Different Types of Tumor Vessels and Hypothesis of “Cavitary” Type of Angiogenesis on the Example of Gastric Cancer

Marina Senchukova¹, Andrew Ryabov², Alexander Stadnikov³

1 Introduction

Angiogenesis is one of the key factors of tumor progression (Folkman, 1998). Activation of angiogenesis is associated with a number of factors among which the special role belongs to vascular endothelial growth factor (VEGF), being expressed by tumoral and stromal cells and influencing the development of new blood vessels and survival of immature ones (Ferrara, 2002). In evaluating of angiogenesis in malignant growth it should be considered that the tumor vessels have some morphological features distinguishing them from usual vessels:

- The tumor vessels are often located chaotically. Typical for them are tortuosity, the formation of vascular rings and pathological partitions, abnormal arteriovenous shunts, vascular lacunae. The size of the vessels varies from a severe dilatation to a sharp narrowing with a possible alternation of expanded and constricted areas (Less et al., 1991; Baluk et al., 2005; Birau et al., 2012; Fukumura et al., 2010);

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• Some authors have noted the absence of pericytes in tumor vessels – the cells that are functionally related to the vascular endothelium and extremely important for the stabilization and maturation of vascular structures (Baluk et al., 2003; Baluk et al., 2005; Morikawa et al., 2002);

• In tumors the vessels (mainly of capillary type) with impaired endothelial lining having a discontinuous basal membrane are frequently observed (Birau et al., 2012; Ribatti et al., 2007);

• The tumor vessels are characterized by the increased permeability playing an important role in the activation of tumor angiogenesis (Dvorak et al., 1995; Nagy et al., 2012);

• In the lumen of blood and lymph vessels of tumor there are often observed tumor emboli, the presence of which is an unfavorable prognostic factor (An et al., 2007; Shen et al., 2009; Yokota et al., 2004).

It is worth noting that the assessment of angiogenesis activity in the tumor is one of the priority tasks in oncology. It is most often determined by the microvessel density (MVD) and the intensity of VEGF expression in tumor (Lazar et al., 2008; Poon et al., 2003; Zhao, 2006). The majority of researchers has pointed to the close relationship of these indicators with the depth of tumor invasion, the presence of metastases in regional lymph nodes (RLN) and the prognosis of the disease (Ding et al., 2006; Ma et al., 2007; Lazar et al., 2008; Poon et al., 2003; Wang et al., 2007). At the same time, tumor vessels are known to be heterogeneous in its origin and morphology and various types of vessels may differ not only in clinical significance, but also in their sensitivity to the antiangiogenic therapy (Birau et al., 2012; Nagy & Dvorak, 2012). Since there is no currently clear classification of tumor vessels by morphology and their role in tumor progression is obscure we decided to investigate this problem on the example of gastric cancer (GC).

2 The Different Types of Tumor Vessels in Gastric Cancer

We investigated samples of tumor and gastric mucosa (GM) in 73 patients with GC who had undergone radical surgery (R0) in the Orenburg Regional Clinical Oncology Center. The average age of the patients was 61.2±9.3 years (from 34 to 78 years, the median – was 61 years). The clinical features of patients included in this study are presented in Table 1.

All specimens were fixed in formalin and embedded in paraffin. Serial sections (4 µm) were stained with Mayer’s hematoxylin and eosin, by van Gieson and immunohistochemically using antibodies to CD34, CD4, CD8, CD20 and CD68. The number of dilated capillaries and the cavitary structures (GS) type-1 were assessed by visual analog way using a low magnification (×100) as none, single (no more than two in the field of view) and multiple (more than two in the field of view). The same manner the presence of CS type-2 was defined. The density of the la-belled lymphocytes (CD4, CD8, CD20) and macrophages (CD68) was calculated on the relative area unit equal to 0.42 × 0.28 mm². MVD was assessed immunohistochemically using antibodies to CD34 (Vermeulen et al., 2002).
### Clinicopathologic variables

<table>
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<th>Percent (%)</th>
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<td>Location of tumor</td>
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<tr>
<td>Middle third</td>
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<td></td>
<td></td>
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</tr>
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<td>Moderate (G2)</td>
<td>14</td>
<td>19.3</td>
</tr>
<tr>
<td>Poorly (G3 – G4)</td>
<td>9</td>
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</tr>
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<td>Signet ring cell carcinoma</td>
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<tr>
<td>T3-4N1M0</td>
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Table 1: Clinicopathologic characteristics of gastric carcinoma cases.

The obtained data were compared with clinical features of GC: stage, size, localization, histological type of tumor, 3-year overall survival (OS) and relapse-free survival (RFS).

The study of special features of angiogenesis by GC allowed us to establish the heterogeneity of vessels of tumor stroma and adjacent GM. We have singled out the following types of the vessels dissimilar in morphology and clinical significance:

#### 2.1 Normal Vessels: Arteries, Veins, Arterioles, Venules and Capillaries

The normal arteries, veins, arterioles and venules were localized in gastric submucosa (GS) and had the average wall thickness 22.24±9.29, 13.26±3.65, 6.33±1.01 and 3.18±0.96 microns respectively. The normal capillaries were localized in both GM and GS and had 5 – 20 microns in diameter. The correlation of normal vessel density with clinical characteristics and long-term results of GC treatment was not revealed.
2.2 Dilated Capillaries of the Lamina Propria of Gastric Mucosa

The described vessels differed from usual capillaries by larger sizes (their diameter was more than 50 microns) and irregular form (Figure 1a). Both usual endothelial cells and the cells with large, light nuclei having a fine-netted chromatin structure took part in their formation. On a cross-section the nuclei of such cells were of the oval or round form (Figure 1b).

![Figure 1](image)

**Figure 1:** The dilated capillaries in the lamina propria of the gastric mucosa. (a): the dilated capillaries (arrows): H&E stain, bars = 100 μm; (b): the cells with large, light nuclei having a fine-netted chromatin structure (arrows) are seen in the endothelial lining of the dilated capillary: staining with CD34, bars = 20 μm.

The Spearman rank correlation analysis ($q$) and gamma correlation coefficient test (gamma) showed that the presence of dilated capillaries in the lamina propria of the GM correlated with sizes tumor ($q = 0.325$, $t = 2.79$, $p = 0.007$), TNM stage (gamma = 0.371, $z = 3.17$, $p = 0.001$), nodal stage (gamma = 0.387, $z = 2.88$, $p = 0.004$), 3-year OS (gamma = -0.467, $z = -2.18$, $p = 0.03$) and RFS (gamma = -0.435, $z = -2.64$, $p = 0.008$).

The increase of the number of the described vessels associated with the tumor size increasing (Table 2) and with the number of metastases in RLN (Table 3) was noted.

In the presence of multiple dilated capillaries in lamina propria of GM the decrease of 3-year OS and RFS of GC patients was recorded (Figure 2a and b).

<table>
<thead>
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<th>Tumor size</th>
<th>The number of dilated capillaries</th>
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**Table 2:** The tumor sizes depending on the number of dilated capillaries in lamina propria of gastric mucosa.
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<td>6</td>
</tr>
<tr>
<td>N2</td>
<td>6</td>
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</tr>
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</table>

\[ \chi^2 = 12.7 \]
\[ p = 0.02 \]

Table 3: The number of the dilated capillaries in lamina propria of gastric mucosa depending on the nodal stage.

Figure 2: Survival of patients depending on the number of dilated capillaries in the lamina propria of gastric mucosa. (a): the curves of 3-year relapse-free surviving, (b): the curves of 3-year overall surviving.
There were no significant differences in the number of dilated capillaries in GM depending on the depth of invasion and histology of tumor.

2.3 The Venous Vessels of the Gastric Submucosa without Muscle Tissue in the Middle Layer

The extremely dilated vessels of this type were characterized by a lack of muscular tissue in the middle layer (Figure 3a). The wall thickness of such vessels ranged from 6.6 to 34.5 microns and the average was 17.43±4.05 microns. Tunica interna was formed by endothelial cells with flattened hyperchromatic nuclei. Vascular adventitia consisted of several layers of cells with elongated, curved nuclei and had a specific connective matrix with a predominance of fibrous structures in it (Figure 3b).

![Figure 3: The vessels of gastric submucosa. (a): the normal arteriola (1) and venula (2), the dilated venous vessel without muscle tissue in the middle layer (3) and the dilated capillary (4): van Gieson’s stain, bars = 100 µm; (b): the absence of muscle tissue in the middle layer of the venous vessel: van Gieson's stain, bars = 20 µm. (c): the dilated venous vessels of muscle type: H&E stain, bars = 100 µm; (d): the vessels of the arterial type with hypertrophied muscle layer (arrows): H&E stain, bars = 100 µm.](image-url)
2.4 The Dilated Venous Vessels of Muscle-Type of the Gastric Submucosa

The vessels of this sort often had an irregular shape and formed the wide vascular lacunae with the stasis of formed elements in their lumen (Figure 3c). The wall thickness of such vessels varied within the wide limits from 18.8 to 54.5 microns and the average size was 32.8±7.1 micrometers.

2.5 The Vessels of the Arterial Type of the Gastric Submucosa with Hypertrophied Muscle Layer

The average wall thickness of these vessels was 56.2±2.6 micrometers. The arterial vessels that prevailed over all others in the number and occupied square were found in 9 samples (14.3%). The described vessels were more often full-blooded, less common - with collapsed walls (Figure 3d).

The correlation of the presence of dilated venous vessels and the vessels of arterial type with the clinical characteristics of GC and long-term results of treatment was not established.

2.6 The Dilated Vessels of Capillary Type of the Gastric Submucosa

In gastric GS adjacent to the tumor the dilated vessels of capillary type with diameter of 100 micron and more were often determined. A distinctive feature of the described vessels was the fact that both the endothelium of normal structure and the cells with large, pale nuclei having a fine-netted chromatin structure took part in their formation (Figure 4a and b). The cells with similar structure were observed not only in the lining of the described vessels but also next to them and around the capillaries located in the lamina propria of GM, and even in their lumen (Figure 4c). The important particularity of the described cells was their ability to form bands and closed structures (Figure 4d and e). Endothelial proliferations were also observed in the lumen of vessels (Figure 4f). The expression of CD34 was completely absent in the most of dilated capillaries (Figure 4g), sometimes it was barely visible or expressed fragmentary (Figure 4h).

The connective tissue surrounding the dilated capillaries was presented by the strands of thin, loose fibrils. The most vessels were free from the content, but there were sometimes vessels with plasma or blood cells. Stromal edema and diapedesis of blood cells were being observed in all the samples under study.

The correlation analysis demonstrated that the dilated capillaries of GS correlated with histologic type (gamma = 0.451, Z = 3.44, p = 0.0006), grade (gamma = 0.341, Z = 3.17, p = 0.002), N category (gamma = 0.536, Z = 4.75, p = 0.000002), 3-year OS (gamma = -0.344, Z = -2.23, p = 0.03) and RFS (gamma = -0.382, Z = -2.75, p = 0.006). The described type of vessels was significantly less common found in the intestinal type of GC, grade G1 and in the absence (N0) of lymph metastases (Table 4).
Figure 4 (a–d): The features of the dilated capillaries of gastric submucosa. (a): the dilated vessel of the capillary type in gastric submucosa: H&E stain, bars = 100 μm; (b): the endothelial cells with the large, pale nuclei having a fine-netted chromatin structure in the lining of the dilated capillary (arrows): H&E stain, bars = 20 μm; (c): the similar cells in the lumen of capillaries (thick arrows) and next to them (thin arrows) in the lamina propria of gastric mucosa: H&E stain, bars = 20 μm; The similar cells form the bands; (d): and the closed structures in gastric.
Figure 4 (e–h): The features of the dilated capillaries of gastric submucosa. (e): H&E stain, bars = 20 μm; (f): the endothelial proliferation in the lumen of dilated capillaries: staining with CD34, bars = 40 μm; The lack of the expression of CD34; (g): and the fragmented expression of CD34; (h): in the endothelial lining of dilated capillaries: staining with CD34, bars = 40 μm.
The number of the dilated capillaries

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<th>multiple</th>
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<tr>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Intestinal type</td>
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<td>66.7</td>
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<tr>
<td>Diffuse-types</td>
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<td>8</td>
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<tr>
<td>G1</td>
<td>19</td>
<td>79.2</td>
<td>1</td>
</tr>
<tr>
<td>G2</td>
<td>7</td>
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<tr>
<td>G3-4</td>
<td>7</td>
<td>36.8</td>
<td>5</td>
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<tr>
<td>Signet ring cell carcinoma</td>
<td>10</td>
<td>41.7</td>
<td>7</td>
</tr>
<tr>
<td>N0</td>
<td>33</td>
<td>70.0</td>
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</tr>
<tr>
<td>N1</td>
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<td>27.3</td>
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<tr>
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**Statistics**

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<tbody>
<tr>
<td>$\chi^2 = 5.39$</td>
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<td>$\chi^2 = 13.49$</td>
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<td>$p = 0.01$</td>
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**Table 4:** The number of dilated capillaries in gastric submucosa depending on the patient characteristics: histology, grade, nodal stage.

The depth of tumor invasion (category T), the degree of atrophy, intestinal metaplasia and dysplasia of GM did not significantly influence the number of vessels of this type. However, the number of dilated capillaries of GS correlated with the MVD ($Q = -0.519$, $t = -2.57$, $p = 0.02$) and CD68 macrophages in GM ($Q = -0.654$, $t = -2.87$, $p = 0.02$). The decrease of the MVD (CD34 +) and the density of macrophages (CD68 +) in GM at the increase of the dilated capillaries number in GS was recorded.

In terms of prognosis more significant appeared to be the fact of the presence of dilated capillaries in GS than the fact of their quantity. The described vessels were detected in 24 of 32 (75%) patients with metastases in RLN and only in 15 out of 49 (30.6%) without metastases ($p = 0.0002$). In the presence of dilated capillaries in GS the decrease of 3-year OS ($p = 0.003$) and RFS ($p = 0.025$) was noted (Figure 5a and b).

At the same time we did not detect the significant differences in the survival rates between the groups of patients with single and multiple dilated capillaries. The 3-year OS was 90.2%1, 61.1%2 and 80%3 ($p^{1-2} = 0.008$, $p^{2-3} > 0.2$ Log-Rank Test) and the RFS was 82.5%1, 58.8%2 and 60.0%3 ($p^{1-2} = 0.049$, $p^{1-3} = 0.042$ Log-Rank Test) in the absence1, in the presence of single2 and in the presence of multiple3 vessels of this type respectively.

### 2.7 The Vessels of “Cavitary” Type

We had described before a new way of angiogenesis on the example of GC that consisted in the formation of CS in the tumor stroma and adjacent GM, being then lined by the be associated with the abruption of tumor cells from their underlying foundation (type-1), with the dilatation of tumor glands, flattening and thinning of the epithelial cells (type-2) and with the formation of CS directly into the GM or the tumor stroma without in-
Figure 5: Survival of the patients depending on the presence of dilated capillaries in gastric submucosa. (a): the curves of 3-year relapse-free surviving; (b): the curves of 3-year overall surviving.

volvement of the tumor cells (type-3). However, in this work it was decided not to consider the features of cavitary angiogenesis type-2 as only the CS type-1 and CS type-3 were associated with the clinical characteristics and prognosis of GC. Besides the existence of “cavitary” vessel type-2 is considered by us to demand an additional confirmation, for example by using double IHC staining. So in this chapter we described only two main types of CS formation corresponding to the first (CS type-1) and third (CS type-2) types of CS in our original work (Senchukova & Kiselevsky, 2014).

The first type of “cavitary” angiogenesis was associated with the abruption of layers of epithelial cells from their underlying foundation and their desquamation into the
lumen of the “obliterated” gastric or tumor glands (Figure 6a). 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 such lining are un-evenly stained by marker and have an uneven surface with a number of protuberances (Figure 6b);

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 (× 100) signification (Figure 6c). 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 (Figure 6d).

We have also pointed out some differences in the morphology of CS type-1 in intestinal and diffuse types of GC (Senchukova et al., 2015). In the intestinal type of GC the formation of CS type-1 was associated with tumor or normal glands where the flaking of epithelial cells from the basement membrane and their desquamation into the lumen of the “obliterated” gastric or tumor glands were being observed (see Figure 6a to c). 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 (Figure 6e). 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 (Figure 6f).

The second type of “cavitary” angiogenesis was associated with the formation of CS directly into the GM or the tumor stroma. This supposition was due to the fact that in the some cases we had observed a characteristic cellular structure of the connective tissue of the lamina propria of GM (Figure 7a), often combining with the expressed phenomena of diapedesis of erythrocytes. 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 (Figure 7b).

The Spearman rank correlation analysis (ρ) and gamma correlation coefficient test (gamma) showed that the number of CS type-1 correlated with histological type (gamma = 0.344, z = 2.51, p = 0.01), degree of tumor differentiation (gamma = 0.318, z = 2.79, p = 0.005), TNM stage (gamma = 0.524, z = 4.27, p < 0.0001), T stage (gamma = 0.666, z = 4.75, p < 0.0001), N stage (gamma = 0.520, z = 4.19, p < 0.0001), 3-year OS (gamma = -0.778, z = -4.64, p < 0.0001) and RFS (gamma = -0.766, z = -5.81, p < 0.0001). The correlation between the number of CS type-1 and CS type-2 was also noted (gamma = 0.577, z = 4.47, p < 0.0001).

The presence of multiple CS type-1 was the most significant factor associated with the prognosis of GC. The multiple CS type-1 were more frequently observed in diffuse type of GC (in 24.4% and 43.7% cases, respectively in intestinal and diffuse types of GC,
**Figure 6:** The morphological features of “cavitary” angiogenesis type-1. (a): the CS type-1 in tumor stroma: H&E stain, bars = 100 µm; (b): the CS type-1 with a partial endothelial lining (black arrows) and dilated vessels located next to them (grey arrows). The cytoplasm of endothelial cells has an uneven surface with a number of protuberances (white arrows): staining with CD34, bars = 40 µm; (c): the CS type-1 without (black arrows) and partial (grey arrows) endothelial lining and the dilated vessels (grey arrows) with tumor emboli in their lumen: staining with CD34, bars = 100 µm; (d): the dilated vessels with tumor emboli in their lumen (black arrows) and tumor glands (grey arrow): staining with CD34, bars = 20 µm; (e): CS type-1 (arrow): staining with CD34, bars = 100 µm; (f): CS type-1 in diffuse type of GC (black arrow). The lining cells have the large, light, oval-shaped nuclei (grey arrow): staining with CD34, bars = 100 µm.
**Figure 7**: The morphological features of “cavitary” angiogenesis type-2. (a): the CS type-2 in the stroma bordering upon tumor tissue (arrows): H&E stain, bars = 100 µm; (b): the CS type-2 with endothelial lining (large arrows). The cytoplasm of the endothelial cells weakly expresses CD34 and has several protuberances growths (small arrows): staining with the anti-CD34, bars = 100 µm.

χ² = 3.42, p = 0.18), in G3-G4 grade (in 14.8%, 42.9%, 62.5% and 37.5% cases, respectively in G1, G2, G3-4 and signet ring cell carcinoma, χ² = 9.64, p = 0.14), in T3-4 stage (in 18.7%, 11.1%, 50% and 100% cases, respectively in T1, T2, T3 and T4 stage, χ² = 15.37, p = 0.001) and in N2 stage (in 18.6%, 44.4% and 66.7% cases respectively in N0, N1 and N2 nodal stage, χ² = 15.74, p = 0.003).

The increase of the number of CS type-1 was accompanied by the increasing of the density of CD68 in GM and tumor stroma. In the presence and absence of multiple CS type-1 the density of CD68 macrophages in GM was respectively 72.6±44.8 and 41.6±15.4 cells on area unit (p = 0.06) and in tumor stroma –68.3±41.7 and 27.2±20.2 cells on area unit (p = 0.15).

3-year OS and RFS were practically identical if the CS type-1 in tumor stroma were single or absent, and significantly worse when the CS type-1 were multiple (Figure 8a and b).

With or without the multiple CS type-1, the 3-year OS was 52.7% and 93.9% respectively (p = 0.0013, OR = 15.0, 95% CI = 2.96 – 76.31), and the RFS was 32.4% and 87.7% respectively (p = 0.0001, OR = 14.93, 95%CI = 4.34 – 51.38).

The CS type-2 correlated in its turn only with the histological type of tumor (gamma = 0.403, z = 2.68, p = 0.008). By the diffuse and intestinal type of GC they were revealed in 55.9% and 44.1% cases respectively (χ² = 3.24, p = 0.07).

The multivariate Cox proportional hazard regression analysis indicated that TNM stage (p = 0.003), nodal stage (p = 0.013), the number of CV type-1 (p = 0.005) were significantly independent prognostic factors in patients with GC.
Figure 8: Survival of the patients depending on the presence of CS type-1 in tumor stroma. (a): the curves of 3-year relapse-free surviving; (b): the curves of 3-year overall surviving.
Angiogenesis is a key factor in tumor progression, directly related to the invasion and metastasis of malignant tumors. The study of its mechanisms attracts attention of many scientists. However, it should be noted that the technical and methodological approaches to the solution of this problem significantly diverge in different researchers. Some researchers give preference to a quantitative evaluation of the angiogenesis activity in tumors, noting that a high MVD in the tumor and a high level of VEGF expression are more frequently observed in advanced malignancy in the presence of metastases and are correlated with poor prognosis (Ding et al., 2006; Erenoglu et al., 2000; Lazar et al., 2008; Ma et al., 2007; Millikan et al., 2003; Poon et al., 2003; Wang et al., 2007).

Other researchers put more emphasis on the study of qualitative changes in the structure of vascular wall and microvasculature, pointing out that the tumor vascular network is heterogeneous in its structure and different types of vessels may respond differently to the use of angiogenesis blockers (Baluk et al., 2005; Birau et al., 2012; Gee et al., 2003; Fukumura et al., 2010; Morikawa et al., 2002; Nagy & Dvorak, 2012; Nagy et al., 2012).

In studies of peculiarities of angiogenesis in patients with GC we used both a quantitative assessment of the activity of tumor angiogenesis (Goddard et al., 2002) and the study of the morphological features of the different types of tumor vessels. Such approach allowed us to single out some types of tumor vessels differed in morphology and their clinical importance, and to formulate a hypothesis of “cavitary” type of angiogenesis by GC (Senchukova & Kiselevsky, 2014).

In the quantitative evaluating of the angiogenesis activity, unlike a number of researchers (Lazar et al., 2008; Poon et al., 2003; Wang et al., 2007), we did not reveal the relationships between MVD in tumor and factors of GC progression. From this point of view our results are closer to the results of the researchers who also found no correlation between MVD in tumor and prognosis by different malignancy (Torres et al., 1999; Zeng et al., 2010; Hillen et al., 2006). Moreover, there is an opinion that the definition of MVD in the tumor is inappropriate to use widely in clinical practice because of apparent defects of this method (Brown et al., 2008).

These limitations are related to the lack of clear standards in the technical aspect of the method based on the subjective assessment of optimal locations to determine "hot spots" as well as the lack of clear criteria for appraisal of the obtained results. A large number of histological types of GC having different ratio of parenchyma and stroma in the tumor makes it difficult to give not only a quantitative estimation of the MVD but the interpretation of the obtained data as well.

The study of the morphological features of tumor stroma vessels and the adjacent GM by GC allowed us to identify several types of abnormal vessels having different prognostic value. More often than others the clearly dilated venous vessels with and without muscle tissue in the middle layer were being defined in GS. We didn’t reveal correlations between the vessels of this type and the factors of GC progression. It can be assumed that
the described vessels are developing from normal vessels as a result of violations of the structure of their basement membrane. In some researches it was shown that the expression of VEGF-A by tumor and stroma cells caused a degradation of the basement membrane of vessels (Nagy et al., 2012). This leads to the remodel of the existing arterioles and venules, to their sharp expansion and increase of vascular permeability playing an important role in tumor angiogenesis.

In contrast to the dilated venous vessels the dilated capillaries of the lamina propria of GM and GS were of great prognostic importance. A distinguishing feature of the described vessels was that both the endothelium of the normal structure and the cells with large, pale nuclei with fine-netted chromatin structure took part in their formation. The presence of dilated capillaries in GM was connected with more advanced stages of GC and with the worsening of the long-term results of GC treatment. As for the dilated capillaries of GS they were observed mainly in patients with lymphatic metastases. Their number was associated with histology, tumor grade, OS and RFS of patients with GC.

The obtained results allow us to suggest that angioblasts should be able to participate in the formation of dilated capillaries. Their involvement in tumor angiogenesis has been described by many researchers (Ahlskog et al., 2003; Asahara et al., 1997; Hillen & Griffioen, 2007; Sussman et al., 2003). The following facts testify to the possible participation of angioblasts in the formation of the described vessels:

- the phenotypical features of the nuclei of cells lining the described vessels: large, pale with fine-netted chromatin structure;
- the lack of expression of CD34 in the newly formed vessels. This can be explained by the fact that the cells lining the dilated capillaries belong to negative CD34 progenitor cells, known as the precursors of CD34 positive cells (Kimura et al., 2004.);
- the presence of the described cells not only in the endothelial lining of vessels, but also around the capillaries located in the lamina propria of GM and in their lumen;
- the ability to form the intravascular growing. This ability of tumor vascular endothelium has been also noted by other investigators who point to the fact that intravascular growing could participate in the formation of new vessels by dividing the capillary lumen (Nagy et al., 2012).

We believe that the revealed changes in GS adjacent to tumor testifying about the active processes of angiogenesis are not casual. It is known that exactly at the border of the tumor and "normal" tissues there are being observed the most active processes associated with the formation of future tumor stroma that determine its further growth and metastasis of malignant neoplasm (Nagy et al., 2012). We consider that a loose connective tissue of the GS is a favorable environment for the formation of a fibrin skeleton of future vessels and their growth as well.

Some scientists have presented evidence that a tumor vasculature is heterogeneous in its structure and different types of vessels may respond differently to the usage of angiogenesis blockers [Holash et al., 1999; Chang et al., 2000; Wang et al., W., 2010; Burri et
The differences in the structure of vessels may be associated with different mechanisms of their formation. These mechanisms include:

- **Sprouting angiogenesis** is a growth of new capillary vessels out of preexisting ones. The process of sprouting angiogenesis involves several sequential steps: the activation of endothelial cells by specific growth factors; the degradation of the extracellular matrix and basal membrane; the invasion of endothelial cells into the surrounding matrix and their migration to the problem areas: the focuses of inflammation, hypoxia, tumor growth (Morikawa et al., 2002; Gee et al., 2003; Baluk et al., 2003; Hillen & Griffioen, 2007).

- **Vessel co-option** is a growth of tumor cells among existing vessels without evoking an angiogenic response (Dome et al., 2002; Holash et al., 1999).

- **Vasculogenic mimicry** is a process of vessel-like structure formation being partially or completely lined with tumor cells (Chang et al., 2000; Folkman, 2001; Li et al., 2010; Maniotis et al., 1999; Wang et al., 2010).

- **Intussusceptive angiogenesis** is a new concept of vessel formation when preexisting vessels split in two new vessels by the formation of transvascular tissue pillar into the lumen of the vessel (Burri et al., 2004).

- **Vasculogenesis** is a formation of new blood vessels with the participation of «endothelial progenitor cells» (EPCs) circulating in blood. It is known that under the influence of tumor mediators, EPCs emerge from the bone marrow into the peripheral blood and can be included in the wall of the existing tumor vessels or participate in the formation of new blood vessels (Asahara et al., 1997; Hillen & Griffioen, 2007; Sussman et al., 2003).

Recently we described a new way of angiogenesis characterized by the formation of CS in the tumor stroma and adjacent GM then being lined by endothelial cells and merged into the blood vessels of the organ (Senchukova & Kiselevsky, 2014). We proposed that there are two main mechanisms of the formation of such CS. The first one is associated with the abruption of tumor cells from their underlying foundation, the second – with the formation of CS directly into the GM or the tumor stroma without involvement of the tumor cells. Analysis of the received data has showed that the first type of “cavitary” angiogenesis plays perhaps a key role in progression of GC. So, the presence of multiple CS type-1 was associated with the diffuse type of GC, grade G3-G4, T3-4 and N2 stages of GC and with the reduction of 3-year RFS and OS. In turn, the presence of CS type-2 was associated with the histological type of GC.

We have also pointed out some differences in the morphology of CS type-1 in intestinal and diffuse types of GC (Senchukova et al., 2015).

We believe that the important role in the formation of the described “cavitary” structures can play inflammatory changes in the tumor stroma and the adjacent GM. It has been known that an active inflammatory process is connected with the increased secretion by immune cells of cytokines, chemokines, growth factors and proteases (Eiro & Vizoso, 2012; Wu & Zhou, 2009) that promote the activation of tumor angiogenesis on the one hand (De Narddo et al., 2008; Pollard, 2009) and influence the adhesive properties of
tumor cells on the other (Mantur & Wojszel, 2008; Reiss et al., 2006). Besides some studies have shown that the immune cells may be directly associated with tumor progression and invasion (Man, 2010; Man et al., 2010; Man et al., 2013; Pawelek & Chakraborty, 2008) 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 (Schreiber et al., 2011).

In 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 favorable 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 – derived prostaglandin E2. The activation of TIM is modulated by local signals within the tumor microenvironment such as tumor necrosis factor-α and hypoxia (Ohta et al., 2004; Sica et al., 2006). They are often found in the vicinity of tumor glands basement membranes, in the places where its integrity is destroyed (Peng et al., 2010). It is logical to assume that the main mechanism of the damaging effect may be associated with the synthesis of matrix metalloprotease (MMP) that are proteolytic enzymes used by cancer to degrade the extracellular matrix (Kamoshida et al., 2012, Kamoshida et al., 2013).

We believe that the previously described “Retraction Artifact” (it is a space between tumor cells and their surrounding stroma) has a direct relationship to the type-1 of “cavitary” angiogenesis. The prognostic significance of “Retraction Artifact” as a factor associated with tumor progression has been mentioned in a number of studies (Acs et al., 2007; Acs et al., 2012; Zaorsky et al., 2012). However, in contrast to the cited sources, we succeeded to link this phenomenon to a previously undocumented type of angiogenesis and to show that tumoral emboli in blood vessels can be formed at the expense of the abruption of tumor cells from their adjacent stroma and of their desquamation directly into the lumen of the vessels of “cavitary” type. It could be assume that the disorder of the adhesive properties of tumor cells is of the key importance in the formation of CS type-1. The focal disruptions in the tumor capsule (Man**, 2010; Man***, 2010) associated with increased immune cell infiltration (Man, 2010; Man et al., 2013) are most likely related to this processes.

It is possible that another important factor associated with the formation of CS type-1 and type-2 is a phenomenon of the increased vascular permeability that on the one hand may influence the process of stroma retraction and on the other - promote the formation of a fibrin matrix and a migration of endothelial cells (Nagy et al., 2012). There is a good reason to believe that parallel processes of formation and lysis of stroma that more actively occurring at the boundary between tumor and adjacent tissue may contribute to the formation of CS type-2.

Concluding the discussion, we would like to point out some differences in the morphology of CS type-1 in intestinal and diffuse types of GC (Senchukova et al, 2015):

- 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 are 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:

• 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 (Forster, 2011).

• The significantly higher levels of MMP-1, MMP-7, VEGF and E-cadherin in diffuse type of GC (Zhou et al., 2010; Kuang et al., 2013).

• A higher incidence of positive expression of integrin beta3 mRNA in diffuse type of GC (Li et al., 2008). It must be noted that integrins are cell adhesion molecules, which mediate cell-cell adhesion or cell-extracellular matrix 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.

4 Conclusion

The results of this study testify that the vessels in the tumor stroma and adjacent GM differ both in structure and clinical significance.

From scientific and practical points of view, two types of vessels the presence of which correlates with clinical characteristics and long-term results of GC treatment are of the most interest: the dilated capillaries of GM and GS and the vessels of “cavitary” type. A distinguishing feature of the dilated capillaries is that both the endothelium of normal structure and the cells with large, pale nuclei with fine-netted chromatin structure take part in their formation. The phenotypical features of the nuclei of cells lining dilated capillaries testify that EPCs could participate in the formation of these vessels. The relationship of the described vessels with the presence of lymph metastases allows them to be considered as a factor in the progression of GC.

As for our hypothesis of the “cavitary” type of angiogenesis, the obtained data indicate that this type of vascular formation possibly plays a key role in the progression of GC. This follows from the fact that the presence of multiple CS type-1 is closely associated with the presence of metastases in RLN and the decrease of RFS and OS of patients with GC. It is conceivable that a violation of the adhesive properties of tumor cells, inflammatory changes in the tumor stroma and the adjacent GM and microvascular hyperpermeability is associated with the formation of CS type-1. We believe that further studies should be carried out to investigate the mechanisms of the formation of the described vessels and their role in progression of GC.
Abbreviations

CS: "cavitary" structures; EPCs: endothelial progenitor cells; GC: gastric cancer; GM: gastric mucosa; GS: gastric submucosa; MMP: matrix metalloprotease; MV: microvessels; MVD: microvessel density; RLN: OS: overall survival; regional lymph nodes; RFS: relapse-free survival; VEGF: TIM: tumor-infiltrating macrophages; vascular endothelial growth factor

References


