Editorial

Molecular classification of gastric cancer

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Gastric cancer is the world's third leading cause of cancer mortality and the most common cancer diagnosed in men in Japan (1). Clinical work-up of gastric cancer relies in part on imaging modalities, including endoscopic ultrasound and CT/PET, and pathologic analysis of tumor biopsies. Gastric cancer is clinically classified as early or advanced stage, with early disease confined to mucosa/submucosa and advanced carcinoma invading into muscularis propria and beyond (2). Gastric carcinoma is subdivided histologically into intestinal type, which is associated with intestinal metaplasia and H. Pylori infection, and diffuse type, which is often linked to familial genetic disorders, such as germline mutations of E-cadherin (CDH1) or mismatch repair genes (Lynch syndrome) (3). In 2010, the World Health Organization (WHO) recognized four major histologic patterns of gastric cancer (tubular, papillary, mucinous, and poorly cohesive), but these classifiers have little clinical utility. Recently, molecular profiling of gastric cancers identified potential driver genes for targeted therapy, such as amplified *ERBB2* (4). Clinical trials such as ToGA study found that trastuzumab (anti-Her2 antibody) plus standard chemotherapy demonstrated significantly improved overall survival in Her2-neu-positive patients compared to chemotherapy alone (5). While these results are encouraging, there is an urgent need to develop robust molecular classifiers of gastric cancer to guide clinical decision-making and tailored therapeutic development.

Recently, the Cancer Genome Atlas (TCGA) project reported a four subtype molecular classification of gastric cancer based on molecular profiling of 295 primary gastric adenocarcinomas (6). The cancers were profiled by copy number analysis (array-based), whole-exome sequencing, DNA methylation profiling (array-based), mRNA sequencing, microRNA sequencing, and/or reverse-phase protein array (RPPA). Roughly one-third of the samples were also profiled by whole genome sequencing. Unsupervised clustering of the data revealed that the gastric cancers could be sub-divided into four groups: (I) cancers with high EBV burden and DNA promoter hypermethylation; (II) cancers with microsatellite instability (MSI), high mutation rate, and promoter hypermethylation; (III) cancers with chromosomal instability (CIN) (i.e., high somatic copy number aberrations); and (IV) cancers with genomic stability (i.e., low copy number aberrations). For clinical decision making, gastric cancers can first be categorized by EBV positivity (group 1, 9% of cases), then by MSI-high status (group 2, 22% of cases), and the remaining cases can be distinguished by copy number aberrations into CIN tumors (group 3, 50% of cases) or genomically stable tumors (group 4, 50% of cases). The distributions of subtypes were similar in patients of East Asian and Western origin.

The EBV-high gastric cancers were largely found in males (81% of cases) and were mostly localized to the gastric fundus and body (7). The cancers were characterized by extreme DNA hypermethylation, distinct from the hypermethylation observed in MSI tumors. EBV-high tumors also showed distinct gene expression profile and mutation spectra compared to the other tumor subtypes. This included *CDKN2A* (*p16*^{INK4A}) promoter hypermethylation, *PIK3CA* mutation (80% of cases), and *ARID1A* mutation (55% of cases) (8). EBV-high tumors also displayed *BCOR* mutation (23% of cases) but only rare *TP53* mutations. Interestingly, EBV-high tumors showed amplification of a 9p24.1 locus, which contained *JAK2*, *CD274* (*PD-L1*), and *PDCD1LG2* (*PD-L2*).

The MSI-high cancers were associated with older age and female gender (56% of cases). These cancers displayed high

mutation rate (greater than 11.4 mutations per megabase) and there were ten genes significantly mutated by base substitution mutation in this group, including *TP53*, *KRAS*, *ARID1A*, *PIK3CA*, *ERBB3*, *PTEN*, and *HLA-B*. Additional genes were mutated by insertions/deletions, such as *RNF43*, *B2M*, and *NF1*. MSI-high cancers displayed alterations in major histocompatibility complex class I genes (such as *B2M* and *HLA-B*), likely for evasion of host immune response (9). While non-MSI-high (i.e., non-hypermutated) tumors also carried mutations in these genes, non-MSI-high tumors in addition displayed mutations in the β-catenin pathway (*APC* and *CTNNB1*), TGF-β pathway (*SMAD4*, and *SMAD2*) and MAPK pathway (*RASA1*, *ERBB2*).

The CIN tumors were largely localized to the gastroesophageal junction and cardia (65% of tumors) and were largely of intestinal-type histology. CIN tumors contained *TP53* mutations (71% of tumors) and displayed amplification of receptor tyrosine kinases, including *EGFR*, *ERBB2* and *ERBB3*. Other genes that were frequently amplified included *CCNE1*, *KRAS*, *MYC*, *CDK6*, *GATA4*, *GATA6*, and *ZNF217*, which are also amplified in other solid tumor types (10). In contrast, the genomically stable tumors were largely of the diffuse histology (73% of cases) and were associated with younger age of onset. Genomically stable tumors contained *ARID1A* mutations and were enriched for *CDH1* somatic mutations and *RHOA* mutations. Translocations that disrupt *RHOA* signaling were also identified, such as the *CLDN18* and *ARHGA26* interchromosomal translocation. Modulation of *RHOA* and its downstream effectors *ROCK1* and *mDIA* may contribute to the lack of cell cohesion seen in the diffuse tumor histology (11).

The molecular characterization of gastric cancer provides insight into personalized treatments for gastric cancer patients. The primary targets identified in EBV-positive tumors were PIK3CA, JAK2, and ERBB2, which have roles in cell proliferation, apoptosis and survival. *PIK3CA* encodes a catalytic subunit (p100α) of the PI3K signaling molecule, and the high incidence of *PIK3CA* mutations in EBV-positive gastric tumors could suggest a targeting strategy for PI3K inhibitors in this subgroup (12). JAK2 is a cytoplasmic tyrosine kinase which facilitates binding and phosphorylation of STATs to regulate cell proliferation, differentiation, and apoptosis. JAK inhibitors have shown clinical utility in oncology and their use may be warranted in EBV-positive gastric cancers (13). Interestingly, the immunomodulators *PD-L1* and *PD-L2* were also amplified in EBV-positive cancers, raising the possibility that PD-1/PD-L1 inhibitors may be targeted to this population.

Several RTK amplifications were observed in CIN tumors, including *EGFR*, *ERRB2*, *ERBB3*, *FGFR2* and *MET*. The *MET* gene displayed exon 2 skipping in approximately 30% of cases (correlating with increased activity), while 17% of cases exhibited skipping in exon 18 and/or 19. This provides a novel biomarker for anti-MET therapeutics, such as Rilotumumab (Amgen). Phase III trials for rilotumumab in combination with chemotherapy are currently underway for MET-positive gastric cancer patients (14). Other amplifications in the CIN sub-group include *VEGFA*, *KRAS/NRAS*, and *CDK6*. Recently, a human monoclonal antibody targeting VEGFR2 (Ramucirumab; Eli Lilly and Company) demonstrated improvement in overall survival in patients with advanced or metastatic gastric cancer (15). Further, as in EBV-positive tumors, amplification of *ERBB2* in CIN tumors may likely be a positive predictor of efficacy of HER2-targeted therapies, such as Herceptin. Cell cycle genes (CCNE1, CCND1, CDK6) were also amplified in CIN tumors, which offer additional targeting strategies for this tumor subtype. CDK4/6 inhibitors are currently in development for a number of cancers types.

In MSI-high tumors, a number of druggable pathways/targets were mutated, such as *PIK3CA*, *ERBB2*, *ERBB3*, and *EGFR*. *PIK3CA* mutations in MSI-high tumors were less dispersed than in EBV-positive tumors and instead occurred at higher incidence at exon 20. Given the high mutation rate of these cancers, the clinical utility of targeted therapeutics in this population remains to be shown. Likewise, few tractable targets were identified in genomically stable gastric cancers. Recurrent mutations were observed in RHOA, CLDN18, and CDH1, which are responsible for cell shape and cell-cell adhesion. While clinical development of novel inhibitors of these targets have been reported, such as the ongoing phase II clinical trial of a monoclonal CLDN18 antibody (Ganymed Pharmaceuticals), additional targets are likely to be found with continued mining of the gastric TCGA datasets.

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