Gastric Cancer Genomics: Advances and Future Directions

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SUMMARY

Improved sequencing technology has led to better understanding of the complex genomic landscape of gastric cancer. Herein, we review the recent advances in gastric cancer genomics and their potential to directly impact the diagnosis and treatment of this cancer.

Advancement in the field of cancer genomics is revolutionizing the molecular characterization of a wide variety of different cancers. Recent application of large-scale, next-generation sequencing technology to gastric cancer, which remains a major source of morbidity and mortality throughout the world, has helped better define the complex genomic landscape of this cancer. These studies also have led to the development of novel genomically based molecular classification systems for gastric cancer, reinforced the importance of classic driver mutations in gastric cancer pathogenesis, and led to the discovery of new driver gene mutations that previously were not known to be associated with gastric cancer. This wealth of genomic data has significant potential to impact the future management of this disease, and the challenge remains to effectively translate this genomic data into better treatment paradigms for gastric cancer. (Cell Mol Gastroenterol Hepatol 2017;3:211–217; http://dx.doi.org/10.1016/j.jcmgh.2017.01.003)

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Gastric cancer continues to remain a major source of morbidity and mortality throughout the world. Recent estimates have indicated that more 950,000 new cases of gastric cancer will be diagnosed per year, with more than 720,000 deaths, making gastric cancer the third most common cancer in the world and the third most common cause of cancer-related mortality.2 Currently, the primary method for classification of gastric cancer is based on its histologic subtype. Lauren’s criteria, the most commonly accepted histologic classification of gastric cancer, separates gastric cancer into 2 major subtypes: intestinal and diffuse. The World Health Organization produced an additional histologic classification for gastric cancer, separating these tumors into categories including tubular, papillary, mucinous, and poorly cohesive/signet ring.3 Despite the ability to classify gastric cancer successfully using these histologic classifications, this information has not led to the development of histologic subtype-specific treatment options.

Over the past decade there have been countless advances in cancer therapy, and many of these gains are related to the development of more personalized therapies for cancer treatment. However, in gastric cancer, although some treatment studies have been successful, such as showing that postoperative chemoradiotherapy is more effective than surgery alone,4 most of the efforts to develop more personalized therapies have proven unsuccessful.5,6 Currently, the only widely used personalized therapy for gastric cancer involves treatment of metastatic human epidermal growth factor receptor 2 (HER2)-positive tumors with the HER2 antibody trastuzumab, the efficacy of which was shown in the Trastuzumab for Gastric Cancer study.7 In this study, patients with metastatic HER2-overexpressing gastric cancers showed increased median overall survival when treated with trastuzumab plus standard chemotherapy compared with standard chemotherapy alone, which led to the approval of trastuzumab for the treatment of metastatic HER2-positive gastric cancer in 2013. Although the use of trastuzumab showed the potential for personalized therapy in gastric cancer, there certainly is room for improved therapeutic options. One way to potentially improve and personalize treatment paradigms for gastric cancer is to better understand the genomics of this disease.

Use of Next-Generation Sequencing to Better Define Gastric Cancer Genomics

Recently, genomic sequencing has become far less expensive, and also has become more efficient, developing even faster than comparable computer technology as predicted by Moore’s Law.8 This has led to next-generation sequencing (NGS) being increasingly used to study nearly all types of malignancies, and gastric cancer is no exception.9,10 There have been 2 recent seminal reports that used NGS to...
sequence large sets of gastric cancer samples to better characterize the genomics of gastric cancer, including a report from The Cancer Genome Atlas (TCGA) as well as a separate study from the Asian Cancer Research Group (ACRG). The TCGA study evaluated 295 treatment-naive primary gastric adenocarcinomas from multiple participating centers, where analysis included whole-exome sequencing, copy number analysis, DNA methylation and RNA analysis, microsatellite instability testing, and, on a select group of tumors, whole-genome sequencing. The study from the ACRG examined 300 primary gastric adenocarcinomas from a single center in Seoul, South Korea. This study used 49 gastric adenocarcinomas that previously underwent study with whole-genome sequencing combined with 251 additional specimens, and then used a combination of gene expression profiling, targeted sequencing of genes of interest, as well as genome-wide copy number microarrays. In addition to these 2 large studies, there also have been multiple smaller studies that have used NGS to better characterize the genomics of gastric cancer. The plethora of data obtained from these recent NGS studies has helped define the genetic landscape of gastric cancer, has led to a contemporary approach to the development of genomically based molecular subtypes of gastric cancer, and has elucidated novel gastric cancer driver mutations, which all may lead to new perspectives on therapeutics.

Development of Genomically Based Molecular Classification Systems for Gastric Cancer

Genomic data have been used to develop molecular classification systems for many types of cancer including colorectal cancer and pancreatic cancer. Although classic classification criteria for gastric cancer has been histologically based (eg, Lauren’s and World Health Organization), recent use of genomic data also has led to the development of novel molecular classification schemes for gastric cancer (Figure 1). First, the TCGA Research Network proposed a classification system that divides gastric cancers into 4 distinct subtypes: Epstein–Barr virus (EBV) positive, microsatellite instability (MSI), genomic stability (GS), and chromosomal instability (CIN). EBV-positive tumors, which represented 9% of the tumors sequenced, showed significant CpG island methylator phenotype, as well as the highest levels of DNA hypermethylation. This observed DNA hypermethylation was consistent with previous reports linking EBV-positive gastric cancers to DNA hypermethylation. All tumors from this class showed CDKN2A (p16INK4A) promoter hypermethylation, but lacked hypermethylation of MLH1. These tumors also had the highest rate (80%) of PIK3CA mutations, showed a high rate of ARID1A mutations (55%), and very infrequently showed any mutations in TP53. Another important characteristic of this group, for therapeutic purposes, was overexpression of programmed death-ligand (PD-L)1/2 in combination with increased immune cell signaling signatures. The second group included tumors with MSI, which resulted in significantly hypermutated tumors. This group of tumors accounted for 22% of the total samples, and showed significant CpG island methylator phenotype, including hypermethylation of the MLH1 promoter. Mutational analysis in this group identified a total of 37 significantly mutated genes including TP53, KRAS, PIK3A, and ARID1A, whereas there were only 25 significantly mutated genes in the non-MSI cancers. The remaining 69% of tumors from the TCGA group were divided based on the presence of extensive somatic copy number aberrations. By using this branch point, the third group defined by the TCGA data is the GS group, which comprised 20% of the total samples. This group of tumors comprised the majority of gastric cancers with diffuse histology, and also had the largest percentage of CDH1 mutations consistent with the abundance of diffuse histology in this group. GS tumors also showed an increase in RHOA mutations and CLDN18–ARHGAP fusions, and increased expression of cell-adhesion pathway genes. Finally, comprising the remaining 50% of the tumors was the CIN group. This group showed marked aneuploidy as well as amplifications of receptor tyrosine kinases (RTKs). This
group also showed a high percentage of TP53 mutations and had primarily intestinal histology.

These tumor subtypes derived from the TCGA data were found to have distinct clinical signatures as well. For primary tumor location, CIN tumors were found more frequently at the gastroesophageal junction and in the cardia, whereas EBV-positive tumors were found more frequently in the fundus and body. In addition, GS tumors were diagnosed at an earlier age (median, 59 y), whereas MSI tumors were diagnosed at an older age (median, 72 y). Gender differences also were appreciated, with MSI tumors being the only group seen more frequently in females (56%), although 81% of EBV tumors were seen in males. Diffuse-type histology was seen most frequently in the GS group, in which 73% of these tumors had diffuse-type histology, however, from an outcomes perspective, none of these 4 subgroups of gastric cancers showed any significant survival differences. In addition to these clinical signatures, some of these TCGA-defined gastric cancer subgroups have analogous subgroups in other gastrointestinal cancers as well, such as the MSI and CIN subgroups that have been noted in colorectal cancer.19,22

The ACRG study led to the development of a different 4-group classification system for gastric cancer, which included the following subtypes: MSI, microsatellite stable (MSS)/epithelial-to-mesenchymal transition (EMT), MSS/TP53+, and MSS/TP53-.12 The MSI subtype represented 23% of the gastric cancers, and showed heavily mutated genetic profiles, with 44% of tumors showing ARID1A mutations and 42% of tumors with a mutation in the phosphoinositide 3-kinase (PI3K)–phosphatase and tensin homolog (PTEN)–mechanistic target of rapamycin (mTOR) pathway. Other heavily mutated genes in this group included KRAS (23%) and ALK (16%). The remaining MSS tumors then were divided based on whether or not they showed gene expression signatures consistent with EMT. Those showing this signature were classified as MSS/EMT gastric cancers, which represented the smallest subset (15%) of samples, and showed the lowest number of mutational events per tumor. The remaining MSS gastric cancers then were divided based on a TP53 activity signature, into those with TP53 activity (MSS/TP53+) and those without TP53 activity (MSS/TP53-). The MSS/TP53+ group comprised 26% of the total samples, and this group had the highest percentage of EBV-positive tumors. Finally, MSS/TP53- comprised 36% of all gastric cancers in this study, and as expected showed the highest prevalence of TP53 mutations (60%).

These 4 gastric cancer subtypes had relevant clinical associations as well, and unlike the TCGA subtypes, the ACRG subtypes showed survival differences that were validated in 3 independent cohorts.12 The MSS/EMT subtype occurred at a younger age than the other subtypes, and the majority of these cancers had diffuse-type histology. These patients also had the worst overall survival, and the highest rate of recurrence, especially involving peritoneal dissemination. In the MSI group, these cancers were found predominantly in the antrum, were diagnosed more frequently at either stage I or II, and were primarily of intestinal-type histology. Given the earlier stage of diagnosis, these patients also had the best overall survival. When recurrences did occur, MSI as well as MSS/TP53- cancers were associated with a higher rate of liver limited recurrences compared with the MSS/EMT and MSS/TP53+ groups.

Of additional interest is that although Helicobacter pylori is well recognized to be an important contributor to gastric cancer pathogenesis,23 H pylori status was not used in either of these classification schemes. In the TCGA study, H pylori infection was found very infrequently in the samples tested.11 In the ACRG study, H pylori was found frequently (43% of 127 samples tested), but there was no association of H pylori infection with any of the defined gastric cancer subtypes. In addition to these 2 larger studies, other smaller studies also have used NGS to develop novel, genomically based classification systems. One such study, based on a cohort of 78 Chinese gastric cancer samples, divided gastric cancers into 2 distinct subtypes, including those with high clonality and low clonality.28 Although developing these new genomically based molecular gastric cancer classification schemes is a significant technologic advance over the use of histologic classifications, the larger question that remains is whether these new subtypes can be used effectively to change treatment paradigms to ultimately improve survival in patients with gastric cancer.

**Strengthening the Importance of Classic Gastric Cancer Driver Mutations**

Mutations in TP53 and CDH1 often were considered classic driver mutations of gastric cancer, even before the NGS era. TP53 codes for the nuclear protein p53, which is a critical tumor suppressor that is responsible for ensuring genome integrity, and whose function can be lost in cancers via a loss-of-function mutation, loss of heterozygosity, or, rarely, methylation.24 Upon activation of p53 in the setting of cellular stress such as DNA damage, oxidative stress, or ionizing radiation, p53 can result in arrest of the cell cycle as well as cellular apoptosis.24 Loss-of-function mutation in the TP53 gene is a common pathogenic genetic alteration in cancers of the gastrointestinal tract, including gastric cancer.25 The TCGA confirmed the high frequency of TP53 mutations, showing that TP53 mutations were the most common mutations found in gastric cancer. TP53 mutations were present in 50% of nonhypermutated gastric cancer samples, and 71% of CIN samples had TP53 mutations.11 However, of additional interest was the lack of TP53 mutation in EBV-positive tumors. In the ACRG series, TP53 mutations were found in a lower percentage of tumors (33%), however, it was still the most commonly mutated gene in this set of gastric cancers.12 Although confirmation of the prevalence of TP53 mutations in gastric cancer by the TCGA and ACRG data is important, this information currently provides no prognostic or therapeutic roles in gastric cancer.

A second classically mutated gene associated with gastric cancer is CDH1, which encodes for the cell adhesion molecule E-cadherin.26 Mutations in CDH1 typically have been associated with a diffuse histologic pattern of gastric cancer, and germline mutation in CDH1 is associated with the autosomal-dominant syndrome hereditary diffuse
gastric cancer, which significantly increases the risk of diffuse gastric cancer as well as lobular breast cancer in affected individuals who carry a germline mutated copy of the CDH1 gene.26,27 From the TCGA data, CDH1 was found to be mutated in 11% of all gastric cancers, with 37% of all genomically stable gastric cancers having a CDH1 mutation.11 Within nonhypermutated tumors, CDH1 was found to be the fourth most commonly mutated gene overall (behind TP53, ARID1A, and PIK3CA). In the ACRG analysis, CDH1 mutation was found in only 2.8% of the MSS/EMT subtype.12 Another smaller series showed CDH1 mutations in 9% of samples.14 Similar to the data with TP53, CDH1 mutations clearly have been validated as frequent mutations in gastric cancer, however, other than in the management of hereditary diffuse gastric cancer families with germline CDH1 mutations, CDH1 mutation status does not alter gastric cancer treatment.

The use of NGS has led to the discovery of other candidate genes with similar functions as TP53 and CDH1, which also may be important in gastric cancer pathogenesis. For example, BRCA2, another maintainer of genome integrity, recently was shown to be mutated in 6% of a Chinese cohort of gastric cancers, and these mutants correlated with longer survival.10 However, this association was not seen in other larger gastric cancer sequencing studies. Of additional interest is also the recent discovery of another cell adhesion gene that functions in the same complex as E-cadherin, CTNNAL1, which codes for α-E-catenin.28 Mutations in this gene have been discovered in hereditary diffuse gastric cancer families, however, similar to BRCA2, a significantly increased frequency of this mutation has not been detected in the larger NGS studies.

The Search for New Driver Mutations in Gastric Cancer

Although NGS has strengthened the role of TP53 and CDH1 as driver mutations in gastric cancer, at this time the presence or absence of these mutations does not alter treatment strategies. Therefore, it also is important to use the data from the NGS studies to determine novel driver mutations that may better impact treatment decisions and outcomes (Figure 2). The major new categories of driver mutations that have been revealed by NGS include chromatin remodeling, cell motility/cytoskeleton, Wnt signaling, and RTK pathway genes.

Chromatin Remodeling

Chromatin remodeling genes, whose products are responsible for regulating chromatin structure to alter DNA accessibility and transcriptional efficiency, frequently are mutated in gastric cancer.11 The most commonly identified chromatin remodeling gene mutation is in ARID1A, which is a putative tumor suppressor that encodes a subunit of the switch-sucrose nonfermentable (SWI–SNF) chromatin remodeling complex.14 This gene was detected initially in 2 smaller gastric cancer sequencing studies.14,15 In one of these reports, 22 gastric cancers and paired normal tissue samples had whole-exome sequencing performed, and showed that 27% of gastric cancers had a mutation in ARID1A.14 This group then looked at ARID1A mutations in a larger set of gastric cancers (109 samples) and found ARID1A mutations in 29% of these samples, primarily in EBV-positive (47%) and MSI (78%) tumors.14 The TCGA confirmed this significance with demonstration of ARID1A mutations in 14% of all nonhypermutated gastric cancers.11 Similar to prior results, these mutations were concentrated primarily in the EBV-positive cancers (55%), as well as hypermutated cancers (44%).11 Similarly, in the ACRG data, ARID1A mutations were found in 18% of gastric cancers.12 In addition to ARID1A, other less commonly mutated chromatin remodeling genes in gastric cancer include other genes of the SWI–SNF complex including ARID1B,16 as well as genes from the mixed-lineage leukemia (MLL) family including those encoding MLL (KMT2A) and MLL3 (KMT2C).15,16

Cell Motility/Cytoskeleton

Another subgroup of frequently mutated genes in gastric cancer are those affecting cell motility and the cytoskeleton, with the most commonly mutated gene in this category being RHOA. RHOA codes for RhoA, which is a Rho guanosine triphosphatase that is part of the Ras superfamily, and has diverse roles within the cell including functions in cell motility and cytoskeleton remodeling, as well as regulation of the cell cycle.29 RHOA was found to be mutated in 25% and 14% of diffuse gastric cancers in 2 separate studies, whereas it was not mutated in intestinal-type gastric cancers.16,17 In the TCGA analysis, RHOA was mutated in 6% of nonhypermutated gastric cancers, with enrichment in the genomically stable subset.11 However, the exact role of RHOA mutations in gastric cancer pathogenesis remains

Figure 2. Commonly mutated pathways in gastric cancers. NGS genomic studies have identified multiple pathways that contain genes that are mutated frequently in gastric cancers.
Wnt Signaling

Wnt signaling is a well-characterized signaling pathway that contributes to tumorigenesis in many cancers, especially of the gastrointestinal tract. The hallmarks of this pathway include increased β-catenin (encoded by CTNNB1) signaling, which often is facilitated by inactivating mutations in APC (encoded by APC). Pathogenic germline mutations in APC, which typically are truncating, lead to familial adenomatous polyposis, which is a condition that is associated with numerous colon polyps, and increases the risk of colon, gastric, as well as other cancers. The TCGA data showed that in nonhypermutated tumors, APC was mutated in 7% of gastric cancers whereas CTNNB1 was mutated in 4% of these tumors. In the ACRG, the mutation rates among all samples were 11% and 3% for APC and CTNNB1, respectively. Another regulator of Wnt signaling is the E3 ubiquitin ligase RNF43, which also was found to be mutated in 3% of nonhypermutated gastric cancers and 33% of the hypermutated gastric cancers in the TCGA data, as well as in other data sets, thus further showing the likely importance of the Wnt signaling pathway in a select set of gastric cancers.

Receptor Tyrosine Kinases

NGS has shown genomic alterations in the RTK pathways, which previously have been shown to be important in the carcinogenesis of gastric cancer. Clinically, this pathway already has garnered some success therapeutically, with the use of trastuzumab for the treatment of HER2-positive gastric cancer as well as the more recent approval of ramucirumab, a monoclonal antibody to vascular endothelial growth factor–receptor-2. As for new targets, PIK3CA, which encodes the p110α subunit of PI3K, is critical for RTK signaling and can be activated by specific point mutations. In the TCGA study, PIK3CA mutations were found in 12% of the nonhypermutated gastric cancers and in 40% of the hypermutated cancers, with a similar percentage of mutations (14%) found in the ACRG study. Based on the TCGA classifications, 80% of the EBV-positive as well as 42% of the MSI cancers had a mutation in PIK3CA. However, in EBV-positive cancers, the PIK3CA mutations were scattered throughout the gene rather than being clustered at classic activating positions, thus requiring further exploration into whether these noncanonical mutations are truly driver mutations of gastric carcinogenesis. The association of EBV-positive cancers with PIK3CA mutations also was appreciated in other reports as well. In addition to PIK3CA, the downstream RTK effector KRAS was mutated in 6% of nonhypermutated tumors in the TCGA study, and 8% of samples in the ACRG study. An additional mechanism of activating downstream RTK effectors includes gene amplification, which was observed with KRAS in 9% of gastric cancers in one series. Although mutations in the ERBB family of genes (including EGFR [ERBB1], HER2 [ERBB2], ERBB3, and ERBB4) were less frequent than PIK3CA or KRAS, their signaling pathways often were found to be activated by gene amplification in multiple different studies.

Other Mutated Genes

In addition to these groups of genes specific to defined pathways, there were also a number of other genes that were mutated in a significant percentage of gastric cancers. Focusing on the data from hypermutated cancers from the TCGA, there were a number of additional frequently mutated genes (>20%) that were not mutated significantly in the nonhypermutated tumors including CIC, ERBB3, PTPN23, VPS13A, BCO1L1, FBXW7, ZBTB20, and HDAC4. Among the nonhypermutated cancers from the TCGA, there were additional genes that were mutated significantly including SMAD4 (8%), which is involved in the transforming growth factor–β signaling pathway. In addition, MUC6, which was mutated in 6% of the TCGA gastric cancers, is important for the production of cytoprotective mucin, in which inactivation may increase the risk of mucosal injury and subsequent carcinogenesis.

Translating Genomic Data Into New Therapeutic Options for Gastric Cancer

Recent NGS studies undoubtedly have provided a wealth of data that has better characterized the genomic landscape of gastric cancer. Further validation of the new genomically based gastric cancer classification schemes as well as the novel gastric cancer driver mutations is needed and may be obtained through larger and more diverse NGS studies on gastric cancer samples. A recent analysis that combined multiple NGS studies for a total analysis of 544 gastric cancer samples showed significant mutational rates in 7 new genes (FBXW7, XIRP2, NBEA, COL14A1, CNBD1, AKAP6, and ITGAV) that had not been shown previously to be significantly mutated in gastric cancer.

More importantly, translating this genomic data into more effective treatments for gastric cancer remains the major challenge moving forward, and there are important translational questions along this line that must be addressed by the field. First, can genomically based classification schemes better stratify gastric cancers to enable use of more effective treatment regimens? The large genomic studies from the TCGA and ACRG, as well as other smaller studies, used NGS to develop novel, well-thought-out, genomically based classification schemes for gastric cancer. The novelty of these schemes are that they classify tumors based on genomic properties, rather than just histologic appearances. Conducting future trials to determine if these molecular classifications can better define response to various
treatment regimens is certainly important. For example, EBV-positive and MSI subtypes of gastric cancer have been shown to have increased expression of PD-L1 and therefore may be good candidates for treatment with immune checkpoint therapy with PD-1/PD-L1 blockade.\textsuperscript{10} In addition, treatment options that previously have been unsuccessful in the treatment of gastric cancer could be re-examined to determine if they may in fact be effective in a specific molecular subtype of gastric cancer.

Second, will targeting somatically mutated driver genes of gastric cancer improve treatment paradigms? Outside of the classic mutations in \textit{TP53} and \textit{CDH1}, recent NGS studies have shown numerous novel candidate driver mutations in gastric cancer. Although the frequency of many of these mutations is low, it remains to be determined whether specifically targeting the pathways in which these mutant genes are involved will serve as an effective therapeutic strategy in a select group of gastric cancers. For example, given the significant increase in \textit{PIK3CA} mutations in EBV-positive tumors,\textsuperscript{11} targeting this pathway in EBV-positive gastric cancers may prove to be effective. In addition, it also remains to be seen if any specific mutational profiles of gastric cancer will be able to help prognosticate response to therapy or overall survival.

Third, can gastric cancer–specific somatic sequencing panels be used to personalize therapy for gastric cancer? Given the dramatic reductions in costs associated with NGS, routine somatic sequencing of all gastric cancer tumor samples could soon become a reality. Although now gastric cancers have HER2 testing performed to decide on the use of trastuzumab, in the future there may be gastric cancer–specific panels of genes to somatically sequence that may help guide personalized therapy.

Fourth, are there applications of this genomic data that may help improve diagnostic capabilities in gastric cancer? Apart from exploitation of genomic vulnerabilities for therapeutic targeting, there also are conceptual potential applications of these data in molecular diagnostics, such as circulating tumor cells, circulating nucleic acids, exomes, and protein-based assays that deserve further exploration.

In conclusion, the field of gastric cancer genomics has been revolutionized by improvements in NGS technology and the widespread application of NGS to studying this cancer. The advances in this field have led to more advanced classification systems for gastric cancer as well novel driver mutations that may be important for its pathogenesis. However, this wealth of information ultimately will only be helpful to gastric cancer patients if it can be applied effectively to improve both treatment paradigms and survival for these patients, and thus there remains a significant amount of work that must be done.

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Conflicts of interest

The authors disclose no conflicts.

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