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Dissection of gastric cancer heterogeneity for precision oncology

Shamaine Wei Ting Ho[1,2] | Patrick Tan[1,2,3,4,5,6]

[1]Cancer Science Institute of Singapore, National University of Singapore, Singapore

[2]Cancer and Stem Cell Biology Program, Duke-NUS Medical School, Singapore

[3]Cancer Therapeutics and Stratified Oncology, Genome Institute of Singapore, Singapore

[4]SingHealth/Duke-NUS Institute of Precision Medicine, National Heart Centre Singapore, Singapore

[5]Cellular and Molecular Research, National Cancer Centre, Singapore

[6]Singapore Gastric Cancer Consortium, Singapore

Correspondence
Patrick Tan, Duke-NUS Medical School, Singapore.
Email: gmstanp@duke-nus.edu.sg

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Abstract

Gastric cancer (GC) remains the fifth most prevalent cancer worldwide and the third leading cause of global cancer mortality. Comprehensive -omic studies have unveiled a heterogeneous GC landscape, with considerable molecular diversity both between and within tumors. Given the complex nature of GC, a long-sought goal includes effective identification of distinct patient subsets with prognostic and/or predictive outcomes to enable tailoring of specific treatments ("precision oncology"). In this review, we highlight various approaches to molecular classification in GC, covering recent genomic, transcriptomic, proteomic and epigenomic features. We pay special attention to the translational significance of classifier systems and examine potential confounding factors which deserve further investigation. In particular, we discuss recent advancements in our knowledge of intra-subtype, intra-patient and intra-tumor heterogeneity, and the pivotal role of the tumor stromal microenvironment.

KEYWORDS
gastric cancer, molecular classification, translational research, tumor heterogeneity, tumor microenvironment

1 | INTRODUCTION

In 2018, gastric cancer was responsible for an estimated one million new cases and 781 000 deaths, representing a significant unmet clinical need.[1] Unravelling the intricate biology underlying GC etiology and progression is fundamental to combating this highly heterogeneous disease. Traditionally, GC classification has been based on histopathological and morphological features. First described by Lauren in 1965, GC can be divided into IGC, DGC and mixed/indeterminate subtypes.[2] These subtypes are known to differ in terms of risk factors and clinical prognosis, where patients with DGC typically experience poor prognosis, poor response to treatment and lower overall survival.[3-6] More recently, new classifications have been proposed, such as the WHO and the closely related Japanese

Abbreviations: ACRG, Asian Cancer Research Group; BC, breast cancer; CAF, cancer-associated fibroblast; CGI, CpG island; ChIP-seq, chromatin immunoprecipitation sequencing; CIMP, CpG island methylator phenotype; CIN, chromosomal instability; CRC, colorectal cancer; CTC, circulating tumor cells; ctDNA, circulating tumor DNA; DGC, diffuse-type gastric cancer; dMMR, mismatch repair deficient; EBV, Epstein-Barr virus; EGC, early gastric cancer; EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; EP, epithelial phenotype; FGFR2, fibroblast growth factor receptor 2; GC, gastric cancer; G-DIF, genomic diffuse; G-INT, genomic intestinal; GS, genomically stable; HDGC, hereditary diffuse-type gastric cancer; HSGAG, heparan sulfate glycosaminoglycans; IGC, intestinal-type gastric cancer; IO, immuno-oncology; MET, MET proto-oncogene, receptor tyrosine kinase; MP, mesenchymal phenotype; MSI, microsatellite instability; MSS/EMT, microsatellite stable with EMT phenotype; PD-L1, programmed death-ligand 1; PD-L2, programmed death-ligand 2; RTK, receptor tyrosine kinase; sCNA, somatic copy number alterations; TCGA, The Cancer Genome Atlas; TIL, tumor-infiltrating lymphocyte; TME, tumor microenvironment; WHO, World Health Organization.

classification, dividing GC into tubular, papillary, mucinous, poorly cohesive/differentiated and signet ring cell subtypes.[7,8] Unfortunately, classifications based on morphology are unable to identify actionable molecular targets. To address this deficiency, high-throughput, large-scale molecular profiling has led to various molecular-based classifications in a bid to uncover subtype-specific dependencies, which may be exploited for therapeutic interventions. Herein, we review the current landscape of molecular classifications in GC, highlighting important aspects relevant to clinical utility. In addition, we discuss confounding factors that could influence the effective use of such classifiers, including tumor heterogeneity and the stromal microenvironment.

2 | MULTIFACETED MOLECULAR CLASSIFICATION OF GC

Comprehensive molecular characterization at the genomic and transcriptomic levels has led to the identification of distinct GC subtypes (Figure 1). More recently, epigenomics has entered the field, with increasing evidence of GC subtypes being characterized by distinct epigenetic hallmarks.[9] An integration of such orthogonal information was best exemplified by TCGA, which led to a classification consisting of four subtypes: EBV, MSI, CIN and GS.[10] These subtypes showed unique molecular features that could potentially guide therapeutic decisions, and have been shown to have prognostic significance, with EBV being associated with the best prognosis, and GS with the worst.[11]

2.1 | Genomics

The genomic landscape of GC has been extensively surveyed, culminating in a near-complete list of alterations including mutations, sCNA and structural variants (reviewed in detail elsewhere[12,13]). Among recurrently mutated genes, *TP53* shows the most frequent mutations, associated with the CIN subtype characterized by a high degree of aneuploidy.[10,14] Other well-known GC driver genes include *ARID1A*, *PIK3CA*, *CDH1* and *RHOA*, the latter two being enriched in Lauren's DGC or TCGA's GS subtype tumors.[10,15] Genomic characterization of the DGC and/or GS subtype has been challenging, owing to their relatively high stromal infiltration and low tumor purity. In this respect, Nanki and colleagues made use of patient-derived GC organoids of pure tumor composition to uncover several *CDH1* mutations likely undetectable in corresponding primary tumors.[16] Furthermore, although *CDH1* and *TP53* have often been considered as independent driver genes in GC, the

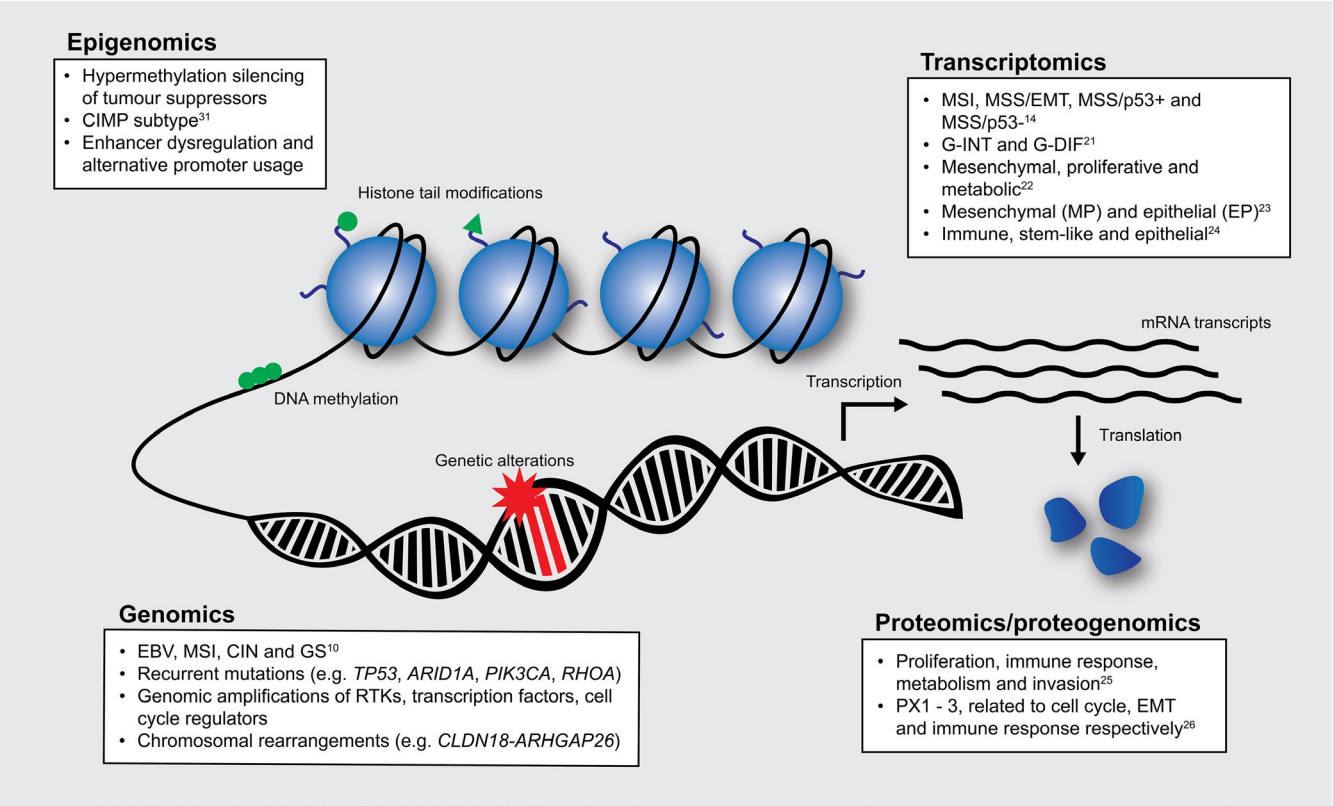

FIGURE 1 Overview of current molecular classifications in gastric cancer including epigenomic, genomic, transcriptomic and proteomic alterations. Key relevant papers are referenced. CIMP, CpG island methylator phenotype; CIN, chromosomal instability; EBV, Epstein-Barr virus; EMT, epithelial-mesenchymal transition; G-DIF, genomic diffuse; G-INT, genomic intestinal; GS, genomically stable; MSI, microsatellite instability; MSS/EMT, microsatellite stable with EMT phenotype; RTK, receptor tyrosine kinase

same study implicated the synergistic oncogenic effect of *CDH1/TP53* co-mutations in conferring an R-spondin independent, hyperactive Wnt phenotype most often observed in DGC. Recent efforts to find genetic variants underlying HDGC beyond *CDH1* have also identified germline mutations in *PALB2*, *BRCA1*, and *RAD51C*, suggesting the importance of homologous DNA recombination in this subtype.[17]

Besides genetic mutations, sCNA represent another important class of oncogenic drivers. Copy number profiling in multiple cohorts have discovered gene amplifications across three major functional categories known to regulate various hallmarks of cancer: components of the RTK/RAS signaling pathway (eg, *EGFR*, *ERBB2*), transcription factors (eg, *MYC*, *GATA4/6*), and regulators of the cell cycle (eg, *CCNE1*, *CDK6*).[10,14,18] Notably, *PD-L1/PD-L2* amplifications were enriched in the EBV-positive tumours in the TCGA study, consistent with their generally elevated PD-L1/PD-L2 expression. However, subsequent studies have highlighted that not all EBV tumors will highly express PD-L1/PD-L2, consistent with additional subtype heterogeneity.[19,20]

2.2 | Transcriptomics and proteomics

Studying the GC transcriptome and proteome provides a comprehensive overview of gene expression and corresponding protein levels. Unsupervised clustering of genome-wide GC transcriptomic profiles has led to the de novo identification of distinct patient groups, including the intrinsic G-INT/G-DIF classification based on in vitro cell lines by Tan et al,[21] the mesenchymal/proliferative/metabolic classification by Lei et al[22] and the ACRG classification of MSI, MSS/EMT, MSS/p53+ and MSS/p53−.[14] More recently, a Korean study led by Oh et al also showed two distinct transcriptomic subtypes in GC, MP and EP, where the MP subtype was correlated with considerably poor survival and resistance to chemotherapy.[23] Another multi-cohort, retrospective study by Cheong et al[24] stratified patients into immune, stem-like and epithelial subtypes which showed promising utility in guiding the choice of post-surgery adjuvant chemotherapy. In terms of the GC proteomic landscape, our understanding is still rudimentary. Of note are two recent large-scale proteomic studies focused on DGC, which independently delineated subtypes of potential prognostic and therapeutic value.[25,26] Nonetheless, these studies remain to be rigorously validated owing to the general lack of comprehensive proteomic datasets.

Unlike CRC where consensus molecular (transcriptomic) subtypes have been formally established, the classification of GC is still very much uncoordinated. However, closer examination of the various transcriptomic-based GC classifications does show similar subgroups sharing common molecular features,[27] especially the consistent identification of a mesenchymal-like subgroup. Indeed, a systematic analysis of two publicly available GC datasets, TCGA RNA-seq and ACRG microarray-based gene expression, showed a high degree of overlap between several of these independently identified subtypes (Figure 2). Notably, the G-DIF, MSS/EMT, MP and stem-like subtypes were observed to experience the worst prognosis in multiple cohorts, highlighting an important patient subset in need of clinical intervention.

2.3 | Epigenomics

Recurrent epigenetic alterations including DNA methylation and histone modifications work together to govern gene expression programs by regulating chromatin structure and accessibility.[28] Dysregulated DNA methylation is one of the best studied epigenetic mechanisms in GC, where several tumor suppressors are transcriptionally silenced by promoter or CGI hypermethylation, including *MLH1*, and *RUNX3*.[29,30] Comprehensive methylation studies have also found a subset of tumors showing CIMP.[10,31] The TCGA study further distinguished between EBV-CIMP and MSI-associated gastric-CIMP. Although the presence of CIMP in GC is well-known, its prognostic significance remains controversial. A recent meta-analysis by Powell and colleagues examined multiple cohort studies testing the association of CIMP status and overall survival in GC and

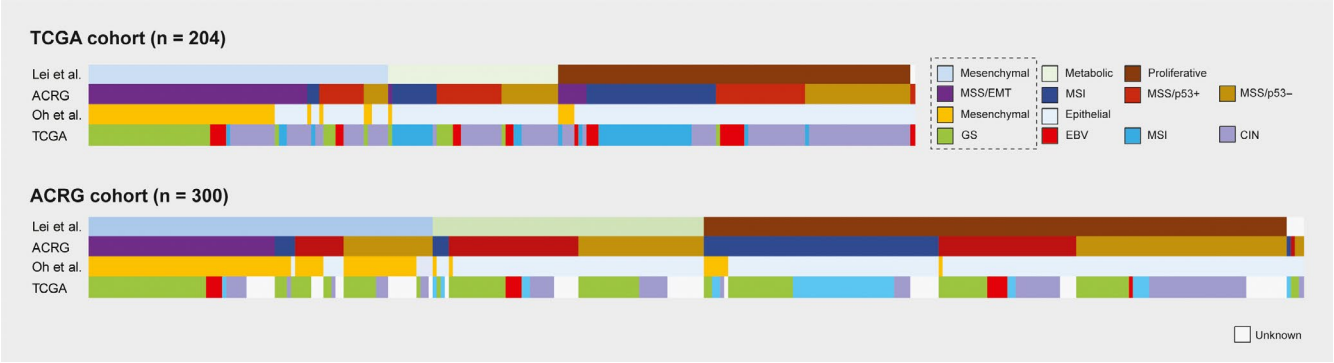

FIGURE 2 Distribution of the various transcriptomic-based (Lei et al,[22] Asian Cancer Research Group [ACRG], Oh et al[23]) and The Cancer Genome Atlas (TCGA)-based subtypes in two independent cohorts. A strong overlap is observed among the Lei et al mesenchymal subtype, ACRG microsatellite stable with epithelial-mesenchymal transition phenotype (MSS/EMT) subtype and Oh et al mesenchymal phenotype subtype. TCGA genomically stable (GS) subtype is comparatively more homogenous in TCGA cohort and overlaps largely with the transcriptomic-based mesenchymal subtypes, unlike in the ACRG cohort. CIN, chromosomal instability; EBV, Epstein-Barr virus; MSI, microsatellite instability

observed markedly divergent results.[32,33] Although five studies reported superior survival rates in CIMP patients, four other studies noted a negative association. These conflicting results are most likely due to lack of a standardized definition of CIMP, small sample sizes, and failure to integrate EBV and MSI subtype information (both are associated with CIMP). Furthermore, the observation that different gene panels used to determine CIMP status results in different prognosis outcomes highlights the possibility of further delineating distinct subtypes of CIMP, and a need to refine the gene panels used to determine CIMP status in order to obtain a consensus definition of CIMP.

Besides DNA methylation, histone modifications are known to mark transcriptional regulatory elements such as promoters and enhancers.[34] Recent work by Muratani et al, Ooi et al and Qamra et al[35-37] using ChIP-seq of various histone modifications reported aberrant enhancer and promoter landscapes in GC distinct from normal gastric tissue counterparts, highlighting their previously under-appreciated roles in disease pathogenesis. One outstanding question from these studies is whether clearly distinct GC subsets are characterized by unique regulatory element landscapes. In this regard, Okabe and colleagues demonstrated pro-oncogenic enhancer dysregulation through redistribution of repressive histone marks following EBV infection in vitro, thereby establishing a potential EBV-induced enhancer landscape unique to this subtype.[38]

3 | FROM BENCH TO BEDSIDE: TRANSLATIONAL AND CLINICAL UTILITY OF CLASSIFIERS

Although significant advancement has been made in distinguishing various molecular subtypes of GC, effective translation of these GC classifiers into clinical practice continues to depend upon two major factors: (i) their execution in the diagnostic laboratory; and (ii) the therapeutic implications of the classifiers. With respect to execution, clinically used genetic or histochemical techniques already exist for the identification of EBV,[39] MSI[40] and *ERBB2*-positivity, whereas stratification of other more complex and heterogeneous subtypes is more technically challenging. For example, follow-up work of the ACRG study developed a mesenchymal subtype 71-gene signature using the NanoString platform, which showed high concordance with the Affymetrix microarray method in identifying patients of the MSS/EMT subtype.[41] Immune/stem-cell/epithelial subtyping by Cheong et al[24] was also easily and robustly implemented using a clinical-grade, four-gene (granzyme B [*GZMB*], tryptophanyl-tRNA synthetase [*WARS*], secreted frizzled-related protein 4 [*SFRP4*], caudal type homeobox 1 [*CDX1*]) PCR-based single-patient classifier. For somatic gene mutations, the development of targeted cancer-gene panels for genomic profiling will also be indispensable for precision oncology. For instance, the Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) gene panel used by Janjigian et al[42] represents one powerful tool that can be applied to prospective genomic profiling of patients for therapeutic decision-making. Such cancer gene panels represent a cost-effective and clinically feasible method that will enable the stratification of patients based on actionable gene alterations associated with FDA-approved targeted therapies, as demonstrated by both Ichikawa et al and Kuboki et al.[43,44]

Apart from technical challenges, it is imperative that classification subtypes are strongly correlated with therapeutic responses, such that they directly inform a patient's course of treatment. For example, with respect to chemotherapy, the study by Cheong et al[24] found that immune-low (GZMB–WARS–) and epithelial-high (CDX1+) GC patients benefited from adjuvant chemotherapy post-surgery. In vitro drug sensitivity testing by Tan et al[21] also identified the relative sensitivity of G-DIF cell lines to cisplatin, and G-INT cell lines to oxaliplatin. This subsequently led to the initiation of the proof-of-concept translational "3G" trial to test the efficacy of the classification in guiding the choice of combining S-1 with either cisplatin or oxaliplatin as first-line therapy in advanced GC.[45] Although the study found no significant clinical utility of the G-INT/G-DIF classifier, post-hoc analysis indicated that patients belonging to the metabolic subtype as defined by Lei et al were most likely to benefit from chemotherapy. Importantly, the 3G trial showed the feasibility of prospective gene-expression profiling in clinical decision-making, having established a reasonable turnaround timeframe from sample collection to molecular classification.

Currently, three targeted therapies have been approved for GC treatment: trastuzumab against erb-b2 receptor tyrosine kinase 2 (ERBB2), ramucirumab against vascular endothelial growth factor receptor 2 (VEGFR2) and pembrolizumab against programmed cell death protein 1 (PD-1). Several other agents targeting diverse oncogenic molecules, especially RTK signaling components (eg, EGFR, FGFR2, MET proto-oncogene, receptor tyrosine kinase), have also been or are currently in clinical trials, although their outlook is uncertain given recent disappointing results.[46] A major reason underlying the multiple failures is the lack of effective patient stratification based on predictive biomarkers. For example, in the case of EGFR inhibition, although phase III trials evaluating the clinical benefits of anti-EGFR therapy have been negative, these studies were conducted in unselected patient cohorts.[47-49] In contrast, an encouraging preliminary investigation by Maron and colleagues found *EGFR* amplification and corresponding overexpression to predict benefit from anti-EGFR therapy.[50] Of note, Kuboki et al[44] highlighted the importance of integrating next-generation sequencing (NGS) with immunohistochemistry in guiding treatment selection, especially in cases of low-level RTK amplification detected by NGS, which does not always correlate with protein overexpression. A thorough understanding of the biological mechanisms underlying oncogenic alterations are likely to reveal further therapeutic opportunities, such as the synergistic effect of SHP2 and MEK2 inhibition in KRAS-amplified GC.[51] Previously discussed molecular classifications also hold implications for targeted therapies, exposing potential vulnerabilities such as PI3K/AKT/mTOR inhibitors in the Lei et al[22] mesenchymal subtype, insulin-like growth factor 1/insulin-like growth factor 1 receptor (IGF1/IGF1R) inhibitors in the Oh et al[23] MP subtype

and nicotinamide phosphoribosyltransferase (NAMPT) inhibitors in the ACRG EMT subtype.[52] These findings, subject to further validation, are especially exciting for the future of precision oncology, given the significant progress made recently in the development of patient-derived organoids as drug-testing platforms, which have shown the potential to recapitulate and predict in vivo patient clinical responses.[53,54]

Converging evidence appears to suggest the presence of "immunogenic" tumor subtypes which benefit most from immunotherapy. For example, MSI-high or dMMR cancers have been predicted to be relatively susceptible to immune recognition due to high levels of mutation-associated neo-antigens.[55] Accordingly, anti-PD-1 pembrolizumab has recently been granted accelerated approval in any cancer type testing positive for MSI or dMMR, signifying a certain proportion of GC that may be amenable to PD-1 inhibition. Pembrolizumab is also currently approved for use in PD-L1-positive advanced GC showing disease progression on or after two or more systemic therapies, although only a subset shows durable responses.[56] EBV-positivity is likely another important biomarker, considering anti-PD-1/PD-L1 responses reported in multiple EBV-positive GC.[42,57,58] Another interesting nominated biomarker is alternative promoter usage. In this respect, Sundar et al[59] showed the preliminary utility of alternative promoters in predicting benefit from PD-1 blockade in metastatic GC, where high alternative promoter burden (AP score) could potentially serve as a negative predictive biomarker.

4 | CONFOUNDING LAYERS OF COMPLEXITIES UNDERLIE GC CLASSIFICATION

4.1 | Tumor heterogeneity: Intra-tumoral, intra-patient and intra-subtype

Recent studies highlight that GC is a complex disease with additional heterogeneity, both intra-tumoral (within a single tumor) and intra-patient (between the primary tumor and its corresponding metastases), where phenotypically and genetically distinct clonal subpopulations coexist (Figure 3).[60] Therefore, molecular characterization of a single tumor tissue biopsy sample is unlikely to provide an accurate reflection of the whole tumor or disease entity, leading to potential misclassification. Intratumoral heterogeneity in GC is arguably most studied in the context of *ERBB2* amplification, where both spatial and temporal heterogeneity have been frequently reported.[61-64] In addition, intratumoral heterogeneity with respect to EBV-positivity, *PIK3CA* mutations,[65] *EGFR* amplification [63] and *FGFR2* amplification[66] have also been reported, all of which hold clinical implications for targeted therapies against these aberrations.

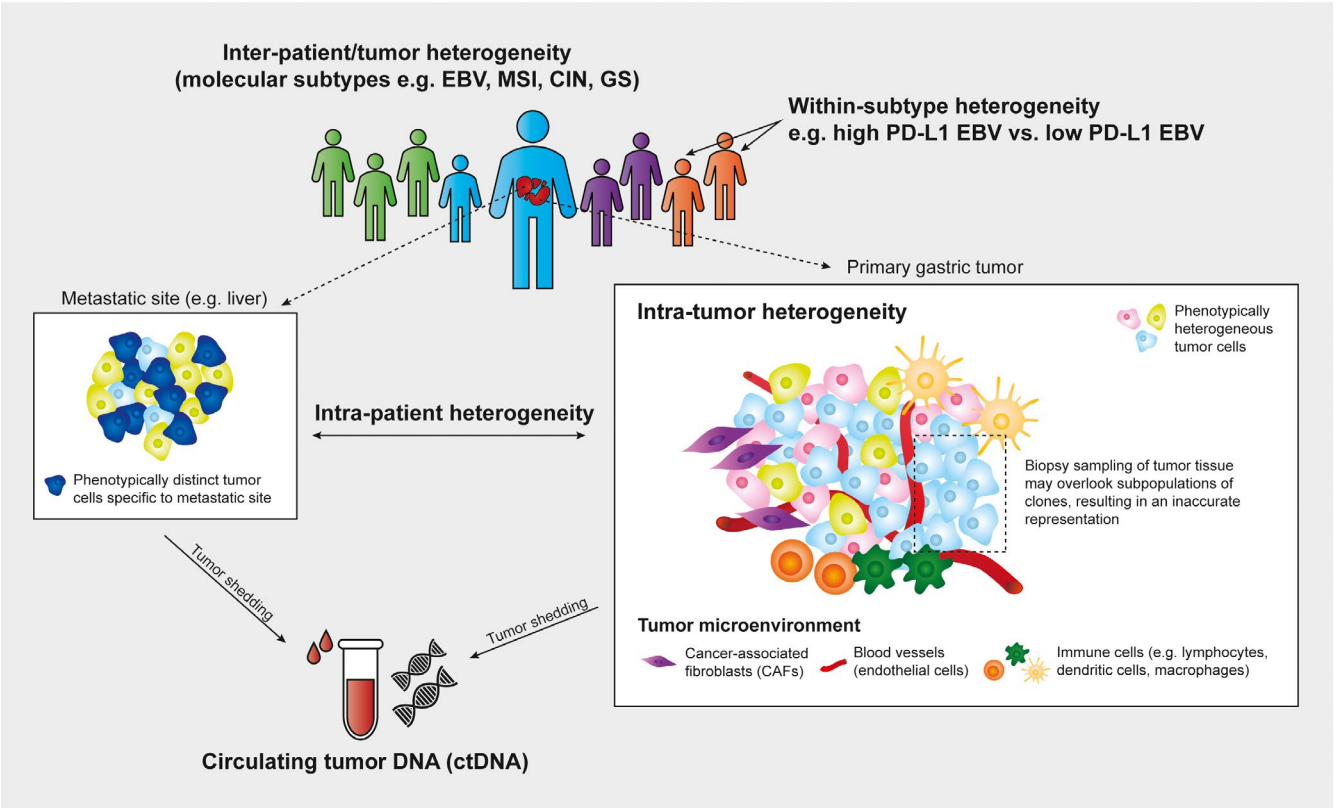

FIGURE 3 Pervasive heterogeneity in gastric cancer between and within patients. Intra-patient and intra-tumoral heterogeneity exist alongside the complex tumor microenvironment. Circulating tumor DNA (ctDNA) can potentially capture heterogeneity within tumors and/or between primary and metastatic lesions. CIN, chromosomal instability; EBV, Epstein-Barr virus; GS, genomically stable; MSI, microsatellite instability; PD-L1, programmed death-ligand 1

In advanced GC, extensive discordance in multiple actionable genomic alterations, including *ERBB2*, *KRAS*, *MET* and *PIK3CA*, between primary tumors and their corresponding synchronous and/or metachronous metastases (intra-patient heterogeneity) has also been observed, further complicating the implementation of systemic targeted therapy.[67-69]

Although the molecular subtypes proposed by TCGA and ACRG have been invaluable for dissecting GC biology, it should also be noted that oncogenic alterations are rarely ubiquitously shared across all members of the subtype (intra-subtype heterogeneity), thereby limiting their clinical applicability. For example, comprehensive genomic profiling by Ichikawa et al[43] showed that frequent genomic alterations in actionable gene targets including *ERBB2* amplification were not exclusive to any TCGA subtype, although they could be enriched in one (eg, *ERBB2* amplification in TCGA CIN subtype). Furthermore, it is important to note that patient cohorts defined by a common biomarker, such as *ERBB2* amplification and/or overexpression, may still show distinct underlying tumor biologies. For instance, Janjigian et al[42] noted substantial heterogeneity in co-occurring *RAS/PI3K* mutations within an *ERBB2*-positive cohort. In particular, *ERBB2*-positive patients harboring co-occurring alterations consistent with RAS/PI3K pathway activation were found to benefit less from trastuzumab treatment.

Clinically, the implications of these heterogeneities are twofold: First, from a diagnostic standpoint, the problem of intra-tumoral/intra-patient heterogeneity threatens to undermine the use of traditional tissue biopsies in diagnostic laboratories, which are typically taken from the primary tumor. Yet, it is not clinically feasible to obtain multiple biopsies for every lesion within a patient. The advent of liquid blood-based biopsies to profile CTC and ctDNA presents a promising non-invasive alternative approach. For instance, an analysis of the PANGEA trial cohort by Pectasides et al[68] found discordant genomic alterations between primary and metastatic tissue in 10 of 28 (36%) patients, of which 87.5% displayed concordance between ctDNA and metastatic tissue suggesting that ctDNA may better represent disease in the advanced setting. ctDNA could also be used to inform the clonality of targetable genomic alterations,[66] capture co-occurring alterations that may predict treatment failure,[67] or longitudinally track a tumor as it undergoes temporal evolution as a result of therapeutic pressure.[70,71] Nonetheless, it should be acknowledged that liquid biopsies are unlikely to be an exhaustive catalogue of tumor traits, as some genomic alterations identified in primary tumor tissue were not found in matched ctDNA.[68] Whether this is a pitfall of ctDNA profiling, or an indication of the lack of clonality of the genomic alteration is still an open but crucial question. Although there are still uncertainties to be resolved, including the technical aspect of a standardized analytical methodology, the anecdotal experiences of liquid biopsies in the clinical management of GC thus far are proof of its utility in tackling the challenge of tumor heterogeneity.[68]

Second, tumor heterogeneity also holds profound implications for therapy selection. For instance, in the PANGEA trial, discordance between primary tumor and metastatic lesion/ctDNA led to a treatment reassignment in nine patients, of which two showed highly encouraging clinical responses.[68] In another clinical trial testing FGFR2 inhibition, durable responses were observed only in high-level *FGFR2* clonally amplified tumors, as assessed by FISH-based in situ heterogeneity mapping.[66] A comparison of paired *FGFR2* expression at baseline and 15 days post-treatment further showed significant decreases in *FGFR2* mRNA only in the sub-clonal, heterogeneously amplified tumor, possibly reflecting clonal selection of non-amplified compartments as a result of therapeutic pressure. In line with such observations, Yan and colleagues recently showed differential drug responses to PARP inhibition in two GC organoids derived from the same patient, with one organoid harboring a *BRCA2* mutation exhibiting greater sensitivity.[54]

4.2 | Tumor microenvironment

Our molecular understanding of tumor-intrinsic features is further complicated by contributions from the TME, including CAF, immune cells and endothelial cells. This was clearly exemplified by Isella et al[72] where the mesenchymal transcriptional subtype of CRC was found to be predominantly driven by stromal components, suggesting a possible superimposition of the EMT process in tumor cells with the inherent mesenchymal traits of stromal cells. In this regard, multiple studies in GC have established the association of stromal content with survival outcomes and/or therapeutic responses.[73-79] Notably, high stromal gene expression consistently predicted poor clinical outcome in several independent GC cohorts,[73,77] which could, in part, explain the poor clinical prognosis of Lauren's DGC subtype which is typically characterized by high stromal infiltration and close interaction between cancer cells and CAF.[80] Digging deeper into the complex interactions and cross-talk between cancer cells and their associated microenvironment therefore holds the potential to reveal actionable targets in this devastating subtype. For example, cross-talk between CAF or bone marrow-derived myofibroblasts and GC cells has been reported to maintain GC stemness[81,82] and invasiveness[83] with critical involvement of transforming growth factor beta signaling, while CAF-derived HGF was found to induce tumorigenesis and metastasis of *MET*-unamplified GC.[84] Ascertaining the intratumoral stromal proportion and characterization of the molecular features may thus prove useful in therapeutic development.

Given the promise of immunotherapy in subsets of GC patients as highlighted earlier, research into the GC immune contexture has also begun to receive greater attention. Several studies have attempted to correlate a tumor's immune profile with prognosis, focusing on T-cell-mediated anticancer immunity.[85] For instance, a study by Morihiro and colleagues reported the combination of PD-L1 and MSI or CD8+ TIL as a useful prognostic biomarker in GC.[86] Another group showed intratumoral infiltration of interleukin-17-producing immune cells to be an independent prognostic biomarker, and predictive of superior response to adjuvant chemotherapy.[87] Interestingly, an analysis of the tumor immune environment of TCGA GC samples found a striking enrichment of B-cell infiltration in the GS/Lauren's DGC subtype.[88] In-depth profiling of the B-cell repertoire in DGC

showed HSGAG to be important B-cell antigens, with naturally occurring anti-HSGAG antibodies exerting potent growth-suppressive effects, thereby opening new avenues for IO in GC.

Meanwhile, the recent advent of single-cell sequencing offers an unparalleled opportunity to interrogate distinct coexisting cell compartments within a tumor at high resolution. For example, single-cell sequencing could help to comprehensively characterize the distinct array of immune cell infiltrates by simultaneously examining both their gene expression profiles and clonal V(D)J immune receptor sequences. Such qualitative differences of the immune cell infiltrate between patients hold the potential to serve as prognostic and predictive biomarkers in the context of IO drugs, and further unlock novel therapeutic IO targets. For instance, single-cell analysis of BC T cells identified a specific tissue-resident memory subset (T_{RM} cells) that appeared to augment immunosurveillance, thereby introducing a potentially ideal target for IO modulation.[89] Notably, single-cell-derived transcriptomic signatures of the T_{RM} cell cluster were found to identify BC patients with significantly improved prognosis. Another similar single T-cell analysis in CRC also showed diverse T-cell subsets, where clonal T_H1-like T cells were enriched in MSI tumors and likely accounted for their positive response towards immunotherapy.[90] Collectively, these results show how understanding the TME at single-cell resolution could open new avenues for identifying distinct patient subsets of prognostic or therapeutic significance.

Single-cell analyses in GC remains scant but is currently actively pursued.[91-93] Of special mention is the recent study led by Zhang et al[94] that mapped the single-cell transcriptomic landscape of premalignant gastric mucosae and early gastric cancers (EGC), identifying clusters of distinct cell types and their unique molecular features at each stage. By investigating the expression profiles of the conserved antral basal gland mucous cell type across different stages of the lesion, the team discovered the acquisition of an intestinal-like stem cell phenotype that could be central in tumorigenesis. Moreover, an in-depth examination of the expression profile of "cancer cell" clusters arising from EGC uncovered a panel of six high-confidence EGC-specific gene markers that could provide a means of accurately diagnosing EGC. Analogous to such analysis, single-cell profiling of distinct GC subtypes could also identify functionally important subtype-specific TME cell types and/or molecular markers that are potentially relevant for diagnosis, prognosis or therapeutic development. Taken together, it would be prudent not only to develop "stroma-aware approaches" in the molecular classification of GC, but also to consider TME components as potential therapeutic targets, as evidenced by the success of VEGFR2 inhibition and immunomodulating therapies.

5 | CONCLUDING REMARKS AND FUTURE OUTLOOK

In conclusion, significant progress has been made in our attempt to understand the molecular heterogeneity underlying GC pathogenesis. Akin to the consensus molecular subtypes of CRC, a formal consolidation of GC subtypes may also prove to be worthwhile in unifying the research community and providing a common basic infrastructure upon which additional complexities could be uncovered. Ultimately, the value of molecular subtyping lies in the effective stratification of distinct patient subsets of predictive value, for which we can then tailor specific treatments to improve patient outcomes.

Although progress within the GC field has mostly stemmed from "static" analysis of large patient cohorts (characterization of a representative tumor section at a single defined time point), we believe a shift in focus towards tracking a tumor's dynamic evolutionary trajectory is on the horizon.[95] The intrinsically heterogeneous nature of tumors is a key enabler of tumor evolution in the face of therapeutic pressure, which ultimately results in resistance and treatment failure. To improve patient outcomes in a durable way, there is thus an urgent need to spatially and temporally monitor tumor heterogeneity and evolution and develop therapies that can overcome resistance mechanisms. This is likely to become the next paradigm of precision oncology, which will also reinvent molecular classification as a dynamic process, rather than set it in stone from the initial diagnosis. Given their minimal invasiveness, liquid biopsies appear promising in addressing this concern.

Finally, our pursuit of precision oncology will hardly be attainable without proper preclinical systems to model the heterogeneous GC landscape and its associated stromal microenvironment. The recent establishment of comprehensive patient-derived organoid biobanks by independent groups, coupled with the rapid development of single-cell sequencing capable of capturing diverse cell types, represents a powerful advancement within the field, and if used effectively, would undoubtedly bring us one step closer towards the goal of precision oncology in GC.

DISCLOSURE

Authors declare no conflicts of interest for this article.

ORCID

Shamaine Wei Ting Ho https://orcid.org/0000-0002-2595-0710

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