its receptors localize in the nucleus, where they function as transcription factors for cyclin D1 and p53 (38). Therefore, HER2 is involved in the regulation of cell proliferation, differentiation, motility and apoptosis (39). Depending on tumor location and subtype, HER2 overexpression or gene amplification ranges from approximately 10% to 30% in GC (36,40,41). HER2 amplification is more often found in intestinal-type tumors and in tumors located in gastroesophageal junction (GEJ) (40,41). In the Trastuzumab for Gastric Cancer (ToGA) trial, 22.1% of the patients enrolled in were HER2 positive. Moreover, tumors in GEJ presented higher rates of HER2 positivity (33.2%) than tumors located in gastric (20.9%). The rates of HER2 positivity in the intestinal subtype GC are 32.4% while those in the diffuse or mixed type GC are 6.1% (42). Various evidences show that HER2 overexpression or amplification is associated with worse prognosis (40,43,44). Although other studies have suggested that HER2 expression may be of no prognostic significance at all (45,46). A recent study attempted to clarify whether HER2 is a prognostic marker in Western patients with metastatic GC. Using currently recommended guidelines, this study determined that HER2 status alone is not an independent prognostic marker (47). Although HER2 is not associated with an adverse prognosis of GC the same as it is in breast cancer, inhibition of the HER2 pathway in patients with tumors overexpressing HER2 has definite clinical benefits. The phase III multicenter, international ToGA trial is well-known as a milestone, as it established trastuzumab as the first biological therapy to confer survival benefits upon patients with GC (48).

Mesenchymal-epithelial transition factor receptor (MET)

MET is the receptor of hepatocellular growth factor (HGF) and play a central role in the process of embryonic development, wound healing and organ regeneration (49). Activated MET undergoes dimerization and phosphorylation, resulting in the activation of multiple pathways, including the phosphatidylinositol-3 kinase (PI3K)-protein kinase B (AKT) pathway and the RAS-mitogen activated kinase pathway. MET may also has cross talk with the EGFR and VEGFR pathways (50,51). It is known that MET kinase mutation or aberrant activation of MET is associated with renal cell carcinomas, and more recently, abnormalities in MET have been implicated in the pathogenesis of other solid tumors, including GC (52). The MET protein is over-expressed in up to 50% of AGC, and the MET gene is amplified in up to 20% of GC (53). MET amplification or over-expression is a poor prognostic marker and is associated with more aggressive disease (53,54). MET overexpression has been demonstrated to be a predictive marker. A phase III trial of rilotumumab, a fully human monoclonal antibody targeting HGF, in addition to chemotherapy is pending.

Other protein markers in the preclinical setting

Except for above-mentioned markers, which have been already in clinical practice, various forthcoming protein biomarkers discovered from microarray and proteomics research have already been evaluated for a relatively long time. Mammalian target of rapamycin (mTOR) is a

protein kinase that participated in regulating cell growth and survival (55). Phosphorylated mTOR expression is considered as a poor prognostic marker because it has been associated with worse disease-free survival (56). Protein p27 inhibits progression from gap 1 to the synthesis phase of the cell cycle. A retrospective study shows that GC tumors that are negative for p27 have been associated with higher rates of lymph node metastasis, a higher proliferative index, and worse OS, indicating that p27 negativity is a potential poor prognostic marker (57). There is a suggestion that metallothioneins are intracellular proteins involved in cell proliferation and apoptosis (58). Low levels of metallothionein 2A have been associated with worse clinical outcomes (59). CD44 and CD133 are both markers of cancer stem cells and are potential poor prognostic markers in GC (60-62). Conversely, patients with higher levels of the proapoptotic protein p53 and low levels of the antiapoptotic protein Bcl-2 may have a better prognosis (63).

Emerging tissue—based biomarkers in gastric cancer (GC)

Genetic biomarkers

Over the last few decades, cancer genomics have been extensively used in biomedical research. Microarray and next generation sequencing has become a very powerful tool for discovering novel tumor biomarkers and treatment target. The use of high throughput whole genome sequencing has brought an improved appreciation of common genetic alterations in GC. With the respect of prognosis, GC gene expression profiling is able to predict which GC patients have good or poor clinical outcomes, thus classifying tumors into intrinsic subtypes and predict the survival of GC patients (64). Several studies show that gene expression techniques can help to predict the risk of recurrence and thus can potentially improve clinical outcome of GC (65,66). Yamada *et al.* identify a 98-gene signature by analyzing 40 GC samples obtained from endoscopic biopsy, showing that the 98-gene signature are significantly correlated with the OS. In addition, PDCD6 was proved to be a prognostic biomarker of GC through a multivariate analysis (67). Lo Nigro *et al.* compared gene expression profiling of three long-term survival cases with that of four normal cases, identifying an 8-gene signature to distinguish long survivors from the control cases (68). Wang *et al.* collected 158 GC patients, among which 33 cases were used as a training set and 125 cases for RT-PCR as a testing set. As a result, 5-gene signature was established for clinical and prognostic (69). Based on the whole genome expression profiling, we also found and validated a 10-gene prognostic marker for OS prognosis of GC patients, which may be used with the TNM staging system as a parallel and complementary approach (70).

Loss of heterozygosity as well as mutations within several proto-oncogenes can lead to microsatellite instability (MSI) (71). Although detection of MSI in tumor tissue samples seems a little complex because it requires a comparison with normal tissue, but it presents a valuable tool for early detection and can also be used for evaluation for prognosis and chemotherapeutic response (71,72). Accumulating evidence suggests that MSI is associated with increased patient survival and a favorable prognosis; accordingly MSI status may be a useful prognostic and predictive marker in GC (73,74).

Epigenetic biomarkers

Cancer is now being recognized mostly an "epigenetic" disease rather than a "genetic" disease, which was once believed to be. The primary processes responsible for epigenetic regulation include DNA methylation, histone modifications and posttranscriptional gene regulation through non-coding RNAs including microRNAs and long ncRNAs (lncRNAs) etc. (75).

DNA methylation

Aberrant DNA methylation in the promoter regions of gene is the most well defined epigenetic hallmark in GC. In general, cancer cells exhibit hypermethylation of the CpG islands of some genes, such as *BRCA1*, *VHL*, *MLH1* and *CDKN2A* (76,77). In contrast, cancer cells exhibit global hypomethylation at many genomic sequences, which can result in chromosomal instability as well as activation of proto-oncogenes (78).

Hypermethylation of CpG islands can lead to silence of neighboring genes and inactivation of tumor suppressor genes in the absence of changes to the genetic sequence of these genes. For instance, promoter methylation of *hMLH1*, a gene encoding a mismatch repair enzyme, has been involved in the development of GC and is closely associated with poor prognosis of GC patients (79). Tumor suppressor gene *p16* is an inhibitor of cyclin-dependent kinase 4 (CDK4) and 6 (CDK6), which bind cyclin D1 and phosphorylate the *retinoblastoma protein* (*Rb*) tumor suppressor genes. It is reported that *p16* methylation affected the overall prognosis in GC regardless whether the patients stay at early-stage or late-stage of the disease (79-81). E-cadherin protein encoded by *CDH1* gene localized mainly to the adherents' junctions of epithelial cells (82).

Loss of E-cadherin is well known for promoting tumor progression through increased proliferation, invasion, and metastasis. *CDH1* is frequently methylated in primary GC, particularly in the poorly differentiated GC and diffuse histotype (83). *CDH1* methylation is believed to be associated with poor prognosis of GC patients (84). The suppressor of cytokine signaling (SOCS)-1 is a protein involved in a negative feedback loop for cytokine signaling, especially the JAK/STAT pathway (85). It has been suggested that SOCS-1 methylation is significantly associated with lymph node metastasis and advanced tumor stage in GC (86). Other well-defined gene methylation that are associated with poor prognosis in GC include: *MGMT* (79,81), *HoxD10* (87), *HAI-2/SPINT2* (88), *DAPK* (89,90), *BNIP3* (89), *RASSF1A* (91), *RAR-β* (91), *Dkk-3* (92) etc.

Several genes involved in tumorigenesis and progression of GC have been found to be hypomethylated, which leads to silence of target genes. For instance, Kwon *et al.* demonstrated that the promoter of *ASCL2*, which encodes a basic helix-loop-helix transcription factor, shows hypomethylation in GC tissues and high expression levels of this gene are correlated with poor survival of GC patients (93).

miRNAs

The miRNAs are noncoding single-stranded RNA molecules that post-transcriptional regulate the expression of targeted mRNAs and therefore act as major regulators of gene expression (94). miRNAs play a central role in cellular differentiation, development, proliferation and apoptosis. In cancer, all of the above-mentioned processes are deregulated due to altered expression of miRNAs, indicating that miRNAs are involved in carcinogenesis and progression of cancer (95).

Using miRNA microarray technology, it has been discovered that thousands of miRNAs are dysregulated in GC. Many of these miRNAs function as tumor promoters or suppressors in GC cells. Besides their potential as diagnostic markers, the expression levels of specific miRNAs can also be used as prognostic markers. Several miRNA expression changes seem to promise poor prognosis. For example, the down-regulation of miR-451 is associated with poor prognosis and the over-expression of miR-451 increased tumor sensitivity to radiotherapy (96). In a very nearly study, miR-630 expression was examined in 236 GC and adjacent normal tissues. Statistic data suggested that miR-630 was elevated in GC tissues and that increased expression of miR-630 was significantly associated with depth of the tumor, lymph node metastasis, distant metastasis and poor OS. These results indicated that miR-630 might serve as a potential marker for the initiation and progression of GC (97). MiRNAs can be combined with other proteins and thus constitute a comprehensive complex marker. An example of this is miR-200c, which combines with GDF15, and is indicative of poor outcomes in GC patients (98).

Long ncRNAs (lncRNAs)

Besides the relatively well-described miRNAs, accumulating knowledge of the non-coding transcriptome has revealed that the genome is also replete with lncRNAs. LncRNAs, once thought to be junk in cells, have attracted increasing attention in various biomedicine fields. However, lncRNA research is still a young field and up to now only a small number of lncRNAs have been partially characterized, some of which have association with GC prognosis. lncRNA HOTAIR functions as a competing endogenous RNA to regulate HER2 expression by sponging miR-331-3p. Expression of HOTAIR plays a role in invasion and the epithelial-mesenchymal transition of GC cells and overexpression of HOTAIR is characteristic of poor prognosis in GC (99,100). Hypoxia inducible factor 1 alpha antisense RNA-2 (HIF1A-AS2) is an antisense long noncoding RNA, which is a natural antisense transcript of HIF-1a. Chen *et al.* reported that upregulation of HIF1A-AS2 was found in GC tissues and significantly correlated with tumor depth, lymph node metastasis, advanced stage and poor prognosis (101). Gastric adenocarcinoma predictive long intergenic noncoding RNA (GAPLINC) enhanced the cell migration and proliferation abilities of GC cells, and overexpression of GAPLINC in GC tissues had a significantly worse prognosis (102). Unlike miRNAs regulate protein-coding RNAs via direct binding, lncRNAs work through guiding chromatin modifiers to the target genes. Studies on the role of lncRNAs in GC have only recently begun, and we are just starting to understand their functions. Nonetheless, this limited evidence still touts the promise that lncRNAs may also have the potential to become biomarkers for diagnosis and prognosis in cancer patients. It is undoubtedly that further studies on noncoding RNAs will reveal a new paradigm in the field of GC research.

Novel protein biomarkers

Paired tissues of gastric tumors and adjacent normal tissues obtained after surgical resection are usually analyzed by 2-DE combined with MALDI or LC-MS/MS analysis to discriminate tumor from normal tissues or early stages from

advanced stages of GC (103). Potential prognostic biomarkers for GC have also been identified using proteomic technology. S100P and S100A6 are calcium-binding proteins. Researches demonstrated that the down regulation of S100P in GC patients is associated with poor outcomes. Moreover, S100A6 was found to interact with annexin A2 and p53 to regulate tumor progression and metastasis (104,105). Our previous study showed that the high expression of S100A6 was associated with tumor local invasion, lymph node metastasis, cancer embolus, distant metastasis and TNM stages ($P<0.05$) (106). Other studies showed that CLIC1 (107), DDX39 (108), 14-3-3β (109), CRIP1, HNP-1, were biomarkers for poor prognosis as well.

Blood biomarkers

Classic serum-based tumor markers

In clinical, the most used tumor markers for GC are carcinoembryonic antigen (CEA), CA19-9, CA72-4. The main limitations of their use are limited organ specificity as well as low sensitivity, which prevent early cancer diagnosis. Several researches have explored the association between these markers and prognosis.

CEA is a glycoprotein attached to the surface of enterocytes and plays a role in programmed cell death and cell adhesion (110). High pre-therapeutic levels of CEA are correlated with the stage of the disease, especially in patients with peritoneal serous carcinoma (111). Normal pre-therapeutic levels promise better survival, in particular in patients receiving perioperative chemotherapy (112). In the case of liver metastasis relapse, the CEA level may increase about 3 months before the radiological diagnosis of the disease (113). Normal postoperative levels for a period within 2 months also correlate with a better OS (114). An increase in its level generally indicates relapse, at least at peritoneal level. Yet, it is less sensitive for other sites of cancer metastasis (115,116).

CA 19-9 protein plays a role in cell adhesion and statistically correlated with lymph node involvement (117), but did not contribute as much as CEA in the identification of operable patients (118). CA 19-9 may be an independent predictive factor for metastatic or recurrent patients and possibly for those undergoing curative surgery as well (119).

CA 72-4 is a glycoprotein found on the surface of tumor cells. In recent years, CA72-4 has attracted more and more attention. Several literatures reported that CA72-4 is the most sensitive and the most specific marker in GC. Furthermore, CA72-4 expression is associated with disease progression, lymph node metastasis and distant metastasis (120,121). It appears that the surge in serum levels best correlates with the stage of lymph node involvement (N category), whereas peritoneal fluid levels correlate with both the N stage and T stage (120,122). Our previous study confirmed that, CA72-4 positive expression has correlation with vascular invasion and III, IV stage. Multivariate Analysis showed that CA72-4 was an independent prognostic factor ($P=0.012$), which is consistent with others' results (122,123).

Innovative blood-based tumor markers

Prognosis for patients with AGC is poor. Early detection plays a key role in reducing the morbidity and mortality. Increasing patient adherence to GC screening will increase the rate of tumors detected at earlier, more treatable disease stages. This urgent need and technological advances resulted in the advent of noninvasive, blood-based GC biomarkers.

Cell-free nucleic acids (cfNAs)

Tumor cell necrosis, apoptosis, and possibly secretion will lead to the release of DNA, RNA, and noncoding RNAs into circulation in cancer patients. Alterations in the concentration and detection of tumor-specific changes in DNA and/or dysregulated RNA expression profiles have been proposed as biomarkers. The development of diagnostic methods based on the detection of cfNAs in circulating is really attractive because they can provide valuable molecular information about the tumor that may be used for diagnostic, predictive, and prognostic purposes (124).

Genetic and epigenetic alterations in circulation

Higher levels of cell-free DNA (cfDNA) in plasma or serum are generally found in cancer patients. To date, there are several reports regarding circulating cfDNA in GC patients. Among those reports, studies with respect to the concentration of circulating cfDNA are rare. In contrast, the detection of genetic and epigenetic alterations in plasma/serum appears to be the most widely used approach. Several studies have used qPCR to quantify the copy number of genes known to be amplified in GC tissues, such as MYC and HER2, in cell-free plasma from GC patients (125,126). An increased MYC/GAPDH ratio in plasma significantly correlated with that in the GC tissues and could distinguish between GC patients and healthy controls (125). However, they might prove to be highly relevant for detecting the

presence or loss of therapeutic targets, and for monitoring treatment efficacy and the course of the disease.

Several other studies have explored the possibility of detecting cancer-associated hypermethylated DNA fragments in the cfDNA of cancer patients. Methylation markers in the bloodstream were first discovered in breast and lung cancer patients in 1999 (127,128). In 2002, the potent application of detecting methylated DNA of death-associated protein-kinase, GSTP1, E-cadherin, p15 and p16 in the serum of GC patients was firstly reported (129). Thereafter, accumulated evidence in this field sprang out. Researchers demonstrated that methylation of several genes could be easily detected in blood circulation; and that this methylation in circulating nucleic acids significantly correlated with methylation levels of these genes in GC tissues and showed diagnostic and prognostic value (129-135). Most recently, Ling *et al.* clearly demonstrated the potent usefulness of detecting methylated XAF1 DNA as a diagnostic as well as prognostic biomarker (136). RUNX3, which plays important roles in both normal developmental processes and carcinogenesis, is frequently inactivated by methylation-induced silencing (137). Hypermethylation of RUNX3 was detected in 45.2% GC patients (138). The quantification of serum RUNX3 methylation can be used as a biomarker for postoperative monitoring of tumor recurrence in these patients (139).

Noncoding RNAs in circulation

Since the discovery of miRNAs and the association of particular miRNAs with GC, intense research efforts have focused on the identification of tumor specific miRNA transcripts as potential blood biomarkers. Evaluation of new miRNA candidate markers, both individually and in panels, in large independent studies is necessary to determine if miRNA markers can be implemented as screening, diagnostic, and/or prognostic tools in the future.

Several groups reported the successful detection of circulating miRNAs and their significance in malignant diseases. It was reported that the miR-200c blood expression levels in GC patients were significantly higher than in normal controls. There was a correlation (P=0.016) with the number of lymph node metastases and the increased expression levels of miR-200c in blood were significantly associated with a poor OS (median OS, 9 *vs.* 24 months; P=0.016) and PFS (median PFS, 4 *vs.* 11 months; P=0.044). Multivariate analyses confirmed that the upregulation of miR-200c in the blood was associated with OS (HR=2.24; P=0.028) and PFS (HR=2.27; P=0.028), independent of clinical covariates (140). MiR-199a-3p is expressed at very high levels in the plasma of GC patients. Of these, miR-199a-3p was significantly associated with lymph node metastasis and progression of primary tumor (T), regional nodes (N), and metastasis (M) (TNM) staging (141). Recently, Li *et al.* investigated the expression level of miR-25 in plasma and GC tissues and found that overexpression of miR-25 in patients was associated with lymph node metastasis. Furthermore, patients with high plasma expression of miR-25 had poor prognoses (142). MiR-25 facilitates GC progression through repression of transducer of ERBB2, 1 and may represent a noninvasive biomarker for GC.

Circulating cfRNA, in particular miRNA, has been found to be remarkably resistant to endogenous and exogenous RNase activity, extreme pH conditions and freeze-thaw cycles. This could be explained by some protective mechanisms, which involve packaging in secretory particles (apoptotic bodies, exosomes etc.) (143). Cancer-derived extracellular vesicles (EV) are considered to be a liquid tumor biopsy because they are found in elevated levels in the circulation and they have been shown to carry cancer cell-derived lipids, proteins, mRNAs, non-coding and structural RNAs and even genomic DNA, which at least partially reflect parental cells and represents attractive shuttles for cancer biomarkers (144). However, there is little data on circulating GC EVs. Considering the current advances in this field, further studies on EVs released in patients with GC are warranted.

Several recent studies reported that lncRNAs whose expression is deregulated in GC tissues can also be reflected in patients' blood (145-148), and thus, they may represent a novel source for circulating biomarker discovery. However, a deeper understanding in their biology, mode of action and mechanism of release into the circulation is required to evaluate their clinical significance. Typically, these non-coding RNAs promote high tumor aggressiveness, and lead to a poor prognosis. In a very newly study, Dong *et al.* identified a GC-associated 3-lncRNA signature (CUDR, LSINCT-5 and PTENP1) in serum. After examining the correlation between the expression levels of the 3-lncRNA in circulation and clinical parameters, they came to a conclusion that GC patients with low value of expression level of the panel of 3 lncRNAs showed higher survival rate than those with high value (149).

Circulating tumor cells (CTCs)

The presence of CTCs in blood is associated with tumor progression or metastasis. With the development of

detection techniques, not only was it shown that presence of CTC in blood correlates with disease state, but also that patients with low CTCs have more favorable median PFS and OS rates when compared to patients with elevated CTCs levels (150,151). In 1869, Ashworth reported the discovery of CTCs for the first time, demonstrating that there existed tumor cells in the blood similar to those in the primary tumors (152). Thereafter, a series of studies have demonstrated the identification and characterization of CTCs in peripheral blood of patients with various malignancies, validating Ashworth's previous remarks. In 2004, prognostic significance of CTCs in breast cancer was first reported by Cristofanilli *et al.* in a multicenter prospective analysis (153). A recent large multi-center analysis in Europe confirmed an independent prognostic value of CTCs in breast cancer patients with regard to PFS and OS with level-one evidence (154).

To date, many researchers have tried to detect CTCs in patients with GC and demonstrated its biological and clinical significance. Since its introduction, RT-PCR technology has become the most widely used approach detect CTCs whose concentration are extremely low in the bloodstream. Accumulating reports have suggested the significance of CTC detection as a prognostic indicator. Yie *et al.* examined survivin-expressing CTCs in peripheral blood, showing that the presence of survivin-expressing CTCs was significantly associated with the degree of tumor penetration, nodal status and disease stages. They concluded that the detection of CTCs expressing survivin mRNA could be used to accurately identify GC patients with high risks of relapse (155). Mimori *et al.* checked one candidate marker, membrane type 1 matrix metalloproteinase (MT1-MMP) mRNA levels, in a qRT-PCR based study involving more than 800 GC patients. As a result, MT1-MMP-expressing CTCs in peripheral blood were indicated to be an independent factor for determining recurrence and distant metastasis of GC (P=0.0018) (156).

One of the advantages that detection of CTCs may have is that it may be used to monitor advanced stage disease in patients who do not have measurable levels of other surveillance markers in blood, such as CEA. However, the research on this type of "liquid biopsy" for gastric detection remains in its infancy, and future research will undoubtedly shed light on clinical applications for CTCs in gastric.

Conclusions

On 21st January 2015, US president Barack Obama called for a Precision Medicine Initiative that select cancer as one of its immediate targets. Subsequently, in March 2015, China announced their Precision Medicine Initiative in search for prediction of hereditary diseases in newborns, investigations on antibiotics resistance, preventive measures development and personalized cancer therapy. Nowadays, precision medicine already took center stage in the world's healthcare goals. The cancer precision medicine will leverage advances in biotechnologies, such as next generation sequencing, proteomics, transcriptome, and epigenetics, to identify precise causes for cancers and develop tailor-fit personalized therapies. As for personalized management of GC, it is now widely appreciated that genomic complexity and heterogeneity determined clinical outcomes. Because many of the current strategies are generally only applicable to a limited number of patients, further improvement in the outcome of AGC patients will depend on the identification of biomarkers in different patient populations. Advances in genomics, transcriptomics and proteomics have already identified large amounts of candidates' biomarkers for GC. In addition, high throughput sequencing technology will undoubtedly accelerate the research in this respect. Also, intense efforts aiming at identifying molecular markers (DNA, RNA or protein) to develop novel, noninvasive biomarker for GC in circulation are underway. Many of these molecular markers have potential to predict prognosis and promise to shift the field to a more individualized approach to GC treatment. Future studies are warranted to settle the controversy surrounding the prognostic values of some of the currently used and newly proposed molecular biomarkers.

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Footnote

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