



The Four Molecular Classifications of Gastric Adenocarcinoma: A Review of What We Already Know

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ABSTRACT

Gastric malignancies are currently the third leading cause of cancer-related deaths worldwide. Several classification proposals have been made, initially morphological and currently molecular, with the latter aiming to integrate neoplasms with specific molecular and prognostic characteristics. The most recent and widely disseminated one subdivides them into 4 groups: EBV-positive adenocarcinomas; adenocarcinomas with high microsatellite instability; adenocarcinomas with chromosomal instability; and genomically stable adenocarcinomas. The objective is to achieve better classification and consequently greater definition in risk stratification, treatment, and prognosis. The high costs involved in the genomic and molecular analyses necessary for complete molecular classification, especially for chromosome instability and genomically stable subtypes, are certainly still limiting factors for the dissemination of molecular classification and the understanding of the expected behavior for each of them.

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Introduction

Gastric malignancies is the third leading cause of cancer-related deaths worldwide, with adenocarcinomas accounting for approximately 90% of cases [1]. According to the American Cancer Society, an estimated 26,890 new cases of gastric malignancies are expected in the United States in 2024 [2]. Over the years, various classification proposals for gastric adenocarcinomas have been put forward, each with its own particularities and justifications. These proposals have evolved from purely morphological characteristics in the past to include Immunohistochemical features and, more recently, molecular characteristics.

Literature Review

One of the most widespread classifications of gastric adenocarcinomas is the Lauren classification, which is purely morphological, easily reproducible, and provides relevant epidemiological and prognostic information. Subsequent analyses seeking to understand and correlate it with potential carcinogenesis mechanisms, biological markers, and responses to chemotherapy regimens have developed over the years [3]. Currently, numerous studies are underway to detect specific molecular alterations that allow the classification of gastric neoplasms into detailed, notably molecular, patterns, aiming for increasingly personalized

therapeutic proposals.

In its latest update of the classification of gastrointestinal tumors, the World Health Organization (WHO) emphasizes a broader morphological classification, separating adenocarcinomas by more detailed cytoarchitectural characteristics. Consideration is also given to the molecular classification proposed by The Cancer Genome Atlas (TCGA) [4], which divides adenocarcinomas into 4 different groups: Epstein Barr Virus associated adenocarcinoma (EBV+), High Micro Satellite Instability (MSI-H), Chromosome Instability (CIN), and Genomically Stable (GS). The goal of this proposal is to unify neoplasms that share specific minimal molecular and/or genetic alterations and then seek adjustments to treatment and prognosis accordingly.

Technological advancement has made it possible to conduct thorough analyses of DNA alterations in cells and propose multiple mechanisms to explain them beyond the action of biological agents such as *Helicobacter pylori*. In colorectal, endometrial, and gastric neoplasms, one of the most researched mechanisms is the DNA Mismatch Repair (MMR) system. This system is responsible for checking the sequence of nitrogenous bases during DNA replication, seeking to identify errors for correction or induction of apoptosis to critical changes, thereby reducing the number

of errors accumulated in cellular replication.

Regarding neoplasms related to infections, identifying the direct association of the Epstein Barr Virus (EBV) with a group of adenocarcinomas with specific genetic and epigenetic alterations is important because they exhibit a less aggressive biological behavior than their Chromosomal Instability (CIN) and Genomic Stability (GS) molecular counterparts, as well as a satisfactory response to immunotherapy. The most recent systematic review published on the topic presents adjusted frequencies of subtypes with rigorous methodology and meta-analysis validating the correlation between EBV+ subtypes with male sex and MSI-H with female sex [5].

The consolidation of knowledge in this area would enable a better understanding of carcinogenesis pathways and provide a basis for the subsequent development of therapeutic targets or specific chemotherapy regimens for each molecular subtype.

Molecular classifications of gastric adenocarcinomas

The two main molecular classifications of gastric adenocarcinomas are those proposed by The Cancer Genome Atlas Program (TCGA) [6] and Asian Cancer Research Group (ACRG) [7]. In 2019, the WHO began to consider this classification, which uses advanced methods of *in situ* hybridization and PCR to distinguish the 4 groups.

Currently, due to the limited availability of molecular methods and the high cost of disseminating this subclassification, some centers opt for alternative, lower-cost methods. The ACRG's proposal serves as an alternative that provides prognostic considerations and classifies gastric adenocarcinoma into Micro Satellite (MSI) unstable tumors, Micro Satellite Stable with Epithelial-to-Mesenchymal Transition type (MSS/EMT), Micro Satellite-Stable TP53 active (MSS/TP53+), and Micro Satellite Stable TP53 inactive (MSS/TP53) [7].

There are still other proposals for immunohistochemical evaluation of these neoplasms as indirect assessment of phenotypes resulting from the most common molecular alterations or even their use as a screening method for potential beneficiaries of personalized therapeutic regimens. What exists as a common point in the proposed classifications is mainly the individualization of EBV+ and MSI-H adenocarcinomas.

Microsatellite instability and gastric adenocarcinomas

MSI is defined as the malfunction of repair proteins in the gene transcription sequence derived from a conventional DNA strand, resulting from genomic instability. This alteration arises from the failure to correct any

errors during gene transcription and the development of an error-prone DNA region, usually in a similar repetition of nucleotides (tandem repeats), known as microsatellites.

This disorder causes a misalignment of the protein produced after the action of DNA polymerase. When these errors accumulate in large numbers, there is the potential for the development of a neoplasm with Micro Satellite Instability High (MSI-H). MSI-H neoplasms typically present a better prognosis when diagnosed at early clinical stages (American Joint Committee on Cancer (AJCC) stages I and II). Microsatellite instability has a heterogeneous distribution in populations, and in gastric neoplasms, it correlates with the Lauren histological subtypes, being more frequent in intestinal-type adenocarcinomas, less common in diffuse types, and rarely in mixed types.

Extensively studied in colorectal and endometrial adenocarcinomas, which are the most common manifestations of Lynch Syndrome (LS), the origin of inefficiency by somatic (acquired) or germline (inherited) pathways is of interest in determining which carcinogenic pathway the neoplasm is related to. Although LS may initially manifest with gastric neoplasms, this finding is rarer when compared to colorectal and/or gynecological neoplasms. In gastric neoplasms, the origin of the deficiency in DNA repair proteins is mainly due to the hypermethylation process of the MLH-1 gene promoter. As a consequence, there is inactivation and disruption of its DNA strand checking process along with its homologue, *PMS-2*. Other genes may also undergo alterations, having been researched in other molecularly similar cancers, such as *hMSH2*, *hMLH1*, *PMS1*, *PMS2*, *hMSH6*, and *hMSH3* genes.

Epstein-barr virus infection and gastric adenocarcinomas

Cellular alterations resulting from EBV infection have long been better understood in the development of lymphoid neoplasms, particularly Hodgkin's lymphoma and post-transplant lymphoproliferative disorders [8]. With consistent reports since the 1990s and the detection of EBV by molecular methods in neoplastic tissue and cells adjacent to the tumor (precursor lesions), the association with poorly differentiated neoplasms with exuberant lymphoid stroma (lymphoepithelioma-like carcinoma) and the carcinogenic mechanisms in glandular epithelial cells were proposed and validated. Despite a lesser understanding of the process in gastric epithelial cells, the genetic and epigenetic effects found are similar to corresponding nasopharyngeal carcinomas, and can be considered well-established [9]. A more prevalent characteristic in EBV-positive adeno-

carcinomas is the *PIK3CA* mutation. Found in 80% of these gastric tumors and approximately 50% of nasopharyngeal carcinomas, this mutation is an example of similarity in action in epithelial cells with distinct differentiations (squamous and glandular) [10].

Considered a low-virulence virus but with the potential to be latent in about 90% of the world's population, EBV-related epithelial neoplasms are a hallmark in oncology [11]. In gastric adenocarcinomas, they account for about 10% of cases and present distinct therapeutic possibilities and prognostic significance.

Viral DNA molecules found in the cytoplasm (episomes) promote mutations in more than 200 host cell genes during gastric cancer development, with the *AKT2*, *CCNA1*, *MAP3K4*, and *TGFBR1* genes highlighted. As a consequence of acting on cells as episomes, direct research of viral genes by molecular methods is the gold standard diagnostic. In gastric carcinomas, nine viral genes are well established: *BARF0*, *BARF1*, *BcLF1*, *BHRF1*, *BLLF1*, *BRLF1*, *EBNA1*, and *LMP2A* [12]. Of these, *BARF1*, *BHRF1*, and *LMP2A* exhibit oncogenic potential, with the latter standing out for participating in the regulation of survivin protein, which grants characteristics of greater cellular resistance and activates the *DNMT3B* gene, the main responsible for the high levels of genetic material methylation observed [12,13].

The epigenetic alterations observed as a result of this dysregulated methylation process have already been demonstrated in 216 genes that are inhibited (down-regulated) due to hypermethylation and 46 hyperactivated (up-regulated) demethylated genes. Other genes are also silenced (knockdown), with *IHH* and *TRABD* standing out, which increase cellular proliferation and are believed to be one of the bases of neoplastic development [12]. When analyzed together, most of the detected alterations occur through the methylation of cytosine-guanine binding islands (CpG islands), subclassifying EBV-positive adenocarcinomas as neoplasms with a high index of CpG Island Methylation (CIMP-H) [13,14]. In the molecular classification, EBV-positive and MSI-H adenocarcinomas are included in this subgroup (CIMP-H) and are generally more prevalent in men, have a better prognosis, earlier diagnosis, with diffuse Lauren histology and lower rates of lymph node metastasis.

It is important to note that the methylation patterns triggered by EBV are broader and distinct, affecting promoter and non-promoter CpG islands, presenting the highest levels of methylation among studies conducted by TCGA. A striking difference between EBV-positive and MSI-H adenocarcinomas is the methylation of the *CDKN2A* (p16) gene in the former and the promoter of *MLH1* in the latter.

Another point of interest for EBV-positive adenocarcinomas is recent studies correlating the expression of apoptosis-inducing ligands [15]. Like adenocarcinomas with microsatellite instability, they have great potential to be treated with immunotherapy. The main alterations caused by EBV in gastric cancer are methylation of cytosine-guanine islands (CpG islets); assembly of programmed cell death ligands (PD-L1/2), and mutation of the *PIK3CA* gene. Apparently, there is no concurrent mutation in the *P53* gene in the EBV-related oncogenic cascade.

The impact of EBV infection on overall survival is still a topic of debate. Most studies conducted were retrospective and had a low level of evidence for definitive conclusions. These tumors represent a relatively small, albeit relevant, subgroup.

Genomic stability and gastric adenocarcinomas

The subgroup termed Genomically Stable exhibits sporadic molecular alterations and is present in small quantities, making characterization difficult [16]. However, this molecular subtype is enriched with the diffuse Lauren histological type (40 out of 55 cases studied by TCGA, 73%) and mutations in genes related to cell mobility and adhesion, particularly in *RHOA* and *CDH1* genes, as well as fusions involving *CLDN18*, *ARHGAP*, and *RHOA* genes [16,18].

Clinically, GS gastric carcinomas tend to be diagnosed at younger ages compared to other groups, with a median age of 59 years according to the TCGA study ($p=0.0000004$) [6]. They present a worse prognosis, lower recurrence-free and overall survival, and greater chemoresistance.

Through TCGA analysis, mutations in the *RHOA* gene were identified in 16 cases, enriched in this molecular subtype (15% of GS cases, $p=0.039$). *In vivo* studies have shown that *RHOA* is involved in cell adhesion and epithelial cell contractility during development and exhibits oncogenic transformation capacity through STAT3 activation *via* its effectors. Besides mutations, potential fusions involving the *RHOA* gene were identified, with the *GPX1* and *RBM6* genes, still without established biological effects [6,16-19].

Sequencing data demonstrated 13 cases of fusions involving the *CLDN18* gene in the TCGA cohort. *CLDN18* is a member of the human claudin family of tight junctions found in gastric epithelium [20]. The *ARHGAP26* (GRAF) gene, encoding a Gtpase Activating Protein (GAP) that acts in the RHO pathway and is involved in cell motility, was the identified partner in 11 cases, and the homologous GAP *ARGGAP6* in 2 other cases [6,18]. Fusions involving the *CLDN18* genes and *RHOA* mutations were mutually exclusive in TCGA analyses.

Germline mutations in the *CDH1* gene lead to hereditary diffuse gastric cancer syndrome [21]. However, in the TCGA case cohort, only somatic mutations were identified (37% of GS cases), except for 2 cases of non-pathogenic polymorphisms identified in germline analysis. The *CDH1* gene encodes E-cadherin, a protein with a fundamental role in cell adhesion and maintenance of cellular differentiation [22].

Although there is an overlap of molecular alterations involving the GS molecular subtype and the diffuse histological type of gastric cancer, analyses of gene mutation patterns may differ. While the diffuse histological type presents alterations related to differentiation, increased aggressiveness, functions such as pluripotency, and cell migration, the GS molecular subtype presents alterations associated with proliferation and the cell cycle [17].

Chromosomal instability and gastric adenocarcinomas

At a molecular level, a chromosome can have errors of different quantities and types in its genes. However, when there is a rapid accumulation of errors in the genes of a chromosome over a short period of time and without effective action of genetic material protection mechanisms, such a chromosome is considered genetically unstable [23,24]. CIN gastric adenocarcinomas generally exhibit an intestinal phenotype, and the most frequent mutations occur in the P53 gene and tyrosine kinase receptors. Some of these receptors are associated with a worse prognosis, higher rates of lymph node metastasis, advanced stages at diagnosis, and low overall survival rates [25]. The most involved tyrosine kinase receptors are *EGFR*, *FGFR2*, *HER2*, and *MET* and some, such as *FGFR2* may also be present in GS adenocarcinomas [26]. Once the oncogenic cascade is initiated in one of these receptors, what is observed is the accumulation and synergy of various processes leading to a high mutational rate in these neoplasms.

Thus, the implementation of specific therapies is challenging for such a heterogeneous group with such broad potential manifestations. When a more specific alteration is detected, already known and consolidated with the potential for individualized intervention such as *HER2*, other possibilities of chemotherapy intervention are opened. Some studies support that the intestinal type is the most common, and the genomic mechanism by which this pathology functions makes it a good candidate for the widely used cisplatin cycle associated with radiotherapy, as it interferes with cell replication, preventing the propagation of abnormalities resulting from chromosomal instability. The pharmacotherapy cycle likely encounters difficulties because the tumor deletes the production of neoantigens to be destroyed by the immune system [26,27].

Conclusion

In many oncological medical services, gastric neoplasm diagnoses are often oversimplified, focusing mainly on morphological and immunohistochemical features, typically categorizing adenocarcinomas. Despite financial constraints, our group is committed to refining molecular subclassifications following TCGA and WHO guidelines. Motivated by scientific responsibility, we are exploring cost-effective alternatives to enhance diagnostic accuracy and treatment efficacy, aiming to bridge the gap between simplified diagnoses and comprehensive molecular profiling.

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Conflict of Interest

The authors declare no conflicts of interest.

References

- [1] Hu B, El Hajj N, Sittler S, Lammert N, Barnes R, Meloni-Ehrig A. Gastric cancer: Classification, histology and application of molecular pathology. *J Gastrointest Oncol* 2012;3(3):251-261.
- [2] Key Statistics About Stomach Cancer. American Cancer Society 2024.
- [3] Ma J, Shen H, Kapesa L, Zeng S. Lauren classification and individualized chemotherapy in gastric cancer. *Oncol Lett* 2016;11(5):2959-2964.
- [4] Nagtegaal ID, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, et al. The 2019 WHO classification of tumours of the digestive system. *Histopathology* 2020;76(2):182-188.
- [5] Santos GO, Nunes WA, Junior WF, Botega LG, Roehe AV. Molecular profile of gastric adenocarcinoma, relevant epidemiological factors-systematic review and meta-analysis relating sex with Epstein-Barr virus and unstable microsatellites subtypes. *Asia Pac J Clin Oncol* 2023.
- [6] Bass AJ, Thorsson V, Shmulevich I, Reynolds SM, Miller M, Bernard B, et al. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 2014;513(7517):202-209.
- [7] Cristescu R, Lee J, Nebozhyn M, Kim KM, Ting JC, Wong SS, et al. Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. *Nature medicine*. 2015 May;21(5):449-56.
- [8] Young LS, Rickinson AB. Epstein-Barr virus: 40 years on. *Nat Rev Cancer* 2004;4(10):757-768.
- [9] Thompson LD, Bishop JA. Update from the 5th edition of the World Health Organization classification of head and neck tumors: Nasal cavity, paranasal sinuses and skull base. *Head Neck Pathol* 2022;16(1):1-8.
- [10] Gulley ML. Genomic assays for Epstein-Barr virus-positive gastric adenocarcinoma. *Exp Mol*

- Med 2015;47(1):e134.
- [11] Rajendra K, Sharma P. Viral pathogens in oesophageal and gastric cancer. *Pathogens* 2022;11(4):476.
- [12] Zhao J, Liang Q, Cheung KF, Kang W, Lung RW, Tong JH, et al. Genome-wide identification of Epstein-Barr virus-driven promoter methylation profiles of human genes in gastric cancer cells. *Cancer* 2013;119(2):304-312.
- [13] Chang MS, Uozaki H, Chong JM, Ushiku T, Sakuma K, Ishikawa S, et al. CpG island methylation status in gastric carcinoma with and without infection of Epstein-Barr virus. *Clin Cancer Res* 2006;12(10):2995-3002.
- [14] Murphy G, Pfeiffer R, Camargo MC, Rabkin CS. Meta-analysis shows that prevalence of Epstein-Barr virus-positive gastric cancer differs based on sex and anatomic location. *Gastroenterology* 2009;137(3):824-833.
- [15] Nakano H, Saito M, Nakajima S, Saito K, Nakayama Y, Kase K, et al. PD-L1 overexpression in EBV-positive gastric cancer is caused by unique genomic or epigenomic mechanisms. *Sci Rep* 2021;11(1):1982.
- [16] Ling Y, Watanabe Y, Nagahashi M, Shimada Y, Ichikawa H, Wakai T, et al. Genetic profiling for diffuse type and genomically stable subtypes in gastric cancer. *Comput Struct Biotechnol J* 2020;18:3301-3308.
- [17] Wong SS, Kim KM, Ting JC, Yu K, Fu J, Liu S, et al. Genomic landscape and genetic heterogeneity in gastric adenocarcinoma revealed by whole-genome sequencing. *Nat Commun* 2014;5(1):5477.
- [18] Aznar S, Valeron PF, del Rincon SV, Perez LF, Perona R, Lacal JC. Simultaneous tyrosine and serine phosphorylation of STAT3 transcription factor is involved in Rho A GTPase oncogenic transformation. *Mol Biol Cell* 2001;12(10):3282-3894.
- [19] Thumkeo D, Watanabe S, Narumiya S. Physiological roles of Rho and Rho effectors in mammals. *Eur J Cell Biol* 2013;92(10-11):303-315.
- [20] Tureci O, Koslowski M, Helftenbein G, Castle J, Rohde C, Dhaene K, et al. Claudin-18 gene structure, regulation, and expression is evolutionary conserved in mammals. *Gene* 2011;481(2):83-92.
- [21] Doherty GJ, Ahlund MK, Howes MT, Moren B, Parton RG, McMahon HT, et al. The endocytic protein GRAF1 is directed to cell-matrix adhesion sites and regulates cell spreading. *Mol Biol Cell* 2011;22(22):4380-4389.
- [22] Graziano F, Humar B, Guilford P. The role of the E-cadherin gene (CDH1) in diffuse gastric cancer susceptibility: from the laboratory to clinical practice. *Ann Oncol* 2003;14(12):1705-1713.
- [23] Heim S, Mitelman F. *Cancer cytogenetics: Chromosomal and molecular genetic aberrations of tumor cells.* John Wiley and Sons 2015.
- [24] Hartwell L. Defects in a cell cycle checkpoint may be responsible for the genomic instability of cancer cells. *Cell* 1992;71(4):543-546.
- [25] Rocken C. Molecular classification of gastric cancer. *Expert Rev Mol Diagn* 2017;17(3):293-301.
- [26] Silva AN, Coffa J, Menon V, Hewitt LC, Das K, Miyagi Y, et al. Frequent coamplification of receptor tyrosine kinase and downstream signaling genes in Japanese primary gastric cancer and conversion in matched lymph node metastasis. *Ann Surg* 2018;267(1):114-121.
- [27] Zhang R, Liu Z, Chang X, Gao Y, Han H, Liu X, et al. Clinical significance of chromosomal integrity in gastric cancers. *Int J Biol Markers* 2022;37(3):296-305.