Molecular Biomarkers in Gastric Cancer

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Abstract

Gastric cancer (GC) represents a serious health problem on a global scale. Despite some recent advances in the field, the prognosis in metastatic GC remains poor. Even in localized disease the adjunctive therapies improve overall survival (OS) by only approximately 10%. A better understanding of molecular biology, which would lead to improved treatment options, is needed and is the basis for this review. Many potential biomarkers of prognostic significance have been identified, including ALDH, SHH, Sox9, HER2, EGFR, VEGF, Hippo/YAP, and MET. However, inhibition of only HER2 protein has led to a modest survival benefit. A new approach to GC treatment, which is a disease influenced by inflammation, is the exploitation of the immune system to fight disease. Two interesting targets/prognostic markers that bear further investigation in GC are PD1 and PDL, particularly given their success in the treatment of other inflammation/immune-associated malignancies. (J Natl Compr Canc Netw 2015;13:e19–e29)

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Gastric cancer (GC) represents a serious health problem on a global scale. It is the second leading cause of cancer-related death worldwide.[1] In the United States, GC is relatively less frequent, with 22,220 new cases and 13,730 cancer deaths occurring in 2014.[2] Between 2002 and 2008, the 5-year relative survival rate was only 27% according to the SEER database.[3]

Despite some recent advances in the field, the prognosis in metastatic GC remains poor. Even in localized disease, the adjunctive therapies improve overall survival (OS) by only approximately 10%.[4–6] Patients often succumb because of the complications of metastases rather than from the primary tumor. A better understanding of molecular biology, which is likely to lead to improved treatment options, is needed and is the basis for this review.

Currently, only 2 molecular subtypes have been identified that are relevant in the clinic. This is based on the overexpression of HER2 protein and/or the amplification of its gene *ERBB2*, thus allowing some advantage from the use of trastuzumab in the first-line setting.[7] This article focuses on the multitude of novel molecular markers with prognostic implications in GC. Table 1 highlights molecular biomarker differences between intestinal and diffuse-type GC; for more detailed discussion, refer to the article by Wadhwa et al.[8]

Potential Novel Prognostic Biomarkers in GC

Aldehyde Dehydrogenase 1

Aldehyde dehydrogenases (ALDHs) are generally regarded as detoxification enzymes that are critical for protecting organisms against various aldehydes that could otherwise be harmful.[9–11] The deficiencies and polymorphisms in various ALDH enzymes can lead

Table 1 Molecular Biomarker Differences Between Intestinal- and Diffuse-Type Gastric Cancer

Biomarker	Role	Genetic Alterations
HER2 (*ERBB2*)	Oncogene	Amplified or overexpressed[124] in intestinal-type gastric cancer
EGFR	Cell migration, adhesion, proliferation	Amplified or overexpressed[125] and activating mutation[126] in intestinal-type gastric cancer
VEGFA	Angiogenesis	Amplified or overexpressed[127] in intestinal-type gastric cancer
NOTCH1	Proliferation	Amplified or overexpressed in intestinal-type gastric cancer
p-mTOR	Cell growth, differentiation, and survival	Amplified or overexpressed in both intestinal- and diffuse-type gastric cancer[97,99]
MMP1, MMP7		Amplified or overexpressed in both intestinal- and diffuse-type gastric cancer[128]
MET	Proliferation and survival	Amplified or overexpressed and activating mutation in diffuse-type gastric cancer[83,129]
TGFB1	Growth, proliferation, differentiation, apoptosis control	Amplified or overexpressed in diffuse-type gastric cancer[130]
SHH/PTCH1/SMO	Control of cell division	Amplified or overexpressed in diffuse-type gastric cancer[131]
HER3 (*ERBB3*)	Control survival and cell cycle	Amplified or overexpressed in diffuse-type gastric cancer[130]
FGFR2	Motogenesis and differentiation	Amplified or overexpressed in diffuse-type gastric cancer[132]
PIK3CA		Amplified or overexpressed and activating mutation in diffuse-type gastric cancer[133]
CDH1	Tumor suppressor, cell-cell interaction	Loss of expression in intestinal-type and loss of function mutation and loss of expression in diffuse-type gastric cancer[134,135]
TP53	Tumor suppressor	Loss of function mutation and loss of expression in intestinal- and diffuse-type gastric cancer[97,99,130]
PTEN	Tumor suppressor	Loss of function mutation and loss of expression in intestinal- and diffuse-gastric cancer[136,137]
ALDH	Stem cell marker, detoxifying enzyme	NR
SOX9	Development and lineage commitment	NR

Abbreviation: NR, not reported.

to many clinical phenotypes.[9,11] Using esophageal cancer cell lines, our group reported that high ALDH-1 labeling index is associated with therapy resistance, aggressive phenotype, and overexpression of genes conferring resistance (*SHH*, *YAP1*, *Gal-3*, and *Hes-1*).[12] ALDH-1 labeling indexes were predictive of pathologic complete response ($P \leq .001$) and extreme treatment resistance ($P \leq .001$), and prognostic of OS ($P = .03$) and progression-free survival ($P = .006$).[12] A Japanese study[13] showed that $ALDH^{high}$ cells exhibited greater tumorsphere-forming ability in vitro and had greater tumorigenic potential in vivo when compared with $ALDH^{low}$ GC cells. Furthermore, cell cultures treated with 5-FU and cisplatin exhibited higher numbers of $ALDH^{high}$ cells and increased Notch1 and Sonic hedgehog (SHH) expression. In another study,[14] researchers identified a subpopulation of CD44+ cells within the tumor of patients with GC, which, when treated with 5-FU, were markedly enriched. Subcutaneous injections of CD44+ GC cells conferred tumorigenicity in SCID mice. Upon enrichment with 5-FU, CD44+ cells harbored increased ALDH expression compared with CD44– cells. Because ALDH is a stem cell marker, it may help identify a subpopulation of patients whose tumors are more likely to be resistant to therapy, and therefore may lead to novel therapeutic approaches.

SOX9

SOX9, a high-mobility group box transcription factor, is required for development and lineage commitment. SOX9 has been reported to be a direct target for Notch signaling[15] and is a documented stem cell marker.[16] Notch signaling and SOX9 have been implicated in cancer development, and most recently our group has shown that SOX9 also plays an important role in esophageal carcinoma.[17] In our study, the loss of an important tumor growth factor (TGF)-β adaptor β2SP in esophageal adenocarcinoma and MEF cells led to activation of Notch signaling and increased expression of SOX9; high levels of nuclear SOX9 expression were associated with poor survival and adverse disease status (lymph node metastasis) in patients with esophageal adenocarcinoma. Using immunohistochemistry, nuclear SOX9 expression was detected in 64 (34.6%) of 185 GCs; however, the authors found no significant relationship between altered expression of SOX9 protein and clinicopathologic parameters, including OS.[18] The role of SOX9 has also been evaluated in terms of its effects on promoting invasion and metastasis in gastric adenocarcinomas.[19] The authors were able to show that higher SOX9 expression was associated with higher stage ($P<.0005$), higher positive lymph node stage ($P<.0005$), and higher tumor stage ($P<.0005$). These results suggest that SOX9 expression is related to tumor progression in gastric adenocarcinoma. Importantly, SOX9 can serve as a therapeutic target through YAP (Figure 1).

YAP and Hippo Pathway

The Hippo pathway is known to control organ size in multiple species (Figure 1). Two kinase cascades participate in this pathway and are formed by MST1/2 and LATS1/2.[20] The activation of these kinases leads to phosphorylation of the downstream transcriptional coactivator YAP/TAZ, thus preventing its interaction with, and therefore transactivation of, the DNA-binding transcriptional factor TEADs/TEF.[21,22] Inactivation of the Hippo pathway leads to an increased cancer risk through increased proliferation and antiapoptotic effects.[23–25]

YAP is a downstream effector in this pathway, which plays an important role in cell proliferation. It has been mostly reported to be an oncoprotein, and its elevated expression and nuclear localization have been frequently observed in human cancers (ie, lung, liver, colon, pancreatic, and breast cancers).[25–27] YAP does not contain any DNA-binding domains. It functions through interaction with TEADs, resulting in the induction of target gene expression, in turn promoting cell proliferation and inhibiting apoptosis. Similar to YAP, the mammalian Vestigial-like proteins VGLL1 though VGLL4 do not contain a DNA-binding domain, and they also exert their transcriptional regulatory functions through pairing with TEADs via their Tondu (TDU) domains.[28] Recently, VGLL4 in *Drosophila* and humans has been shown to be a transcriptional repressor that inhibits YAP-induced overgrowth and tumorigenesis.[29–31]

Increased YAP mRNA levels in GC samples were previously reported to correlate with lymphatic metastasis and tumor TNM stage.[32] In a more recent study,[33] YAP mRNA levels were found to be associated with not only tumor size, differentiation, and stage, but also *Helicobacter pylori* infectious status ($P<.05$). YAP-positive rates were much higher in GC and dysplasia than in normal tissues ($P<.01$). Consistently, the protein levels of YAP also tend to increase in higher-grade tumors. This same article found that the average ratio of YAP to VGLL4 at mRNA levels in higher-grade tumor samples is significantly higher than in lower-grade tumor samples ($P<.05$). Moreover, the YAP/VGLL4 ratio at mRNA level is strongly correlated with GC clinical factors, including lymphatic invasion, tumor size, TNM stage, and *H pylori* infectious status. Immunohistochemical analysis showed that among patients with YAP-positive GC, those with VGLL4-positive expression have a better clinical outcome (survival time, 39 months) when compared with VGLL4-negative expression (survival time, 16 months).[33] These new results suggest that the YAP/VGLL4 ratio can be used as a prognostic marker in GC. Interestingly, the investigators found that a peptide mimicking this function of VGLL4 potently suppressed tumor growth in vitro and in vivo, which may lead to new therapeutic targets in GC.[33]

SHH

The SHH signaling pathway plays a critical role in stem cell maintenance and specifying patterns of cell growth and differentiation during embryonic development.[34] SHH activates its signaling by binding to the receptor, Patched (Ptch), which relieves Ptch-mediated repression of a downstream membrane protein related to G protein–coupled receptors, Smoothened (Smo). On activation, Smo promotes nuclear translocation of a family of transcription factors (Ci

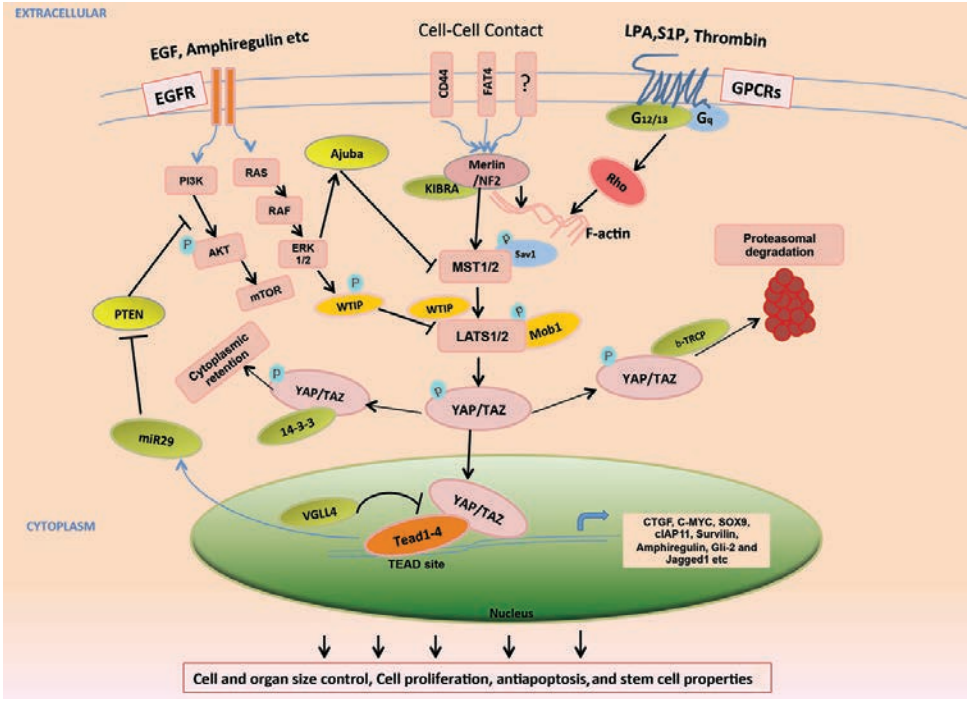

Figure 1 The Hippo/YAP pathway and the crosstalk with epidermal growth factor receptor (EGFR) and G-protein–coupled receptor (GPCR) signaling. Two kinase cascades participate in this pathway and are formed: MST1/2 and LATS1/2. The activation of these kinases leads to phosphorylation of the downstream transcriptional coactivator YAP/TAZ, thus preventing its interaction with and therefore transactivation of the DNA-binding transcriptional factor TEADs/TEF. Inactivation of the Hippo pathway leads to an increased cancer risk through increased proliferation and antiapoptotic effects.
YAP functions through interaction with TEADs, resulting in the induction of target gene expression, in turn promoting cell proliferation and inhibiting apoptosis. Similar to YAP, the mammalian vestigial-like proteins VGLL1 through VGLL4 do not contain DNA-binding domain and they also exert their transcriptional regulatory functions through pairing with TEADs via their TONDU (TDU) domains. YAP/TAZ binding to TEADs leads to the increased expression of amphiregulin, which in turn activates EGFR leading to PI3K/mTOR and Ras/Raf pathway activation. Activation of EGFR and RAS activates the Yorkie homologue YAP, and EGFR-RAS-MAPK signaling promotes phosphorylation of the Ajuba family protein WTIP, and also enhances WTIP binding to the Warts and LATS. The YAP pathway can also be activated through interaction of GPCR and activation of Rho GTPase, leading to increased assembly of F-actin. PTEN is regulated by miR-29, and YAP–TEAD binds to the promoter of mir-29 and promotes its transcription. miR29, through its inhibition of PTEN, inhibits the PI3K/Akt pathway.

in *Drosophila* and Gli [Gli1, Gli2, and Gli3] in vertebrates), and subsequently activates target genes through Gli.[35] In a recent study, GC specimens were classified into 2 groups according to their SHH score using immunohistochemistry.[36] The SHH overexpression group included more patients with early GC compared with the low SHH expression group (25.9% vs 74.1%; *P*=.000). In addition, the survival time of patients with SHH overexpression was significantly prolonged (69.27 ± 1.39 months) compared with that of patients with low SHH expression (61.23 ± 2.04 months; log-rank test, *P*=.03).[36] SHH has also been implicated in radiation resistance. It was shown that the SHH signaling was extensively activated in esophageal cancer cells and residual tumors after chemoradiotherapy, and the temporal kinetics of SHH signaling preceded increases in proliferation biomarker expression and tumor size during tumor regrowth.[37] In a second article, Sims-Mourtada et al[38] showed that the inhibition of SHH signaling increases the response of cancer cells (including esophageal adenocarcinoma, androgen receptor–positive prostate carcinoma, and squamous cell carcinoma) to multiple structurally unrelated chemotherapies. In an immunohistochemical analysis of 178 primary human gastric tumor biopsies, SHH expression was found to be positively correlated with lymph node metastasis, high lymphatic vessel density, and poor prognosis.[39] In mouse xenograft models of human GC, enforced expression of SHH significantly enhanced the incidence of lung metastasis compared with nonexpressing controls. Furthermore, phosphoinositide 3-kinase (PI3K)/Akt inhibition blocked SHH-induced epithelial-mesenchyme transition, the activity of matrix metalloproteinase 9 (MMP-9), and lymphangiogenesis, reducing tumor invasiveness and metastasis.[39]

Prognostic Biomarkers/Molecules Undergoing or Tested in Clinical Trials

Fibroblast Growth Factor Receptor

Fibroblast growth factor (FGF) triggers the autophosphorylation of FGF receptor (FGFR) at a key tyrosine residue in an activation loop of the tyrosine kinase domain, resulting in a structural change of the tyrosine kinase domain from an inactive to an active form.[40] The activated tyrosine kinase domain of FGFR then phosphorylates other tyrosine residues, ultimately leading to catalysis of phosphatidylinositol diphosphate (PIP2) to diacylglycerol (DAG) and inositol triphosphate (IP3).[41] Activated FGFR phosphorylates FGFR substrate 2 (FRS2) to recruit the GRB2 adaptor molecule.[42] FGF signals are transduced to the RAS-MAPK or PI3K/Akt signaling cascades. SRC tyrosine kinases are also activated by FGF signals.[43] FGF-induced RAS-MAPK activation is involved in cellular proliferation, whereas FGF-induced PI3K/Akt activation is involved in cellular survival.[44]

In particular, somatic *FGFR2* mutations have been reported in lung, gastric, and ovarian cancers.[45–47] *FGFR2* amplification has been associated with tumor cell proliferation and survival of GC cell lines.[48] Furthermore, *FGFR2* amplification may correlate with a poor prognosis for patients with GC.[49] In a recent genomic survey of GC using high-resolution single nucleotide polymorphism (SNP) arrays, *FGFR2* copy number gain was found in 9.3% of tumors and was more common than *EGFR* (7.7%), *HER2* (7.2%), or *MET* (4.3%) copy number gains.[50] Another large screening study, including Caucasian (n=408) and Korean (n=356) GC samples,[51] found that 5.9% of the samples presented *FGFR2* amplification. The *FGFR2* amplification was significantly associated with lymph node status ($P<.0007$). *FGFR2* amplification was found to be a prognostic biomarker of shorter OS in both cohorts. Jung et al[52] also showed the presence of *FGFR2* amplification in Korean GC samples (4.5%), and its association with a higher TNM stage and a shorter OS by univariate analysis.[52] FGFR is currently a target of interest in the treatment of GC, and FGFR inhibitors have been developed (dovitinib and AZD4547).[50,53] These inhibitors have demonstrated efficacy in GC cell lines and xenograft models in vivo. Whether this will translate into clinical efficacy remains to be seen and is being tested in an ongoing phase II trial comparing AZD4547 with paclitaxel in the second-line setting in patients with *FGFR2* polysomy or amplification (ClinicalTrials.gov identifier: NCT01457846).

HER2

In GC, *ERBB2* amplification or *HER2* overexpression has been reported in 7% to 34% of the tumors,[54–56] particularly in the gastroesophageal junction carcinomas (proximal) and in intestinal-type GC.[54,56,57] The prognostic value of HER2-positivity in advanced GC is, however, a controversial issue. Reports and a meta-analysis have indicated that *ERBB2* amplification is associated with poor prognosis and aggressive disease,[55,56,58–60] whereas other reports show no difference in prognosis when compared with HER2-negative tumors.[61–63] Two recent studies showed that HER2 status is not an independent prognostic biomarker in early gastroesophageal adenocarcinoma.[64,65]

Although the prognostic value of HER2 expression remains in question, a modest survival advantage in patients with HER2-positive tumors treated with trastuzumab has been established.[7,66,67] The Trastuzumab for Gastric Cancer (ToGA) trial (Table 2) randomized 584 patients whose tumors overexpressed HER2 to receive a fluoropyrimidine (5-FU or capecitabine) plus cisplatin with or without trastuzumab.[8] Addition of trastuzumab to chemotherapy increased the OS from 11.1 to 13.8 months (hazard ratio [HR], 0.74; 95% CI, 0.60, 0.91; $P=.0046$). On extended follow-up, the HR of OS for the addition of trastuzumab decreased to 0.80,[68] indicating that, although real, the response to trastuzumab may be short-lived. Based on the results of this trial, the addition of trastuzumab to chemotherapy has become the standard of care in patients whose tumors overexpress HER2. Neither the LoGIC nor the TYTAN trials (in the first- and the second-line settings, respectively) evaluating the use of lapatinib (dual epidermal growth factor receptor [EGFR] and HER2 tyrosine kinase inhibitor) met their primary end points of OS.[57,69]

Currently, 2 ongoing phase III trials are testing the benefit of alternative agents to HER2 in GC. Pertuzumab (JACOB trial), a monoclonal antibody that binds to a different epitope of HER2 than trastuzumab, is being studied,[70] and has already proven to be effective in HER2-positive breast cancer.[71] An ongoing randomized trial is also investigating the predictive value of trastuzumab emtansine (T-DM1),

Table 2 Major Phase III Trials Using Targeted Therapy in Advanced Gastric Cancer

Trial	N	Treatment Arms	HR for OS (*P*)	Survival Comparison
First-line				
Bang et al[7]	584	CX, CF, and trastuzumab vs CX and CF	0.74 (.0046)	OS: 13.8 vs 11.1 mo
Ohtsu et al[91]	774	CF vs CF and bevacizumab	0.87 (.1002)	OS: 10.1 vs 12.1 mo PFS: 5.3 vs 6.7 mo
Lordick et al[78]	904	CX vs CX and cetuximab	1.004 (.9547)	OS: 10.7 vs 9.4 mo
Waddell et al[79]	553	EOC vs mEOC–P	1.37 (.013)	OS: 11.3 vs 8.8 mo
Hecht et al[69]	545	CapeOx and lapatinib vs CapeOx and placebo	0.91 (.35)	OS: 12.2 vs 10.5 mo
Second-line				
Ohtsu et al[100]	656	BSC and placebo vs BSC and everolimus	0.90 (.1244)	OS: 4.3 vs 5.4 mo
Fuchs et al[93]	355	BSC and ramucirumab vs BSC	0.776 (.047)	OS: 5.2 vs 3.8 mo
Wilke et al[94]	665	Paclitaxel and ramucirumab vs ramucirumab	0.807 (.0169)	OS: 9.63 vs 7.36 mo
Bang et al[57]	261	Lapatinib and paclitaxel vs paclitaxel	0.84 (.2088)	OS: 11.0 vs 8.9 mo

Abbreviations: BSC, best supportive care; CapeOx, capecitabine and oxaliplatin; CF, cisplatin and 5-FU; CX, cisplatin and capecitabine; EOC, epirubicin, oxaliplatin, and capecitabine; HR, hazard ratio; mEOC–P, modified-dose EOC plus panitumumab; OS, overall survival; PFS, progression-free survival.

an antibody–drug conjugate incorporating HER2-targeted antitumor properties of trastuzumab with the cytotoxic activity of the microtubule-inhibitory agent DM1 (GATSBY trial; ClinicalTrials.gov identifier: NCT01641939), another strategy proven to be effective in HER2-positive breast cancer.[72]

Epidermal Growth Factor Receptor

EGFR (encoded by *EGFR*) is another member of the HER family that, unlike HER2, is activated by the binding of specific ligands, including EGF and TGF-α. In a study using fluorescence in situ hybridization (FISH) assays, 365 GC samples were analyzed and the incidence of *EGFR* amplification was found to be 4.9%. A second study, using SNP assays, found that 7.7% of GCs harbored *EGFR* amplification.[50] A larger study examined 511 GC samples for EGFR overexpression by immunohistochemistry and FISH and reported that 27.4% of the samples were positive for EGFR protein expression by immunohistochemistry, but only 2.3% were amplified when detected by FISH.[73] EGFR overexpression has been shown to be a prognostic indicator of worse outcome in GC.[74,75] Although EGFR immunohistochemical or FISH positivity has been associated with the presence of lymph node metastasis, higher stage, and poor survival, after multivariate analysis, only EGFR immunohistochemical positivity remained a poor prognostic factor.[73] However, a recent study has also found conflicting data, in that EGFR expression using immunohistochemistry was correlated with improved progression-free survival and OS,[76] whereas a meta-analysis found that EGFR was not an independent predictor for survival in patients with GC.[77]

EGFR inhibitors have been tested in multiple clinical trials; however, the results in GC have been disappointing. The EXPAND trial did not achieve its primary end point, with the median progression-free survival for the capecitabine-cisplatin plus cetuximab arm being 4.4 months compared with 5.6 months for the capecitabine-cisplatin arm (HR, 1.09; 95% CI, 0.92–1.29; *P*=.32).[78] The REAL-3 study was terminated prematurely because a statistically significantly lower OS was noted in patients treated with modified epirubicin/oxaliplatin/capecitabine (EOC) and panitumumab. The final analysis of this study[79] showed a median OS of 11.3 months in patients allocated EOC versus 8.8 months in those allocated EOC and panitumumab (HR, 1.37; 95% CI, 1.07–1.76; *P*=.013). It is possible that these trials were negative because they did not appropriately enrich the patient population likely to benefit from this treatment.

MET (Hepatocyte Growth Factor Receptor)

MET (encoded by *MET*) belongs to the hepatocyte growth factor receptor (HGFR) family. *MET*

amplification and/or overexpression of its protein product has long been implicated in the pathogenesis of GC, with many reports based on gene copy number, RNA expression, and/or protein expression, supporting its role as a poor prognostic factor.[80–84] Nevertheless, the prevalence of *MET* amplification in GC varies widely in the literature, from 0%[85] to 21%.[81] This discrepancy is greatly attributed to the methodology used to detect gene amplification/copy number gain and/or protein expression. Depending on the method used, different *MET* amplification prevalences have been further reported.[80,81,83,84] Nonetheless, in all of these studies, the patients with GC with polysomic and/or amplified *MET* demonstrated poorer disease-free survival and OS compared with the nonpolysomic *MET*. In a small study with crizotinib, 2 of 4 patients with 5 or more *MET* copy number gains had a longer response duration than those with fewer than 5 copies of the gene.[83] Furthermore, rilotumumab (AMG 102), a fully human monoclonal antibody, demonstrated longer OS for patients whose tumors had high total c-MET expression.[86] The RILOMET-1 phase III study (ClinicalTrials.gov identifier: NCT01697072), testing the efficacy of rilotumumab in combination with chemotherapy as a first-line treatment of metastatic gastric and gastroesophageal junction adenocarcinoma, and the MetGastric study (ClinicalTrials.gov identifier: NCT01662869), testing the efficacy of onartuzumab versus placebo combined with chemotherapy in MET-positive metastatic GC, were recently terminated because they did not meet safety/efficacy end points and the protocol-defined futility criteria would likely have been met at the planned interim analysis in March 2015. An interesting study of single-agent AMG 337 (oral MET tyrosine kinase inhibitor) in 90 patients with advanced solid tumors included 13 individuals with MET-amplified gastroesophageal adenocarcinomas. Of these 13 patients, 8 had partial or near-complete responses, indicating that small molecule tyrosine kinase inhibitors still hold some promise in this disease.[87]

Angiogenic Pathway Markers

Angiogenesis, the growth of new blood vessels, is an important aspect of tumorigenesis. It is primarily modulated by the vascular endothelial growth factor A (VEGF-A) and its receptors VEGFRs, particularly types 1 and 2. In GC, expression of VEGF-A and VEGFR was reported in 40% and 36 % of cases, respectively.[74] VEGF-A expression in GC and serum has been associated with poor prognosis, lymph node involvement, and metastasis, in both resectable and advanced disease.[88] Increased VEGF levels were also correlated with decreased OS (P=.009), and in a multivariate analysis were found to be an independent prognostic factor for OS.[89] A recent meta-analysis also confirmed that VEGF-A overexpression indicates a poor prognosis for OS and disease-free survival in patients with GC.[90] The GC trials testing the usefulness of VEGF therapy have been somewhat conflicting. Bevacizumab failed to demonstrate an OS benefit in patients with advanced gastric and gastroesophageal junction adenocarcinoma.[91] A subsequent retrospective biomarker analysis of the AVAGAST trial showed that patients with high baseline plasma VEGF-A levels and with low baseline expression of neuropilin-1 seemed to have an improved OS.[92] It is important to note that neither of these biomarkers has been validated. The recently published REGARD trial demonstrated a marginal improvement in median OS in the second-line setting with the addition of ramucirumab (anti-VEGFR2 antibody).[93] Subsequently, the RAINBOW trial showed that the addition of ramucirumab to paclitaxel significantly prolonged the primary end point of OS from a median of 7.36 to 9.63 months (P=.0169).[94] At the 2015 ASCO Gastrointestinal Cancers Symposium, Tabernero et al[95] presented the findings regarding the exposure/response relationship of ramucirumab in patients from the REGARD and RAINBOW trials. In these 2 trials, longer OS was seen in patients with higher levels of ramucirumab exposure (concentration minimum at the end of dose one). This association also corresponded with progression-free survival. Clearly, more studies are needed to develop an optimized ramucirumab exposure strategy.

PI3K-mTOR Pathway

The phosphatidylinositol-3-kinase (PI3K)/mTOR pathway represents one common final convergence signalling pathway originated by the activation of several receptor tyrosine kinases (RTKs). Activation of the PI3K/mTOR pathway in GC has been demonstrated in preclinical studies,[96,97] and its de-

regulation has been associated with increased lymph node metastasis and decreased survival in patients with GC.[98,99] The GRANITE-1 study testing the efficacy of everolimus did not achieve its primary end point of OS (5.4 months with everolimus and 4.3 months with placebo; HR, 0.90; 95% CI, 0.75–1.08; *P*=.124).[100]

Oncogenic mutations in *PIK3CA* (gene encoding the alpha p110 catalytic subunit of PI3K) have been observed in GC, constitutively activating the PI3KA/mTOR pathway. Studies in GC reported a *PIK3CA* mutation frequency of 5% to 25%.[101–105] One of these studies reported *PIK3CA* amplification in 67% of GCs[104] and an association with poor prognosis, indicating that this is a major mechanism leading to activation of the PI3K/mTOR pathway in GC.

Future Directions

The future of GC treatment, which is a disease influenced by inflammation, likely lies in the exploitation of the immune system to fight disease. This can be achieved through reprogramming T cells, the use of antibody conjugates (such as T-DM1) to target tumor cells more specifically, gene editing, the use of cancer vaccines, and the inhibition of checkpoint receptors.

To this end, the other interesting targets/biomarkers are programmed death ligand (PDL) and programmed death-1 (PD-1). PD-1 is a potent immunoregulatory molecule expressed on activated T and B lymphocytes, natural killer T cells, and monocytes.[106] It can attenuate activation of T cells and suppress cytokine secretion through delivering inhibitory signals, and may also induce immune tolerance to tumor after interaction with its 2 ligands, programmed death-1 ligands (PD-Ls) 1 and 2.[107,108] PD-Ls expression has been reported extensively in various tumors, including esophageal cancer.[109–112] In addition, upregulation of PD-Ls in several carcinomas can contribute to tumor evasion from the host immune system and is correlated with unfavorable clinical prognosis of many tumors, and gastric and esophageal cancer.[112–115] Previous studies have shown that blocking the PD-1/PD-L pathway can result in efficient antitumor T-cell responses and a better control of tumor.[116–118] Given that the immune system plays an important role in GC, these biomarkers are of interest in this disease.

Of 2 recent studies using whole genome and exome sequencing, one identified *TP53*, *CDKN2A*, *SMAD4*, *ARID1A*, *PIK3CA*, and chromatin-modifying factors as driver mutations,[119] whereas the other identified *TP53*, *ARID1A*, *CDH1*, *MUC6*, *CTNNA2*, *GLI3*, and *RNF34* as important.[120] Interestingly, 2 studies found *RHOA* mutations in diffuse-type tumors but not in intestinal-type tumors.[120,121] In one cohort, all of the tumors with *RHOA* mutations were HER2-negative; this finding may be useful in the treatment of patients with HER2-negative tumors for whom no targeted therapies are yet available.[121] Recently, The Cancer Genome Atlas (TCGA) analysis uncovered 4 genotypes of gastroesophageal adenocarcinoma[122]: Epstein-Barr virus (EBV)–containing GC (10%), in which *PIK3CA* alterations are common; tumors showing microsatellite instability (20%); "chromosomally unstable" (50%; most common); and "genomically stable" (20% contain diffuse histology and 30% have *RHOA* signaling alterations). Unfortunately, these discoveries are not sufficient to change treatment strategies and more work is clearly needed.

With the advent of cancer genomics, many new mutations/potential biomarkers will likely be found to play a role in tumorigenesis and to subsequently affect prognosis. However, the sheer complexity of the genome is staggering, and genetic abnormalities do not always translate into functional/protein aberrations. In a recent article, Vogelstein et al[123] proposed dthat the whole idea of eradicating cancer through targeting metastatic disease is impossible. What is known is that carcinogenesis is a process that takes decades to develop, and involves the accumulation of multiple genetic alterations that in turn affect the function of numerous pathways. What should be clear is that more work toward the discovery of "driver mutations" is necessary and that the cure of cancer is unlikely to come from increasing drug development, but rather that early cancer prevention and diagnosis (eg, blood biomarker testing) are likely to be much more fruitful. In patients for whom this is not possible and who develop metastatic disease, the exploration of predictive (rather than prognostic) biomarkers is necessary to better direct care.

Conclusions

Understanding of molecular mechanisms of diseases such as GC, which are prevalent in the East and

Asia, lags behind that of diseases such as breast and colon cancer, which are common in the West. Better algorithms to identify driver mutations are needed, but, more importantly, prevention and the development of novel biomarkers for early diagnosis are the keys to managing GC.

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