Editorial

Gastric cancer—a convergence of genomic heterogeneity

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Gastric cancer (GC) is the 5th most common cancer worldwide and the 3rd leading cause of global cancer mortality (1). In 2012, an estimated 723,000 deaths were ascribed to this disease. The incidence of GC is known to vary widely across geographical regions, being particularly common in East Asian countries (e.g., South Korea, Japan and China) and parts of Latin America, while remaining relatively infrequent in Western countries (1). The underlying reasons for these geographical differences are likely multi-factorial, and may involve differences in infectious etiology (e.g., *Heliobacter pylori*, *Epstein Barr Virus*), environmental risk factors (diet, obesity), and population-specific differences in host genetic polymorphisms in genes such as *IL1β* and *TLR4* that can contribute to GC risk.

GC is a particularly deadly disease for many reasons. First, the disease typically presents late and most patients are diagnosed with advanced stage disease. With the exception of Japan and South Korea where the disease incidence is sufficiently high to warrant population-based screening, screening for early GC detection is not routinely performed in most countries (2). Second, there is as yet no universally accepted treatment regimen for GC, and different countries and treatment centers typically offer distinct treatment options. For example, patients in the USA with early GC are usually managed with adjuvant chemoradiation, while patients in Europe are treated with perioperative chemotherapy and in Japan GC patients have only recently begun to be managed with adjuvant chemotherapy as the standard-of-care. Third, GCs from individual patients are known to show a high degree of heterogeneity at the histologic and molecular level, which can likely influence disease aggressiveness and response to therapy. Most GCs are adenocarcinomas, and based on their microscopic appearance, several classification schemes for GC have been proposed. The most widely used GC classification system is the Lauren classification (3) which classifies GCs into "intestinal", "diffuse", and "mixed" subtypes. Other GC classification schemes include the World Health Organization classification, subdividing GCs into papillary, tubular, mucinous and poorly cohesive subtypes (4), and other classifications such as those of Ming and Goseki. A common limitation of these classification schemes is that there can be significant inter-observer variability between pathologists, often leading to difficulties in reproducibility.

The advent of molecular biology prompted scientists to derive a GC classification based on molecular criteria. Compared to classification schemes based on tumor morphology, classification schemes using molecular data have the potential advantage that they can be based on key driver alterations responsible for the tumor phenotype, which may suggest options for therapy. A good example of a clinically-useful molecular marker in GC is genomic amplification or overexpression of the HER2 receptor tyrosine kinase, occurring in 10% of GCs. In randomized clinical trials, it has been shown that HER2-positive GCs are treatable with the anti-HER2 monoclonal antibody traztuzamab (Herceptin) (5).

The quest to achieve a genomic classification of GC started in the early 2000s, when several investigators attempted to use emerging genomic technologies such as DNA microarrays to classify GC based on global patterns of gene expression (6-8). While informative in highlighting the widespread molecular heterogeneity of GC, the clinical relevance of the subtypes revealed in these early studies remained unclear. Nevertheless, the scientific contributions of these earlier studies should be appreciated, as they were able to reveal new genes related to GC prognosis (e.g.,

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PLA2G2A) and the role of these genes in modulating canonical oncogenic pathways such as Wnt signaling (8,9). More recently, newer studies have attempted to increase the number of samples profiled so as to better capture the inherent heterogeneity of GC (10) and integrating the resultant gene expression data with other levels of molecular information, such as copy number alterations and DNA methylation patterns (11). Meta-analysis approaches integrating data from multiple GC studies have also been reported, which have highlighted a role for the tumor microenvironment in influencing GC prognosis (12).

The recent study by the USA TCGA project represents the field's largest and most comprehensive attempt to achieve an integrated molecular classification scheme for GC (13). In the TCGA GC study, the investigators performed multi-tiered genomic profiling of 295 GCs using multiple molecular platforms, including exome and RNA-sequencing, methylation, miRNA and protein pathway profiling, and microsatellite instability (MSI) testing. >100 GCs were also subjected to whole-genome sequencing, which can highlight recurrent chromosomal structural variations. The tumors analyzed were surgical resection specimens from patients who had not received prior chemotherapy, and of the 295 GCs approximately 25% were from patients of Asian ethnic descent while the remaining 75% were from non-Asian patients. A number of the key findings from the TCGA study are now presented. Through studies such as these, the field is now seeing, for the first time, a convergence in our understanding of the exact nature of GC heterogeneity.

The main overall finding of the TCGA study is that there are four main molecular subtypes of GC, identified through unbiased genomic clustering of the combined molecular data. These subtypes are chromosomal instability (CIN), MSI, genomically stable (GS), and Epstein-Barr Virus (EBV). Supporting their robustness, variations in the type of clustering algorithm used still yielded these four subtypes. In terms of prevalence, the CIN, GSS, MSI and EBV groups represent approximately 50%, 22%, 20% and 8-9% of GCs respectively.

The CIN subgroup is marked by high levels of chromosomal aneuploidy, involving both broad chromosomal and also focal genomic amplifications and deletions. Tumors in this are enriched in *TP53* mutations, which may explain the observed chromosomal instability since one key function of the P53 pathway is to maintain genomic integrity. In terms of tumor morphology, the CIN GC subtype is enriched in Lauren's intestinal type tumors. More relevant to tumor biology, this subtype is associated with frequent amplifications in genes related to receptor tyrosine kinase RTK/RAS signaling, including *HER2*, *EGFR*, *MET*, *FGFR2*, and *RAS* genes (*KRAS/NRAS*). Supporting previous studies (14), many of these RTK/RAS amplifications occurred in a mutually exclusive fashion pointing to a common mutual dependence on RTK/RAS oncogenic signaling in these tumors. For many of these RTK/RAS components, several pharmacological therapies are already in clinical trials. Besides RTK/RAS genes, other genes recurrently amplified in CIN-positive GCs include *VEGFA*, a mediator of angiogenesis that is of particular note given the role of anti-angiogenic therapy in GC (15) and various transcription factors such as GATA factors (*GATA4*, *GATA6*). Interestingly, somatic copy-number amplification of GATA transcription factors appears to be largely restricted to gastrointestinal malignancies. It is possible that these transcription factors may represent "lineage-survival" factors, which can function to reawaken early developmental programs and collaborate with RTK/RAS signaling to drive GC tumorigenesis (16,17).

The second subtype (MSI) is characterized by a lack of overt chromosomal alterations but substantial microsatellite instability. Tumors in this subtype have a very high level of DNA mutations (50 mutations/Mb, compared to 5-10 mutations/Mb for the other three subtypes). MSI-positive GCs are also frequently associated with a high degree of DNA methylation, which can lead to G-CIMP (Gastric-CpG Island Methylator Phenotype) and promoter methylation-induced silencing of key driver genes such as *MLH1*. Interestingly, while *RAF* (*V600E*) mutations are commonly observed in MSI-positive colon cancer, such *RAF* mutations are not present in MSI-positive GCs, indicating that MSI GCs are not exactly identical to MSI colorectal tumors. Instead, the investigators discovered recurrent mutations in the *ERBB3* RTK in these hypermutated tumors, suggesting a role for RTK/RAS signaling in MSI-positive GC as well. Interestingly, MSI-positive hypermutated GCs, by virtue of producing a large complement of frame-shifted and altered proteins, may be particularly sensitive to the effects of the immune system. Reflecting the importance of evading the immune system in this GC subtype, MSI-positive GCs also exhibited mutations in key genes related to antigen presentation such as *HLA-B* and *B2M*, which may reduce the overall immunogenicity of MSI-positive cancer cells.

The third subtype, referred to as GS (or "genomically stable") corresponds to a subtype which lacks both high

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levels of chromosomal amplifications and microsatellite instability. The absence of these two features points to a new driver process of carcinogenesis in this tumor type. The TCGA authors found that unlike CIN and MSI GCs, GS-type GCs are associated with diffuse-type GC. At the histologic level, diffuse-type GCs are often recognized as loosely attached patches of tumor cells, intermingled amidst a highly infiltrative tumor stroma. Prior to this study, the only gene known to be disrupted in diffuse-type GCs was the E-cadherin *CDH1*, of which germline mutations are known to cause familial diffuse-type GC. In one of the key findings of the TCGA study, the authors discovered that besides *CDH1* mutations, GS-type GCs also displayed *RhoA* hot-spot mutations. This discovery of *RhoA* mutations has also independently confirmed by two other studies (18,19). The *RhoA* mutations all localize to the N-terminus, and are predicted to modulate downstream Rho signaling, a cellular pathway regulating cell shape and motility. Further supporting a role for altered Rho signaling in GS-type GCs, structural variant analysis also revealed recurrent *CLDN18-ARHGAP26* fusion genes in this subtype, where *ARHGAP26* is another regulator of the Rho signaling pathway. In GS-type GCs, the *RhoA*, *CLDN18-ARHGAP26*, and *CDH1* mutations all occurred in a large mutually exclusive manner, highlighting disruption of cell adhesion and motility as major deregulated processes in the gestation of GS-type/diffuse-type GCs. The 4th subtype, "EBV", is particularly fascinating. Nine to ten percent of GCs are caused by EBV infection, and previous studies studying EBV-positive GC have hinted that these GCs may have unique molecular features, chiefly the presence of an exceptionally high degree of DNA methylation (20). This hypermethylation pattern was indeed confirmed in the TCGA study, and remarkably among all tumor types studied by TCGA to date, EBV-positive GC appears to exhibit the highest levels of global DNA hypermethylation. It is intriguing to consider why these EBV-associated GCs might exhibit such high degrees of methylation, surpassing levels observed in even in G-CIMP tumors. It is possible that such hypermethylation might represent a cellular reaction to viral infection. Another striking feature of the EBV-positive GCs is that they appear enriched in *PIK3CA* mutations, although it should be noted that these mutations are not confined to the canonical activating exons 9 and 20 regions, but are distributed throughout the *PIK3CA* gene. An important area of future research will be to test if PIK3CA signaling is indeed consistently deregulated in EBV-positive GCs. EBV-positive GCs also typically exhibit a high lymphocytic infiltrate. Reflecting the importance of immune function in this subtype, the TCGA team discovered that EBV-positive GCs exhibited a specific 9p24 amplification, which contains the genes *JAK2*, a known master regulator kinase of immune cells, and amplification of genes encoding the immune checkpoint ligands *PD-L1* and *PD-L2*. By examining RNA-sequencing data, the authors further confirmed that *PD-L1* and *PD-L2* are highly expressed in these tumors. These results immediately raise the exciting possibility that EBV-positive GCs might be especially amenable to immune checkpoint modulation.

While the TCGA study primarily focused on abnormalities specific to each subtype, it is also important to note that there also exist molecular aberrations shared by multiple subtypes, which may reveal additional molecular vulnerabilities. For example, by combining data from multiple subtypes, genes such as the gastric mucin *MUC6* and *BCOR*, encoding a BCL6 corepressor, were highlighted as new recurrently mutated GC driver genes. *FGFR2* gene amplifications were observed in both the CIN and GS groups, suggesting that this specific RTK may play a tumorigenic role in both tumor subtypes, and represent a therapeutic target. Another interesting gene mutated across GC subtypes and with significant therapeutic implications is *RNF43* (a regulator of the Wnt pathway), associated with sensitivity to Wnt-pathway targeting compounds. Both MSI and EBV positive GCs also share several molecular commonalties, such as *ARID1A* mutations and CIMP (21,22) albeit to different degrees. MSI and EBV-positive GCs may thus represent a common "epigenetic subtype" that could be targeted by epigenetic compounds such as DNA demthylating agents (23).

Like most seminal studies, the success of this TCGA study raises a host of further questions to be answered. For example, while the molecular distinctive of these four subtypes is not in doubt, their clinical relevance remains unclear with respect to clinically important features such as patient prognosis and therapy selection. Preliminary analysis conducted by the TCGA authors suggests that patients belonging to the four subtypes may not show obvious differences in patient survival. Future work could thus focus on how these subtypes relate to patient outcomes in clinical trial cohorts, where the treatments and patient populations are more accurately defined. Another important question involves defining optimal treatment options for each subtype, based on their predicted molecular vulnerabilities. For example, as previously mentioned EBV-positive GCs may be more susceptible to immune checkpoint modulation

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and JAK inhibitors, while CIN tumors may be more susceptible to RTK/RAS directed therapies. Answering this question will require the generation and availability of accurate preclinical models, including cell lines, patient derived xenogratfs, and genetically engineered mouse models, that accurately recapitulate the molecular features of the four subtypes. Finally, it is also critical to address the extent to which these four subtypes are recapitulated in different patient populations. Analysis suggests that the prevalence of these four subytpes do not differ dramatically between GC patients of Asian and non-Asian origin, consistent with earlier studies showing a high degree of similarity in the repertoire of somatic alterations between Asian and non-Asian GC patients (24). However, it is also known that Asian GC patients do exhibit improved survival compared to non-Asian patients, and in recent randomized Phase III clinical trials, Asian and non-Asian patients were found to differ in clinical benefit despite standardized treatments (25). An unexplored question is thus to discover potential molecular differences between Asian and non-Asian GC populations.

In conclusion, the TCGA gastric study represents a major milestone in the GC field, as it has confirmed the existence of at least four robust and distinct molecular subtypes, underpinned by highly specific alterations at the mutation, gene amplification, and epigenetic level. Through the comprehensive nature of this molecular profiling effort, it has also enabled the integration of many earlier findings previously described in the literature, such as observation of EBV-associated hypermethylation and the molecular distinctiveness of MSI and CIN GCs. Finally, the study revealed a host of novel findings related to GC, most notably *RhoA* mutations in GS-subtype GCs and *PD-L1/L2* amplification in EBV-positive GC. In closing, while the number of GCs analyzed in this paper is extensive, it should be noted that more GCs are still being profiled by the TCGA team. As such the current study should be viewed as a "snapshot" of a rapidly evolving field. Undoubtedly, the TCGA study sets the stage for much future work in the GC field to come.

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References

1. Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 Lyon, France 2013. [International Agency for Research on Cancer]. Available online: http://globocan.iarc.fr
2. Leung WK, Wu MS, Kakugawa Y, et al. Screening for gastric cancer in Asia: current evidence and practice. Lancet Oncol 2008;9:279-87.
3. Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathol Microbiol Scand 1965;64:31-49.
4. Bosman FT, Carneiro F, Hruban RH, et al. WHO Classification of Tumours of the Digestive System. 4th ed. International Agency for Research on Cancer (IARC); 2010.
5. Bang YJ, Van Cutsem E, Feyereislova A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet 2010;376:687-97.
6. Boussioutas A, Li H, Liu J, et al. Distinctive patterns of gene expression in premalignant gastric mucosa and gastric cancer. Cancer Res 2003;63:2569-77.
7. Tay ST, Leong SH, Yu K, et al. A combined comparative genomic hybridization and expression microarray analysis of gastric cancer reveals novel molecular subtypes. Cancer Res 2003;63:3309-16.
8. Leung SY, Chen X, Chu KM, et al. Phospholipase A2 group IIA expression in gastric adenocarcinoma is associated with prolonged survival and less frequent metastasis. Proc Natl Acad Sci U S A 2002;99:16203-8.
9. Ganesan K, Ivanova T, Wu Y, et al. Inhibition of gastric cancer invasion and metastasis by PLA2G2A, a novel beta-catenin/TCF target gene. Cancer Res 2008;68:4277-86.
10. Tan IB, Ivanova T, Lim KH, et al. Intrinsic subtypes of gastric cancer, based on gene expression pattern, predict survival and respond differently to chemotherapy. Gastroenterology 2011;141:476-85, 485.e1-11.
11. Lei Z, Tan IB, Das K, et al. Identification of molecular subtypes of gastric cancer with different responses to PI3-kinase inhibitors and 5-fluorouracil. Gastroenterology 2013;145:554-65.
12. Wu Y, Grabsch H, Ivanova T, et al. Comprehensive genomic meta-analysis identifies intra-tumoural stroma as a predictor of survival in patients with gastric cancer. Gut 2013;62:1100-11.
13. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma.

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Nature 2014;513:202-9.
14. Deng N, Goh LK, Wang H, et al. A comprehensive survey of genomic alterations in gastric cancer reveals systematic patterns of molecular exclusivity and co-occurrence among distinct therapeutic targets. Gut 2012;61:673-84.
15. Fuchs CS, Tomasek J, Yong CJ, et al. Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (REGARD): an international, randomised, multicentre, placebo-controlled, phase 3 trial. Lancet 2014;383:31-9.
16. Sulahian R, Casey F, Shen J, et al. An integrative analysis reveals functional targets of GATA6 transcriptional regulation in gastric cancer. Oncogene 2014;33:5637-48.
17. Chia NY, Deng N, Das K, et al. Regulatory crosstalk between lineage-survival oncogenes KLF5, GATA4 and GATA6 cooperatively promotes gastric cancer development. Gut 2014. [Epub ahead of print].
18. Kakiuchi M, Nishizawa T, Ueda H, et al. Recurrent gain-of-function mutations of RHOA in diffuse-type gastric carcinoma. Nat Genet 2014;46:583-7.
19. Wang K, Yuen ST, Xu J, et al. Whole-genome sequencing and comprehensive molecular profiling identify new driver mutations in gastric cancer. Nat Genet 2014;46:573-82.
20. Matsusaka K, Kaneda A, Nagae G, et al. Classification of Epstein-Barr virus-positive gastric cancers by definition of DNA methylation epigenotypes. Cancer Res 2011;71:7187-97.
21. Wang K, Kan J, Yuen ST, et al. Exome sequencing identifies frequent mutation of ARID1A in molecular subtypes of gastric cancer. Nat Genet 2011;43:1219-23.
22. Zang ZJ, Cutcutache I, Poon SL, et al. Exome sequencing of gastric adenocarcinoma identifies recurrent somatic mutations in cell adhesion and chromatin remodeling genes. Nat Genet 2012;44:570-4.
23. Zouridis H, Deng N, Ivanova T, et al. Methylation subtypes and large-scale epigenetic alterations in gastric cancer. Sci Transl Med 2012;4:156ra140.
24. Su X, Zhan P, Gavine PR, et al. FGFR2 amplification has prognostic significance in gastric cancer: results from a large international multicentre study. Br J Cancer 2014;110:967-75.
25. Ohtsu A, Shah MA, Van Cutsem E, et al. Bevacizumab in combination with chemotherapy as first-line therapy in advanced gastric cancer: a randomized, double-blind, placebo-controlled phase III study. J Clin Oncol 2011;29:3968-76.

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