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REVIEW ARTICLE

Gastric molecular classification and practice in immunotherapy

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Abstract

Gastric cancer (GC) is a highly heterogeneous malignancy with a high incidence worldwide; the prevalence of GC is relatively higher in China than in other countries. Treatment of advanced GC has been slow to develop due to lack of a proper classification system to guide clinical practice. With the development of molecular biology techniques, the molecular classification of GC has been established and may have applications in guiding precise and personalized therapy. To date, three or four molecular classifications for GC have been recognized; these include Singapore, the Cancer Genome Atlas (TCGA) Research Network, and Asian Cancer Research Group (ACRG) classifications. Here, we review the development of molecular classifications and characteristics of different subtypes, and discuss the applications of molecular classifications in clinical practice, with a focus on immunotherapy.

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Gastric cancer (GC) is the third leading cause of global cancer-related mortality, and the prevalence of GC in China is higher than that in other countries around the world, with morbidity and mortality rates of 42.5% and 45%, respectively [1]. According to cancer statistics for 2015 published by China's National Cancer Center, GC ranks second of all tumors in estimated morbidity and mortality rates and ranks first for both parameters in rural areas [2].

Treatment methods for advanced GC have been slow to develop, and chemotherapy remains the backbone of therapeutic strategies. In contrast to melanoma, non-small cell lung cancer, and breast cancer, targeted therapies and immunotherapies have not yet been extensively applied. This discrepancy could be related to the lack of proper molecular classifications to guide clinical practice. Traditional clinicopathological classification systems have mainly included Lauren and World Health Organization (WHO) classifications. These classifications depend on cell and tissue morphology observed under a microscope and they are influenced by many subjective factors. Moreover, classifying results cannot accurately reflect the biological behaviors of tumors [3]. With the development of genomics, transcriptomics, proteomics, and metabolomics, molecular classification of GC has emerged and it shows potential in guiding precise, personalized therapy.

Molecular classification

The concept of tumor molecular classification was first proposed in the 1990s and referred to classifying tumors using information obtained from comprehensive molecular analysis [3]. After this concept was proposed, hundreds of thousands of studies have investigated molecular classifications in different types of tumors, generating huge amounts of data and facilitating the establishment of several different molecular classifications. To date, three or four GC molecular classifications have been recognized.

In 2011, Tan et al. identified two intrinsic GC subtypes (G-INT and G-DIF) by analyzing the gene expression profiles of 37 GC cell lines and validated these subtypes in primary tumors from 521 patients in four independent cohorts. They found that two intrinsic subtypes were associated with patient survival and response to chemotherapy [4].

In 2013, Lei *et al* compared gene expression patterns among 248 gastric tumors and identified three major subtypes (proliferative, metabolic, and mesenchymal). The subgroups exhibit differences in molecular and

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genetic features and response to therapy and have also been shown to be associated with Lauren and Tan classifications. Cancer cells of the metabolic subtype are more sensitive to 5-fluorouracil, whereas cancer cells of the mesenchymal subtype include cells with features of cancer stem cells and are particularly sensitive to phosphatidylinositol 3-kinase/AKT/mammalian target of rapamycin (mTOR) inhibitors *in vitro*, providing important insights into clinical treatment strategies [5].

In 2014, the Cancer Genome Atlas (TCGA) Research Network published their molecular classification in Nature [6]. After comprehensive molecular evaluation of 295 primary gastric adenocarcinomas using single nucleotide polymorphism array somatic copy-number analysis, whole-exome sequencing, mRNA sequencing, miRNA sequencing, array-based DNA methylation profiling, and reverse-phase protein arrays, they proposed a molecular classification dividing GC into four subtypes: tumors positive for Epstein-Barr virus (EBV+), microsatellite unstable tumors (MSI), genomically stable tumors (GS), and tumors with chromosomal instability (CIN). The clinical and molecular characteristics are summarized in Table 1.

In 2015, the Asian Cancer Research Group (ACRG) published a new molecular classification in Nature Medicine [7]. The group procured 300 primary GC tumor specimens, and, through analysis of data from next-generation sequencing, they classified GC into four subtypes: mesenchymal-like type (epithelial mesenchymal transition [EMT]), microsatellite-unstable type (MSI), p53 (TP53)-active type (MSS/p53+), and TP53-inactive type (MSS/p53–). ACRG also validated these subtypes in independent cohorts in order to provide a consistent and unified framework for further clinical and preclinical translational research. Characteristics of the four subtypes are summarized in Table 2.

Practice in immunotherapy

Current approaches to GC management largely consist of endoscopic detection, followed by gastrectomy and chemotherapy (CT) or chemoradiotherapy (CRT) in a neoadjuvant or adjuvant setting. For advanced GC, the efficacy of CT and CRT is not satisfactory. One of the key reasons for observed heterogeneity in response to treatments is a one-size-fits-all approach to treatment. With the development of next-generation sequencing and bioanalysis techniques for large datasets, we are entering the age of precision medicine [8]. The aim of precision medicine is to improve efficacy and reduce adverse reactions through screening patients for genes, biomarkers, phenotypes, and social psychological characteristics [9]. Traditional clinicopathological classifications cannot fully and accurately reflect the biological heterogeneity of tumors; therefore, the appropriateness of some treatment strategies is unclear. The development and establishment of molecular classifications may provide a solid foundation for precision treatment.

Targeted therapy and immunotherapy are the two important components of precision medicine and show some overlap. After the ToGA study demonstrated that trastuzumab in combination with chemotherapy for human epidermal growth factor receptor 2 (HER2)-positive advanced GC as a first-line regimen was superior to chemotherapy alone [10], several receptor tyrosine-kinase (RTK)-targeted drugs were investigated in GC. However, the majority of these studies yielded negative results. A retrospective analysis suggested that one important reason for the failure is the absence of biomarker-driven trials or the methodology of biomarker selection [11]. In summary, tumor cell-targeted therapies have not been sufficiently established in GC, and further studies are needed.

As has been observed in other cancer types, the use of immunotherapy approaches may improve outcomes in patients with GC. Generally speaking, immunotherapy includes active immunotherapy (e.g., cancer vaccines and immunogenes), passive immunotherapy (e.g., adoptive immune cell transfer and some monoclonal antibodies), and nonspecific immunomodulator therapy (e.g., cytokines and checkpoint inhibitors) [12–14]. Checkpoint inhibitor therapy is currently a research hotspot that is relatively mature and well recognized, and molecular classification may have great potential value in guiding clinical practice in this field. Using genomic technology in GC in an effort to improve our understanding and the stratification of GC on a genetic and molecular level, TCGA classification has revealed that patients with EBV-positive and MSI subtypes may be the appropriate population for immunotherapy approaches. EBV-positive tumors, possibly derived from viral stimulation, may show amplification of genes that encode the immunosuppressant proteins programmed death ligand (PDL) 1 and 2. KEYNOTE-012 and CheckMate-032 trials have suggested a trend toward improved response rates and progression-free survival (PFS), with higher levels of PD-L1 overexpression, although the validity of PD-L1 as a robust biomarker of response should be confirmed in additional studies [12, 15, 16]. The MSI subgroup is characterized by gene promoter hypermethylation and displays a high mutational load, including alterations in major histocompatibility complex (MCH) class I genes.

To date, checkpoint inhibitors targeting PD-1, PD-L1, and T lymphocyte antigen (CLTA)-4 have had a major impact on clinical practice. These three types of checkpoint inhibitors have been evaluated in multiple tumor types with confirmed responses in GC.

Table 1 TCGA classification and characteristics

Subtype	Clinicopathological characteristics	Percentage (%)	Molecular characteristics
EBV+	Tend to be male, present in the gastric fundus or body	9	Mutations in *PI3KCA* (80% nonsilent), *ARID1A*, *BCOR*; rare *TP53* mutations; EBV-CIMP; higher prevalence of DNA hypermethylation; *CDKN2A* ($p16^{INK4A}$) hypermethylation; lack *MLH1* hypermethylation; *JAK2*, *ERBB2*, *CD274 (PD-L1)*, *PDCD1LG2 (PD-L2)* amplification; PD-L1/2 overexpression; immune cell signaling
MSI	Tend tobe female, diagnosed at relatively older ages	22	MSI-high status; hypermutations (*PIK3CA*, *ERBB3*, *ERBB2*, *EGFR*, *B2M*, *HLA-B*); lack targetable amplification; no *BRAF* V600E hmutation; hypermethylation; *MLH1* silencing (*MLH1* promoter hypermethylation); gastric-CIMP; mitotic pathway
GS	Diagnosed at an earlier age, diffuse histology	20	Mutations in CDH1, RHOA; CLDN18-ARHGAP fusion; cell adhesion; few other clear treatment targets.
CIN	Elevated frequency in the geal gastroesopha junction/cardia, intestinal histology	50	Genomic amplification of RTKs, RTK-RAS activation; elevated phosphorylation of EGFR; recurrent amplification of the gene encoding ligand VEGFA; *TP53* mutation (71%); elevated p53 expression.

PI3KCA: phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α; ARID1A: AT-rich interactive domain-containing protein 1A; BCOR: BCL-6 corepressor; CIMP: CpG island methylator phenotype; CDKN2A: cyclin dependent kinase inhibitor 2A; MLH1: mutL homolog 1; JAK2: Janus kinase 2; ERBB2: erb-b2 receptor tyrosine kinase 2; PDCD1LG2: programmed cell death 1 ligand 2; PD-L1/2: programmed dead ligand 1/2; EGFR: epidermal growth factor receptor; B2M: beta-2-microglobulin; HLA-B: human leukocyte antigen-B; CDH1: cadherin 1; RHOA: Ras homolog A; CLDN18: Claudin-18; ARHGAP: Rho GTPase activating protein; RTKs: receptor tyrosine kinases; VEGFA: vascular endothelial growth factor A

Table 2 ACRG classification and characteristics

Subtype	Clinicopathological characteristics	Percentage (%)	Molecular characteristics
EMT	Diffuse-type predominant (> 80%); younger age; poorer prognosis; higher chance of recurrence (63%); most recurrence for peritoneal seeding	15.3	Lower number of mutation events; less CDH1 expression; no RHOA mutations
MSI	Intestinal subtype (60%); predominantly in the antrum, diagnose at early stage; best prognosis; lower chance of recurrence (23%); higher percentage of liver-limited metastasis recurrence	22.7	Loss of MLH1; DNA hypermutation; hypermutation, such as mutations in KRAS, PI3K-PTEN-mTOR pathway, ALK, and ARID1A genes; enrichment of PIK3CA H1047R mutations
MSS/p53+	Moderate prognosis; more frequent EBV infection	26.3	p53 activation; relatively higher prevalence of mutations in APC, ARID1A, KRAS, PIK3CA, SMAD4
MSS/p53–	Moderate prognosis	35.7	Highest prevalence of TP53 mutations; recurrent focal amplifications in ERBB2, EGFR, CCNE1, CCND1, MDM2, ROBO2, GATA6, and MYC with corresponding increases in mRNA and protein expression levels

CDH1: cadherin1; RHOA: Ras homolog gene family, member A; MLH1: mutL homolog 1; KRAS: kirsten rat sarcoma viral oncogene homolog; PI3K: phosphatidylinositol-4,5-bisphosphate 3-kinase; PTEN: phosphatase and tensin homolog; mTOR: mammalian target of rapamycin; ALK: anaplastic lymphoma kinase;ARID1A: AT-rich interactive domain-containing protein 1A; PIK3CA: phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α; APC: amino acid-polyamine-organocation; SMAD4: mothers against decapentaplegic homolog 4; ERBB2: human epidermal growth factor receptor 2; EGFR: epidermal growth factor receptor; CCNE1: cyclin E1; CCND1: cyclin D1; MDM2: mouse double minute 2 homolog; ROBO2: Roundabout homolog 2; GATA6: GATA-binding factor 6

Anti-PD-1 drugs

Pembrolizumab is a highly specific, humanized monoclonal IgG4 antibody against PD-1. Its activity has been and is being investigated in many phase I to III clinical trials, including GC cohorts, investigating drug therapy or use in combination with chemotherapy or other monoclonal antibodies targeting HER2 or vascular endothelial growth factor receptor 2 (VEGFR2) (ClinicalTrials.gov identifier: NCT02335411 [KEYNOTE 059], NCT02370498 [KEYNOTE 061], NCT0249458 [KEYNOTE 062], NCT02443324, NCT02563548, NCT02318901) [17]. In the phase Ib KEYNOTE 012 trial, 39 patients treated with pembrolizumab showed an overall response rate (ORR) of 22% by investigator review, with a 6-month PFS of 24% and median duration of response of 6 months [15]. Another monoclonal antibody against

PD-1, nivolumab, was investigated in the phase Ib/II CheckMate 032 trial, in which the activity of single-agent nivolumab or nivolumab plus ipilimumab (an anti-CTLA-4 monoclonal antibody) was explored in multiple tumor types, with the initial report on the GC cohort presented at GI ASCO 2016 [16, 18]. Irrespective of PD-L1 status, patients were treated with single-agent nivolumab, and the ORR was 14%, with a median duration of response of 7.1 months. Further analysis showed that the ORRs in patients with PD-L1-positive (≥ 1% cutoff) and -negative tumors were 27% and 12%, respectively [12]. Two trials of nivolumab are currently ongoing (ClinicalTrials.gov identifier: NCT01928394 and NCT02267343).

Anti-PD-L1 drugs

Three anti-PD-L1 antibodies, i.e., atezolizumab (MPDL3280A), dervalumab (MEDI4736), and avelumab (MSB0010718C), were evaluated in GC and showed confirmed responses [19–21]. In a phase Ib trial, avelumab in two GC cohorts achieved ORRs of 15% with a median PFS of 11.6 weeks and 7.3% with a median PFS of 14.1 weeks [21].

Anti-CTLA-4 drugs

Tremelimumab and ipilimumab are two anti-CTLA-4 monoclonal antibodies tested in several trials. In a small phase II trial, tremelimumab was used as second-line therapy for GC, but the ORR (5%) and median overall survival (OS; 4.8 months) did not meet the expected results [22]. A phase Ib/II trial to combine tremelimumab and dervalumab in refractory GC is ongoing (ClinicalTrials.gov identifier: NCT02340975). In a recently completed phase II trial, ipilimumab was used as a maintenance drug after first-line CT and showed a shorter PFS (2.9 versus 4.9 months) but longer OS (16.8 versus 12.1 months), but the longer OS did not reach statistical significance (NCT01585987). The results of the CheckMate-032 trial for the combination of ipilimumab and nivolumab and the NCT01928394 trial for the combination of ipilimumab and nivolumab for refractor GC have not yet been published [16, 23].

Discussion

From 2011 to 2015, GC molecular classifications were rapidly established; TCGA and ACRG classifications are the most commonly used classifications and provide important information for molecular diagnosis, personalized therapy, and development of targeted and immunotherapy drugs. Molecular classifications can permit stratification of patients according to genomic and proteomic information, providing insights into clinical guidance. In theory, it is more reasonable to develop targeted therapies for diseases with the same molecular aberrations than to treat cancers with similar morphologies using CT. Thus, we predict that traditional treatment strategies based on tumor phenotypes will be replaced by precision medicine based on features of genomic aberrations [3].

Targeted therapy in GC has only succeeded in a few trials; this lack of efficacy can be attributed to the highly heterogeneous nature of GC caused by protein expression, gene amplification, and gene mutations and to insufficient selection of patient groups by biomarkers. As immunotherapy gradually becomes a major research focus, similar problems will arise. Immune responses are dynamic, and there is still a lack of consensus on optimal assaying techniques, such as for adequate definition of PD-L1 positivity, with trials using differing antibodies and staining cut-off points [24]. Several studies have discussed immune-related gene expression signatures as promising biomarkers [25–26]. Based on the tumor microenvironment, a framework for classifying tumors according to tumor-infiltrating lymphocytes (TILs) and PD-L1 expression has been proposed [27]. However, all of these ideas must be evaluated in further studies.

Additionally, problems associated with resistance mechanisms to targeted therapies and immunotherapies, alterations in molecular phenotypes, activation of bypass signaling, advantages and disadvantages of monoclonal antibodies and small molecular inhibitors, concomitant and combined medicines for targeted therapy and immunotherapy or traditional therapy, and understanding and targeting of the tumor microenvironment are all linked to molecular changes. Thus, further studies are required to obtain critical genomic, transcriptomic, proteomic, and metabolomic data.

A macroscopic description of diseases will not cover all the characteristics of different groups, although a driver gene can exhibit similarities in a series of diseases. The development of molecular biology techniques may help us to identify new methods to recognize and diagnose sickness. We predict that treatment patterns for GC and all other tumors may be replaced with personalized therapies based on molecular classifications, allowing the realization of truly meaningful precision medicine.

Conflicts of interest

The authors indicated no potential conflicts of interest.

References

1. Ferlay J, Soerjomataram I, Dikshit R, *et al*. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer, 2015, 136: 359–386.
2. Chen W, Zheng R, Baade PD, *et al*. Cancer statistics in China, 2015. CA Cancer J Clin, 2016, 66: 115–132.
3. Yu YY. Evaluation of traditional pathological classification at molecular classification era for gastric cancer. Chin J Gastrointest Surg, 2014, 17: 18–20.

4. 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: 476–485.
5. 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: 554–565.
6. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. Nature, 2014, 513: 202–209.
7. Cristescu R, Lee J, Nebozhyn M, *et al*. Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. Nat Med, 2015, 21: 449–456.
8. Collins FS, Varmus H. A new initiative on precision medicine. N Engl J Med, 2015, 372: 793–795.
9. Jameson JL, Longo DL. Precision medicine--personalized, problematic, and promising. N Engl J Med, 2015, 372: 2229–2234.
10. 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: 687–697.
11. Fontana E, Smyth EC. Novel targets in the treatment of advanced gastric cancer: a perspective review. Ther Adv Med Oncol, 2016, 8: 113–125.
12. Davidson M, Chau I. Immunotherapy for oesophagogastric cancer. Expert Opin Biol Ther, 2016, 16: 1197–1207.
13. Butterfield LH. Cancer vaccines. BMJ, 2015, 350: h988.
14. Melero I, Gaudernack G, Gerritsen W, *et al.* Therapeutic vaccines for cancer: an overview of clinical trials. Nat Rev Clin Oncol, 2014, 11: 509–524.
15. Muro K, Bang YJ, Shankaran V, *et al.* Relationship between PD-L1 expression and clinical outcomes in patients (Pts) with advanced gastric cancer treated with the anti-PD-1 monoclonal antibody pembrolizumab (Pembro; MK-3475) in KEYNOTE-012. 2015.
16. Le DT, Bendell JC, Calvo E, *et al*. Safety and activity of nivolumab monotherapy in advanced and metastatic (A/M) gastric or gastroesophageal junction cancer (GC/GEC): Results from the CheckMate-032 study. 2016.
17. Muro K, Bang Y, Shankaran V, *et al*. A phase 1B study of pembrolizumab (Pembro; MK-3475) in patients (PTS) with advanced gastric cancer. Ann Oncol, 2014, 25 (suppl 4).
18. Callahan MK, Bendell JC, Chan E, *et al*. Phase I/II, open-label study of nivolumab (anti-PD-1; BMS-936558, ONO-4538) as monotherapy or combined with ipilimumab in advanced or metastatic solid tumors. J Clin Oncol, 2014.
19. Herbst RS, Gordon MS, Fine GD. A study of MPDL3280A, an engineered PD-L1 antibody in patients with locally advanced or metastatic tumors. 2013.
20. Segal NH, Antonia SJ, Brahmer JR, *et al.* Preliminary data from a multi-arm expansion study of MEDI4736, an anti-PD-L1 antibody. Journal of Clinical Oncology. 2014.
21. Chung HC, Arkenau HT, Wyrwicz L, *et al*. Avelumab (MSB0010718C; anti-PD-L1) in patients with advanced gastric or gastroesophageal junction cancer from JAVELIN Solid Tumor phase 1b trial: analysis of safety and clinical activity. 2016.
22. Ralph C, Elkord E, Burt DJ, *et al.* Modulation of lymphocyte regulation for cancer therapy: a phase II trial of tremelimumab in advanced gastric and esophageal adenocarcinoma. Clin Cancer Res, 2010, 16: 1662–1672.
23. Janjigian YY, Bendell J, Calvo E, *et al*. Phase 1/2, Open-Label Study of Safety and Activity of Nivolumab Alone or With Ipilimumab in Advanced and Metastatic Gastric Cancer: CheckMate 032. 2016.
24. Topalian SL, Taube JM, Anders RA, *et al*. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. Nat Rev Cancer, 2016, 16: 275–287.
25. Ribas A, Robert C, Hodi FS, *et al*. Association of response to programmed death receptor 1 (PD-1) blockade with pembrolizumab (MK-3475) with an interferon-inflammatory immune gene signature. J Clin Oncol, 2015, 33: 3001.
26. Bang YJ, Chung HC, Shankaran V, *et al*. Clinical outcomes and their correlation with gene expression in patients with advanced gastric cancer treated with pembrolizumab (MK-3475): KEYNOTE-012. Ann Oncol, 2015, 26: 117–121.
27. Teng MW, Ngiow SF, Ribas A, *et al.* Classifying cancers based on T-cell infiltration and PD-L1. Cancer Res, 2015, 75: 2139–2145.

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