Gastric Cancer Genetics and Its Implications for Diagnosis, Prognosis, and Treatment of the Disease

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Gastric cancer (GC) is an aggressive disease and the fifth most common cancer worldwide with a variable geographical distribution. GC has a very low survival rate, mainly because of its heterogeneous presentation, multifactorial etiology, and late diagnosis. It is well established that various risk factors contribute to the development of the disease, including salty diet, smoking, and excessive alcohol consumption. Importantly, interactions between genetic and environmental traits trigger the activation of key signaling pathways, influencing gastric cell behavior towards neoplastic transformation and progression. Despite important advances in our understanding of GC, it remains a major health burden owing to epidemiological and therapeutic limitations. This study aimed to provide a comprehensive overview of the genetic landscape of GC phenotypes and molecular biomarkers for diagnosis and prognosis. In particular, we discuss the advances in genomic knowledge and technology that have yielded comprehensive information on the genetics of GC and classified it from a histological to a molecular perspective. Therefore, targeted and immune-based therapies have been developed, highlighting the challenges associated with intratumoral and interpatient heterogeneity. Finally, we explored potential research avenues on the intricacies of GC and identified accurate biomarkers for improved cancer screening and stratification. The development of innovative approaches to tackle relevant molecules is needed for GC management.

Keywords Gastric cancer; Cancer genetics; Molecular profile; Diagnostic.
**INTRODUCTION**

Gastric cancer (GC) is an aggressive disease and the fifth most common malignancy worldwide. Despite extensive research and recent breakthroughs in medicine, GC has a very low survival rate, making it the fourth leading cause of cancer-related death worldwide.\(^1,2\) The incidence of GC differs globally, with a higher prevalence in Eastern Europe, South America, and Eastern Asia than in North European and East African countries.\(^3,4\) Men are more prone to gastric carcinogenesis than women.\(^1,2\)

According to the histological pattern, GC has been classified into intestinal, diffuse, and indeterminate subtypes based on the Lauren's classification.\(^5\) The intestinal type is characterized by well-differentiated glandular structures and accounts for approximately 50%–60% of cases. This subtype exhibits aneuploidy or frequent genetic alterations that affect mitotic and relevant oncogenic signaling pathways. In contrast, the diffuse type is rarer (30%–40%) and displays an undifferentiated pattern of poorly cohesive cells, often with a signet ring cell morphology. A mixture of intestinal- and diffuse-type components is observed in a small proportion of GC cases, which are classified as the indeterminate type. A different classification system was proposed by the World Health Organization in 2010 and updated in 2019. This complex scheme recognizes four major histological patterns, tubular, papillary, mucinous, and poorly cohesive, followed by several subdivisions in each category.\(^6\)

To some extent, the heterogeneous presentation of GC is associated with its multifactorial etiology. It is well established that various risk factors contribute to disease development.\(^7\) For instance, salty diet, smoking, and excessive alcohol consumption are regarded as modulators of gastric cell behavior that can promote genetic alterations and subsequent neoplastic transformations.\(^7,8\)

Among environmental factors, *Helicobacter pylori* is recognized as type 1 carcinogenic being associated with approximately 80% of all GC cases worldwide.\(^9,10\) This pathogen promotes chronic gastritis and increases the pH of the stomach, enabling the colonization of the gastric epithelium by opportunistic microbes.\(^11\) The preferential binding of *H. pylori* to the gastric mucosa is mediated by the bacterial blood group antigen-binding adhesin (BabA), sialic acid-binding adhesin (SabA), and Lewis antigen-binding adhesin present in the gastric cell membrane.\(^12-14\) This initiates a gradual process of histological alteration, known as the Correa cascade, from chronic to atrophic gastritis, intestinal metaplasia, dysplasia, and eventually to invasive carcinoma.\(^15,16\) Progression to GC is strongly associated with bacterial virulence factors and genetic polymorphisms in the host.\(^17,18\) Among these, cytotoxin-associated gene A (CagA) and vacuolating cytotoxic gene A (VacA) genotypes are correlated with severe clinical outcomes and increased incidence in Western countries.\(^19,20\)

The Epstein-Barr virus (EBV) has also been associated with gastric adenocarcinoma, although only a small proportion of infected individuals progress to GC.\(^21,22\) EBV-positive tumors are distinguishable from EBV-negative tumors based on genetic alterations and evident clinicopathological features, including the formation of lumps or ulcers accompanied by lymphocyte infiltration.\(^23\) Interestingly, other environmental factors, such as eating salty or spicy foods, cigarette smoking, excessive coffee consumption, and drinking high-temperature beverages, are also considered risk factors for developing EBV-positive GC, corroborating the relevance of repeated tissue injury in the disease.\(^24,25\)

Although most cases of GC are sporadic, genetic predisposition plays an important role in its development. Accordingly, germline mutations in tumor suppressor genes may trigger familial aggregation of GC and early onset manifestations.\(^26,27\)

Herein, we aimed to provide a comprehensive overview of the genetics underlying GC phenotypes and how this knowledge can aid in the diagnosis and overall patient management. We outlined the challenges associated with the heterogeneity of the disease and explored potential research avenues to advance our understanding of the etiology of GC and development of alternative therapeutics.

**GENETICS OF GASTRIC CANCER**

In recent years, improved genomic knowledge and technology have yielded a large amount of information on the genetics of tumors, challenging the classification paradigm from a histological to a molecular perspective. This has not only revealed the molecular mechanism of cancer but has also contributed to innovative biomarkers and targeted therapeutics. In 2014, The Cancer Genome Atlas Research Network proposed the molecular classification of GC into four distinct subtypes: EBV-positive, microsatellite instability (MSI), chromosomal instability (CIN), and genomically stable (GS) (Fig. 1).\(^28\)

EBV-positive gastric carcinomas are mostly located in the gastric fundus and more prevalent in male patients. These tumors are characterized by an extensive mutation burden, including recurrent mutations in genes involved in the phosphatidylinositol 3-kinase (PIK3CA) pathway or gene amplification of Janus-associated kinase 2 (JAK2) and HER2 receptor tyrosine kinase 2 (ERBB2).\(^28\) In addition, these malignancies typically present mutations in chromatin remodeling genes, such as AT-rich interactive domain-containing protein 1A (ARIDIA)
and B-cell lymphoma 6 corepressor (BCOR), and overexpression of programmed death ligand-1/2 (PD-L1/2). Extensive DNA methylation and the subsequent silencing of cyclin-dependent kinase inhibitor 2A gene (CDKN2A) has also been reported in EBV-positive tumors.

MSI subtype tumors are typically found in the distal stomach of older patients and are associated with *H. pylori* infection and intestinal metaplasia. These tumors display a hypermutation profile resulting from promoter methylation and transcriptional silencing of the DNA mismatch repair gene MLH1. Dysfunction of the mismatch repair system leads to high mutation rates and inactivation of tumor suppressor genes or oncogenes. The most frequently mutated genes are PIK3CA and epithelial growth factor receptor (EGFR). To escape immune-surveillance triggered by the mutation burden, these tumors express high levels of PD-L1/2 with concomitant low levels of major histocompatibility complex class I (MHC1).

In contrast to EBV-positive and MSI subtypes, GS tumors are characterized by lower rates of somatic mutations, earlier onset, and diffuse histology. Mutations usually affect the CDH1 and RHOA genes, decreasing cell-cell adhesion and promoting cell motility. Genomic rearrangements, such as the CLDN18::ARHGAP26 fusion, can also be detected and are strongly associated with RHOA inhibition and loss of epithelial integrity.

CIN carcinomas exhibit intestinal histology and are often located in the gastroesophageal junction or cardia of the stomach. These gastric tumors exhibit severe chromosomal abnormalities that can result from four distinct mechanisms: inaccurate chromosome segregation during mitosis, cell cycle checkpoint defects, oncogene-induced mitotic stress, and replication stress. CIN tumors exhibit high aneuploidy rates and are characterized by tumor protein 53 (TP53) mutations, the most recurrent genetic alterations, which impair cell division/death control. Genomic amplification of receptor tyrosine kinases (EGFR, RAS, FGFR, ERBB2, and MET), VEGFA (VEGFR2 ligand), and cell cycle mediators (CCNE1, CCND1, and CDK6) are also described in the CIN subtype.

In addition to sporadic cases, 10% of GC cases arise in familial settings. GC is the primary clinical manifestation of hereditary diffuse gastric cancer (HDGC), gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS), and familial intestinal gastric cancer (FIGC) syndromes (Fig. 2).

HDGC is an autosomal-dominant syndrome characterized by a significant lifetime risk of diffuse gastric and lobular breast cancer.
cancers, although congenital malformations such as cleft lip/palate have also been described as part of the disease spectrum.39,40 In this setting, GC is highly aggressive, with neoplastic cells diffusely infiltrating the gastric wall and spreading into the peritoneal cavity and distant sites.41 To date, pathogenic germ-line mutations of \( \text{CDH1} \) and \( \text{CTNNA1} \) genes are the only proven cause of HDGC.42,43 \( \text{CDH1} \) encodes E-cadherin, a transmembrane protein responsible for homophilic binding between adjacent cells, whereas \( \text{CTNNA1} \) codifies for α-catenin, a pivotal molecule for linkage between E-cadherin and cytoskeleton.44,45 Thus, it is clear that the loss of either of these proteins affects intercellular junction and maintenance of epithelial structure and function.

\( \text{CDH1} \) and \( \text{CTNNA1} \) germline variants are evenly distributed throughout the gene, and no definite genotype-phenotype correlations can be established.42 Because mutations are inherited from one parent, every cell from the progeny carries a functional and a defective copy of the gene. Complete gene inactivation occurs upon somatic loss of the second allele (Knudson’s two-hit hypothesis) through hypermethylation, mutation, or deletion.46,47

GAPPS was first recognized in 2012; it is characterized by fundic gland polyposis with focal dysplasia and intestinal or mixed-type adenocarcinoma.48 For diagnosis, >100 gastric polyps should be detected, and polyposis elsewhere in the gastrointestinal tract, namely colorectal or duodenal, should be excluded. Syndrome etiology is attributed to germline single nucleotide mutations in the promoter region 1B of the tumor suppressor gene \( \text{APC} \), which lead to decreased \( \text{APC} \) expression.49,50 Second-hit events encompass truncating mutations or loss of the wild-type allele, resulting in biallelic inactivation of \( \text{APC} \) gene.49,50

In contrast to HDGC and GAPPS, the pathogenesis of FIGC remains largely unknown, possibly because of the broad categories of cancer that can arise in this setting.51 In addition to an increased risk of intestinal-type GC, colorectal, and breast carcinomas, 15 other cancer phenotypes were observed in family members with FIGC. These comprise lung, laryngotracheal, hepatobiliary and liver cancers, as well as leukemia, among other malignancies.49,51 Moreover, studies addressing the genetic factors underlying FIGC are scarce, and suggest a polygenic cause. For instance, heterozygous mutations in the immune response-related gene \( \text{IL12RB1} \) were reported in a Dutch family in which several members were affected by the intestinal-type GC.52 The implementation of next-generation sequenc-
ing approaches identified TP53 as a potential susceptibility gene for FIGC, while MSI was detected in 38% of cases.51

GC may also develop in the context of other hereditary cancer syndromes, albeit at a lower frequency. These include Li-Fraumeni syndrome, familial adenomatous polyposis, Peutz-Jeghers syndrome, Lynch syndrome, hereditary breast/ovarian cancer syndrome, MUTYH-associated adenomatous polyposis, juvenile polyposis syndrome, and Cowden syndrome.53

**DIAGNOSTIC APPROACHES**

During its initial stages, GC often presents with no noticeable symptoms. As the disease progresses, common symptoms may include difficulty in swallowing (dysphagia), asthenia, indigestion, vomiting, weight loss, early satiety, and iron deficiency anemia.2,54 However, these symptoms are not specific, and there is little urgency in determining their causes. Consequently, approximately 60% of individuals with GC are ineligible for curative treatment due to late diagnosis or underlying health conditions.54,55

Traditionally, GC diagnosis relies on endoscopic examination and subsequent histological analysis of gastric biopsies.56 Examination of tissue features includes not only the epithelial component but also elements of the tumor microenvironment, namely infiltrating lymphocytes and the surrounding extracellular matrix.57,58 Recently, deep learning methods have emerged as promising complementary tools for improving the detection of cancer cell traits, which can promptly affect screening and therapeutic strategies. For instance, deep learning was able to effectively predict the MSI status directly from H&E-stained histology slides.59 The classification of gastric and colon biopsy specimens has also been achieved using convolutional neural networks, supporting routine histopathological diagnoses.60,61 Likewise, the implementation of multiplexed immunofluorescence imaging approaches provides a framework for the quantitative analysis of spatial interactions and immune phenotypes in gastric biopsies and resection specimens.62,63

Contrary to tissue biopsies, which can be expensive, carry potential risks for patients, and only provide information on a small portion of the tumor, liquid biopsies offer a non-invasive approach and a broader overview of the heterogeneity and complexity of the disease.54,65 Several tumor-derived components have been detected in the bloodstream of patients with cancer, including circulating tumor DNA (ctDNA), circulating tumor cells (CTC), and exosomes. While CTCs and exosome content can provide relevant molecular and functional data, ctDNA is easy to isolate, more sensitive, and constitutes a superior source of genetic information.64,65 In particular, ctDNA has been proposed for the identification of HER2 amplification, whose detection rates within tumor tissues are hampered by GC heterogeneity.66,67

**MOLECULAR BIOMARKERS**

The challenges associated with the management of GC are intratumoral and intertumoral heterogeneity. Therefore, remarkable efforts have been made to identify effective molecular markers for diagnosis and prognosis.

Currently, biomarkers such as carcinoembryonic antigen (CEA) and carbohydrate antigens CA19-9 and CA72-4 are used in clinical pipelines to predict GC recurrence. Nonetheless, these methods lack specificity and sensitivity and are often used in combination with other tumor markers.68,69

The tyrosine-kinase membrane receptor HER2 has predictive value for treatment of advanced gastric carcinoma with HER2 inhibitors. This protein, encoded by the **ERBB2** gene located on chromosome 17, belongs to the EGFR family and can activate survival and proliferative signaling pathways without the need for a binding ligand.70,71 The activation mechanism of this oncogene occurs primarily upon amplification or, in rare cases, by gene fusion. **HER2** amplification occurs in 6%–23% of patients, predominantly in the CIN molecular subtype, encompassing the intestinal type and well-differentiated tumors.72

HER2 status is usually evaluated by immunohistochemistry and its expression is scored from 0 (no presence of HER2 amplification) to 3 (HER2 overexpression). In cases where HER2 staining remains unclear, in situ hybridization with directed probes should be performed.73 HER2-amplified GC is associated with an aggressive phenotype and poor outcome; therefore, this marker is considered a negative prognostic factor.74,76

Another emerging biomarker is PD-L1, a binding ligand for the membrane programmed death 1 (PD-1) protein, which is expressed in immune cells. The interaction between PD-L1 and PD-1 impairs the immune response and induces the apoptosis of cytotoxic T cells, thereby allowing tumor progression.77 Thus, this molecule has become valuable for the prognosis and assessment of potential sensitivity to PD-1/PD-L1 inhibitors in various cancers. In GC, PD-L1 is differentially expressed across distinct subtypes, exhibiting higher levels in MSI and EBV-positive tumors.78,79 Similar to HER2 evaluation, PD-L1 is assessed by immunohistochemistry in gastric biopsies and tumors, where PD-L1 overexpression is associated with an increased ability to evade the immune system and, consequently, poor survival rates.80

Approximately 15%–30% of gastric tumors display MSI. Microsatellites are short tandem repeats (1–6 nucleotides) s
Tumors exhibiting 10%–29% unstable microsatellite regions fall into the MSI-high group. MSI is primarily caused by promoter hypermethylation and epigenetic silencing of MLH1 gene. A comprehensive analysis conducted in South Korea revealed that over 63% of MSI-high GC cases harbored mutations in genes, such as TGFBR2, CEP164, MIS18BP1, RNPC3, KIAA2018, CNOT1, and CCDC150. MSI is detected through immunohistochemistry or polymerase chain reaction (PCR) in gastric biopsies or by PCR/next-generation sequencing molecular analysis in plasma-derived cell-free ctDNA. Interestingly, MSI-high tumors show a better prognosis than those presenting MSI-low phenotypes, which is attributed to increased levels of tumor-infiltrating lymphocytes and consequently higher immunogenicity.

Fibroblast growth factor receptor 2 (FGFR2) has been suggested as a biomarker for predicting long-term recurrence and lower overall survival, given its involvement in several cancer hallmarks, namely mitogenesis, differentiation, cell proliferation, migration, and angiogenesis. FGFR2 is systematically amplified in metastatic GC, and its overexpression (31%) is more common than that of EGFR (24%), HER2 (12%), and MET (25%).

More recently, claudin 18.2 has arisen as a selective molecular marker of diffuse-type GC. In normal gastric mucosa, the expression of this protein is limited to tight junctions, which become exposed and overexpressed in gastric adenocarcinoma cells following the disruption of cell-cell adhesion. Claudin 18.2 overexpression is related to a higher probability of tumor invasion limited to the mucosa and submucosa; hence, it is regarded as a predictive biomarker in resectable GC. Corroborating these data, low claudin 18.2 levels were described in more aggressive and invasive tumors.

Ultimately, knowledge gathered regarding the identification of accurate biomarkers and their biological significance is essential for cancer screening and stratification as well as for devising innovative therapeutic interventions.

**TREATMENT STRATEGIES**

Despite the important developments in our understanding of GC, very little has been translated into successful therapeutics. For patients with early stage GC, surgery remains the primary line of treatment, whereas patients with advanced GC rely on chemotherapy alone or combined with targeted and immunotherapies. Standard chemotherapy involves a platinum-fluoropyrimidine doublet, usually involving oxaliplatin or cisplatin and fluoropyrimidines administered as an infusion (5-FU) or orally (capecitabine or tegafur-gimeracil-oteracil [S-1]). Conventional treatment results in a median overall survival of approximately 12 months, reflecting high toxicity and drug resistance. In a phase 3 randomized trial, a triplet of docetaxel, cisplatin, and S-1 was evaluated, which yielded a debatable benefit and increased toxicity.

The past decade has witnessed remarkable progress in cancer treatment using immunotherapy and targeted therapy regimens. For instance, patient stratification based on the HER2 status revealed the possibility of combining chemotherapy with trastuzumab, with increased response rates and overall survival up to 16 months. Trastuzumab is a monoclonal antibody that binds specifically to the extracellular domain of the receptor, blocking downstream survival and proliferative signaling pathways such as PI3K/AKT. Approval of this antibody for unresectable or metastatic GC represented a paradigm shift in the management of HER2-positive tumors. Notably, the role of anti-HER2 targeted therapy in the perioperative setting remains under investigation, although current guidelines do not recommend its administration, given the extensive adverse effects and absence of significant improvement in patient well-being.

Immune checkpoint blockade using the anti-PD-1 monoclonal antibody nivolumab, in addition to chemotherapy, resulted in significant improvements in overall survival and progression-free survival. In addition to PD-L1 expression, high MSI and tumor mutation burden are also indicative of immunotherapy response. Increased mutation rates can induce the formation of immunogenic neoantigens that are easily recognized by effector immune cells. Furthermore, EBV-positive tumors exhibit an excess of CD8+ tumor-infiltrating lymphocytes, supporting the use of immune-based therapies. PD-1 inhibitors, such as nivolumab and pembrolizumab, have been evaluated as the first- or second-line treatment, either as monotherapy or in combination with other agents. In particular, the potential benefits of immune checkpoint inhibitors along with anti-HER2 therapies have been observed in patients with HER2-sensitive GC.

Anti-angiogenic therapies using vascular endothelial growth factor receptor 2 (VEGFR2) antibodies following the first-line chemotherapy have also been investigated. VEGFR2 is a tyrosine kinase receptor involved in angiogenesis, autocrine survival, and migration signaling. Upon binding to its ligand VEGF, it stimulates neighboring neovascularization, increasing the availability of oxygen and nutrients to malignant cells. According to the REGARD trial, the anti-VEGFR2 ramucirumab improved overall survival compared with the best sup-
portive care, whereas in the RAINBOW study, the combination of ramucirumab and paclitaxel improved survival compared with paclitaxel alone. However, anti-VEGFR2 therapeutics did not provide benefits when combined with platinum- or fluoropyrimidine-based chemotherapy. In addition, it is well established that the angiogenic factor VEGF and its downstream signaling pathways yield an anti-inflammatory phenotype and immune evasion. Therefore, a synergistic mechanism involving anti-VEGFR2 and immunotherapy has been proposed to enhance antitumor immunity.

Innovative therapies targeting relevant biomarkers remain in demand for GC management. In particular, claudin 18.2 and FGFR2 have gained attention for the design of novel regimens, either alone or in combination with other agents. Anti-claudin and anti-FGFR2 antibodies are already available and currently being tested in combination with chemotherapy in trials for gastric or gastro-esophageal junction adenocarcinomas.

**CONCLUSION**

GC remains a major health burden due to the epidemiological and therapeutic challenges associated with late diagnosis. Future research should focus on the implementation of endoscopic screening programs, as well as on community literacy to ameliorate the associated risk. Moreover, efforts should be made to develop less invasive tools for early disease detection.

The main barrier in the near future is to overcome the intratumoral and interpatient heterogeneity for patient stratification and drug development. Advances in the molecular landscape of GC highlight the need for a more rigorous selection of patients for a given treatment while revealing the remarkable capacity of tumors to overcome therapeutic assault. Understanding the biological intricacies of GC will certainly reveal unforeseen susceptibilities that must be addressed for the benefit of patients.

**Authors’ Contribution**

Conceptualization: José Carlos Machado. Funding acquisition: José Carlos Machado, Joana Figueiredo. Investigation: all authors. Writing—original draft: José Pedro Santos. Writing—review & editing: José Carlos Machado, Joana Figueiredo. Approval of final manuscript: all authors.

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