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The Morphological Features of "Cavitary" Type Angiogenesis in Diffuse and Intestinal Types of Gastric Cancer and Its Relationship with Tumor-Infiltrating Immune Cells

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Authors' contributions

This work was carried out in collaboration between all authors. Author MS proposed a hypothesis and participated in the evaluation of the results of histological, immunohistochemical and statistical studies and wrote the first draft of the manuscript. Authors AR and TK helped and prepared the data. Authors OT helped in histological and immunohistochemical studies. Author AS co-ordinated the research project. All authors read and approved the final manuscript.

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ABSTRACT

Background: Previously we have described the "cavitary" type of angiogenesis by gastric cancer (GC) consisting of the formation of "cavitary structures" (CS) in tumor stroma, which are then lined by endothelial cells and merged into the blood vessels of the organ. The morphological features of the "cavitary" type of angiogenesis in intestinal and diffuse types of GC and the relations of CS with the tumor-infiltrating immune cells, was the purpose of this study.
Materials and Methods: The samples of tumor and adjacent gastric mucosa (GM) in 73 patients with GC who had undergone radical surgery were being studied. The sections were stained with hematoxylin and eosin and immunohistochemically using antibodies to CD34, CD4, CD8, CD20 и CD68.
Results: The differences of "cavitary" type of angiogenesis in the intestinal and diffuse types of GC are only associated with CS type-1 that are formed as a result of the abruption of epithelial cells from the underlying stroma. In the intestinal type of GC the basis for the formation of CS type-1 are the tumor glands. The wall of such CS is most likely the basement membrane bordering the connective tissue. In the diffuse type of GC the CS type-1 are presented as the structures limited from outside by the tumor cells. In their lumen the fragments of tumor tissue having the same structure as the surrounding one are being detected. The performed analysis showed that the number of CS type-1 was associated with the density of CD68, whereas the presence of CS type-2 – with the presence of lymphoid follicles (LF) and B-cell infiltrations at the boundary of tumor and GM. The density of CD68 in GM was higher in cases with multiple CS type-1 (72.6±47.0 vs. 41.6±15.4 cells per unit area, P= .03). In turn, CS type-2 were more often met in the presence of multiple LF (72,3% vs. 33,3%, P= ,04) and B-cell infiltrations (90% vs. 26,3%, P= ,001).
Conclusion: The obtained data testify about the relation of CD20 lymphocytes and CD68 macrophages with the "cavitary" type of angiogenesis.

Keywords: Angiogenesis; gastric cancer; prognosis of gastric cancer; tumor-Infiltrating lymphocytes; tumor-Infiltrating macrophages; tumor progression; vessels morphology.

ABBREVIATIONS

CS: "Cavitary Structure"; ECM: Cell-extracellular Matrix; EGF: Epidermal Growth Factor; GM: Gastric Mucosa; GC: Gastric Cancer; IGH: Immunohistochemically; H&E: Hematoxylin and Eosin; LF: Lymphoid Follicles; MMP: Matrix Metalloproteinase; MVD: Microvessel Density; OS: Overall Survival; PD-ECGF: Platelet Endothelial Cell Growth Factor; RFS: Relapse-free Survival; TIM: Tumor-Infiltrating Macrophages; TGF: Transforming Growth Factor; VEGF: Vascular Endothelial Growth Factor.

1. INTRODUCTION

Angiogenesis is one of the key factors of tumor progression associated with processes of invasion and metastasis of malignant tumors [1-4]. The majority of researchers have pointed out a close relationship of angiogenesis activity with the depth of tumor invasion, the presence of metastases in regional lymph nodes (RLN) and the prognosis of the disease, including the patients with GC [2-4,5-6]. Evaluation of the angiogenesis activity in tumor that most often determined by the microvessel density (MVD) or lymphatic vessel density and by the severity of the vascular endothelial growth factor (VEGF) expression [7-11], is widely used both in scientific purposes and in clinical practice. At the same time, it is known that the tumor vessels are heterogeneous, and the various types of vessels may differ not only in origin, morphology and clinical significance, but also in their sensitivity to the anti-angiogenic therapy [12], and in this context the studies in this direction are very promising.

Previously we described a new type of vessel formation by GC named by us the "cavitary" type of angiogenesis [13]. We assumed that the formation of tumor vessels can be associated with the generation of "cavitary structures" (CS) in tumor stroma or the adjacent GM, their lining by endothelial cells and their merger into the blood vessels of the organ. We have also assumed that there are two main types of the formation of the described CS. The first one can be associated with the abruption of epithelial cells from their underlying foundation, the second - with the formation of CS directly in the lamina propria of the GM or in the tumor stroma, without the involvement of the epithelial cells. The

analysis of clynical significance of the “cavitary” type of angiogenesis has shown that the presence of multiple CS type-1 in tumor stroma is associated with the diffuse type, poor differential forms of GC, advanced stage and nodal stage N2, and is accompanied by the decrease of overall survival rate from 93.9% to 52.7% (P= ,001) and relapse free survival rate from 87.7% to 32.4% (P< ,001). In its turn, the CS type-2 are associated only with the histological type of GC (P= ,008). In the diffuse type of GC the CS type-2 are more often met than in intestinal type. The multivariate Cox proportional hazard regression analysis has indicated that TNM stage (P= ,003), nodal stage (P= ,013), the number of CS type-1 (P= ,005) are significantly independent prognostic factors in patients with GC.

Considering that the formation of the “cavitary” vessels can differ depending on the histology type of GC and can be associated with inflammatory changes in the tumor stroma and the adjacent GM, we decided to study the morphological features of CS in the intestinal and diffuse types of GC and their relationship with the tumor-infiltrating immune cells.

2. MATERIALS AND METHODS

2.1 Patients

73 patients with GC who had undergone radical surgery (R0) in the Orenburg Regional Clinical Oncology Center between January 2009 and July 2010 were included in this prospective study. Subtotal distal resection was performed in 56 cases (76.7%), subtotal proximal resection - in 10 cases (13.7%), and gastrectomy - in 7 cases (9.5%). D2 volume lymphadenectomy was performed in all patients, with D3 elements - in 38 patients (52.0%).

The clinical features of patients included in this study are presented in Table 1. The average age of the patients was 61.2±9.3 years (from 34 to 78 years, the median – was 61 years). Patients with decompensation of chronic diseases, acute infection pathology, severe allergic processes were not included in the study as well as the ones who received corticosteroids, antihistamines, non-steroidal anti-inflammatory drugs and neoadjuvant chemotherapy.

2.2 Histological Analysis

After removal of stomach a greater curvature of the organ was opened and biopsy samples were taken from the tumour and the adjacent macroscopically non-tumorous mucosa at a distance of 3-5 cm from the proximal tumour margin. The specimens of GM and tumor were fixed in buffered formalin, embedded in paraffin. 4 μm thick slices were stained with Mayer’s hematoxylin and eosin (H&E). Histological slides were studied by light microscopy (Optika B-350 microscope, connected to a ScopeTek DCM500 camera, Italy). The histological type of GC was assessed according to the Lauren's classification. The number of CS type-1 (none, single – no more than two in the field of view, and multiple – more than two in the field of view) and the presence of CS type-2 were assessed by visual analog way using a low magnification (x100).

Table 1. Clinicopathologic characteristics of gastric carcinoma cases

Clinicopathologic variables	Number of cases (n)	Percent (%)
Gender		
Male	43	58.9
Female	30	41.1
Location of tumor		
Upper third	14	19.2
Middle third	18	24.7
Lower third	39	53.4
Total cancer	2	2.7
Lauren classification		
Intestinal type	41	56.2
Diffuse type	32	43.8
Differentiation		
Well (G1)	27	36.9
Moderate (G2)	14	19.3
Poorly (G3-G4)	9	12.3
Signet ring cell carcinoma	23	31.5
Nodal status		
pN0	43	59.9
pN1	9	12.3
pN2	21	28.8
Depth of invasion		
pT1	16	21.9
pT2	18	24.7
pT3	36	49.3
pT4	3	4.1
Stage (TNM)		
T1-2N0M0	34	46.6
T3N0M0	9	12.3
T3-4N1M0	9	12.3
T3-4N2M0	21	28.8

2.3 Immunohistochemistry

The sections for immunohistochemistry (IGH) were dewaxed and rehydrated by sequential

immersion in xylene and graded ethanol and water. For antigen retrieval, the sections boiling for 10 min in citrate buffer (pH 6) and endogenous peroxidase activity was blocked with 30 mL/L hydrogen peroxide solution. Adjacent slides were incubated at room temperature with the anti-CD4 Ab-8 (Clone 4B12, Thermo Fisher Scientific) mouse monoclonal antibodies in diluted at 1:20, the anti-CD-8 Ab-1 (Clone C8/144B, Thermo Fisher Scientific) mouse monoclonal antibodies in diluted at 1:25, the anti-CD20 epitope specific rabbit antibodies (Thermo Fisher Scientific) in diluted at 1:400, the anti-CD68 (macrophage marker) Ab-3 (CloneKP1, Thermo Fisher Scientific) mouse monoclonal antibodies in diluted at 1:100 and the anti-CD34 (QB–END/10, Novocastra Laboratories Ltd) monoclonal antibodies in diluted at 1:50. The time of antibodies incubation was according to the manufacturer protocol. The visualization system has included DAB (UltraVision LP Detection System HRP Polymer & DAB Plus Chromogen) and Hematoxylin counterstaining. For negative control sections, primary antibody was replaced with phosphate-buffered saline and processed in the same manner.

The density of the labelled lymphocytes (CD4, CD8, CD20) and macrophages (CD68) was calculated on the relative area unit equal to 0.42 x 0.28 mm² using the magnification x 400. In each image the areas of the epithelial and stromal components were calculated and glandular lumen were subtracted. For each immune cell subset six images with the most abundant infiltration were taken up from both tumor stroma and adjacent GM. At the boundary of the GM and the tumor, the number of lymphoid follicules (LF) (no, a single - no more than two in the field of view and multiple - more than two in the field of view) and the presence of the focal CD20-cell infiltrates were also evaluated using a low magnification (x100). MVD was assessed immunohistochemically using antibodies to CD34, in accordance with the international consensus on the methodology and criteria for quantitative evaluation of angiogenesis in human solid tumors [14]. Microvessel count was performed in the areas with the highest vascularization ("hot spots") using a focused study of high magnification (x 400) in three consecutive fields. A single, countable microvessel was defined as any brown-stained endothelial cell (or cluster) clearly separated from the adjacent microvessels.

2.4 Statistics

Statistical analysis was performed using the Statistica 6.0 software. The density of the lymphocytes, macrophages and MVD were expressed as Mean ± SD. Kruskal-Wallis and Mann-Whitney U nonparametric tests were used to compare the value of density of cells and MVD. The correlations between different data were evaluated using nonparametric Spearman's rank correlation or gamma correlation. Chi-square tests were carried out to analyze the difference of distribution among the categorized data. A value of $P<0.05$ was considered statistically significant.

3. RESULTS

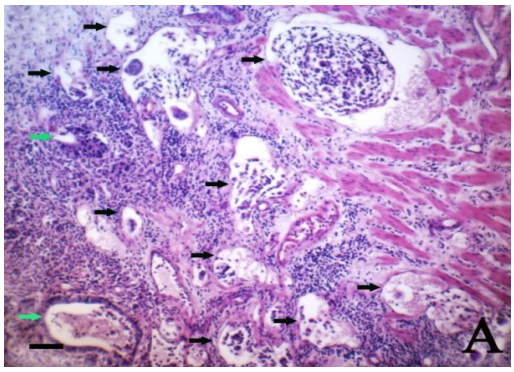
3.1 The Morphological Features of "Cavitary" Type of Angiogenesis in Intestinal and Diffuse Types of Gastric Cancer

The first type of CS formation (CS type-1) is associated with the abruption of epithelial or tumor cells from their underlying foundation and can be observed in the tumor tissue (Fig. 1A) and in the adjacent GM as well (Fig. 1B). By IHC staining the internal surface of the described CS can be fully or partially lined by endothelial cells expressing CD34 (Fig. 1C).

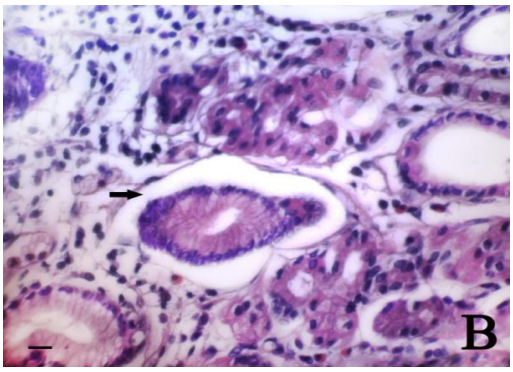
We have noted two main signs specific to this type of angiogenesis:

1. The presence of CS with a partial endothelial lining. The cells of the lining are unevenly stained by marker and have an uneven surface with a number of protuberances (Fig. 1D);
2. The CS without endothelial lining and CS with a partial endothelial lining as well as the dilated vessels located next to them are simultaneously detected in the samples of tumor tissue by low (x100) signification (Fig. 1E). We believe that these vessels are directly related to "cavitary" angiogenesis type-1. In the lumen of such vessels the tumoral or epithelial emboli are often detected and erythrocyte margination is observed (Fig. 1F).

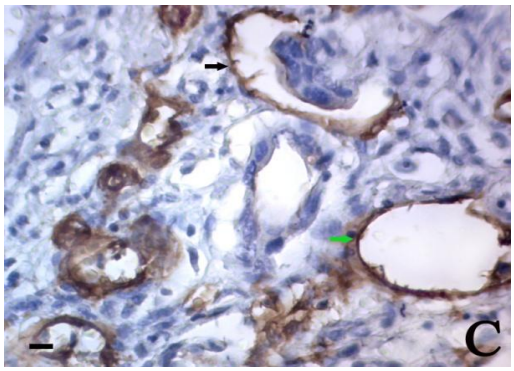
We have pointed out some differences in the morphology of CS type-1 in intestinal and diffuse types of GC. In the intestinal type of GC the formation of CS type-1 was associated with tumor or normal glands where the flaking of



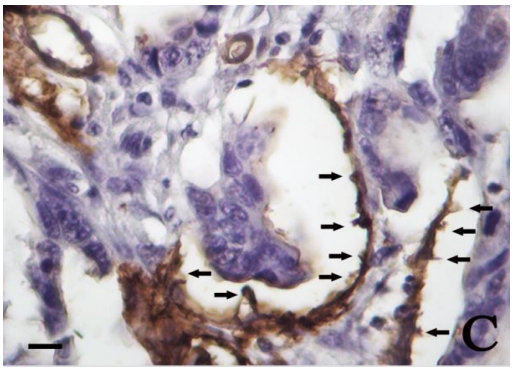
A. Tumor glands (green arrows) and CS type-1 (black arrows)
bars = 100 μm



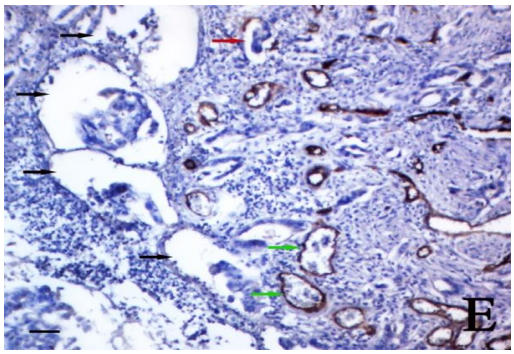
B. The CS type-1 in the gastric mucosa (arrow),
bars = 20 μm



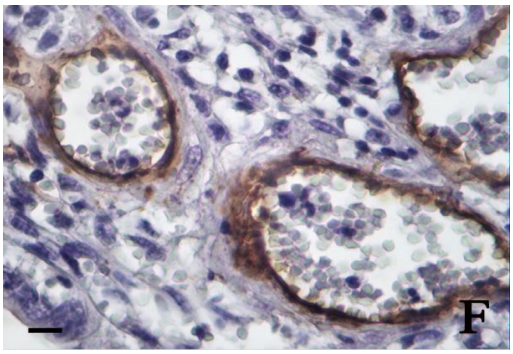
C. The CS type-1 with a complete (green arrow) and partial endothelial lining (black arrow),
bars = 20 μm



D. The cytoplasma of lining cells in CS type-1 have a number of protuberances (arrows),
bars = 20 μm



E. The CS type-1 without endothelial lining (black arrows), CS type-1 with a partial endothelial lining (red arrow) and the dilated vessels with tumoral emboli in their lumen (green arrows)
bars = 100 μm



F. The dilated vessels with tumoral emboli in their lumen and erythrocyte margination
bars = 20 μm

Fig. 1. The morphological features of "cavitary" angiogenesis type-1 in intestinal type of gastric cancer
Fig. A-B: H&E stain. Fig. C-F: immunoperoxidase staining with anti-CD34 monoclonal antibody

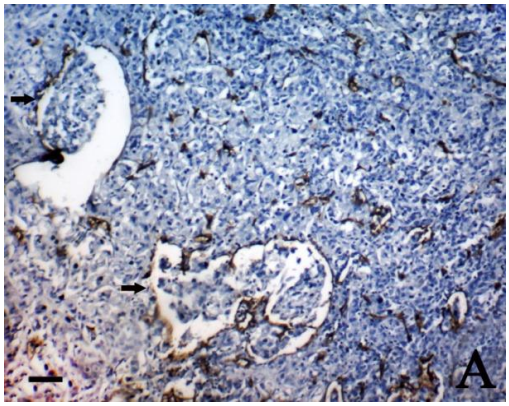
epithelial cells from the basement membrane and their desquamation into the lumen of the "obliterated" gastric or tumor glands were being observed (see Fig. 1A – 1E). The wall of such CS is most likely the basement membrane bordering the tumor stroma.

In the diffuse type of GC the CS were presented by the structures limited from outside by the tumor cells (Fig. 2A). In their lumen the fragments of tumor tissue having the same structure as the surrounding one were being detected. The cytoplasm of cells lining such CS did not often express CD34 and was difficult to be distinguished on the light-optical level. The cells with large, light, oval-shaped nuclei are sometimes observed in the structure of such endothelial lining (Fig. 2B).

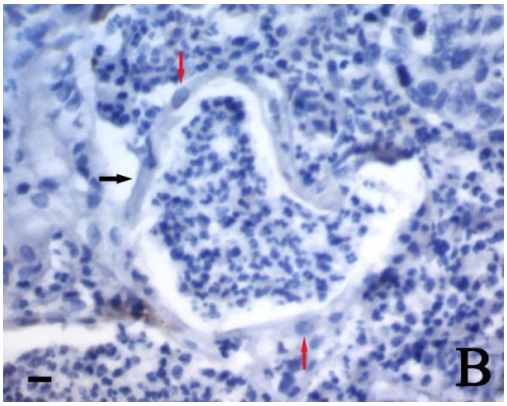
The second type of CS formation (CS type-2) was associated with the formation of CS directly into the GM or the tumor stroma, without involvement of the tumor cells. This supposition is due to the fact that in the some cases we observed a characteristic cellular structure of the connective tissue of the lamina propria of GM (Fig. 3A), often combining with the expressed phenomena of diapedesis of erythrocytes and associating with a number of clinically relevant factors. Most often than not the described CS were observed in the GM at the level of gastric pits or directly in the stroma bordering upon tumor tissue. Sometimes the cavities with endothelial lining were revealed. The cytoplasm of the cells of such lining weakly expressed CD34 and was characterized by the presence of a number of protuberances and intracavitary growths (Fig. 3B).

3.2 The Relations of "Cavitary Structures" with the Tumor-Infiltrating Immune Cells

The study of the associations between the features of "cavitary" angiogenesis and the density of tumor-infiltrating immune cells showed that the number of CS type-1 correlated with the density CD68 macrophages in GM (*ρ=0,506, t=2,42, P= 0,03*) whereas the number of CS type-2 – with density of CD20 lymphocytes in tumor (*ρ=0,449, t=2,31, P= ,03*). The increase of the number of CS type-1 has been accompanied by the increasing of the CD68 density in GM and tumor stroma. In the presence of multiple CS type-1 in tumor stoma compared to the cases without them or with single ones, the density of CD68 macrophages in GM was 72,6±44,8 and 41,6±15,4 cells on area unit (*P= ,06*) and the density of CD68 macrophages in tumor stroma - 68,3±41,7 and 27,2±20,2 cells on an area unit (*P= ,15*). At the same time, the presence of CS type-2 was associated with a higher density of CD20 lymphocytes in tumor stroma (42.3±24.5 vs. 25.1±15.0 cells on an area unit in the presence and in the absence of the CS type-2 respectively, *P= .04*).

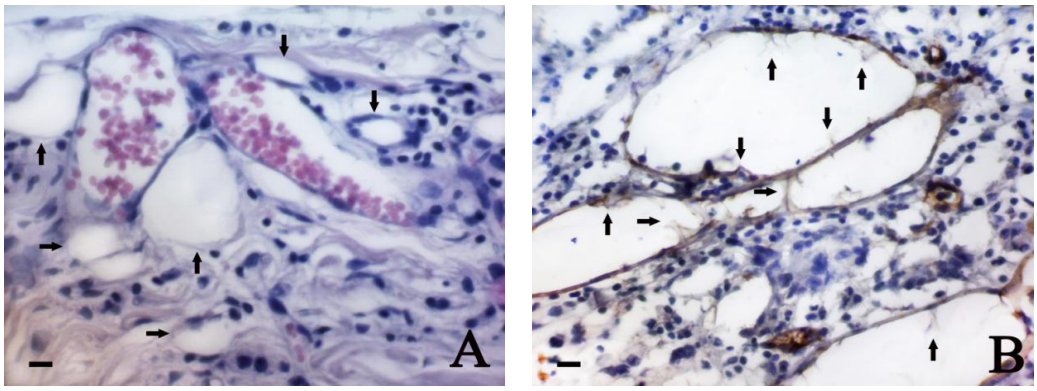


A. The CS type-1 (arrows) with partial endothelial lining,
bars = 100 μm



B. The CS type-1 (black arrow). The lining cells have the large, light, oval-shaped nuclei (red arrows),
bars = 20 μm

Fig. 2. The morphological features of "cavitary" angiogenesis type-1 in diffuse type of gastric cancer
Immunoperoxidase staining with anti-CD34 monoclonal antibody



A. The CS type-2 in the stroma bordering upon tumor tissue,
bars = 20 μm

B. The CS type-2 in the lamina propria of the gastric mucosa at the level of gastric pits [13]. The cytoplasm of the lining cells has a number of protuberances (arrow)
bars = 20 μm

Fig. 3. The morphological features of "cavitary" angiogenesis type-2
Fig. A: H&E stain. Fig. B: immunoperoxidase staining with anti-CD34 monoclonal antibody

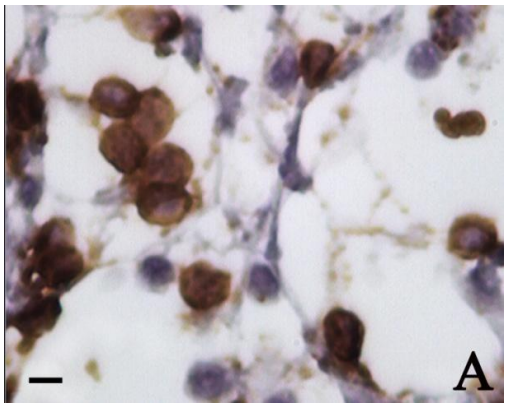
Particularly, we would like to note the special aspects of the expression of CD20 at the boundary of GM and tumor. We noted three different types of structures associated with the expression of this marker: the individual cells, focal infiltrates and LF. The individual cells were of two types: the round shape cells with a narrow rim of cytoplasm on the periphery of the nucleus, having a clear and smooth contours (Fig. 4A), and the irregularly shaped cells with the indistinct contours having a plurality of cytoplasmic processes. As a result, the expression of the marker in such cells seemed to be fragmented (Fig. 4B).

The focal CD20-cell infiltrates consisted mainly of cells of the second type. Directly in the projection of such infiltrates or in a close proximity to them the CS type-1 (Fig. 4C) and type-2 (Fig. 4D) were often observed.

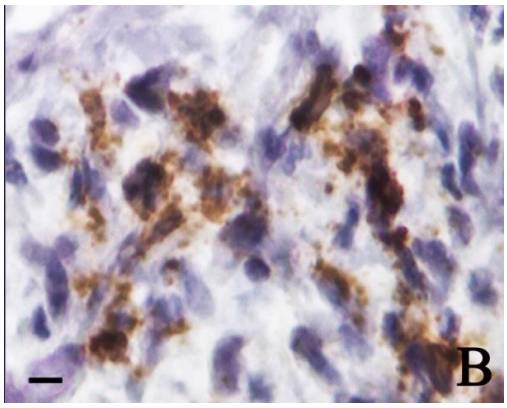
In the presence of focal CD20-cell infiltrates the CS type-2 were detected in 90% patients, while in the absence - in 26,3% only ($\chi 2 = 10,6$, $P= ,001$). Simillarly, in cases without LF or with single ones the CS type-2 were revealed in 33,3% patients, whereas in cases with multiple LF – in 72,3% ($\chi 2=4,24$, $P= ,04$). A significant correlation (*gamma=0,815, z=3,09, P= ,002*) between the density CD20 lymphocytes in GM and the presence of focal CD20 cells infiltrates at the boundary of GM and tumor was also noted. However, the correlations between the density of CD4- and CD8-lymphocytes and the number of CS type-1 and type-2 were not revealed.

4. DISCUSSION

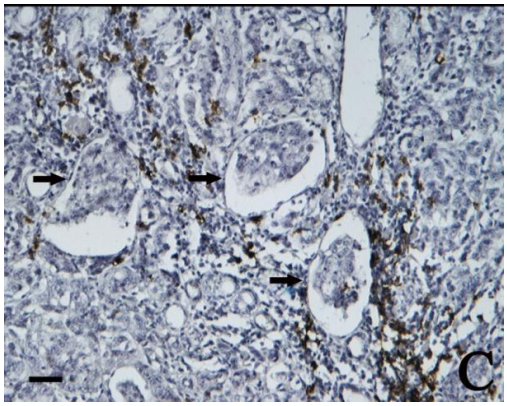
Despite a large number of studies pointing to the importance of angiogenesis in the progression of malignant tumors [3,6,15,16], the study of its mechanisms still has been attracting attention of many scientists. Previously, we have described a new way of angiogenesis on the example of GC that consists in the formation of CS in the tumor stroma and adjacent GM, being then lined by the endothelium and merged into the blood vessels of the organ [13]. It was established that this type of angiogenesis played perhaps a key role in tumor progression. According to our data, the presence of multiple CS type-1 being formed by the abruption of tumor cells from their underlying foundation and their desquamation into the lumen of the forming CS, was a significant criterion associated with the tumor size, the depth of tumor invasion, the presence of lymph metastases and long-term results of GC treatment. In turn, the presence of CS type-2 was associated with the histological type of GC. In this research we studied the morphological features of "cavitary" type of angiogenesis depending on the histological type of tumor and the correlations of CS with the tumor-infiltrating immune cells. In was noted that the differences in the morphology concerned only the CS type 1 and consisted in the following:



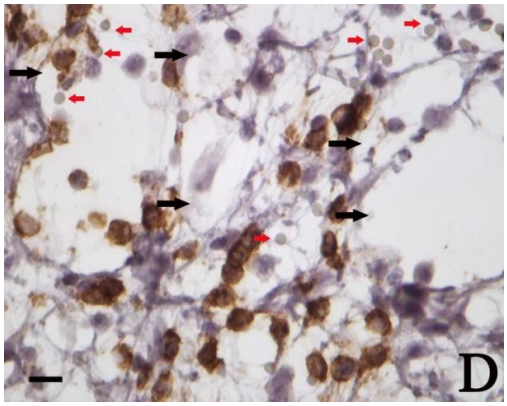
A. The expression of CD20 in the cells having a round shape with a clear and smooth contours
bars = 20 μm



B. The expression of CD20 in the cells having an irregularly shape with the indistinct contours and the fragmented expression of marker
bars = 20 μm



C. The CS type-1 is in the area of focal CD20-cell infiltration
bars = 100 μm



D. The peculiar to CS type-2 the cellular structure of the connective tissue (black arrows) combining with the diapedesis of erythrocytes (red arows) is in the area of focal CD20-cell infiltration
bars = 20 μm

Fig. 4. The features of CD20 expression on the boundary of gastric mucosa and tumor
The immunoperoxidase staining with anti-CD20 monoclonal antibody

- In the intestinal type of GC the desquamated epithelium of tumor glands was observed in the lumen of CS, while in the diffuse type – there were fragments of tumor tissue.
- In the intestinal type of GC the wall of CS was likely the basement membrane bordering the tumor stroma, while in the diffuse one - the tumor cells.

We believe that the revealed features were associated with the differences of the biological properties of the tumor cells themselves and their microenvironment. Worthy of note are some features of intestinal and diffuse types of GC which, in our opinion, are able to influence the mechanisms of the formation of CS type-1:

- A higher level of nitric oxide synthase (iNOS) expression in diffuse type of GC [17,18]. There were marked the correlations of iNOS expression in tumor cells with intratumor and peritumor blood microvessel density and lymphatic vessel

density, tumor size, invasion depth, lymph node involvement, TNM stage and survival of patients with GC [19,20].

- The increased synthesis of thrombospondin in diffuse adenocarcinomas. Thrombospondin-4 is a glycoprotein of the extracellular matrix involved in the regulation of the adhesive properties of tumor cells. Its highest intensity of expression was observed within the extracellular matrix surrounding the tumor cells in the fields of high tumor cell density and invasion [21].
- The significantly higher levels of matrix metalloproteinase-1 (MMP-1), MMP-7, VEGF and E-cadherin in diffuse type of GC [22,23].
- A higher incidence of positive expression of integrin beta3 mRNA in diffuse type of GC [24]. It must be noted that integrins are cell adhesion molecules, which mediate cell-cell adhesion or cell-extracellular matrix (ECM) adhesion and are essential for invasion and metastasis of carcinoma cells. These authors have demonstrated the relationship between integrin β3 mRNA and VEGF protein expression, MVD and 5-year survival rate of gastric carcinoma patients.

We can assume that these features lead to a different character of the relationship of tumor cells and stroma in intestinal and diffuse types of GC [25], and, as a consequence, to different mechanisms of CS type-1 formation. As for CS type-2, the differences in their morphology depending on the histological type of GC have not been revealed. Their formation occurs without tumor cells, and therefore it can be assumed that the other factors associated with inflammatory changes in the tumor stroma and the adjacent tissue may be involved in this process.

It should be noted that the role of tumor-infiltrating immune cells in activation of tumor angiogenesis was confirmed in a large number of studies [16,26]. It is known that an active inflammatory process is connected with the increased secretion by immune cells of cytokines, chemokines, growth factors and proteases [27,28] that promote the activation of tumour angiogenesis on the one hand [29,30], influence the adhesive properties of tumour cells on the other [31,32]. Besides, some studies have shown that the immune cells may be directly associated with tumor progression and invasion [33-35], and that the type and density of immune cells in the tumor tissue may be one of the most reliable parameters for predicting a patient's clinical outcome in certain types of cancer [36].

According to the data of our research, the presence of CS type-1 was associated only with the density of CD68 macrophages. It has been documented that the tumor-infiltrating macrophages (TIM) are favourable to the activation of tumor angiogenesis at the expense of the increasing production of mediators that promote angiogenesis, such as vascular endothelial growth factor (VEGF) and cyclo-oxygenase-2 (COX-2)–derived prostaglandin E2. The activation of TIM is modulated by local signals within the tumor microenvironment such as tumor necrosis factor α (TNF-α) and hypoxia [37-40]. Moreover, macrophages are believed to be directly involved in the formation of CS type-1. They are often found in the vicinity of tumor glands basement membranes, in the places where its integrity is destroyed [25]. It is logical to assume that the main mechanism of the damaging effect may be associated with the synthesis of matrix metalloprotease (MMP) by macrophages, and especially of MMP-2 and MMP-9. It has been established that MMPs are the most important group of proteolytic enzymes used by cancer to degrade the ECM. In some studies the co-culture of tumor cells with monocytes has been noted to be accompanied by the enhanced production of matrix MMP-9, being diminished by the depletion of fibronectin from the conditioned media [41,42].

Equally interesting is the relationship between the presence of CS type-2 and the features of CD20 expression. The presence of CS type-2 was associated with the presence of the multiple LF and the focal CD20-cell infiltrates at the boundary of GM and tumor. It should be noted that the role of B-lymphocytes in tumor progression has been insufficiently studied. However, some studies have shown that B-lymphocytes may also be involved in the activation of angiogenesis. For example, in patients with rheumatoid arthritis the direct correlations of a total number of B cells with the serum visfatin levels were found [43]. In synovium, visfatin was predominantly expressed in the lymphoid aggregates and interstitial vessels. Adding of recombinant vistafin to the culture medium with fibroblast induced high amounts of chemokines such as IL-8 and MCP-1, proinflammatory cytokines such as IL-6, and matrix metalloproteinases such as MMP-3 [44].

In malignant tumors it was noted that visfatin significantly and dose-dependently up-regulated gene expression and protein production of VEGF and MMPs [45-48]. In GC the increased levels of visfatin in plasma were associated with invasion depth, lymph node metastasis, distant metastasis, peritoneal dissemination, tumor size and tumor node metastasis stage and were correlated with the worsening of prognosis [49].

Also very interesting are the studies showing the activation of signal transducer and activator of transcription 3 (STAT3) in the TIL-B [50,51]. It is known that STAT3 is crucial for tumor angiogenesis, since Stat3 directly regulates expression of VEGF and others pro-angiogenic genes in tumors [52,53]. In particular, it was found that the density of tumor-infiltrating B cells correlates with expression levels of VEGF, MMP9 and HIF1a [50,51]. The increasing of B cells with activated Stat3 was observed in gastric, lung, liver and prostate cancers and was associated with deterioration of the long-term results of treatment [50].

It should also be noted those studies that have shown the essential role of B cells in regulation of macrophage phenotype. In the experiment in vitro and *in vivo* it was revealed that B1 lymphocytes expressing IL-10 induced an M2 polarization of tumor-associated macrophages that was associated with immunosuppression, promotion of tumor angiogenesis and metastasis. [54,55].

5. CONCLUSION

The features of “cavitary” type angiogenesis in intestinal and diffuse types of GC have been revealed. We believe that they may be associated with different mechanisms of the formation of CS type-1, perhaps, owing to the lack of basement membranes and overexpression of adhesion molecules in diffuse type of GC. The obtained data also testify about the relation of CD20 lymphocytes and CD68 macrophages with the "cavitary” type of angiogenesis. We believe that further researches should be carried out to the study of the mechanisms of CS formation and the role of tumor-infiltrating immune cells in "cavitary" type of angiogenesis.

ETHICAL APPROVAL

The study was performed in accordance with the Helsinki Declaration, internationally recognized guidelines, and the privacy of patients was protected by decoding of data, according to the privacy regulations of the Orenburg regional oncologic clinic (Russia, Orenburg). Written informed consent was obtained from the patient and the protocol was approved by the Institutional Review Board of Orenburg State Medical University (Russia, Orenburg).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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