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# Pathomorphological and molecular genetic features of diffuse gastric cancer

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**ABSTRACT**

Gastric cancer (GC) is the fifth most common type of cancer in the world and the third leading cause of death from cancer. GC is a multi-factorial and morphologically heterogeneous disease. Currently, several morphological classifications of GC are used, however, for diagnosis, it is necessary to take into account not only the morphological type of the tumor, but also its molecular subtype. According to the literature, the intestinal type of GC is most often associated with effects of environmental factors and is usually found in older age groups in men, while diffuse gastric cancer (DGC) is a genetically determined disease which is more common in younger patients, with the same frequency among men and women.

This review covers in detail GC, its classification by P.A. Lauren (1965), and its molecular subtypes characterized during the Cancer Genome Atlas project and examines the impact of certain risk factors on the pathogenesis of the disease, such as *H. pylori* infection or Epstein – Barr virus. A separate section in this analytical work is dedicated to expression of the PD-L1 marker by tumor cells and the use of this parameter for prognosis and therapy of this disease. An essential part of the work is discussion of the features of intestinal and diffuse types of gastric cancer, which reflect not only the differences in classifications used in modern diagnosis, but also the relationship between the pathological pattern and the molecular subtype of gastric cancer.

**Key words:** diffuse gastric cancer, epidemiology, molecular genetic diagnosis, classification, immunotherapy.

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# Патоморфологические и молекулярно-генетические особенности диффузного типа рака желудка

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**РЕЗЮМЕ**

Рак желудка (РЖ) занимает пятое место в мире по распространенности среди всех злокачественных новообразований и является третьей по значимости причиной смертности от онкологических заболеваний.

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РЖ является мультифакториальным, морфологически неоднородным заболеванием. В настоящее время используется несколько морфологических классификаций РЖ, однако для постановки диагноза требуется учитывать не только морфологический тип опухоли, но и ее молекулярный подтип. По данным литературы, РЖ интестинального типа чаще всего ассоциирован с действием факторов окружающей среды и, как правило, встречается в старших возрастных группах у мужчин. Диффузный тип рака желудка (ДТРЖ) является в большей степени генетически детерминированным заболеванием и чаще встречается у более молодых пациентов, при этом с одинаковой частотой среди мужчин и женщин.

В данном обзоре подробно освещается тема РЖ, его классификация по P.A. Lauren (1965), его молекулярным подтипы, охарактеризованные в Атласе ракового генома (The Cancer Genome Atlas), а также рассматривается влияние определенных факторов риска на патогенез заболевания, таких как инфицирование *H. pylori* или вирусом Эпштейна – Барр. Отдельную роль в данной аналитической работе занимает вопрос экспрессии опухолевыми клетками маркера PD-L1 и использование данного параметра для прогнозирования и терапии этого заболевания. Немаловажной частью работы является обсуждение особенностей интестинального и диффузного типов рака желудка, которые отражают не только различия используемых в современной диагностике классификаций, но и взаимосвязь патоморфологической картины с молекулярным подтипом рака желудка.

**Ключевые слова:** рак желудка диффузного типа, эпидемиология, молекулярно-генетическая диагностика, классификация, иммунотерапия.

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## INTRODUCTION

Gastric cancer (GC) is the fifth most common type of cancer in the world and the third leading cause of death from cancer. Morbidity and mortality differ depending on the geographical region. In countries like Japan, China, Korea, and Chile, GC ranks first in morbidity and mortality [1]. In Russia in 2018, 21,279 men and 15,662 women were diagnosed with GC. The average age of patients upon diagnosis was 67.5 years, and the incidence rate was 25.4 cases per 100,000 population. In 2017, 16,572 people died from gastric cancer in Russia. The average age of patients was 68.7 years, and the mortality rate per 100,000 population was 18.97 [2].

## DEFINITION

In 1965, P.A. Lauren proposed a classification of GC, which contained intestinal, diffuse, and mixed histotypes [3]. GC is a multi-factorial and histologically heterogeneous disease. For example, intestinal GC is most often associated with environmental factors (diet, smoking, obesity, alcohol consumption) and *Helicobacter pylori* (*H. pylori*) infection [4], which, in turn, leads to development of chronic gastritis, followed by atrophic gastritis, intestinal metaplasia, dysplasia, and carcinoma through the Correa cascade [5].

The intestinal type occurs more frequently in older men, is usually macroscopically represented by a tumor with an exophytic type of growth and a tendency to ulceration, and microscopically consists of cells that form glands. Diffuse gastric cancer (DGC) is less associated with environmental factors and inflammatory diseases: the role of *H. pylori* in the pathogenesis of DGC is still debatable. However, a number of studies conducted in the UK and Japan showed the presence of *H. pylori* outside the tumor tissue in approximately 32% of cases in patients younger than 40 years with morphologically confirmed DGC [6].

DGC is considered to be a more genetically determined disease associated with loss of heterozygosity on p17 chromosome, p53 mutation or loss of heterozygosity, and mutation or loss of E-cadherin [7]. DGC is more common in younger patients with the same frequency among men and women. 1–3% of all cases of DGC are related to a proven heredi-

tary genetic syndrome that causes a mutation in the E-cadherin gene (*CDH1*) located on chromosome 16 — hereditary diffuse gastric cancer (HDGC). For this category of patients, gastrectomy is recommended as a method of preventive treatment [8].

## MOLECULAR CLASSIFICATION OF GASTRIC CANCER

According to the Cancer Genome Atlas (TCGA) project, there are four molecular subtypes of GC [9]:

1. Associated with the Epstein – Barr virus (EBV-associated subtype), which accounts for about 9% of cancer cases.

2. Caused by microsatellite instability (MSI subtype): this subtype accounts for about 22% of cancer cases.

3. With genome stability (GS subtype): this subtype accounts for about 20% of cancer cases.

4. Caused by chromosomal instability (CIN subtype): this subtype accounts for about 50% of cancer cases.

*EBV-associated molecular subtype of GC (Epstein – Barr Virus-Positive Gastric Cancer)*. Epstein – Barr virus (EBV) is a human γ-herpesvirus that is characterized by pronounced tropicity to the lymphatic system and has the ability to persist in the human body. Given the lymphotropicity of EBV after penetration into B-lymphocytes, the virus causes their uncontrolled proliferation. EBV also has pronounced tropicity to the gastric mucosa, which has a developed lymphatic system [10–12].

The International Agency for Research on Cancer (IARC) classifies EBV as a Group 1 carcinogen. According to a number of researchers, only latent EBV infection can be associated with various types of human neoplasms and hemoblastosis.

EBV-associated gastric adenocarcinoma was first described by A.P. Burke in 1990. It is known that about 90,000 people annually develop EBV-associated gastric carcinoma, which is about 10% of all cases of gastric cancer [13]. The association between *H. pylori* and EBV is not fully understood, but there is no doubt that in a group of patients with no history of *H. pylori* infection or who have undergone successful eradication of the pathogen, EBV may be a leading factor in chronic inflammation in the gastric mucosa and cause a risk of malignant neoplasms [14–16].

*GC caused by microsatellite instability (MSI)*. Microsatellite instability (MSI) is a phenotype characterized by an increased probability of mutations occurring as a result of impaired repair system of incorrectly paired DNA bases. Following the impaired repair in cells, errors during DNA replication accumulate, which leads to emergence of new microsatellites.

When using a standard panel that includes BAT26 and BAT27 mononucleotides and D2S123, D5S346, and D17S250 dinucleotide repeats [17], microsatellite instability can be divided into 3 levels: high MSI (MSI-H), low MSI (MSI-L), and microsatellite stability (MSS). According to the literature, MSI-H-associated carcinoma, depending on the ethnic group, occurred in 5–50% of cases, and MSI-L- and MSS-associated carcinomas are known to be localized in the gastric antrum. According to the histological classification proposed by Lauren, they belong to the intestinal type of GC and have a good prognosis with rare metastasis, compared to MSI-H [18].

*GC with genome stability (Genomically Stable, GS)*. GC with a stable genome has a lower mutation load compared to other molecular subtypes and occurs at a relatively young age. This molecular subgroup is characterized by a diffuse histological type according to Lauren, as well as a large number of mutations in RhoA and CDH1 (as mentioned earlier, CDH1 is associated with inherited DGC).

*GC caused by chromosomal instability (CIN)*. Chromosomal instability is a type of genome instability in which non-clonal karyotype changes are observed in the daughter generations of dividing cells, namely, loss or acquisition of chromosomes and their sections. Cancer with chromosomal instability is characterized by extremely high frequency of chromosomal abnormalities and their high diversity [19]. In GC, a high CIN level is always associated with a poor prognosis.

In a study conducted by A. J. Bass et al. [9], the molecular subtype of GC was correlated with its localization. Each molecular subtype of GC could have any localization, but CIN-associated tumors were more common in the gastrointestinal tract and gastric cardia (65%); most EBV-associated tumors were found in the bottom or body of the stomach (62%); MSI-associated tumors were less often detected in the gastrointestinal tract and cardia, but

with approximately the same frequency were found in other parts of the stomach; GS-tumors were found with approximately the same frequency in all parts of the stomach, and this subtype was mostly presented by DGC (according to Lauren). At the same time, GS-tumors were diagnosed at an earlier age (the average age was 59 years), while MSI-associated tumors were diagnosed at a relatively older age (the average age was 72 years). Patients with MSI-associated tumors were usually women (56%), and most cases of EBV-associated tumors were detected in men (81%).

## PD-L1 AND GASTRIC CANCER

Programmed cell death-1 (PD-1) is a membrane protein of the immunoglobulin superfamily involved in differentiation of immune cells. PD-1 plays an important role in negative regulation of the immune system by preventing activation of T-lymphocytes, which reduces autoimmunity and increases self-tolerance [20]. The protein has two ligands: PD-L1 and PD-L2. In tumor cells that express PD-L1, this ligand is involved in mechanisms of tumor escape from immune control. Determining the expression of PD-L1 makes it possible to identify a group of patients who are most likely to benefit from therapy by immune checkpoint inhibitors. A fairly good result of anti-PD-L1 therapy was achieved for non-small cell lung cancer.

A relationship between PD-L1 expression and prognosis in GC is still the subject of debate. According to a study by S. Boger et al. [21], patients with PD-L1-positive tumor cells had improved disease prognosis. However, according to H. Chang et al. [22], high PD-L1 expression was an unfavorable prognostic factor, and A. Kawazoe et al. [23] did not consider PD-L1 as a prognostic factor at all. It is important to keep in mind that all of the above-presented research data were obtained from patients of the Asian ethnic group, and, therefore, the results cannot be applied to the general population, since the response to therapy / drugs in different ethnic groups may not be the same.

According to the experiments by C. Ma. et al. [24] and S. Derks et al. [25], EBV- and MSI-associated GCs most often showed greatly positive PD-L1 expression or overexpression of PD-L1, which was a significant adverse prognostic factor. Another study [9] showed that in the molecular EBV- and MSI-associated subgroups of GC, pronounced lymphocytic infiltration in the tumor stroma was found. Therefore, these subtypes can be classified as GCs with severe lymphoid stroma (medullary carcinoma). The lymphoid stroma in these tumors has a large number of CD8+ T cells that can cause a strong anti-tumor inflammatory response. In addition, positive PD-L1 expression was associated with a significant increase in the number of CD8+ T cells at the edge of the invasive tumor front.

S. Derks et al. [25] observed a difference in the nature of infiltration of PD-L1-positive cells depending on the molecular subtype of the tumor. In EBV- and MSI-associated gastric carcinomas, PD-L1 positive cells had the ability to penetrate into the center of the tumor in contrast to MSS-associated gastric carcinomas, in which PD-L1 positive cells remained mainly at the edge of the tumor invasion area. Based on this result, patients with EBV- and MSI-associated gastric carcinomas may be the main candidates for therapy with PD-1 inhibitors.

According to H. Saito et al. [26], five-year survival in patients with and without positive PD-L1 expression differed by 48.9% and 80.7%, respectively. In addition, PD-L1 positive expression was observed in the older age group of patients, and by the histological type, positive expression was more often detected in undifferentiated gastric carcinomas.

L. Wang et al. [27] studied the correlation between HER2 status and PD-L1 expression. According to this research, HER2-positive patients had positive PD-L1 expression in 24.2% of cases, while HER2-negative patients had positive PD-L1 expression in 39.0% of cases. Based on the data obtained, HER2-negative patients may be the best candidates for targeted anti-PD-1 therapy. However, the authors drew attention to false negative results that were associated with the biopsy technique: prominent PD-L1 expression was detected at the edge of the invasive front of the tumor and not in its center.

According to H. Fukamachi et al. [28], following the study of DGC by gene expression profiling, the authors distinguished two DGC clusters. The first cluster is represented by DGC with a stable genome (GS), which can be interpreted as “primary” DGC.

The second cluster is represented by DGC with microsatellite instability (MSI) and chromosomal instability (CIN), which was defined by the authors as DGC developed from the intestinal type. In addition, an analysis of the expression of mTOR and PD-L1 in each individual cluster was performed, which showed more pronounced expression in the second cluster (MSI and CIN). Based on these data, it can be assumed that in a group of patients with DGC, which has developed from the intestinal type of GC, it is possible to use mTOR and PD1 inhibitors for treatment.

## *HELICOBACTER PYLORI* AND DIFFUSE GASTRIC CANCER

The International Agency for Research on Cancer (IARC) classified *H. pylori* as a Group I carcinogen (strong carcinogen) in 1994 [29, 30]. Initially, *H. pylori* infection was thought to be associated mainly with the intestinal type of GC, while for DGC, the underlying factors in the pathogenesis were genetic abnormalities. However, if we do not consider cases of inherited DGC, numerous studies report a significant role of *H. pylori* and Epstein – Barr virus in the occurrence of sporadic DGC [31–33]. Serological studies also confirmed that *H. pylori* is associated with both histological types of GC.

A number of studies showed that patients with a low *H. pylori* IgG titer are more likely to develop an intestinal type of GC, while patients with a high *H. pylori* IgG titer have a high risk of developing DGC [34–36]. There is evidence that *H. pylori* is able to inhibit factors responsible for cell adhesion and, thus, participate in the pathogenesis of DGC. Y. Yang et al. demonstrated disintegration of E-cadherin by *H. pylori* SS1 and 26695 strains. It was found that the SS1 strain more effectively disintegrated E-cadherin after 12 and 24 hours [37]. After penetration of *H. pylori* into the gastric epithelium, nonphosphorylated binding of CagA to E-cadherin occurs, which leads to separation of the E-cadherin / β-catenin complex and causes accumulation of β-catenin in the cytoplasm and nucleus, ultimately activating a β-catenin-dependent gene involved in cancer progression [38]. Aberrant activation of β-catenin disrupts normal apical junctional complexes, which leads to loss of cell polarity [39].

## MORPHOLOGY OF DIFFUSE GASTRIC CANCER

In a study by H.E. Lee et al., the differences in the morphology of hereditary and sporadic DGC were identified. Based on the material presented by 11 cases of gastrectomy in patients with inherited DGC and a genetically confirmed mutation in the CDH1 gene, the tumor cells were morphologically divided into three groups.

Group 1 included well-differentiated, large signet ring cells with a large amount of cytoplasm, a small nuclear-cytoplasmic ratio, and flattened and eccentrically located nuclei with moderate atypia. They were located under the surface epithelium and had positive expression to mucicarmine and pCEA and negative expression to p16 and CDX2.

Group 2 contained well-differentiated, small signet ring cells with a smaller amount of cytoplasm, with more rounded and hyperchromic nuclei with pronounced signs of atypia, and a high nuclear-cytoplasmic ratio. These cells were located in their own mucosa and had negative expression to mucicarmine, pCEA, p16, and CDX2.

Group 3 included poorly differentiated, small signet ring cells with positive expression to p16 and negative expression to CDX2. As a control group, material from 20 cases of gastrectomy in sporadic DGC was used. No morphological features were identified for them, but positive expression to p16 and CDX2 was noted [40].

In the work by H.H. Wong and P. Chu [41], the authors considered the features of immunohistochemical diagnosis in the group of gastrointestinal cancers. For DGC, positive expression to CDX-2, CK7, and HepPar-1 in approximately 70% of cases was reported. About half of the cases had positive expression to CK20, MUC2, and MUC5AC. Moreover, negative expression to MUC1 and E-cadherin was detected. According to the authors, morphological cases of low-grade adenocarcinoma with pronounced lymphoplasmacytic infiltration may be positive for EBV.

## CONCLUSION

Currently, it is important not only to timely diagnose GC, but also to find a personalized approach to each patient. An extremely important aspect of diagnosis (verification) is a comprehensive

approach to the study of surgical material using traditional optical research methods with mandatory periodic acid Schiff (PAS) and Alcian Blue staining of the material and counting of the number of signet ring cells, expressed as a percentage, as well as detection of *H. pylori*, and immunohistochemistry using a panel of antibodies (CDX-2, CK7, CK20, HepPar-1, MUC1, MUC2, MUC5AC, HER2, mTOR, PD-L1, and E-cadherin).

A molecular genetic study of the material is required to determine the molecular subtype of GC and correlate the data obtained with the expression of such antibodies as HER2, PD-L1, and mTOR for more accurate determination of the cohort of patients who can benefit from therapy with mTOR and PD1 inhibitors. Only this comprehensive approach to diagnosis of GC can give specialists more clear understanding of the disease and help in choosing a treatment strategy for such patients.

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## Authors contribution

Mikhaleva L.M., Midiber K.Yu. – conception and design. Pechnikova V.V., Vasyukova O.A. – analysis and interpretation of data. Mikhaleva L.M. – substantiation of the manuscript and critical revision for important intellectual content. Mikhaleva L.M., Midiber K.Yu., Pechnikova V.V., Vasyukova O.A., Gushchin M.Yu. – final approval of the manuscript for publication.

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