Gastric cancer is a complex disease that is caused by interactions among multiple genetic and extrinsic factors. The heterogeneous characteristics of gastric cancer make it difficult to select suitable treatment options for individual patients. According to a clinicopathologic perspective, gastric cancer is divided into two subtypes, intestinal or diffuse, which is also known as Lauren’s classification. This classification helps to understand the pathogenesis of gastric cancer, but it is insufficient as a predictor of disease prognosis and drug treatment. Thus, more elaborate subgrouping of individual patients is required to develop a personalized therapeutic regimen.

In efforts to stratify gastric cancer patients, recent next-generation sequencing (NGS) studies have identified previously unrecognized molecular subtypes of gastric cancer. As a representative study, The Cancer Genome Atlas (TCGA) network classified gastric cancer into four subtypes, including Epstein-Barr virus (EBV)-positive, microsatellite instable (MSI), genomically stable (GS), and chromosomal instability (CIN) subtypes (1). This study used both sequencing and array-based approaches by investigating exome sequences, copy-number alterations, gene expression, DNA methylation, and protein activities in gastric cancer (2). As a result, this classification provides the most well-defined gastric cancer molecular subtypes to date. Nonetheless, more research is needed to determine significantly mutated genes, druggable targets, or prognosticators that represent each subtype.

In a recent report published in Cancer Research, Li et al. attempted to define novel molecular subtypes of gastric cancer using methodologies different from those of TCGA (3). In contrast to previous clustering strategies where transcriptome or methylome profiles were generally used, this study used only mutation data for clustering. First, a classification was conducted based on mutation loads by analyzing a total of 544 gastric cancer patients combined from five previous whole genome or whole exome studies. This unsupervised clustering stratified gastric cancer into two subtypes, referred to as regular-mutated (2.4 mutations/Mb; range from 0 to 8.3) and hyper-mutated (20.5 mutations/Mb; range from 9.6 to 200.2). The hyper-mutated subtype from Li et al. overrepresented MSI patients, but this subtype appears to be slightly different from TCGA’s MSI subtype, as the hyper-mutated phenotype can be generated by defects of various genomic integrity maintenance mechanisms as well as mismatch repair, which is related to the MSI phenotype. Importantly, previous elegant studies from Rizvi et al. and Le et al. demonstrated that patients with high mutation loads showed sensitive responses to therapeutic blockade against immune checkpoints such as PD-1 (4,5). Thus, the MSI subtype from TCGA or the hyper-mutated subtype from Li et al. would clinically benefit from immune checkpoint blockade with PD-1 inhibitors (i.e., pembrolizumab) (5), suggesting the clinical relevance of the hyper-mutated subtype from Li et al.

The different mutation loads between subtypes indicate that tumors from different subtypes may go through different mutation processes during tumor evolution. In fact, mutation signatures between the regular- and hyper-mutated subtypes were distinct. Notably, the regular-mutated subtypes acquired six times more mutations at TpCpA/T sequences than the hyper-mutated subtype (3). Given that TpCpA/T is a sequence motif for the DNA cytosine deaminase APOBEC3B (6), this result suggested...
that APOBEC3B may be involved in the mutation loads of the regular-mutated or microsatellite stable (MSS) subtypes. Moreover, the number of APOBEC signature mutations positively and significantly correlated with the APOBEC3B mRNA level. However, these data were interpreted with caution because the APOBEC signature of the hyper-mutated subtype can be attenuated by other predominant mutation processes, such as a deficiency in mismatch repair.

Supporting this suspicion, the APOBEC3B mRNA levels were comparable between two subtypes. Therefore, the fact that the APOBEC signature more strongly contributes to the mutation processes of the regular-mutated subtype may be true, but we cannot say that the APOBEC signature is not important in the hyper-mutated subtype.

Li et al. further clustered the regular-mutated subtype into two groups, referred to as C1 and C2. This clustering was performed based on a binary mutation status matrix of significantly mutated genes that were found by three algorithms, MutSigCV, MutSigCL, and MutSigFN (7).

The C1 cluster contained a significantly high proportion of TCGA's CIN subtype patients, whereas the C2 cluster was enriched with TCGA's GS subtype patients. Reflecting these patterns, TP53 mutations, which are highly detected in the CIN subtype, were enriched in the C1 cluster, and RHOA and CDH1 mutations, which are the representative mutations of the GS subtype and diffuse-type gastric cancer, were overrepresented in the C2 cluster. More importantly, the C2 cluster displayed poorer prognostic outcome than the C1 cluster, suggesting that the high proportion of GS subtype or diffuse-type GC within the C2 cluster could result in poor survival rates. However, multivariate analysis revealed that C1/2 clusters alone have prognostic value independent of Lauren's classification and the TNM staging. For easier C1/2 clustering, Li et al. defined eight genes (TP53, ARID1A, CDH1, PIK3CA, XIPR2, APC, ERBB2, and RHOA) as a classifier. Because the eight-gene classifier showed enough power to discriminate C1/2 clusters and acted as a significant independent prognostic marker, this method could be applied to gastric cancer patients for clinical uses.

Mutation data combined from 544 gastric cancer patients enabled detection of previously unrecognized significantly mutated genes with a high statistical power. In the regular-mutated subtype, 31 significantly mutated genes were identified. In addition to well-known cancer driver genes such as TP53, ARID1A, CDH1, PIK3CA, APC, RHOA, SMAD4, ERBB4, KRAS, ERBB2, and CTNNB1, Li et al. found unreported significantly mutated genes, including XIPR2, NBEA, COL14A1, AKAP6, CNBD1, and ITGAV. NBEA is located on chromosome 13 and is frequently deleted in multiple myeloma (8,9). COL14A1 is down-regulated by promoter hyper-methylation in renal cell carcinoma (10). AKAP6 and CNBD1 are mutated in esophageal adenocarcinoma and gastroenteropancreatic neuroendocrine tumors, respectively (11,12). The functional roles of XIPR2, compared to those of other genes, have not been elucidated in the cancer field. Because XIPR2 is one of the eight genes used in the classifier for C1/2 clustering, preferential functional validation is required to demonstrate the activity and role of XIPR2 in gastric cancer.

Although this study revealed novel aspects of gastric cancer subtypes, there are several limitations. One limitation of this study is the lack of functional validation of the novel significantly mutated genes that were identified. Another limitation is that the statistical power to detect less frequent mutations (below 2%) is still not high enough with the current 544 samples. Interestingly, Lim et al. recently analyzed a total of 629 gastric cancer patients and identified several significantly mutated genes (i.e., DHFR, GHSR, GLI3, GRM8, KIF2B, and PREX2) (2) not reported in the Li et al. analysis. With more samples, more significantly mutated genes are expected to be discovered. As another limitation, it is unclear whether the subtyping from Li et al. is superior to the TCGA's gastric cancer subtypes. For instance, the C1 cluster from Li et al. contained a significantly enriched number of ARID1A and PIK3CA mutations, which were included in TCGA's EBV subtype, suggesting that the C1 cluster may be a mixture of TCGA's EBV subtype and other subtypes. Even four subtypes from TCGA may oversimplify the complexity of gastric cancer. Therefore, further efforts to categorize each molecular subtype from TCGA and Li et al. may be required to facilitate precision medicine of gastric cancer patients.

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Footnote

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