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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

REVIEW

Gastric Cancer in the Era of Precision Medicine

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SUMMARY

The following pages provide a summary of current knowledge regarding the genomics of gastric cancer (GC), with a particular emphasis on how new genomic knowledge informs precision medicine and personalized therapies.

Gastric cancer (GC) remains the third most common cause of cancer death worldwide, with limited therapeutic strategies available. With the advent of next-generation sequencing and new preclinical model technologies, our understanding of its pathogenesis and molecular alterations continues to be revolutionized. Recently, the genomic landscape of GC has been delineated. Molecular characterization and novel therapeutic targets of each molecular subtype have been identified. At the same time, patient-derived tumor xenografts and organoids now comprise effective tools for genetic evolution studies, biomarker identification, drug screening, and preclinical evaluation of personalized medicine strategies for GC patients. These advances are making it feasible to integrate clinical, genome-based and phenotype-based diagnostic and therapeutic methods and apply them to individual GC patients in the era of precision medicine. *(Cell Mol Gastroenterol Hepatol 2017;3:348–358; http://dx.doi.org/10.1016/j.jcmgh.2017.02.003)*

Keywords: Gastric Cancer; Cancer Genomics; Molecular Classification; Preclinical Models.

Gastric cancer (GC) is the fifth most common cancer worldwide and the third leading cause of cancer death in developed countries, with 984,000 new cases and 841,000 deaths occurring globally in 2013.[1] The incidence and mortality of GC are declining, in part because of improved *Helicobacter pylori* eradication and cancer screening. However, adenocarcinoma of the gastric cardia is increasing in North America and Europe,[2,3] and the incidence of non-cardia GC among whites aged 25–39 years has increased 1.67-fold in the United States during the past 2 decades.[4] Moreover, most GC cases are diagnosed at advanced stages, with consequent poor outcome; treatment is mostly restricted to cytotoxic chemotherapy. Thus, there is an urgent need to improve our understanding of the pathogenesis of GC and to identify more effective, less toxic therapeutic strategies. GC is multifactorial, with complex host genetic and environmental factors contributing to its development. GC is also highly heterogeneous; it is customarily divided into 2 main histologic subtypes, intestinal and diffuse, which are based on the Lauren classification.[5] However, the use of anti–human epidermal growth factor receptor-2 monoclonal antibody, trastuzumab, and anti–vascular endothelial growth factor receptor-2 monoclonal antibody, ramucirumab, has shifted the previous histopathologic paradigm to incorporate new genetic and molecular features.[6,7] Recently, remarkable advances in next-generation sequencing (NGS) technologies have defined the genomic landscape of GC[8–10]; studies of microRNAs (miRNAs) and long noncoding RNAs (lncRNAs)[11,12] as well as novel preclinical models (such as patient-derived tumor xenografts [PDX] and patient-derived organoids) have largely filled the gap between cancer genetics and phenotype.[13–17] These advances have made it possible to integrate traditional, genome-based and phenotype-based diagnostic and therapeutic methods with application to individual GC patients in the era of precision medicine.

Etiologic Factors in Gastric Carcinogenesis

Environmental Factors

Among clinical risk factors for GC, which include smoking, high-salt diet, high intake of meats, and bile reflux, infection with *H pylori* is a leading factor, especially in distal GC.[18–21] On the basis of improved estimates from prospective studies, 89% of new non-cardia GC cases are attributable to *H pylori* worldwide.[22] *H pylori*–mediated gastric carcinogenesis involves several mechanisms: cytotoxin-associated gene A, vacuolating cytotoxin A–induced chronic inflammation, oxidative damage, genomic instability, and epigenetic changes in gastric epithelial cells.[18,23–26] Interestingly, an

Abbreviations used in this paper: **CIMP, CpG island methylator phenotype; CIN, chromosomally unstable/chromosomal instability; EBV, Epstein-Barr virus; GAPPS, gastric adenocarcinoma and proximal polyposis of the stomach; GC, gastric cancer; GTPase, guanosine triphosphatase; HDGC, hereditary diffuse gastric cancer; hPSC, human pluripotent stem cell; lncRNA, long noncoding RNA; LOH, loss of heterozygosity; miRNA, microRNA; MSI, microsatellite unstable/instability; MSI-H, high microsatellite instability; MSS/EMT, microsatellite stable with epithelial-to-mesenchymal transition features; NGS, next-generation sequencing; PDX, patient-derived tumor xenografts; TCGA, The Cancer Genome Atlas; TGF, transforming growth factor.**

Most current article

2352-345X
http://dx.doi.org/10.1016/j.jcmgh.2017.02.003

inverse relation between *H pylori* infection and the risk of proximal GC has been observed in Western countries.[27]

Epstein-Barr virus (EBV) occurs in 2%–20% of GC, with a worldwide average of 10%.[28] In EBV-associated GC, latent membrane protein 2A activates DNA methyltransferase 1 by inducing phosphorylation of STAT3, thereby causing CpG island hypermethylation of the PTEN promoter.[29] Specific EBV transcripts, including latent genes and viral miRNAs, also have oncogenic properties such as increased cell proliferation and motility, impairment of apoptosis, and increased chemoresistance.[30]

Host Factors

Hereditary cancer syndromes linked to 1%–3% of GC consist of 3 principal syndromes: hereditary diffuse gastric cancer (HDGC), gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS), and familial intestinal GC.[31] Germline mutations in *CDH1*, *CTNNA1,* and other tumor suppressor genes, including *BRCA2*, *STK11,* and *SDHB*, have been identified in HDGC.[32] *CDH1* mutations are prognostic genetic markers in HDGC. GAPPS is characterized by autosomal dominant transmission of fundic gland polyposis restricted to the proximal stomach, without evidence of colorectal or duodenal polyposis or other hereditary gastrointestinal cancer syndromes.[33] GC is also increased in other heritable syndromes, such as Li-Fraumeni syndrome with germline mutation of *TP53*, Peutz-Jeghers syndrome with frameshift mutation in *STK11*, hereditary nonpolyposis colorectal cancer with germline DNA mismatch repair gene mutation, and familial adenomatous polyposis with germline *APC* mutation.[31,34]

From Histologic to Molecular Classification

GC has long been categorized by using histomorphologic classification systems. According to the Lauren classification, GCs are divided into 2 main subtypes, intestinal and diffuse.[5] However, these histologic classifications are not sufficient to reflect the molecular and genetic characteristics of GC or to develop personalized treatment strategies in the era of precision medicine.

Recently, advances in genomic technology and high-throughput analysis have helped reveal the molecular genetic landscape of GC (Figure 1). Several molecular classification systems have been proposed, and distinct molecular subtypes have been identified.[8–10,35–37] In 2014, a landmark study by The Cancer Genome Atlas (TCGA) proposed 4 subtypes: (1) EBV-positive (8.8%), (2) microsatellite unstable/instability (MSI, 21.7%), (3) genomically stable (19.7%), and (4) chromosomally unstable/chromosomal instability (CIN, 49.8%).[8] Most EBV-positive tumors occurred in male patients and in the gastric fundus or body, displaying extreme DNA hypermethylation and amplification of *JAK2* and *PD-L1/2*, with 80% harboring non-silent *PIK3CA* mutations. All EBV-positive GCs displayed *CDKN2A* promoter hypermethylation, while lacking the *MLH1* hypermethylation characteristic of the MSI-associated CpG island methylator phenotype (CIMP).[8,38] Strong interleukin-12–mediated signaling signatures suggested a robust immune cell presence in this subtype. In contrast, MSI-subtype tumors tended to occur in female patients, diagnosed at advanced ages, and characterized by elevated mutation rates, including mutations of genes encoding targetable oncogenic signaling proteins. The genomically stable subtype lacked numerous molecular alterations, correlated well with the Lauren diffuse histologic variant, but harbored mutations of *RHOA* or fusions involving RHO-family guanosine triphosphatase (GTPase)–activating proteins. The active GTP-bound form of *RHOA* activates *STAT3* to promote tumorigenesis.[39] Finally, CIN subtype tumors were frequent at the gastroesophageal junction/cardia, correlated well with the Lauren intestinal histologic variant, showed marked aneuploidy, and harbored focal amplifications of

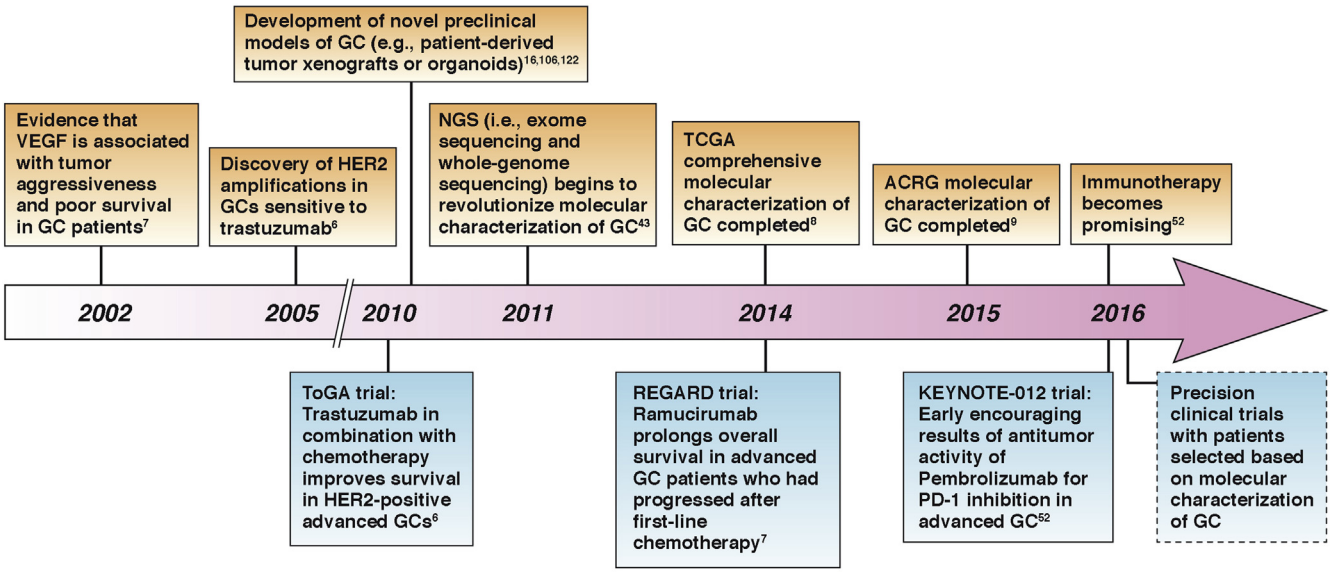

Figure 1. Timeline of selected major developments in GC (*above arrow*) and related clinical trials (*below arrow*) in recent years.

receptor tyrosine kinases (*RTKs*), in addition to recurrent *TP53* mutations and RTK-RAS activation.[8]

In 2015, the Asian Cancer Research Group proposed a new classification system associated with distinct genomic alterations, disease progression, and prognosis across multiple GC cohorts.[9] On the basis of whole-genome sequencing, gene expression profiling, genome-wide copy number microarrays, and targeted gene sequencing, 4 molecular subtypes were identified: (1) MSI, (2) microsatellite stable with epithelial-to-mesenchymal transition features (MSS/EMT), (3) MSS/TP53 mutant (MSS/TP53+), and (4) MSS/TP53 wild-type (MSS/TP53–).[9] One strong point of this study was the availability of long-term follow-up data, enabling association of this molecular classification with clinical outcome. The postoperative surveillance program for recurrence in the Asian Cancer Research Group study consisted of performing follow-up exams every 6 months until 5 years after the date of surgery.[9] MSI tumors were hypermutated, intestinal, usually antral, and diagnosed at clinical stage I/II. MSI tumors had the best prognosis; their recurrence rate after surgical resection of primary GC was the lowest among all 4 subtypes (22%). MSS/TP53+ tumors were linked to EBV infection and had the next best prognosis, followed by MSS/TP53- tumors. MSS/EMT tumors occurred at a younger age, mostly diagnosed at clinical stage III/IV, and were the Lauren diffuse histologic type. The MSS/EMT subtype had the worst prognosis and the highest recurrence rate (63%), with recurrences located mostly in the peritoneal cavity.[9]

Continuing to refine molecular classification, regular mutated (2.4 mutations/megabase; range, 0–8.3) and hypermutated (20.5 mutations/megabase; range, 9.6–200.2) GC types were identified in another recent study.[10] The regular mutated type was further subclassified into 2 subgroups, C1 and C2. The first subgroup, C1, was enriched in mutations of *TP53*, *XIRP2*, and *APC* and was associated with a significantly better prognostic outcome, whereas C2 was overrepresented by mutations in *ARID1A*, *CDH1*, *PIK3CA*, *ERBB2*, and *RHOA*. Furthermore, consistent with the Asian Cancer Research Group study, this research team observed that *CDH1* mutations were associated with a worse outcome in diffuse-type GC, independent of disease stage.[10] Because *ARID1A* is frequently mutated in both EBV and MSI subtypes, its mutation alone is not likely to constitute an alternative GC pathway.

Molecular Genetic Profiling of Gastric Cancer

Somatic Mutations in Gastric Cancer

The mutational landscape of GC has been deciphered by using large-scale analyses of data from genomic, expressional, and mutational profiling studies, especially potential clinically relevant driver mutations (Figure 2). Approximately 16.4% of GC cases exhibit hyperdense mutation frequencies.[10]

Genomic instability, and thus mutability, endows cells with genetic alterations, which in turn aid tumorigenesis and tumor progression.[40] As the guardian of the genome, *TP53* plays a central role in maintenance of genome integrity.[41] *TP53* mutations allow the accumulation of genetic alterations, leading to genomic instability. As the most frequently mutated gene, *TP53* mutations occurred in about 50% of GC and 71% of CIN subtype tumors, on the basis of TCGA data.[8] *BRCA2* is also involved in maintaining genomic integrity. *BRCA2* mutations were identified in 8% of the Tianjin (northern Chinese) cohort, validated in the TCGA cohort, and associated with significantly better survival.[37]

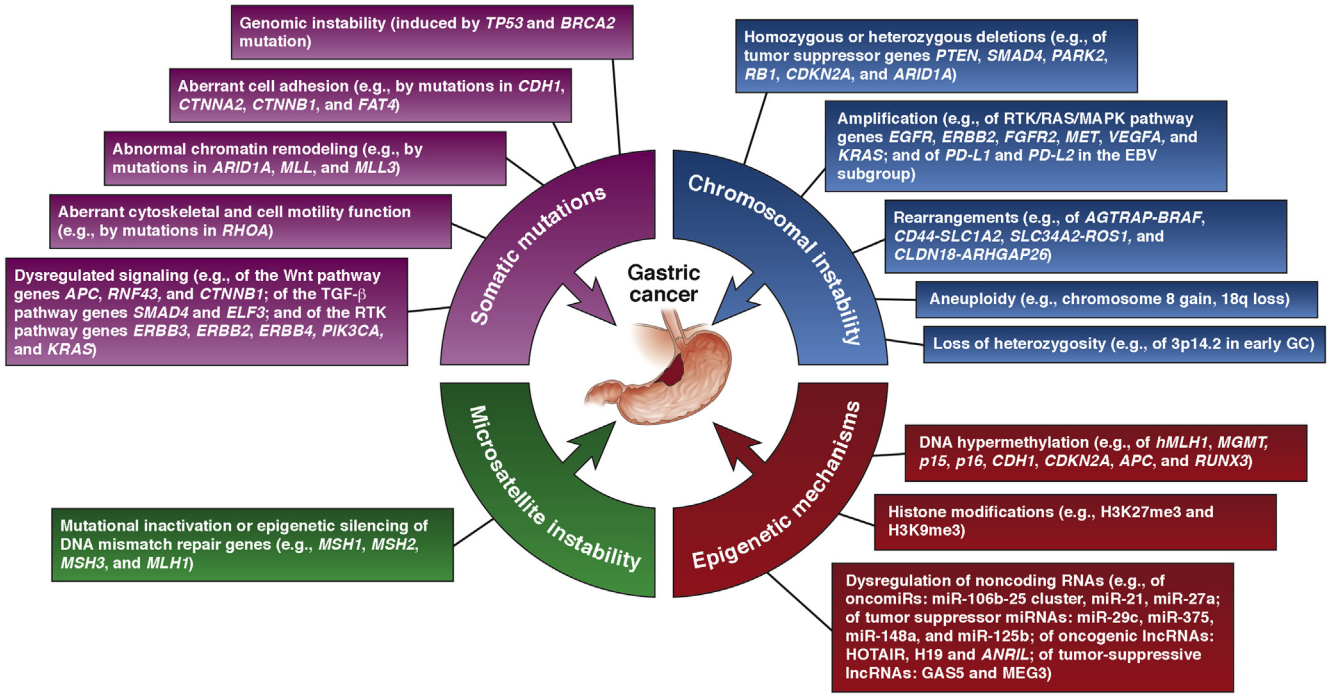

Figure 2. Genetic and epigenetic characteristics of GCs.

Recurrent somatic mutations in cell adhesion genes (eg, *CDH1*, *CTNNA2*, *CTNNB1,* and *FAT4*) and chromatin remodeling genes (eg, *ARID1A*, *MLL*, and *MLL3*) have been identified as the most commonly perturbed pathways in GC.[8–10,42–44] Among mutated genes in GC exomes, cell adhesion was the most significantly enriched biological process, consistent with a marked tendency of GC toward loss of cell-cell adhesion.[42,43] *CDH1* mutations occurred in 11.6% of regular mutated GC and constituted a significant negative prognostic factor in diffuse-type GC, because they were associated with shortened patient survival independent of TNM staging.[10] Nineteen percent to 40% of HDGC families have germline *CDH1* mutations.[32] *CTNNA2,* encoding a component of cell adhesion complexes, was mutated in 6.4% of MSS-subtype GCs,[44] whereas *CTNNB1* was mutated in 3.1% of regular mutated GCs.[10] Additional *CTNNA2* and *CTNNB1* functions include regulation of β-catenin signaling during early embryonic development.[8,44] *ARID1A* encodes a member of the SWI-SNF protein complex, which represses gene activity via chromatin remodeling. An interesting finding was the inverse relationship between *ARID1A* and *TP53*; *TP53* mutations were uncommon in both MSI and EBV subtypes, which possessed frequent *ARID1A* mutations (83% and 73%, respectively).[43] Moreover, *ARID1A* mutations were associated with better prognosis in a stage-independent manner. Thus, independent of *TP53*, mutation of *ARID1A,* encoding a chromatin-remodeling enzyme, may constitute an alternative GC developmental pathway, with distinct clinical behavior.[42,43]

RHOA, belonging to the Rho GTP family, mediates anoikis, focal adhesions, and cell adherens junctions.[35,44] Residues Arg5, Tyr42, Gly17, and Leu57 are *RHOA* mutational hotspots.[8,35,44] *RHOA* mutation, specifically in the diffuse-type or GS subtypes, showed a predilection for the antrum and body, with fewer *TP53* mutations.[8,35,44]

Dysregulated signaling, including the canonical Wnt, transforming growth factor (TGF)-β, and RTK pathways, in each of which several mutated genes are involved, has also been identified on the basis of NGS data. *APC* and *RNF43* are associated with truncating or inactivating mutations as negative regulators of the Wnt pathway.[8,44] *CTNNB1* encodes β-catenin; its mutation enhances resistance of intranuclear β-catenin to degradation, leading to continuous Wnt signaling.[8,45]

Wnt pathway-mutated GCs exhibited longer survival than *CDH1*-mutated GCs in Chinese patients.[37] Mutations of *SMAD4* and *ELF3*, in the TGF-β pathway, were critical drivers in both the MSI and MSS subtypes.[44] *ERBB3* mutations occurred principally in hypermutated GC, with recurrent mutation sites or COSMIC-reported sites, whereas *ERBB2* was significantly mutated in non-hypermutated GC, occurring at known hotspots.[8] *ERBB4* and its ligand *NRG1* were mutated in 10% of GC; *ERBB4* mutations occurred in both the receptor and kinase domains, activating both ERBB4 and PI3K-AKT signal transduction.[37] *PIK3CA* was frequently mutated in EBV-positive or MSI subtypes.[8,9] *KRAS*, an important downstream molecule in RTK signaling, was mutated in MSI subtype tumors.[9]

Chromosomal Instability

The dynamic process of CIN constitutes a major event in tumor progression. By involving gains or losses of entire chromosomes or fractions of chromosomes, CIN leads to altered DNA copy number (aneuploidy), amplification, deletion, loss of heterozygosity (LOH), or rearrangement. CIN is common in GC; gastric tumors with a high level of CIN, rather than with *TP53* mutation, were more likely to benefit from cisplatin-based neoadjuvant chemotherapy.[46] Specific gains or losses of chromosomes are also associated with tumor type and progression. Frequent chromosome 8 gain occurred in MSI subtype, whereas 18q loss prevailed in EBV-positive GC.[44] Copy number gains at 8q, 17q, and 20q occurred in intestinal-type GC, whereas gains at 12q and 13q occurred in diffuse-type tumors.[47,48] Gains at 8q23.3 and 8q23.2 were most frequently associated with early GC, whereas gains at 8q24.21 and 8q24.3 were associated with advanced GC.[49] Finally, gain of 1q32.3 and loss of 18q22.1 were associated with poor clinical outcome.[50]

Frequent focal amplifications of *EGFR*, *ERBB2*, *FGFR2*, *MET*, *VEGFA,* and *KRAS* are involved in RTK/RAS/MAPK signaling and were associated with increased tumor cell proliferation, differentiation, apoptosis, adhesion, and migration.[8,9,51] The utilization of trastuzumab, a monoclonal antibody targeting *ERBB2*, is a successful example of translational genetic profiling in GC. One international randomized controlled trial (ToGA) demonstrated that human epidermal growth factor 2–positive advanced GC patients exhibited prolonged survival when treated with trastuzumab plus chemotherapy.[6] Recurrent amplifications at 9p24.1, the locus containing *CD274* and *PDCD1LG2,* which encode the immunosuppressant proteins PD-L1 and PD-L2, were enriched in the EBV subgroup.[8] A recent open-label phase 1b trial (KEYNOTE-012) demonstrated manageable toxicity and promising antitumor activity of anti–PD-1 antibody (pembrolizumab) in advanced GC.[52]

Additional amplified genes in GC comprise oncogenic transcription factors, including *MYC*, *GATA4*, *GATA6,* and *KLF5*, and cell-cycle regulators such as *CCNE1*, *CCND1*, and *CDK6*.[8,9,51,53]

Focal genomic deletions in tumor suppressor genes such as *PTEN*, *SMAD4*, *PARK2*, *RB1*, *CDKN2A*, and *ARID1A* have been identified in GC.[8,9,51] LOH pattern also differed significantly between early and advanced GCs; the most frequent LOH was at 3p14.2 in early GCs, whereas in advanced GCs the most frequent LOH occurred at 11q24.3-25, 11q23.2-24.1, 11q14.1, and 12p11.21-13.33.[49]

Although the creation of fusion genes via genomic rearrangement is less reported in solid than in liquid tumors, fusion genes have been identified in GC. In one study, approximately 1%–2% of GCs harbored RAF pathway gene rearrangements wherein exon 8 of *BRAF* was fused with exon 5 of *AGTRAP*.[54] *CD44-SLC1A2* and *SLC34A2-ROS1* fusions were also identified in GCs.[55,56] Interchromosomal rearrangements between claudin 18 (*CLDN18*) and Rho GTPase-activating protein 6 (*ARHGAP26*) were exclusively identified in the GS subtype in the TCGA study[8]; this fusion resulted in impaired epithelial integrity and wound healing.[57]

Microsatellite Instability

MSI is characterized by alterations in length within short repeated DNA sequences (microsatellites), resulting from mutational inactivation or epigenetic silencing of DNA mismatch repair genes (eg, *MSH1*, *MSH2*, *MSH3*, and *MLH1*).[58] These mutations include coding region frameshift mutations caused by MSI, which can drive oncogenesis by inactivating tumor-suppressor genes or disrupting other noncoding regulatory sequences.[59,60] MSI, a major type of genetic instability in cancer, occurs in a substantial portion of GCs.[61–63] On the basis of the frequency of mutations within microsatellite markers, GC can be classified as MSS or MSI, which includes high-frequency (MSI-H) and low-frequency subtypes. In Korean GC-derived cell lines and primary tissues, the number of microsatellite mutations causing insertions or deletions in gene-encoding regions was 4-fold to 6-fold higher in MSI-H than in MSS samples, which was based on genome-wide and transcriptome-wide analyses of mutations associated with MSI.[62] MSI-H GC is characterized by older age, mostly female, distal location, and better survival.[64] Mismatch repair–deficient cancers may stimulate the immune system, making them susceptible to immune checkpoint blockade therapy. This theory was confirmed in a recent clinical trial in which patients with MSI-positive tumors across 6 cancer types, including 1 case of GC treated with a PD-1 inhibitor (pembrolizumab), had improved outcomes.[65]

Epigenetic Mechanisms

Epigenetic mechanisms play important roles in the pathogenesis of GC, including DNA methylation (regional hypermethylation and global hypomethylation), histone modification, chromatin remodeling, and dysregulation of noncoding RNAs (eg, miRNAs or lncRNAs). Gene silencing mediated by regional hypermethylation in gene promoter regions, almost exclusively associated with CpG islands, has been widely studied in GC.[66–68] Some silenced genes play key roles in malignant transformation, affecting expression of various proteins or noncoding RNAs. Tumor-suppressor genes including *hMLH1*, *MGMT*, *p15*, *p16*, *CDH1*, *CDKN2A*, *APC*, *RUNX3*, *DAPK*, and *BNIP3*, which are involved in DNA repair, cell-cycle control, cell adhesion/invasion, cell proliferation, and apoptosis, are inactivated by promoter methylation.[66,69] *hMLH1* inactivation by promoter hypermethylation is a predominant cause of DNA mismatch repair deficiency and results in frequent MSI in sporadic cancers.[9,66] The majority of MSI-H GCs have *hMLH1* hypermethylation, whereas this alteration is uncommon in MSS GCs.[66] Methylation profiles also differ between the diffuse and intestinal types of GC; for example, *CDH1* and *p14* hypermethylation occurred more frequently in the diffuse type, whereas *p16* hypermethylation occurred mostly in the intestinal type.[66] Several tumor-suppressor miRNAs, including miR-124a, miR-199a, miR-34b, and miR-129, were silenced by DNA hypermethylation of their promoter CpG islands in GC.[70–72] Hypermethylation may also determine GC prognosis. In one study, methylation of *GFRA1*, *SRF*, and *ZNF382* was associated with a low risk of metastasis and longer overall survival in GC patients.[73] Another study that used genome-wide methylation analysis in the gastric CIMP phenotype demonstrated that CIMP GCs were associated with widespread hypermethylation, young patient age, and worse survival outcomes, independent of tumor stage.[68] CIMP GCs tended to harbor mutations in the oncogenes *CTNNB1*, *ERBB2*, *KRAS*, and *PIK3CA*.[74] Similarly, reduced expression of hypermethylated *BRINP1*, *SGCE,* and *MLH1* was significantly associated with favorable survival in GC.[75] Moreover, infectious agents such as *H pylori*, EBV, and the infection-associated inflammatory response all induce altered DNA methylation.[76–78] EBV-positive GCs exhibited extreme CIMP, confirmed by the TCGA study.[8]

Histone modifications including methylation, acetylation, phosphorylation, and ubiquitination can affect gene expression in several cancer types.[79,80] The tumor suppressor *RUNX3* is hypoxically silenced by histone modification during GC progression.[81] In one study comparing H3K27me3 variations at the genome-wide level by ChIP-chip, 128 genes displayed significant H3K27me3 differences between GC and adjacent normal tissue.[82] Similarly, hundreds of altered promoters and predicted enhancers were identified by comparing multiple histone modifications in 5 GCs and matched normal tissues, on the basis of nanoscale chromatin immunoprecipitation sequencing.[83] Finally, high levels of H3K9me3 trimethylation were associated with poor outcome in GC patients.[84]

There is accumulating evidence suggesting that epigenetic and genetic defects in noncoding RNAs play crucial roles in tumor initiation, progression, invasion, and metastasis. This finding is particularly evident for miRNAs and lncRNAs. Overexpressed oncogenic microRNAs that target tumor suppressor genes can promote progression, resist apoptotic signals, and promote cell invasion and tumor metastasis.[11,71] Upregulation of the miR-106b-25 cluster dysregulates *E2F1* activity and impairs TGF-β–dependent cell-cycle arrest and apoptosis by suppressing *p21* and *Bim* in GC.[85] Similarly, by directly inhibiting expression of *RECK* and *PTEN,* overexpressed miR-21 leads to enhanced cell proliferation and invasion.[86,87] Overexpression of miR-27a is associated with metastasis of GC to lymph nodes.[88] In contrast, tumor suppressor miRNAs such as miR-29c, miR-375, miR-148a, and miR-125b are downregulated in GC, targeting *ITGB1*, *JAK2*, *ROCK1*, and *MCL1*, respectively.[89–93]

LncRNAs comprise another type of RNA that contributes to cancer, with oncogenic or tumor-suppressive functions. Knockdown of the oncogenic lncRNA HOTAIR reduces invasiveness and reverses epithelial-mesenchymal transition in GC cells, and high expression levels of HOTAIR are significantly associated with advanced tumor stage, lymph node metastasis, and poor survival in GC patients.[94] Similarly, overexpression of the lncRNA H19 enhances carcinogenesis and metastasis mediated by direct upregulation of *ISM1* and indirect suppression of *CALN1* expression via miR-675 in GC.[95] LncRNA H19, which induces cell proliferation, can also function via *TP53* inactivation and apoptosis inhibition.[96] Moreover, high *ANRIL* expression correlated significantly with advanced TNM stage and poor overall survival, promoting proliferation of GC cells by

epigenetically silencing miR-99a/miR-449a.[97] Finally, decreased expression of the tumor-suppressive lncRNAs GAS5 and MEG3 indicated a poor prognosis and promoted cell proliferation in GC,[98,99] whereas conversely, ectopic expression of MEG3 inhibited GC cell proliferation, promoted apoptosis, and modulated *TP53* expression.[99]

More recently, circular RNAs, characterized by the formation of a covalently closed continuous RNA loop, are drawing renewed attention in cancer research.[100,101] Moreover, noncoding RNAs are stable in bodily fluids such as serum, plasma, gastric juice, and even in exosomes, making them promising GC biomarkers.[102–105]

Preclinical Models of Gastric Cancer

The development of targeted anti-tumor drugs has been hampered by a paucity of effective preclinical models that can reliably reflect the complexity and heterogeneity of human tumors. In this context, PDX and organoids are attractive as offering effective tools for genetic evolution studies, biomarker identification, drug screening, and preclinical evaluation of personalized medicine strategies.[16,106]

PDX models of human GC constructed by using subcutaneous or orthotopic implantation of surgical tissues or gastroscopic biopsies have the advantage of recapitulating most of the histology and somatic genetics of primary patient tumors.[107–110] Moreover, orthotopic implantation of intact GC tissue can lead to primary and metastatic tumor growth mimicking that seen in patients.[107] In one study using PDX models generated by subcutaneous implantation, CD44v8-10 was verified as a GC stem cell marker.[111] In another study, in vivo high-throughput screening using a $1 \times 1 \times 1$ experimental design (a "one animal per model per treatment" approach) with PDX models assessed population responses to 62 treatments across 6 indications including GC[112]; these latter data demonstrated the reproducibility and clinical translatability of PDX clinical trials by identifying associations between a genotype and a drug response and established mechanisms of resistance.[112] Similarly, on the basis of genomically defined GC PDX models, combination therapy of irinotecan with a *BCL2L1*-targeted drug was confirmed to effectively reduce tumor size.[113] Moreover, inhibition of fibroblast growth factor receptor 2 signaling by AZD4547 induced dose-dependent GC regression in *FGFR2*-amplified PDX models (SGC083).[114] PDX models can also be used to provide a surrogate assessment of efficacy and histologic investigation for tumor immunotherapy. In PDX models with transferred peripheral blood mononuclear cells from the same patient, combination therapy with anti-hCD137 and anti-hPD1 antibodies (urelumab and nivolumab, respectively) significantly slowed tumor growth.[115] Although these data are promising, practical challenges remain to improving use of PDX models in cancer research, including delay between murine engraftment time and patient treatment schedule, lymphomagenesis of human tumors in mice, and cost.[16,116] In one large breast cancer PDX model study, even within the first murine passage, all models showed moderate drift to dramatic clonal selection during tumor growth.[117]

Organoids are miniature replicas of tissues cultured three-dimensionally in a semi-solid extracellular matrix and growth factor–enriched medium.[118,119] Organoids sustain high levels of architectural and physiological similarity to native organ systems, superior to traditional two-dimensional homogeneous cell lines.[120] Additional advantages of organoids are that they are self-organizing, easy to handle, acceptable in cost, accessible to genetic engineering, and amenable to large-scale drug screening with shorter turnaround times.[14,121]

Gastric organoids can be developed from pyloric glands and $Lgr5^{+ve}$ stem cells,[122] from corpus glands and $Troy^{+}$ chief cells,[123] or from human pluripotent stem cells (hPSCs).[13] Most recently, Wnt/β-catenin signaling was shown to promote gastric fundus specification in organoids developed from hPSCs.[17] In one study using microinjection, gastric organoids mounted a nuclear factor kappa B–driven inflammatory response to *H pylori* infection, and the strength of this response depended on the differentiated cell types contained within the organoids.[124] A quasi-immortal tissue culture model has also been developed in the form of gastric spheroids, which can serve as a suitable model of *H pylori* infection because of the formation of dense planar cultures of polarized epithelial cells after being transferred into two-dimensional culture.[125] In a study using pluripotent stem cell–derived gastric organoids to model human disease pathogenesis, *H pylori* induced robust activation via tyrosine phosphorylation of c-Met and a 2-fold increase in epithelial cell proliferation. Cytotoxin-associated gene A played a pivotal role in this process, forming a complex with the c-Met receptor.[13] In another related study, gastric organoids exhibited dysplasia and readily generated adenocarcinomas in mice characterized by activating mutations in *KRAS* or loss of *TP53*.[126] The potential metastatic role of *TGFBR2* loss-of-function mutations was shown in $CDH1^{-/-}$; $TP53^{-/-}$ murine epithelial-mesenchymal organoids used to model hereditary GC, with short hairpin RNA knockdown of *TGFBR2*.[127] A critical role of *RHOA* function in mediating anoikis in diffuse-type gastric carcinogenesis was confirmed in mouse intestinal organoids containing stably expressed *RHOA* mutations.[44] Thus, organoids constitute a robust model system that may facilitate personalized therapy development by enabling high-throughput drug screening to identify gene-drug associations, as well as by testing specific individual responses to different therapeutic agents.[128]

Precision Medicine in Gastric Cancer

Although thus far only 2 targeted molecular therapeutic agents, trastuzumab and ramucirumab, have been approved by the Food and Drug Administration, a better and deeper understanding of genomic and epigenomic characteristics of GC, coupled with analyses of cancer phenotypes by using novel preclinical models, will hopefully lead to treatment optimization in the appropriate patient at the appropriate time (Figure 3). New molecular classification systems now provide a critical basis for the design of precision medicine clinical trials. Similarly, continuously updated molecular genetic profiling of GC has yielded promising new

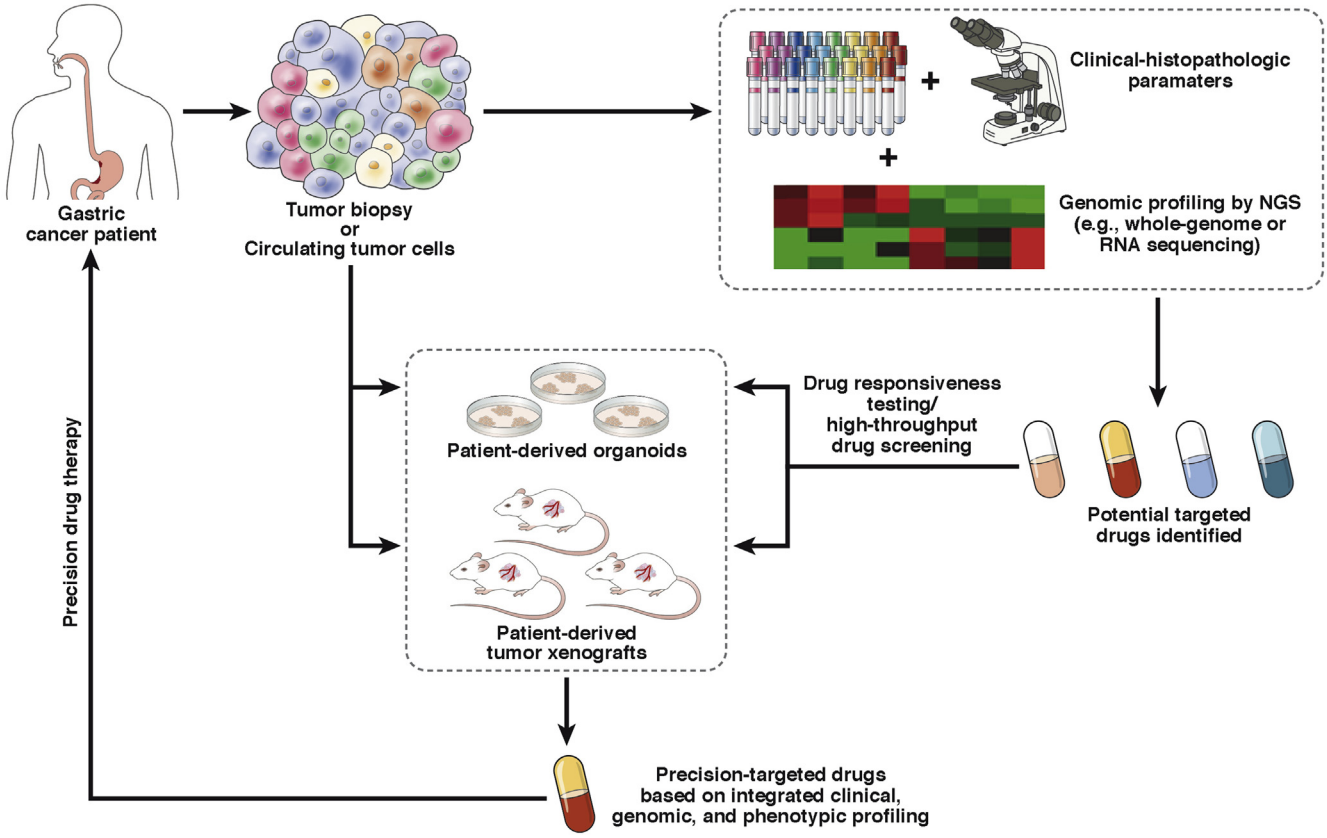

Figure 3. Integrated precision medicine strategy for GC. Tumor specimens obtained by biopsy or circulating tumor cells are subjected to clinical-histopathologic examination and genomic analysis by NGS. Potential targetable genomic alterations are identified. Simultaneously, patient-derived tumor models (eg, xenografts or organoids) are established from the same specimens. Effective, safe therapies are then chosen by using high-throughput screening in these model systems. Finally, the patient receives optimized therapy based on this integrated strategy.

therapeutic targets such as RTKs or RAS and PI(3)-kinase signaling proteins.[8,9,129] Although various new agents are still being investigated for targeted GC therapy, several ongoing clinical trials are already targeting *STAT3*, *c-MET*, *mTOR*, *CLDN18.2*, and PD-1/PD-L1.[52,130,131] Some early results of these trials have been encouraging, particularly the multicenter phase 1b trial of pembrolizumab (KEYNOTE-012), which showed durable remissions in a subset of patients with PD-L1–positive advanced GC identified by using a prototype assay (sequencing was not performed in that study).[52] Meanwhile, targeting epigenetic regulators such as methyltransferases, demethylases, and miRNAs for cancer therapy are also under investigation. One ongoing clinical trial will evaluate combination therapy with vorinostat (a histone deacetylase inhibitor) and radiotherapy in GC (ClinicalTrials.gov identifier: NCT01045538).[132] Furthermore, integration of GC genotype and phenotype by using PDX and patient-derived organoid models promises to assist clinical trial design and personalized medicine strategies in the future.[16,106] However, much work remains to be done, considering that trastuzumab is thus far the only biomarker-driven therapy in clinical practice and especially in view of numerous negative clinical trials such as the LOGiC and TyTan trials of lapatinib (a tyrosine kinase inhibitor targeting HER2 and EGFR),[133,134] the RILOMET-1 trial of rilotumumab, and the METGastric trial of onartuzumab (targeting c-MET).[135,136] Notably, in this context, it should be emphasized that ramucirumab is not a biomarker-driven drug.[137]

Conclusions

Because of the pronounced interpatient and intratumor heterogeneity of GC, the current uniform treatment strategy used in virtually all patients seems suboptimal. With the advent of NGS and novel preclinical model strategies, the study and treatment of GC are undergoing a radical shift toward precision medicine. Recent genomic and epigenomic profiling studies are now beginning to form a crucial foundation on which to build both improved molecular understanding of and better targeted therapies for GC. Combined with new PDX and organoid models, integrated traditional, genome-based and phenotype-based strategies promise to open new vistas for precision medicine applications in GC.

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Received January 10, 2017. Accepted February 13, 2017.

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Conflicts of interest
The authors disclose no conflicts.

Funding
Dr Meltzer is an American Cancer Society Clinical Research Professor, the Myerberg/Hendrix Professor of Gastroenterology at Johns Hopkins, and is supported by USPHS grants CA190040 and CA211457. Xi Liu is supported by the Key Science and Technology Program of Shaanxi Province, China, 2015SF128.