# Epithelial-mesenchymal transition in main types of gastric carcinoma

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*Introduction.* The rapid development of basic science enabled us to significantly expand our understanding of various intercellular interactions. Epithelial-mesenchymal transition (EMT) is known to play a key role in certain tissue formation in the embryonic period. However, recent data show that EMT can also be observed in some pathological conditions, in particular, in various neoplasm development. This suggests that there are a number of alternative and fundamentally new mechanisms for the tumor formation and progression. Thus, EMT, which occurs in carcinomas, increases the invasiveness, immunoresistance, immunity to therapy, and the metastatic potential. Knowledge of EMT features and their timely recognition in morphological tumor diagnosis is of great predictive importance for patients. The aim of the research was to study the morphological features of epithelial-mesenchymal transition in the main types of gastric cancer.

*Materials and methods.* We studied specimens of gastric carcinomas (N=64) including 31 cases of diffuse type, 19 cases of intestinal type, and 14 cases of mixed type.

*Results.* All cases of the diffuse carcinoma group showed spread EMT features, which appeared already in the mucosa and completed with positive vimentin expression in 93.5% of cases. The malignant cell proliferative activity was low; however, in 29% of cases we detected areas of moderate or even high activity. In the intestinal type gastric cancer, EMT developed as a result of tumor progression, it arose more often in the deeper layers and was incomplete and focal. As a rule, the proliferative activity of tumor cells was high and moderate. Vascular invasion occurred more often in diffuse type (90.3%), less often in mixed type (71.4%), and even less often in the intestine type (55.8%) gastric carcinoma.

*Conclusion.* The variety of morphological features of EMT, its frequency, prevalence, completeness, and sequence in the development of various types of gastric cancer determines the features of their clinical manifestation and influences their further management.

**Keywords:** gastric cancer, diagnosis, histological main types, EMT, morphopathology

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## Эпителиально-мезенхимальный переход в основных типах рака желудка
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*Введение.* Стремительное развитие фундаментальной медицины позволило значительно расширить наши представления о различных межклеточных взаимодействиях. Известно, что эпителиально-мезенхимальная трансформация (ЭМТ) играет ключевую роль при формировании некоторых тканей в эмбриональном периоде. Тем не менее, по последним данным, процессы ЭМТ могут наблюдаться и при некоторых патологических состояниях, в частности при развитии разных новообразований. Это наталкивает на мысль, что существует целый ряд альтернативных и принципиально новых механизмов формирования и прогрессии опухолевых заболеваний. Так, ЭМТ, возникающая при карциномах, усиливает инвазивность, иммунорезистентность, невосприимчивость к терапии и метастатический потенциал последних. Знание особенностей подобных трансформаций и своевременное распознавание таких процессов при морфологической диагностике опухолей очень предиктивно значимы для пациентов. Целью настоящего исследования является изучение морфологических особенностей

эпителиально-мезинхимального перехода в основных типах рака желудка.

*Материалы и методы.* Изучены образцы 64 случаев рака желудка: 31 случай диффузного типа, 19 случаев кишечного типа и 14 случаев смешанного типа.

*Результаты.* Во всех случаях диффузного рака наблюдались признаки распространенной ЭМТ, которая появляется уже в слизистом слое и в 93,5% случаев завершается экспрессией виментина. Пролиферативная активность злокачественных клеток была низкой, но в 29% случаев отмечались участки умеренной или даже высокой активности. При кишечном типе рака желудка ЭМТ развивается в результате прогрессии опухоли, чаще в более глубоких слоях, и носит неполный и очаговый характер. Пролиферативная активность опухолевых клеток была, как правило, высокой и умеренной. Сосудистая инвазия чаще встречалась при раке диффузного типа (90,3%), реже – при раке смешанного типа (71,4%) и еще реже – при кишечном типе рака желудка (55,8%).

*Выводы.* Разнообразие морфологических особенностей ЭМТ, ее частота, распространенность, полнота и последовательность в развитии при различных видах рака желудка определяют особенности их клинического проявления.

**Ключевые слова:** рак желудка, диагностика, основные гистологические типы, ЭМТ, морфология

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## Introduction

Epithelial-mesenchymal transition (EMT) occurs during embryonic development, organogenesis, regeneration (wound healing), chronic inflammation with organ fibrosis, and in neoplasms [1–6]. The basic nature of this process is the loss of main epithelial features such as cell-cell adhesion, spindle shape acquisition by epithelial cells, diminished expression of cytokeratins, emerging capacity to express vimentin, and α-smooth muscle actin (α-SMA) with ability to migrate.

EMT drastically enhances carcinoma invasiveness and its metastatic potential, increases cellular viability preventing cells from apoptosis and aging, and also provides them with stem cell features, immunoresistance, and unresponsiveness to radiation and chemotherapy, which leads to aggressive behavior of these tumors [4, 6, 7]. Many routine cancer treatments may stimulate EMT and enhance invasion and metastasis [8].

Therefore, in tumor morphologic diagnosis, it is important to establish the EMT presence, its spread, and completeness. Completeness means that a cell not only diminishes or loses its epithelial features, but also gains those of mesenchymal tissues such as vimentin and α-SMA expression, and cell discohesion and motility [9, 10].

P. Lauren classification (1965) subdivides gastric carcinomas into 2 main histological types: diffuse and intestinal [11]. The so-called mixed type carcinoma has features of both types. These carcinomas differ in their etiology, pathogenesis, morphology, and presumably EMT specificities. Initially, gastric carcinoma develops in the mucosa with subsequent invasion into the submucosa, the muscularis propria, and the subserosa. That allows an investigator to monitor the effects of morphological changes, including EMT features, as the tumor progresses.

## Materials and methods

We studied specimens of 64 patients with gastric carcinomas, including 31 of diffuse type, 19 of intestinal type, and 14 of mixed type. Gross examination and sampling were performed: we excised strips from cardiac to pyloric margin of the tumors measuring approximately 1 cm in width and comprising the entire gastric wall thickness. The strips were consequently cut in pieces and fixed in formalin. The slides were made from paraffin-embedded blocks with subsequent staining with hematoxylin and eosin and alcian blue at a pH of 1.0 and 2.5 to detect sulfated and nonsulfated mucins. The PAS reaction was also performed. We used single pieces from both the center and the periphery of the tumors to assay them immunohistochemically. The specimens were cut into 4-μm sections, subsequently deparaffinized in xylene, and hydrated. After PBS rinsing, endogenous peroxidase activity was blocked by 3% hydrogen peroxide in 100% methanol. The slides were incubated with diluted anti-E-cadherin antibody, AE1/AE3, cytokeratin 18, vimentin, α-SMA, Ki-67, and then secondary antibody. We utilized the visualization system EnVision FLEX (Dako, Copenhagen, Denmark).

Two criteria were used to evaluate EMT: prevalence (focal and widespread) and completeness. Incomplete EMT was accompanied by the loss of cell communication, change in shape to a fibroblast-like, decrease in or absence of epithelial marker expression (AE1/AE3, SC18, and E-cadherin), and complete acquisition of mesenchymal marker expression (vimentin and/or α-SMA).

By the presence of tumor emboli in the vessels, we accessed the tumor invasive potential, based on the work of T. Mita, T. Shimoda (2001), according to which tumor invasion of the lymphatic vessels in gastric cancer (GC) was the most significant determinant (OR 8.68) for lymph node metastases, which dramatically worsens the prognosis [12]. The invasiveness was compared with the EMT presence, its prevalence, and completeness. The proliferative activity index (Ki-67 expression) was estimated semi-quantitatively as weak (up to 15% positively stained cells), moderate (up to 40%), and high (above 40%).

Statistical processing was performed in the MedStat package using basic methods of mathematical statistics. Statistical analysis of the indicators obtained as a result of processing stabilograms showed that their distribution differed from normal (at the level of $p<0.001$), in accordance with which, when conducting further statistical analysis, nonparametric criteria were used. The reliability of the relationship or differences in the indicators (p) were determined, and the nonlinear Kendall correlation coefficient (Tau) was used to assess the degree and reliability of the relationship between two related samples.

### Results

Patients with main histological types of GC differed in age: 42% of diffuse type patients were fewer than 50 years old, but, at the same time, intestinal and mixed type patients comprised 21.4% and 21% in this age group, respectively.

All cases of diffuse GC showed the spread of EMT features. In the gastric mucosa, the tumor diffusely exhibited detachment of malignant cells from each other. Small areas where the connection between the cells was preserved in the deeper layers of the gastric wall were noted in 12 examined cases; more often they were observed in a form of short trabeculae, small groups, and, less often, in a form of solid, gland-like structures. In such areas, membranous and/or cytoplasmic expression of E-cadherin was preserved as well as it was expressed in some malignant cells. In 8 cases, more commonly in the deeper layers of the gastric wall (the muscularis propria and the subserosa), we discovered malignant cells with a greater degree of atypia (big nuclei with dispersed chromatin, diminished amount of cytoplasm, and decreased mucin secretion).

All cases of diffuse GC type displayed changes in the shape of malignant cells: small signet ring cells became stellate spindle fibroblast-like cells. In 7 out of 31 cases (22.6%), elongated fibroblast-like malignant cells were detected in mucosa basal layers adjacent to the muscularis mucosa; in all other cases, we found them in the submucosa and deeper layers of the gastric wall. These cells often expressed cytokeratins, i.e. pancytokeratin AE1/AE3 or cytokeratin 18. However, in some malignant cells, cytokeratin expression was decreased to the point of complete loss. The loss of cytokeratin expression occurs even in the mucosa, but it may be selective. For example, it was absent in big signet ring cells, but still preserved in small malignant cells (Fig. 1 A). Already in the basal layer of the gastric mucosa, fibers of extracellular matrix (ECM) appeared next to fibroblast-like malignant cells in bigger numbers than in other parts of the gastric mucosa. In the submucosa, due to enlarged ECM volume, cancer acquired scirrhous features.

Almost in every case of diffuse GC type (29 out of 31 cases), EMT was complete. In other words, the expression of mesenchymal markers such as vimentin and α-SMA was detected in round signet ring cells as well as in fibroblast-like malignant cells (Fig. 1 B).

The change of cellular form and acquisition of mesenchymal phenotype–one of the main EMT features–have been connected to an increase in tumor invasiveness and metastatic potential (Fig. 1 C) [9, 10, 13]. We studied tumor invasiveness by the presence of intravascular malignant cells. In diffuse cancer cases, they were found in 28 out of 31 cases (90.3%).

*Table | Таблица*

**Comparison of the EMT frequency, prevalence, and completeness and tumor cell vascular invasion in different histological GC types |**
**Сопоставление частоты, распространенности и полноты ЭМТ с инвазией сосудов опухолевыми клетками при разных гистологических типах рака желудка**

| EMT \| ЭМТ | Diffuse gastric carcinoma EMT 31 of 31 \| Диффузный рак желудка ЭМТ – 31 из 31 | | Intestinal gastric carcinoma EMT 18 of 19 \| Кишечный рак желудка ЭМТ – 18 из 19 | | Mixed gastric carcinoma EMT 14 of 14 \| Смешанный рак желудка ЭМТ – 14 из 14 | |
|---|---|---|---|---|---|---|
| | with invasion \| с инвазией | no invasion \| без инвазии | with invasion \| с инвазией | no invasion \| без инвазии | with invasion \| с инвазией | no invasion \| без инвазии |
| Prevalent EMT \| Распространенная ЭМТ | 28 | 3 | – | – | 3 | – |
| Local EMT \| Очаговая ЭМТ | – | – | 11 | 7 | 6 | 5 |
| Complete EMT \| Полная ЭМТ | 28 | 1 | – | – | 3 | – |
| Incomplete EMT \| Неполная ЭМТ | – | 2 | 11 | 7 | 7 | 4 |

As a rule, the morphology of the intraluminal tumor and the tumor outside the vessels was identical. Only in 5 cases of signet ring small-cell cancer, the intraluminal carcinoma had composition of a solid tumor (Fig. 1 C), with one of them having gland-like structures suggesting the presence of EMT.

In all cases, the proliferative activity of malignant cells was low with a proliferation index below 5%, but in 9 cases
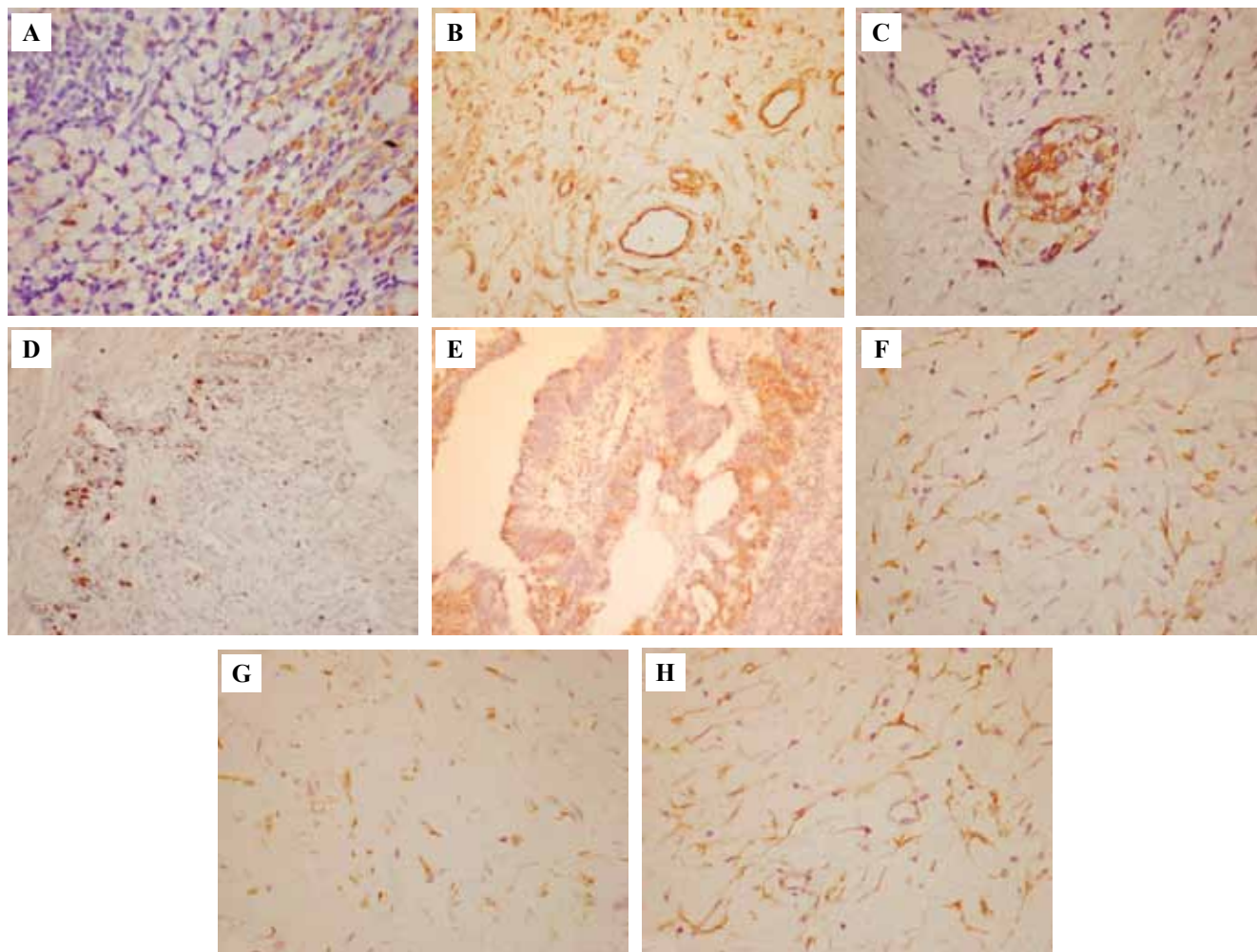


*Fig. 1*. Immunohistochemical features of Epithelial-mesenchymal transition (EMT) in gastric cancer (GC).
A – selective loss of cytokeratin AE1/AE3 expression in signet cells but its preservation in small malignant cells (immunohistochemical [IHC] assay with antibodies to pancytokeratin AE1/AE3, ×400)]; B – complete EMT in diffuse type GC, vimentin expression in signet cells and fibroblast-like malignant cells (IHC with vimentin antibodies, ×400), C – invasion of fibroblast-like malignant cells with expression of cytokeratin 18 in the vessel wall. Intravascular malignant cells became round or signet cells (IHC with vimentin-18 antibodies, ×400), D – low proliferative activity in diffuse type GC, increased in the invasion margin (IHC with Ki-67 antibodies, ×200), E – prominent cellular atypia and high proliferative activity in intestinal type GC (IHC with Ki-67 antibodies, ×200), F – fibroblast-like cells of mixed type GC, expressing cytokeratins AE1/AE3 (IHC with pancytokeratin AE1/AE3 antibodies, ×400), G – vimentin expression in the same cells (IHC with vimentin antibodies, ×400), H – α-SMA expression in the same cells (IHC with anti-α-SMA antibody, ×400)

*Рис. 1*. Иммуногистохимические особенности эпителиально-мезенхимальной трансформации (ЭМТ) при раке желудка (РЖ).
А – избирательная потеря экспрессии цитокератинов AE1/AE3 в перстневидных клетках, но сохранение ее в небольших злокачественных клетках (ИГХ с антителами к панцитокератину AE1/AE3, ×400), B – полная ЭМТ в РЖ диффузного типа, экспрессия виментина в перстневидных и фибробластоподобных злокачественных клетках (окраска антителами к виментину, ×400), C – инвазия фибробластоподобных злокачественных клеток с экспрессией цитокератина 18 в стенке сосуда; внутрисосудистые злокачественные клетки стали круглыми или перстневидными (окраска антителами к виментину-18, ×400), D – низкая пролиферативная активность РЖ диффузного типа, повышенная в крае инвазии (окраска антителами к Ki-67, ×200), E – выраженная клеточная атипия и высокая пролиферативная активность при РЖ кишечного типа (окраска антителами к Ki-67, ×200), F – фибробластоподобные клетки РЖ смешанного типа, экспрессирующие панцитокератин AE1/AE3 (окраска антителами к панцитокератину AE1/AE3, ×400), G – экспрессия виментина в тех же клетках (окраска антителами к виментину, ×400), H – экспрессия α-актина гладких мышц в тех же клетках (окраска антителами к гладкомышечному α-актину, ×400)

there were areas of moderate proliferative activity up to 20% and even high activity up to 46%. Almost in all cases, the increase in proliferative activity occurred in areas with small glandular and solid elements in deep layers of the gastric wall (most often the muscular one), at the margin of invasion, where cellular atypia was rising (Fig. 1 D).

At the tissue level, intestinal type GC was represented by well-differentiated adenocarcinoma with preservation of cell-to-cell connection and positive expression of E-cadherin. In 14 out of 19 cases, cellular atypia and proliferative activity (Fig. 1 E) were prominent or moderate and prominent; in 4 cases, moderate; and only in 1 case, mild and moderate. In the last case, the main features of EMT, i.e. cell-to-cell connection loss and cellular form change, were absent. Nevertheless, this tumor had small areas with high proliferative activity (proliferation index 35–40%) without decrease in pancytokeratin AE1/AE3 expression.

In the remaining 18 cases of intestinal type GC, EMT was noted; however, as opposed to diffuse type, it was focal and incomplete with negative vimentin expression. The foci of EMT were recognized at the invasion lateral margin or deeper, at the border with unchanged tissue; in only 3 cases, they occured in basal layers of mucosa. They were characterized by changing cell-to-cell connections with formation of trabeculae, small groups, and separated cells, commonly round, less often stellate or fibroblast-like. Uncommonly, weakly expressed ECM was produced in only one case showing sclerosis in the serosa and tumor, i.e. concentric growth of connective tissue around malignant glands with abnormal polar differentiation such as mucin secretion at the basal pole of malignant cells with preserved cell-to-cell connection. In two more cases of intestinal type GC, mucin was produced at the basal pole of malignant cells and extracted into the stroma within the foci of EMT; no significant ECM was produced.

Mixed type GC was characterized by diverse histology. We observed adenocarcinomas of different grades at the tissue level and solid areas of undifferentiated polymorphic cancer, with 2 cases demonstrating small foci of signet ring carcinoma. EMT occurred in all cases and was mostly focal; only in 3 cases, it was diffuse. In the first case, it started into the submucosa and in the second one, into the muscularis propria. In the third case, it was starting into the subserosa and was complete: prominent fibrosis elongated markedly inside and almost indistinguishable from fibroblasts cells were expressing pancytokeratin AE1/AE3 (Fig. 1 F), vimentin (Fig. 1 G), and α-SMA (Fig. 1 H). A few of those cells were even expressing desmin.

In this and two other cases of focal EMT, it was complete, i.e. malignant cells expressed vimentin. It can be concluded that EMT is complete only in those foci where there is no synchronism between cytokeratin expression loss and vimentin expression appearance or where vimentin is detected in solid and glandular structures with preserved positive E-cadherin expression; in other words, with relatively "incomplete" EMT. When malignant cells change their form and size to such extent that they look like fibroblasts,

they produce prominently EMC, lose cytokeratin expression, while acquiring vimentin and α-SMA expression, so that these cells become indistinguishable from fibroblasts and myofibroblasts. The first EMT signs were detected in the submucosa in 9 out of 14 cases and in the muscularis propria in 5 out of 14 cases. In all 14 cases, we noted the cell discohesion with the entire loss of E-cadherin expression or only its cytoplasmic expression. The pancytokeratin AE1/AE3 expression was already decreased in trabeculae of malignant cells up to complete loss, but it could be preserved in separated cells or even in elongated fibroblast-like cells (Fig. 1 F). In all cases, the cellular form inside EMT foci was changed: they became stellate and fibroblast-like. In 2 cases, elongated shape of malignant cells was detected even in glandular walls before their separation. Seven cases showed fibroblast-like separated cells being surrounded by a significant number of thin ECM fibers.

In 10 out of 14 cases of mixed type GC (71.4%) we found vascular invasion.

## Discussion

Diffuse and intestinal types GC differ from each other in their etiology and pathogenesis. Intestinal type develops through *Helicobacter pylori*-associated gastritis, intestinal metaplasia, and dysplasia, but diffuse type is apparently unrelated to *H. pylori* arising from morphologically normal gastric mucosa [14, 15]. Hereditary predisposition to GC has been established. Thus, the closest relatives of GC patients have a 2-fold increased risk of developing all forms of this tumor, whereas the relatives of diffuse type GC patients have a 7-fold higher risk compared to that of the control group [16]. Among diffuse type GC cases, there is a familial (hereditary) subgroup associated with E-cadherin mutation [17–19] and discohesive growth. In hereditary and sporadic diffuse type GC, genetic and/or epigenetic alteration of *CDH1* encoding E-cadherin are frequently detected [20].

All cases of diffuse type GC in our material showed that such an EMT feature as discohesion of malignant cells appears already in the mucosa.

Although *H. pylori* may potentiate EMT by different pathways in gastric carcinoma [21–23], in intestinal type GC, EMT develops as a result of tumor progression. Uncommonly, its features occur already in the mucosa, more often in the deeper layers.

The cellular form alteration and acquisition of fibroblast-like shape in diffuse type GC occurred in the mucosa near the muscularis mucosa in 22.6% of cases, where, according to B. Humar et al. [18], the activation of oncogene *c-Src* occurs already after malignant cell discohesion and this process has been associated with the submucosa invasion. In the intestinal type, the cellular form change appeared in the deeper layers, sometimes even with preserved cell-to-cell connection in the gland, but, at the same time, stroma invasion was seen.

In cases of diffuse type GC, malignant cells lost cytokeratin expression already in the mucosa, but the loss

might be selective: it might be absent in signet ring cells but preserved in small cells. Surprisingly, in malignant cells of diffuse type GC, the cytokeratin expression was often conserved in markedly elongated fibroblast-like cells in the submucosa and the muscularis propria, and in one case of the mixed type, even in the subserosa. Simultaneously, it often decreased and disappeared in trabeculae and groups of malignant cells in EMT foci of intestinal and mixed types.

Vimentin expression in malignant cells, which is considered to be complete EMT, was focally detected in almost all cases of diffuse type GC (93.5%), only in 3 cases of the mixed type, and was never observed in the intestinal type. It was recognized that vimentin expression correlated significantly with aggressive tumor behavior and unfavorable prognosis, but, on the other hand, cytokeratin expression loss may not correlate with malignant phenotype [9, 10, 24].

Abnormal polar differentiation occurred in all cases of diffuse type GC and was widespread because anomalous cellular adhesion and separation of cells lead to the loss of polarity. In cases of the intestinal type, abnormal polar differentiation was focal in a few cells detached from glands, whereas in 3 cases, the polarity loss was detected throughout the entire EMT and the mucin spread into the stroma. The mucin plays a crucial role in EMT development. Thus, the protein part of transmembrane mucin MUC-1 is considered to be an oncogene [25]. Hyaluronic acid, which is the main component of nonsulfated mucin, may induce EMT in tumors [26].

Therefore, cell discogesia and loss of polarity resulting from changes in cell contacts may not be the first EMT sign in cases of intestinal type GC compared to those of diffuse type GC. They are rather characterized by a decrease in cytokeratin expression, a change in cell shape, and a violation of polarity with the preservation of glandular morphology.

As we have already stated, many morphologic EMT features–separation of cells from each other, acquisition of fibroblast-like shape, and expression of mesenchymal markers–promote vascular invasion [27]. In our research, this process occurred more often in diffuse type GC (in 28 out of 31 cases, or 90.3%), less often in mixed type GC (in 10 out of 14 cases, or 71.4%), and even less often in intestinal type (in 11 out of 19 cases, or 57.8%). At the same time, in diffuse and mixed types, complete EMT (with vimentin and α-SMA expression) was accompanied by vascular invasion in 96.5% and 100% of cases, respectively. In our study, EMT completeness correlated with vascular invasion in the diffuse type (Tau=0.802, p<0.01) and in GC in general (Tau=0.510, p<0.01).

The relationship between proliferation and EMT is a difficult issue. The cell transformation from small signet ring to fibroblast-like shape in diffuse type GC occurs in basal parts of the mucosa, where, according to B. Humar et al. [18], oncogene *c-Src* activates, proliferation increases, and EMT manifests, which is necessary for malignant cells to invade through the muscularis mucosa [19]. In the intestinal type, we noted pancytokeratin expression loss

as an EMT feature, in foci of high proliferative activity. Consequently, high proliferative activity is required for EMT development. However, malignant cells after EMT are noticeable for low proliferative activity, which is typical for this process. During embryonic development and organogenesis there is an interchange of processes of cellular proliferation with an increase in their volume throughout mesenchymo-epithelial transition (MET) and processes of proliferative decreasing and elevated cellular mobility in EMT such as gastrulation [3, 4, 6]. In our cases, diffuse type GC with spread EMT showed low proliferation index (Ki-67<5%), but in 9 cases there were areas of moderate proliferative activity, where cell-to-cell connection and E-cadherin expression were preserved. Probably, these are MET foci that may explain tumor growth in the gastric wall. It is also established that low proliferative index estimated with Ki-67 correlates with invasiveness, metastases, and worst prognosis GC [28].

## Conclusion

A large number of EMT inductors and possible post-translational gene regulation form the basis for the diversity of morphological EMT features and their consequent development in different histological types of gastric cancer. These inductors activate different pathways and networks of signal transduction including many EMT-related transcription factors and their cooperation whereas post-translational gene regulation releases programs of structural and functional changes of malignant cells. EMT spread, completeness, and speed of development in these types of gastric cancer define their clinical behavior.

**Author contributions**
Conceived the study and designed the experiment – I.V. Vasilenko, R.B. Kondratyk, I.S. Grekov, A.M. Yarkov.
Collected the data and performed the analysis – R.B. Kondratyk, I.V. Vasilenko, A.M. Yarkov, I.S. Grekov.
Wrote the paper – R.B. Kondratyk.
Edited the manuscript – I.V. Vasilenko, R.B. Kondratyk, I.S. Grekov, A.M. Yarkov.

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## References/Литература

1. *Мнихович М.В., Вернигородский С.В., Буньков К.В., Мишина Е.С.* Эпителиально-мезенхимальный переход, транс-дифференциация, репрограммирование и метаплазия: современный взгляд на проблему. Вестник Национального медико-хирургического Центра им. Н.И. Пирогова. 2018;13(2):145–152.
*Mnihovich MV, Vernigorodskij SV, Bunkov KV, Mishina ES.* Epithelially-mesenchimal transition transdifferentiation, reprogramming and metaplasia: modern view on the problem. Bulletin

of Pirogov National Medical & Surgical Center. 2018;13(2):145–152 (In Russ.).

2. *Богуш Т.А., Калюжный С.А., Четыркина М.Р., Ястребова М.А., Щербаков А.М., Мамичев И.А. и др.* Экспрессия виментина в культурах клеток эпителиальных опухолей человека. Успехи молекулярной онкологии. 2018;5(2):24–30. DOI: 10.17650/2313-805X-2018-5-2-24-30.
   *Bogush TA, Kaliuzhny SA, Chetyrkina MR, Yastrebova MA, Scherbakov AM, Mamichev IA et al.* Vimentin expression in human cell lines of epithelial tumors. Advances in Molecular Oncology. 2018;5(2):24–30 (In Russ.). DOI: 10.17650/2313-805X-2018-5-2-24-30.

3. *Nieto MA.* The snail superfamily of zinc–finger transcription factors. Mol Cell Biol. 2002;3(3):155–66. DOI: 10.1038/nrm757.

4. *Nieto MA.* Epithelial-mesenchymal transitions in development and disease: old views and new perspectives. Int J Dev Biol. 2009;53(8–10):1541–7. DOI: 10.1387/ijdb.072410mn.

5. *Radisky DC.* Epithelial-mesenchimal transition. J Cell Sci. 2005;118(Pt. 19):4325–6. DOI: 10.1242/jcs.02552.

6. *Thiery JP, Acloque H, Huang RY, Nieto MA.* Epithelial–mesenchymal transition in development of disease. Cell. 2009;139(5):871–90. DOI: 10.1016/j.cell.2009.11.007.

7. *Weinberg RA.* Mechanisms of malignant progression. Carcinogenesis. 2008;29(6):1092–5. DOI: 10.1093/carcin/bgn104.

8. *de Wever O, Pauwels P, Craene B, Sabbah M, Emami S, Redeuilh G et al.* Molecular and pathological signatures of epithelial–mesenchymal transitions at the cancer invasion front. Histochem Cell Biol. 2008;130(3):481–94. DOI: 10.1007/s00418-008-0464-1.

9. *Kato Y, Yashiro M, Noda S, Tendo M, Kashiwagi S, Doi Y et al.* Establishment and characterization of a new hypoxia-resistant cancer cell line, OCUM-12/Hypo, derived from a scirrhous gastric carcinoma. Br J Cancer. 2010;102(5):898–907. DOI: 10.1038/sj.bjc.6605543.

10. *Kim MA, Lee HS, Lee HE, Kim JH, Yang HK, Kim WH.* Prognostic importance of epithelial-mesenchymal transition-related protein expression in gastric carcinoma. Histopathology. 2009;54(4):442–51. DOI: 10.1111/j.1365-2559.2009.03247.x.

11. *Lauren P.* The two histological main types of gastric carcinoma: diffuse and so-called intestinal types carcinoma. An attempt at a histo-clinical classification. Acta Pathol Microbiol Scand. 1965;64:31–49. DOI: 10.1111/apm.1965.64.1.31.

12. *Mita T, Shimoda T.* Risk factors for lymph node metastasis of submucosal invasive differentiated type gastric carcinoma: clinical significance of histological heterogeneity. J Gastroenterol. 2001;36(10):661–8. DOI: 10.1007/s005350170028.

13. *Dai YH, Tang YP, Zhu HY, Lv L, Chu Y, Zhou YQ et al.* ZEB2 promotes the metastasis of gastric cancer and modulates epithelial mesenchymal transition of gastric cancer cells. Dig Dis Sci. 2012;57(5):1253–60. DOI: 10.1007/s10620-012-2042-6.

14. *Hohenberger P, Gretshel S.* Gastric cancer. Lancet. 2003; 362(9380):305–15. DOI: 10.1016/s0140-6736(03)13975-x.

15. *Schier S, Wright NA.* Stem cell relationship and the origin of gastrointestinal cancer. Oncology. 2005;69 (Suppl1):9–13. DOI: 10.1159/000086625.

16. *Lehtola J.* Family study of gastric carcinoma with special reference to histological types. Scand J Gastroenterol Suppl. 1978;50:3–54. PMID: 360367.

17. *Baber ME, Save V, Carneiro F, Dwerryhous S, Lao-Sirieix P, Hardwick RH et al.* Histopathological and molecular analysis of gastrectomy spesimens from hereditary diffuse gastric cancer patients has implications for endoscopic surveillance of individuals at risk. J Pathol. 2008;216(3):286–94. DOI: 10.1002/path.2415.

18. *Humar B, Fukuzawa R, Blair V, Dunbier A, More H, Charlton A et al.* Destabilized adhesion in the gastric proliferative zone and c-Src kinase activation mark the development of early diffuse gastric cancer. Cancer Res. 2007;67(6):2480–9. DOI: 10.1158/0008-5472.CAN-06-3021.

19. *Humar B, Guilford P.* Hereditary diffuse gastric cancer: a manifestation of lost cell polarity. Cancer Sci. 2009;100(7):1151–7. DOI: 10.1111/j.1349-7006.2009.01163.x.

20. *Shimada S, Mimata A, Sekine M, Mogushi K, Akiyama Y, Fukamachi H et al.* Synergistic tumour suppressor activity of E-cadherin and p53 in a conditional mouse model for metastatic diffuse-type gastric cancer. Gut. 2012;61(3):344–53. DOI: 10.1136/gutjnl-2011-300050.

21. *Buti L, Spooner E, Van der Veen AG, Rappuoli R, Covacci A, Ploegh HL.* Helicobacter pylori cytotoxin-assosiated gene A (CagA) subverts the apoptosis-stimulating protein of p53 (ASPP2) tumor suppressor pathway of the host. Proc Natl Acad Sci USA. 2011;108(22):9238–43. DOI: 10.1073/pnas.1106200108.

22. *Yin Y, Grabowska AM, Clarke PA, Whelband E, Robinson K, Argent RH et al.* Helicobacter pylori potentiates epithelial:mesenchymal transition in gastric cancer: links to soluble HB-EGF, gastrin and matrix and matrix metalloproteinase-7. Gut. 2010;59(8):1037–45. DOI: 10.1136/gut.2009.199794.

23. *Zhu Y, Jiang Q, Lou X, Ji X, Wen Z, Wu J et al.* MicroRNAs upregulated by CagA of Helicobacter pylori induce intestinal metaplasia of gastric epithelial cells. PLoS One. 2012;7(4):e35147. DOI: 10.1371/journal.pone.0035147.

24. *Iwatsuki M, Mimori K, Fukagawa T, Ishii H, Yokobori T, Sasako M et al.* The clinical significance of vimentin-expressing gastric cancer cells in bone marrow. Ann Surg Oncol. 2010;17(9):2526–33. DOI: 10.1245/s10434-010-1041-0.

25. *Kufe DW.* Mucins in cancer: function, prognosis and therapy. Nat Rev Cancer. 2009;9(12):874–85. DOI: 10.1038/nrc2761.

26. *Chow G, Tauler J, Mulshine JL.* Cytokines and grow factors stimulate hyaluronan production: role of hyaluronan in epithelial to mesenchymal-like transition in non-small cell lung cancer. J Biomed Biotechnol. 2010;2010:485468. DOI: 10.1155/2010/485468.

27. *Christiansen JJ, Rajasekaran AK.* Reassessing epithelial to mesenchymal transition as a prerequisite for carcinoma invasion and metastasis. Cancer Res. 2006;66(17):8319–26. DOI: 10.1158/0008-5472.CAN-06-0410.

28. *Lee HE, Kim MA, Lee BL, Kim WH.* Low Ki-67 proliferation index is an indicator of poor prognosis in gastric cancer. J Surg Oncol. 2010;102(3):201–6. DOI: 10.1002/jso.21583.

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