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Research Article

New Classification of Tumor Microvessels and the Risk of Regional Metastasis in Squamous Cell and Glandular Cancers

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Abstract

Previously, we proposed a new classification of tumor microvessels (MVs) on the basis of their morphological features and clinical significance. The aim of this study was to summarize the obtained results and establish the predictive value of different types of tumor MVs for assessing the risk of metastasis to regional lymph nodes (RLNs) in glandular and squamous cell carcinomas. *Materials and methods.* A total of 385 archival samples of gastric cancer stages I--III, breast cancer stages I--IIIA, cervical squamous cell carcinoma stages I--IIA, and lung squamous cell carcinoma stages I--IIIA were studied. The tumor sections were processed routinely and subjected to immunohistochemistry with antibodies against cluster of differentiation 34 (CD34) and podoplanin. To assess independent predictors of the risk of metastasis to RLNs, correlation analysis and univariate and multivariate logistic regression analyses were performed. Statistical analysis was performed via Statistica 10.0 software. *Results*. For patients with gastric cancer and breast cancer, the independent predictors of a high risk of metastasis to RLNs are T2 ($p=0.007$) and T3 ($p<0.00001$) stages, tumor grade 3 ($p=0.0002$), the presence of lymphovascular invasion (LVI) ($p=0.044$) and peritumoral retraction clefts ($p=0.008$). For patients with squamous cell carcinoma of the cervix and lung, independent predictors of a high risk of metastasis to RLNs are the T2 ($p=0.01$) and T3 ($p=0.007$) stages, the presence of LVI ($p=0.0014$), dilated capillaries (DCs) of the "contact type" ($p=0.0007$), capillaries in the tumor solid component ($p=0.046$) and peritumoral retraction cleftings ($p=0.0006$). *Conclusion*. The results of the present study indicate that when assessing the risk of metastasis to RLNs, it is advisable to consider the presence of peritumoral retraction clefting and LVI in both glandular and squamous cell carcinomas. In addition, in squamous cell carcinomas, the accuracy of assessing the risk of metastasis to RLNs can be increased by taking into account "contact-type" DCs and capillaries in the solid component of the tumor.

Keywords

Gastric Cancer, Breast Cancer, Cervical Squamous Cell Carcinoma, Lung Squamous Cell Carcinoma, Tumor Microvessels, Regional Metastases

1. Introduction

In 1971, Judah Folkman first proposed the brilliant hypothesis that the formation of tumor microvessels (MVs) is a necessary condition for the growth of malignant neoplasms [1]. Further studies have shown that tumor angiogenesis is

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also associated with tumor metastasis and can be a target for antitumor therapy [2, 3]. Research on tumor angiogenesis is actively continuing, and over the past half century, much work has been done to study the mechanisms of tumor angiogenesis:

The key factors that initiate tumor angiogenesis processes, such as hypoxia, hypoxia-inducible factor-1, and vascular endothelial growth factor, have been identified. In addition, the ability to induce tumor angiogenesis has been established for fibroblast growth factor, angiopoietins, epidermal growth factor, platelet-derived growth factor, transforming growth factors, hepatocyte growth factor, insulin-like growth factor, nitric oxide, and their receptors [4-6].

Various mechanisms of tumor microvessel formation have been established, including sprouting angiogenesis [7, 8], vasculogenic mimicry [9-11], vasculogenesis [12-14], tumor vessel co-option [15, 16], and intussusceptive angiogenesis [17-19].

The main characteristics of tumor MVs, such as variability in vessel diameter, incomplete structure of their walls and random arrangement, are described. The basement membrane of tumor MVs has a discontinuous structure and is weakly associated with pericytes. The endothelial cells (ECs) lining tumor MVs are weakly associated with each other, can overlap with each other, often have an irregular shape with ruffled margins and numerous cytoplasmic projections and are characterized by high inter- and intratumoral heterogeneity [13, 20-24]. The described features of tumor MVs contribute to the passage of fluid and fibrin into the surrounding tissue and the formation of tumor stroma, and an increase in interstitial pressure promotes tumor metastasis [20, 21, 25, 26].

One of the main goals of tumor angiogenesis studies is the possibility of using the obtained results in clinical practice. Currently, a number of targeted drugs have been developed to block the action of factors that activate tumor angiogenesis. Unfortunately, the results of the use of angiogenesis blockers in the treatment of cancer patients have not met expectations [27-29]. The insufficient effectiveness of angiogenesis blockers is believed to be associated with the heterogeneity of tumor MVs, which have different origins and structures and respond differently to angiogenic therapy [8, 21, 23, 30, 31]. There are several classifications of tumor MVs, which are based mainly on the degree of vessel maturity as a factor associated with tumor sensitivity to antiangiogenic therapy [21, 23, 30, 32, 33]. However, most of these classifications do not consider the variety of tumor MVs or their role in tumor progression. Thus, the study of the morphology of different types of tumor MVs and their correlations with key factors of tumor progression has not lost relevance.

Since 2011, our team has been actively studying the morphological features of tumor MVs in malignant neoplasms. We studied tumor MVs in glandular cancers of the stomach and breast and squamous cell cancers of the cervix and lung. As a result of the analysis, we described 8 types of tumor MVs and structures with partial endothelial linings (SPELs). The new classification of tumor MVs is based on characteristics such as size, shape, contours, localization, features of the endothelial lining, intensity of staining of the cytoplasm of ECs with the markers CD34 and podoplanin (PDPN), characteristics of the EC nuclei and the content of the MV lumen. A detailed description of different types of tumor MVs is presented in our previously published works [34-38]. In short, we distinguished the following types of tumor MVs:

1) normal MVs, capillaries with a diameter of 5–40 μm with a normal endothelial lining;
2) dilated capillaries (DCs), MVs of regular shape with a diameter greater than 40 with a normal endothelial lining;
3) atypical dilated capillaries (ADCs), MVs with a diameter greater than 40 μm, with unclear contours due to the chaotic arrangement of the lining ECs;
4) structures with partial endothelial lining (SPELs), cavity structures, the walls of which are only partially lined with chaotically arranged ECs;
5) DCs with weak expression of CD34, MVs with a diameter greater than 40 μm, located in loose irregular connective tissue, and lined by ECs with very weak, sometimes barely noticeable cytoplasmic expression of CD34;
6) contact-type DCs, MVs, the walls of which are in direct contact with tumor cells, with no connective tissue layer between them;
7) capillaries in the tumor solid component, linear capillaries with collapsed walls located directly in the solid component of the tumor; and
8) capillaries in lymphoid and polymorphic cell infiltrates, MVs located in lymphoid and polymorphic cell infiltrates with a very thin endothelial lining and lymphocytes in their lumen.

Normal MVs, DCs, ADCs, SPELs and DCs with weak expression of CD34 are observed in both glandular and squamous cell carcinomas. At the same time, DCs of the contact type, capillaries in the solid component of the tumor and capillaries in lymphoid and polymorphic cell infiltrates were detected only in squamous cell carcinomas.

We found that the DCs with weak expression of CD34 and capillaries in the solid component of the tumor were blood vessels, since these MVs were not stained with antibodies against podoplanin (PDPN). In contrast, all capillaries in lymphoid and polymorphic cell infiltrates are stained with PDPN, i.e., they are lymphatic vessels. Other types of tumor MVs can be both blood and lymphatic vessels. Importantly, the described tumor MVs not only differed in structure but were also associated with different clinicopathological characteristics of malignant neoplasms. When the correlations of the described MVs with the presence of metastases in RLNs, the most significant clinical factor determining the prognosis of the disease, were assessed, it was found that, in

patients with gastric cancer and squamous cell lung cancer, the number of ADCs and SPELs in the tumor was greater in patients with metastases in the RLNs than in patients without them. In addition, in squamous cell lung cancer, the presence of capillaries in the solid component of the tumor is also associated with the risk of metastases in RLNs [34-38]. However, it is unclear whether these MVs are independent predictors of the risk of regional metastasis and whether there are differences in the prognostic significance of different types of tumor MVs between glandular and squamous cell carcinomas. Thus, the aim of this study was to establish the predictive significance of different types of tumor MVs for assessing the risk of metastasis to RLNs in glandular and squamous cell carcinomas.

2. Materials and Methods

2.1. Patients

This study is a summary analysis of the results of immunohistochemical (IHC) examination of 385 archival samples of stage I--III gastric cancer, stage I--IIIA breast cancer, stage I--IIA cervical squamous cell carcinoma and stage I--IIIA lung squamous cell carcinoma. A detailed description of the studies performed is provided in our earlier works [34-38]. Briefly, all the studies were retrospective, case−control studies. The inclusion criteria for the study were as follows: (1) the surgery was performed at the first stage of treatment; (2) the surgery was radical (R0); and (3) the condition of the archival material, allowing for high-quality histological and IGH examinations. The study did not include archival blocks (1) from patients who received neoadjuvant therapy preceding surgical treatment; (2) from patients who consumed antihistamines or nonsteroidal drugs; (3) from patients who had stage IV cancer; or (4) from patients whose material for morphological examination was poor quality. Studies were conducted in accordance with the Declaration of Helsinki principles and ethical and legal requirements. The study protocols were approved by the Ethics Committee of Orenburg State Medical University (Orenburg, Russia). The characteristics of the patients whose archival blocks were examined in this study are presented in Table 1.

Table 1. *Patient characteristics.*

	N (%)
Age years (M±σ)	57.9 ± 11.1
Diagnosis	
Gastric cancer	74(19/2%)
Breast cancer	66(17.1%)
Squamous cell cervical cancer	65(16.9%)
Squamous cell lung cancer	180(46.7%)
Sex	
Men	221(57.4%)
Women	164(42.6%)
T stage	
T1	128(33.2%)
T2	160(41.6%)
T3	92(23.9%)
T4	5(1.3%)
N stage	
N0	191(49.6%)
N1	114(29.6%)
N2	80(20.8%)
Stage	
I	141(36.6%)
II	126(32.7%)
III	118(30.6%)
Grade	
G1	140(36.4%)
G2	158(41.0%)
G3	87(22.6%)

2.2. Immunohistochemistry

IGH staining was performed on an Autostainer 480 (Thermo Fisher Scientific Ltd., Vantaa, Finland) or a fully automated BOND-MAX staining system (Leica Biosystems Melboume Pty Ltd., Australia) in the pathology department of the Orenburg Regional Oncology Center (Orenburg, Russia). For the study, archival paraffin blocks of surgical material were used. Four-micron-thick sections were stained with an antibody against cluster of differentiation 34 (CD34) and an antibody against PDPN according to the manufacturers' protocols. Marker expression was assessed as described previously [34-38].

For analysis, the density of normal MVs; the density of DCs, ADCs and SPELS; the presence or absence of DCs with weak CD34 expression; contact-type DCs; capillaries in lymphoid and polymorphic cell infiltrates; and the presence or absence of lymphovascular invasion (LVI) were determined. We also assessed the presence or absence of a peritumoral retraction cleft, since we believe that this phenomenon is associated with a specific type of angiogenesis. Figures 1-4 show examples of the described types of tumor MVs in various malignant neoplasms.

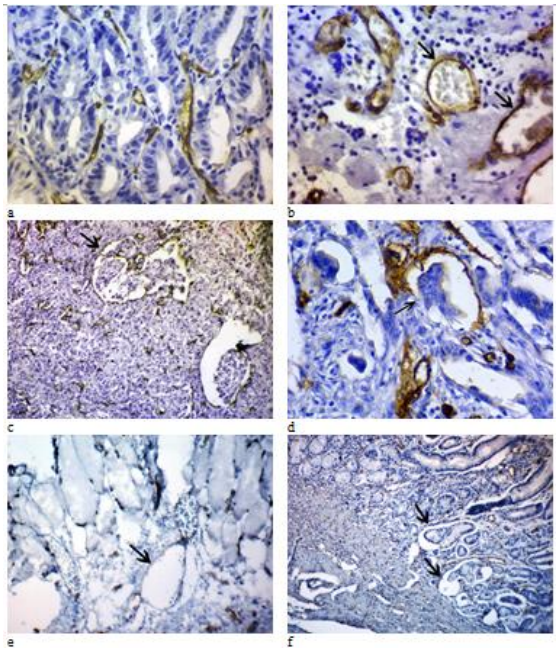

Figure 1. *Different types of tumor microvessels and structures with partial endothelial linings and the phenomenon of peritumoral retraction clefting in gastric cancer. a: normal microvessels; b: dilated capillaries (arrows); c: atypical dilated capillary (arrow); d: structure with partial endothelial lining (arrow); e: dilated capillary with weak expression of CD34 (arrow); f: phenomenon of peritumoral retraction clefting (arrows). Staining with antibodies against CD34; magnification: a, b and d – 800x; c and f – 200x; e – 400x.*

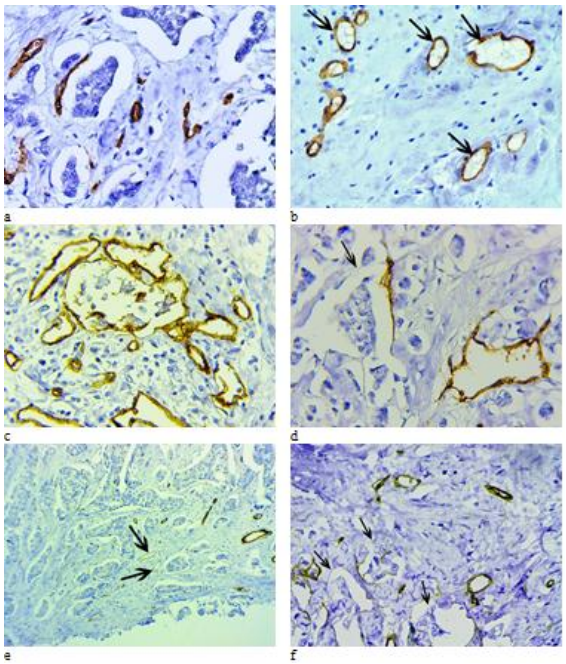

Figure 2. *Different types of tumor microvessels and structures with partial endothelial linings and the phenomenon of peritumoral retraction clefting in breast cancer. a: normal microvessels; b: dilated capillaries (arrows); c: atypical dilated capillaries; d: structure with partial endothelial lining (arrow); e: dilated capillaries with weak expression of CD34 (arrows); f: phenomenon of peritumoral retraction clefting (arrows). Staining with antibodies against CD34; magnification: a, c and d – 800x; b and f – 400x; e – 200x.*

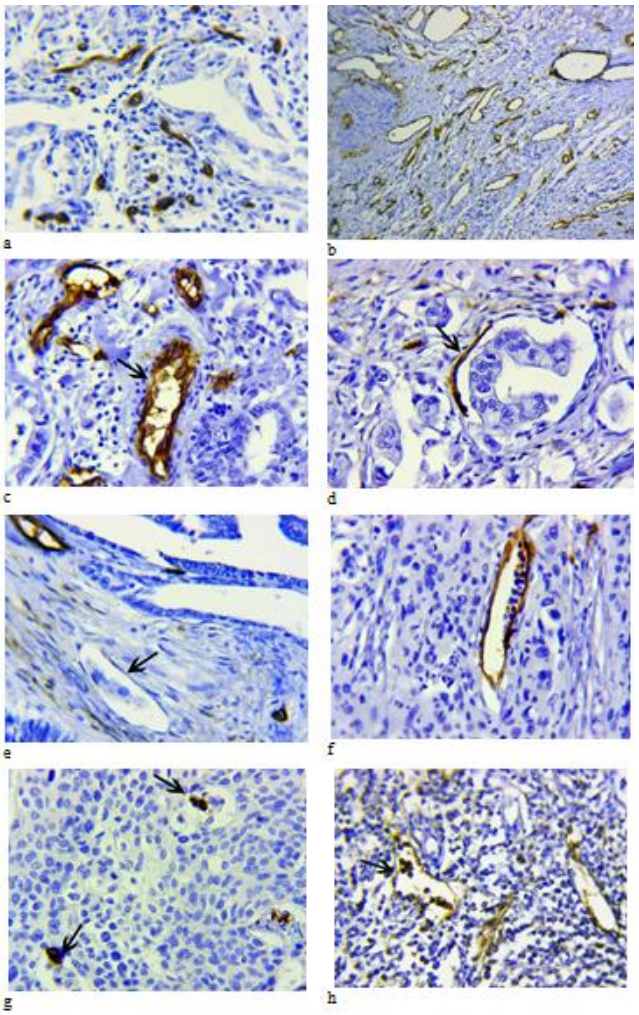

Figure 3. *Different types of tumor microvessels and structures with partial endothelial linings in cervical squamous cell carcinoma. a: normal microvessels; b: dilated capillaries; c: atypical dilated capillaries (arrow); d: structure with partial endothelial lining (arrow); e: dilated capillaries with weak expression of CD34 (arrow); f: contact-type dilated capillaries; g: capillaries in the solid component of the tumor (arrows); h: capillaries in lymphoid and polymorphic cell infiltrates (arrow). Staining: a--e and g – with antibodies against CD34, f and h – with antibodies against PDPN; magnification: a, c--h – 800x; b – 200x.*

2.3. Statistical Analysis

All analyses were conducted via Statistica 10.0 software. Correlations between biomarkers were quantified via Spearman's rank correlation coefficients or nonparametric gamma correlation. To identify potential risk factors for cancer metastasis to RLNs, all covariates that were found to be significant in the correlation analysis were included in univariate and multivariate logistic regression analyses. A value of $p < 0.05$ was considered to indicate statistical significance.

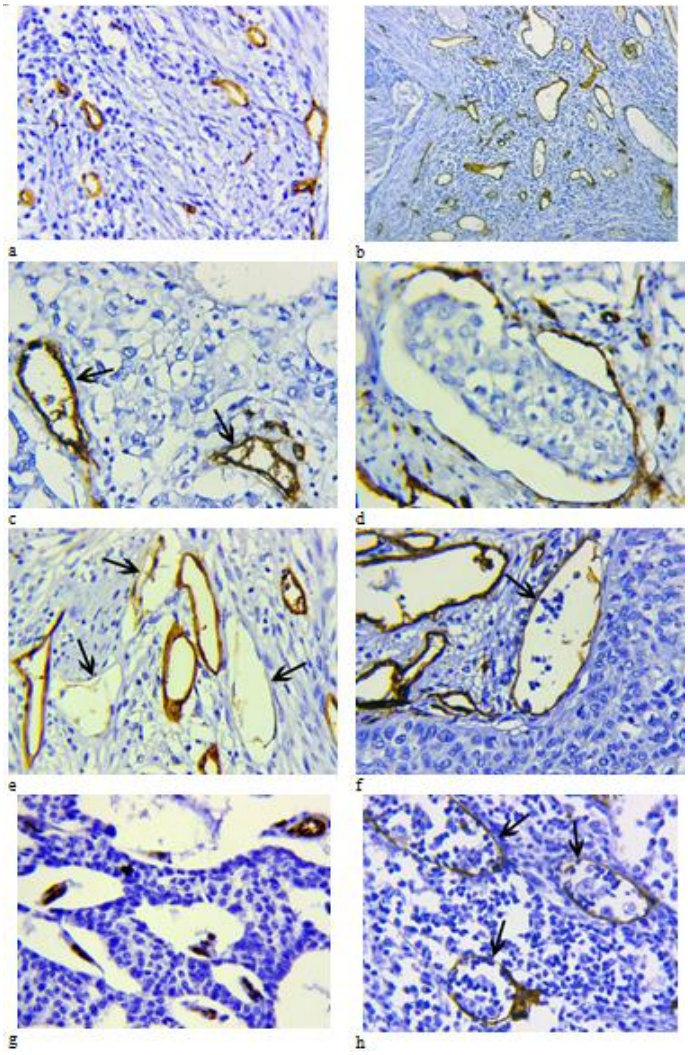

Figure 4. *Different types of tumor microvessels and structures with partial endothelial linings in lung squamous cell carcinoma. a normal microvessels; b: dilated capillaries; c: atypical dilated capillaries (arrows); d: structure with partial endothelial lining; e: dilated capillaries with weak expression of CD34 (arrows); f: contact-type dilated capillary (arrow); g: capillaries in the solid component of the tumor; h: capillaries in lymphoid and polymorphic cell infiltrates (arrows). Staining with antibodies against CD34; magnification: a, c--h – 800x; b – 200x*

3. Results

The results of the correlation analysis used to establish the relationships among the tumor MVs, the clinical and pathological characteristics of malignant neoplasms and the risk of regional metastasis are presented in Table 2.

Table 2. *Results of the correlation analysis.*

Characteristics of malignant tumors	Gamma coefficient	P
Histology	0,680515	0.0000*
T stage (Gamma Correlations)	0,659919	0.0000*
Tumor grade (Gamma Correlations)	0,330748	0.0000*
MVD	0,025287	0.792
DCs	0,213919	0,191
ADCs	0,095410	0,323

Characteristics of malignant tumors	Gamma coefficient	P
SPELs	0,389671	0,0004*
DCs with weak expression of CD34	0,538965	0.0000*
Contact-type DCs (in squamous cell carcinomas)	0,549409	0.0000*
Capillaries in the tumor solid component (in squamous cell carcinomas)	0,249118	0.005*
Capillaries in lymphoid and polymorphic cell infiltrates (in squamous cell carcinomas)	-0,047619	0,911
Peritumoral retraction clefting	0,547855	0.0000*

Thus, according to the obtained results, histology, T stage, tumor grade, SPELs, DCs with weak expression of CD34, and peritumoral retraction clefting were included in the univariate and multivariate analyses. In squamous cell carcinoma, contact-type DCs, capillaries in the solid component of the tumor, and capillaries in lymphoid and polymorphic cell infiltrates were additionally included in the analysis. The results of univariate and multivariate analyses for the overall group of patients and separately for glandular and squamous cell carcinomas are presented in Tables 3-5.

Table 3. *Results of univariate and multivariate analyses for the overall group of patients.*

Characteristics	Univariate analysis OR (95%CI)	*P* value	Multivariate analysis OR (95%CI)	*P* value
Histology				
Squamous cell cervical cancer	1	-	1	-
Breast cancer	12.07(3.93-37.05)	0.0000*	1.37(0.35-5.34)	0.649
Gastric cancer	10.17(3.34-30.93)	0.0000*	0.761(0.18-3.15)	0.707
Squamous cell lung cancer	41.20(14.24-119.18)	0.0000*	3.84(1.09-13.62)	0.0361*
T stage				
T1	1	-	1	-
T2	7.64(4.42-13.2)	0.0000*	3.53(1.78-7.03)	0.0003*
T3	12.06(6.42-22.65)	0.0000*	7.27(3.02-17.47)	0.0000*
Tumor grade				
G1	1	-	1	-
G2	0.85(0.49-1.45)	0.549	1.20(0.60-2.39)	0.603
G3	3.14(1.96-5.06)	0.0000*	1.75(0.91-3.38)	0.094
LVI				
No	1	-	1	-
Presence	5.99(3.83-9.35)	0.0000*	3.29(1.89-5.72)	0.0000*
Structures with partial endothelial lining				
No	1	-		
Presence	1.44(0.96-2.16)	0.07		
DCs with weak expression of CD34				
No	1	-	1	-
Presence	1.53(1.03-2.29)	0.037*	0.95(0.56-1.63)	0.851
Peritumoral retraction clefting				

Characteristics	Univariate analysis OR (95%CI)	*P* value	Multivariate analysis OR (95%CI)	*P* value
No	1	-	1	-
Presence	3.65(2.39-5.58)	0.0000	2.17(1.23-3.84)	0.0073*

Thus, in the overall group of patients, in univariate analysis, breast cancer, gastric cancer and squamous cell lung cancer; T2 and T3 stages; tumor grade 3; LVI; DCs with weak expression of CD34; and peritumoral retraction clefting were associated with a high risk of regional metastasis. However, in the multivariate analysis, only squamous cell lung cancer, T2 and T3 stage, LVI and peritumoral retraction clefting were associated with a high risk of metastasis.

Table 4. *Results of univariate and multivariate analyses in the group of patients with breast cancer and gastric cancer.*

Characteristics	Univariate analysis OR (95%CI)	*P* value	Multivariate analysis OR (95%CI)	*P* value
Histology				
Breast cancer	1	-		
Gastric cancer	0.87(0.44-1.70)	0.684		
T stage				
T1	1	-	1	-
T2	3.68(1.40-9.69)	0.0082	4.79(1.53-14.96)	0.007
T3	13.93(4.80-40.42)	0.0000	24.85(6.05-102.12)	0.0000
Tumor grade				
G1	1	-	1	-
G2	1.07(0.49-2.32)	0.866	0.34(0.11-1.05)	0.0612
G3	8.28(2.60-26-37)	0.0003*	16.49(3.74-72.67)	0.0002
LVI				
No	1	-	1	-
Presence	3.32(1.60-6.89)	0.0013	2.54(1.03-6.31)	0.0440*
Structures with partial endothelial lining				
No	1	-	1	-
Presence	2.26(1.10-4.65)	0.0268*	0.88(0.18-4.20)	0.8595
DCs with weak expression of CD34				
No	1	-		
Presence	1.33(0.66-2.61)	0.4120		
Peritumoral retraction clefting				
No	1	-	1	-
Presence	2.43(1.12-5.28)	0.0251*	1.81(1.08-2.36)	0.0077

In the group of patients with glandular cancers, in univariate analysis, T2 and T3 stages, tumor grade 3, LVI, structures with partial endothelial lining and the presence of peritumoral retraction clefting were associated with a high risk of regional metastasis. However, in the multivariate analysis, only T2 and T3 stage, tumor grade 3, the presence of LVI and peritumoral retraction clefting were independent predictors of a high risk of regional metastasis.

Table 5. *Results of univariate and multivariate analyses in patients with squamous cell carcinomas of the cervix and lung.*

Characteristics	Univariate analysis OR (95%CI)	*P* value	Multivariate analysis OR (95%CI)	*P* value
Histology				
Squamous cell cervical cancer	1	-	1	-
Squamous cell lung cancer	56.83(17.04-189.60)	0.0000	4.88(0.99-27.26)	0.07
T stage				
T1	1	-	1	-
T2	12.56(6.27-25.19)	0.0000	3.85(1.37-10.78)	0.0104*
T3	11.31(5.12-24.98)	0.0000	5.44(1.58-18.67)	0.0071*
Grade				
G1	1	-	1	-
G2	0.79(0.37-1.67)	0.5346	0.82(0.34-1.97)	0.6620
G3	2.97(1.66-5.28)	0.0002	1.05(0.39-2.87)	0.9180
LVI				
No	1	-	1	-
Presence	9.33(5.21-16.73)	0.0000	3.62(1.64-7.98)	0.0014*
Structures with partial endothelial lining				
No	1	-		
Presence	1.44(0.85-2.43)	0.1700		
DCs with weak expression of CD34				
No	1	-	1	-
Presence	1.67(1.01-2.78)	0.0473*	0.99(0.45-2.17)	0.9822
DCs of "contact type"				
No	1	-	1	-
Presence	3.44(2.01-5.87)	0.0000*	4.10(1.82-9.26)	0.0007*
The capillaries in the tumor solid component				
No	1	-	1	
Presence	1.66(1.02-2.81)	0.0471*	3.24(1.44-7.30)	0.0046*
Peritumoral retraction clefting				
No	1	1	1	-
Presence	6.36(3.62-11.17)	0.0000*	4.19(1.85-9.51)	0.0006*

The most significant association between the risk of regional metastasis and different types of tumor MVs was observed in patients with squamous cell carcinomas. In univariate analysis, the risk of regional metastasis was associated with lung cancer, T2 and T3 stages, tumor grade 3, the presence of LVI, DCs with weak expression of CD34, DCs of the "contact type", capillaries in the solid tumor component and the presence of peritumoral retraction clefting. In multivariate analysis, independent predictors of the risk of regional metastasis were T stage, the presence of LVI, DCs of "contact type", capillaries in the solid tumor component and peritumoral retraction clefting. Histology and tumor grade were not associated with the risk of regional metastasis.

4. Discussion

One of the key factors of tumor progression is metastasis to RLNs. The number and location of regional metastases influence overall survival and relapse-free survival in patients with malignant neoplasms, including those with gastric cancer [39, 40], breast cancer [41, 42], cervical cancer [43, 44], and lung cancer [45, 46]. Although tumor angiogenesis is currently considered a key factor associated with regional and distant metastasis, many questions remain that have not been answered. In most studies, the correlations between angiogenesis activity and the risk of regional metastasis were assessed in accordance with the tumor microvascular density (MVD), which was determined by IGH using markers such as CD34, CD31, CD133, endocan, and PDPN. However, the data concerning the relationship between tumor MV density and the risk of regional metastasis are quite contradictory. Some authors have reported that high tumor MVD is associated with an increased risk of regional metastasis [47-49] or decreased survival in patients with malignant neoplasms [50-52]. Other authors have not found a convincing association between tumor MVD and the risk of regional metastasis [53, 54]. Moreover, some studies have noted that, for example, hypovascular pancreatic tumors with low MVD and fibrous stroma are more aggressive than tumors with increased MVD [55]. Some researchers have noted that the presence of high endothelial venules in tumors is associated with a low risk of regional metastasis and a better prognosis in various malignancies [56]. In addition, a number of studies have noted that a high density of CD31-positive MVs is associated with a better prognosis in patients with malignancies [57], including cervical cancer [58]. Some authors noted that only certain types of tumor MVs are important for disease prognosis, such as collapsed MVs in non-small cell lung cancer [59], or noted the importance of determining the distance from tumor cells to MVs to assess the risk of regional metastasis [60]. A number of studies have noted a relationship between endothelial markers and the risk of regional metastasis [61-63].

In this way, the results of studies assessing the clinical significance of angiogenesis are contradictory because tumor vessels are heterogeneous and differ in origin, morphology, and clinical significance [8, 21, 23, 30, 31]. Currently, several classifications of tumor MVs have been developed on the basis of their degree of maturity [21, 23, 30, 32, 33]. However, these classifications do not reflect the diversity of tumor MVs or their relationship with factors associated with malignant neoplasm progression. As an alternative, we have proposed a new classification of tumor MVs on the basis of their morphological features and correlations with the clinicopathological characteristics of malignant neoplasms [34-38]. In this study, we summarize the obtained results and establish the predictive value of different types of tumor MVs for assessing the risk of regional metastasis in glandular and squamous cell carcinomas.

Analysis of the predictive value of different types of tumor MVs revealed that common independent predictors of regional metastasis for glandular and squamous cell carcinomas are the T stage of the disease, the presence of LVI and peritumoral retraction clefting. According to the univariate analysis, tumor histological structure was the most significant factor associated with the risk of metastasis to RLNs. However, in multivariate analysis, tumor histological structure lost its predictive value since the risk of regional metastasis depends primarily on the T stage of cancer.

In squamous cell and glandular cancers, significant differences in the predictive value of different markers associated with angiogenesis have been noted. In glandular cancers, in univariate analysis, in addition to the above factors, structures with partial endothelial linings are associated with the risk of regional metastasis. However, in multivariate analysis, these markers lost their predictive value. In contrast to glandular cancers, in squamous cell carcinomas, in both univariate and multivariate analyses, independent predictors of a high risk of regional metastasis are "contact-type" DCs, capillaries in the solid component of the tumor and peritumoral retraction clefting. Notably, DCs with weak expression of CD34 and tumor grade were associated with the risk of regional metastasis only in univariate analyses.

Of particular interest is the phenomenon of peritumoral retraction clefting. This phenomenon manifests as the presence of a cavity without an endothelial lining around the tumor glands or tumor cell clusters [64]. Several studies have pointed to the diagnostic and prognostic significance of this artifact, establishing that the presence of retraction clefts may be associated with a high risk of metastasis to RLNs and a poor prognosis in various malignancies, including breast cancer [65-67]. Our interest in this phenomenon is because we assume that there is a special type of angiogenesis, which we call the “cavity” type of angiogenesis and which explains a possible mechanism for the formation of tumor cell clusters in tumor MVs [21]. We suggest that as a result of a violation of the adhesive properties of tumor cells, their retraction from the underlying stroma may occur with the formation of retraction clefts with tumor cells in the lumen. The inner surface of the retraction clefts may subsequently be partially or completely lined by ECs. The endothelium-lined structures can then merge with blood or lymphatic vessels, which leads to the entry of tumor cell clusters into the lumen of the vessels. The characteristic features of the described method of tumor MV formation are the presence of peritumoral retraction clefting, SPELs, and vessels with tumor cell clusters in the lumen. In gastric cancer, breast cancer, squamous cell cervical cancer, and squamous cell lung cancer, the presence of peritumoral retraction clefting is correlated with the presence of metastases in the RLN and is associated with a high risk of disease recurrence [34-38]. Some studies have demonstrated that circulating tumor cell clusters are associated with a greater risk of metastasis and relapse than single circulating tumor cells are.

5. Conclusion

Thus, the results of this study indicate that when assessing the risk of regional metastasis, it is advisable to consider the presence of peritumoral retraction clefting in both glandular and squamous cell carcinomas. In addition, in squamous cell carcinomas, the accuracy of assessing the risk of regional metastasis can be increased by taking into account "contact type" DCs and capillaries in the solid component of the tumor. Close contact of the described vessels with tumor cells apparently facilitates their invasion into the lumen of blood vessels. Given the data on the role of circulating tumor cell clusters in the progression of malignant neoplasms, the study of the role of peritumoral retraction clefting in their formation is of undoubted interest. Our findings have several limitations related to the single-center nature of the study, significant heterogeneity of patients by histology and stage of the disease, and the descriptive nature of the characteristics of different types of tumor MVs. Many questions remain regarding the origin of different types of tumor MVs and the cells lining these vessels. We believe that further study of the characteristics of different types of tumor MVs will contribute to a better understanding of the mechanisms of tumor progression associated with metastasis and the identification of promising directions for their treatment.

Abbreviations

ADCs	Atypical Dilated Capillaries
CD	Cluster of Differentiation
DCs	Dilated Capillaries
ECs	Endothelial Cells
IGH	Immunohistochemistry
LVI	Lymphovascular Invasion
MVD	Microvascular Density
MVs	Microvessels
PDPN	Podoplanin
RLNs	Regional Lymph Nodes
T	Tumor
SPELs	Structures with Partial Endothelial Linings

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Author Contributions

Marina Senchukova is the sole author. The author read and approved the final manuscript.

Conflicts of Interest

The author declares no conflicts of interest.

References

[1] Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med. 1971 Nov 18; 285(21): 1182-6. https://doi.org/10.1056/NEJM197111182852108

[2] Folkman J. Tumor angiogenesis and tissue factor. Nat Med. 1996 Feb; 2(2): 167-8. https://doi.org/10.1038/nm0296-167

[3] Folkman J. Role of angiogenesis in tumor growth and metastasis. Semin Oncol. 2002 Dec; 29(6 Suppl 16): 15-8. https://doi.org/10.1038/nm0296-16710.1053/sonc.2002.37263

[4] Teleanu RI, Chircov C, Grumezescu AM, Teleanu DM. Tumor Angiogenesis and Anti-Angiogenic Strategies for Cancer Treatment. J Clin Med. 2019 Dec 29; 9(1): 84. https://doi.org/10.3390/jcm9010084

[5] Schito L. Hypoxia-Dependent Angiogenesis and Lymphangiogenesis in Cancer. Adv Exp Med Biol. 2019; 136: 71-85. https://doi.org/10.1007/978-3-030-12734-3_5

[6] Guo M, Niu Y, Xie M, Liu X, Li X. Notch signaling, hypoxia, and cancer. Front Oncol. 2023 Jan 31; 13: 1078768. https://doi.org/10.3389/fonc.2023.1078768

[7] Al-Ostoot FH, Salah S, Khamees HA, Khanum SA (2021) Tumor angiogenesis: Current challenges and therapeutic opportunities. Cancer Treat Res Commun 28: 100422. https://doi.org/10.1016/j.ctarc.2021.100422

[8] Dudley AC, Griffioen AW (2023) Pathological angiogenesis: mechanisms and therapeutic strategies. Angiogenesis 26(3): 313-347. https://doi.org/10.1007/s10456-023-09876-7

[9] Maniotis AJ, Folberg R, Hess A, Seftor EA, Gardner LM, Pe'er J, Trent JM, Meltzer PS, Hendrix MJ (1999) Vascular channel formation by human melanoma cells in vivo and in vitro: vasculogenic mimicry. Am J Pathol 155(3): 739-52. https://doi.org/10.1016/S0002-9440(10)65173-5

[10] Sun B, Zhang D, Zhao N, Zhao X (2017) Epithelial-to-endothelial transition and cancer stem cells: two cornerstones of vasculogenic mimicry in malignant tumors. Oncotarget 8(18): 30502-30510. https://doi.org/10.18632/oncotarget.8461

[11] Barnett FH, Rosenfeld M, Wood M, Kiosses WB, Usui Y, Marchetti V, Aguilar E, Friedlander M (2016) Macrophages form functional vascular mimicry channels in vivo. Sci Rep 6: 36659. https://doi.org/10.1038/srep36659

[12] Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM (1997) Isolation of putative progenitor endothelial cells for angiogenesis. Science 275(5302): 964-7. https://doi.org/10.1126/science.275.5302.964

[13] Fisher DT, Muhitch JB, Kim M, Doyen KC, Bogner PN, Evans SS, Skitzki JJ (2016) Intraoperative intravital microscopy permits the study of human tumour vessels. Nat Commun 7: 10684. https://doi.org/10.1038/ncomms10684

[14] Yu M, Men HT, Niu ZM, Zhu YX, Tan BX, Li LH, Jiang J (2015) Meta-Analysis of Circulating Endothelial Cells and Circulating Endothelial Progenitor Cells as Prognostic Factors in Lung Cancer. Asian Pac J Cancer Prev 16(14): 6123-8. https://doi.org/10.7314/apjcp.2015.16.14.6123

[15] Liu ZL, Chen HH, Zheng LL, Sun LP, Shi L (2023) Angiogenic signaling pathways and anti-angiogenic therapy for cancer. Signal Transduct Target Ther 8(1): 198. https://doi.org/10.1038/s41392-023-01460-1

[16] Kuczynski EA, Vermeulen PB, Pezzella F, Kerbel RS, Reynolds AR (2019) Vessel co-option in cancer. Nat Rev Clin Oncol 16(8): 469-493. https://doi.org/10.1038/s41571-019-0181-9

[17] Burri PH, Hlushchuk R, Djonov V (2004) Intussusceptive angiogenesis: its emergence, its characteristics, and its significance. Dev Dyn 231(3): 474-88. https://doi.org/10.1002/dvdy.20184

[18] Ribatti D, Djonov V (2012) Intussusceptive microvascular growth in tumors. Cancer Lett 316(2): 126-31. https://doi.org/10.1016/j.canlet.2011.10.040

[19] Ribatti D, Tamma R, Annese T, d'Amati A, Ingravallo G, Specchia G (2023) Vascular Growth in Lymphomas: Angiogenesis and Alternative Ways. Cancers (Basel) 15(12): 3262. https://doi.org/10.3390/cancers15123262

[20] Klein D (2018) The Tumor Vascular Endothelium as Decision Maker in Cancer Therapy. Front Oncol 8: 367. https://doi.org/10.3389/fonc.2018.00367

[21] Nagy JA, Dvorak HF (2012) Heterogeneity of the tumor vasculature: the need for new tumor blood vessel type-specific targets. Clin Exp Metastasis 29(7): 657-62. https://doi.org/10.1007/s10585-012-9500-6

[22] Hashizume H, Baluk P, Morikawa S, McLean JW, Thurston G, Roberge S, Jain RK, McDonald DM (2000) Openings between defective endothelial cells explain tumor vessel leakiness. Am J Pathol 156(4): 1363-80. https://doi.org/10.1016/S0002-9440(10)65006-7

[23] Birau A, Ceausu RA, Cimpean AM, Gaje P, Raica M, Olariu T (2012) Assessement of angiogenesis reveals blood vessel heterogeneity in lung carcinoma. Oncol Lett 4(6): 1183-1186. https://doi.org/10.3892/ol.2012.893

[24] Milosevic V, Edelmann RJ, Winge I, Strell C, Mezheyeuski A, Knutsvik G, Askeland C, Wik E, Akslen LA, Östman A (2023) Vessel size as a marker of survival in estrogen receptor positive breast cancer. Breast Cancer Res Treat 200(2): 293-304. https://doi.org/10.1007/s10549-023-06974-4

[25] Rofstad EK, Galappathi K, Mathiesen BS (2014) Tumor interstitial fluid pressure-a link between tumor hypoxia, microvascular density, and lymph node metastasis. Neoplasia 16(7): 586-94. https://doi.org/10.1016/j.neo.2014.07.003

[26] Tomita T, Kato M, Hiratsuka S (2021) Regulation of vascular permeability in cancer metastasis. Cancer Sci 112(8): 2966-2974. https://doi.org/10.1111/cas.14942

[27] Pezzella F, Gatter K, Qian CN (2016) Twenty years after: the beautiful hypothesis and the ugly facts. Chin J Cancer 35: 22. https://doi.org/10.1186/s40880-016-0087-1

[28] Pezzella F, Ribatti D (2022) Vascular co-option and vasculogenic mimicry mediate resistance to antiangiogenic strategies. Cancer Rep (Hoboken) 5(12): e1318. https://doi.org/10.1002/cnr2.1318

[29] Petrovic N (2016) Targeting Angiogenesis in Cancer Treatments: Where do we Stand? J Pharm Pharm Sci 19(2): 226-38. https://doi.org/10.18433/jpps.v19i2.27608

[30] Sitohy B, Chang S, Sciuto TE, Masse E, Shen M, Kang PM, Jaminet SC, Benjamin LE, Bhatt RS, Dvorak AM, Nagy JA, Dvorak HF (2017) Early Actions of Anti-Vascular Endothelial Growth Factor/Vascular Endothelial Growth Factor Receptor Drugs on Angiogenic Blood Vessels. Am J Pathol 187(10): 2337-2347. https://doi.org/10.1016/j.ajpath.2017.06.010

[31] Ribatti D, Pezzella F (2021) Overview on the Different Patterns of Tumor Vascularization. Cells 10(3): 639. https://doi.org/10.3390/cells10030639

[32] Sitohy B, Nagy JA, Jaminet SC, Dvorak HF (2011) Tumor-surrogate blood vessel subtypes exhibit differential susceptibility to anti-VEGF therapy. Cancer Res 71(22): 7021-8. https://doi.org/10.1158/0008-5472.CAN-11-1693

[33] Raica M, Cimpean AM, Ferician OC, Ferician AM (2021) Tumour-associated Angiogenesis and Intermediate Blood Vessels in Renal Cell Carcinoma. Cancer Diagn Progn 1(3): 231-234. https://doi.org/10.21873/cdp.10031

[34] Senchukova M, Kiselevsky MV (2014) The "cavitary" type of angiogenesis by gastric cancer. Morphological characteristics and prognostic value. J Cancer 5(5): 311-9. https://doi.org/10.7150/jca.8716

[35] Senchukova MA, Nikitenko NV, Tomchuk ON, Zaitsev NV, Stadnikov AA (2015) Different types of tumor vessels in breast cancer: morphology and clinical value. Springerplus 4: 512. https://doi.org/10.1186/s40064-015-1293-z

[36] Senchukova MA, Makarova EV, Shurygina EI, Volchenko NN (2020) Morphological Characteristics and Clinical Significance of Different Types of Tumor Vessels in Patients with Stages I-IIA of Squamous Cervical Cancer. J Oncol 2020: 3818051. https://doi.org/10.1155/2020/3818051

[37] Senchukova MA, Kalinin EA, Volchenko NN. Different types of tumor microvessels in stage I-IIIA squamous cell lung cancer and their clinical significance. World J Clin Oncol. 2024 May 24; 15(5): 614-634. https://doi.org/10.5306/wjco.v15.i5.614

[38] Senchukova, M. A. (2024). Morphological Features and Clinical Significance of Tumor Microvessels in Glandular and Squamous Cell Cancers. In: Interdisciplinary Cancer Research. Springer, Cham. https://doi.org/10.1007/16833_2024_297

[39] Jeong O, Jung MR, Kang JH. Prognostic Value of the Anatomic Region of Metastatic Lymph Nodes in the Current TNM Staging of Gastric Cancer. J Gastric Cancer. 2021 Sep, 21(3): 236-245. https://doi.org/10.5230/jgc.2021.21.e22

[40] Purich K, Skubleny D, Ghosh S, Bédard ELR, Stewart KC, Johnson ST, Haase E, McCall M, Schiller D. A prospective Canadian gastroesophageal cancer database: What have we learned? Can J Surg. 2023 Aug 8; 66(4): E422-E431. https://doi.org/10.1503/cjs.005122

[41] Cetin IA, Akay SU, Caglar Ozkok HB, Sengoz M. Lymph node ratio as an independent prognostic factor for breast cancer-related mortality in patients with node-positive breast cancer. J Cancer Res Ther. 2020 Oct-Dec; 16(6): 1387-1392. https://doi.org/10.4103/jcrt.JCRT_1034_19

[42] Zarean Shahraki S, Azizmohammad Looha M, Mohammadi Kazaj P, Aria M, Akbari A, Emami H, Asadi F, Akbari ME. Time-related survival prediction in molecular subtypes of breast cancer using time-to-event deep-learning-based models. Front Oncol. 2023 Jun 5; 13: 1147604. https://doi.org/10.3389/fonc.2023.1147604

[43] Wang M, Ma M, Yang L, Liang C. Development and validation of a nomogram for predicting pelvic lymph node metastasis and prognosis in patients with cervical cancer. Front Oncol. 2022 Sep 2; 12: 952347. https://doi.org/10.3389/fonc.2022.952347

[44] Kwon J, Eom KY, Kim YS, Park W, Chun M, Lee J, Kim YB, Yoon WS, Kim JH, Choi JH, Chang SK, Jeong BK, Lee SH, Cha J. The Prognostic Impact of the Number of Metastatic Lymph Nodes and a New Prognostic Scoring System for Recurrence in Early-Stage Cervical Cancer with High Risk Factors: A Multicenter Cohort Study (KROG 15-04). Cancer Res Treat. 2018 Jul; 50(3): 964-974. https://doi.org/10.4143/crt.2017.346

[45] Guo D, Ni Y, Lv X, Zhang Z, Ye P. Distribution and prognosis of mediastinal lymph node metastases of nonsmall cell lung cancer. J Cancer Res Ther. 2016 Oct; 12(Supplement): 120-125. https://doi.org/10.4103/0973-1482.191613

[46] Łochowski MP, Chałubińska-Fendler J, Szlachcińska A, Łochowska B, Brzeziński D, Kaczmarski J, Kozak J. Prognostic Value of Lymph Node Ratio (LNR) in Patients with Postoperative N2 Feature in Non-Small Cell Lung Cancer (NSCLC). J Clin Med. 2024 Aug 5; 13(15): 4570. https://doi.org/10.3390/jcm13154570

[47] Goyal AV, Shukla S, Acharya S, Vagha S, Jajoo S. Correlation of microvessel density with histopathological parameters of carcinoma breast. Indian J Med Res. 2023 Oct 1; 158(4): 417-422. https://doi.org/10.4103/ijmr.ijmr_1588_22

[48] Wu Y, Chen D, Luo Y, Wang J, Gong H, Li J, Jiang L. Expression and clinical significance of SYNE3 in non-small cell lung cancer. Am J Transl Res. 2024 Sep 15; 16(9): 4436-4449. https://doi.org/10.62347/ZHBP7145

[49] Zhang Y, Qu H. Expression and clinical significance of aquaporin-1, vascular endothelial growth factor and microvessel density in gastric cancer. Medicine (Baltimore). 2020 Sep 4; 99(36): e21883. https://doi.org/10.1097/MD.0000000000021883

[50] Hu X, Liu H, Ye M, Zhu X. Prognostic value of microvessel density in cervical cancer. Cancer Cell Int. 2018 Oct 3; 18: 152. https://doi.org/ 10.1186/s12935-018-0647-3

[51] Zhao K, Zhao T, Yang R, Liu J, Hu M. Peroxiredoxin 2 as a potential prognostic biomarker associated with angiogenesis in cervical squamous cell cancer. Oncol Lett. 2024 May 16; 28(1): 328. https://doi.org/10.3892/ol.2024.14461

[52] Zhang T, Nie J, Liu X, Han Z, Ding N, Gai K, Liu Y, Chen L, Guo C. Correlation Analysis Among the Level of IL-35, Microvessel Density, Lymphatic Vessel Density, and Prognosis in Non-Small Cell Lung Cancer. Clin Transl Sci. 2021 Jan; 14(1): 389-394. https://doi.org/10.1111/cts.12891

[53] Abbasi A, Ghaffarizadeh F, Mojdeganlou H. Prognostic Significance of Microvessel Density in Invasive Ductal Carcinoma of Breast. Int J Hematol Oncol Stem Cell Res. 2023 Apr 1; 17(2): 100-105. https://doi.org/10.18502/ijhoscr.v17i2.12646

[54] Kubik S, Jesionek-Kupnicka D, Stawiski K, Fijuth J, Moszynska-Zielinska M, Kuncman L, Pajdzinski M, Gottwald L. Clinical significance of CD34, podoplanin and Ki-67 expression in patients with locally advanced squamous cell cervical carcinoma. J Obstet Gynaecol. 2022 Aug; 42(6): 2248-2254. https://doi.org/10.1080/01443615.2022.2037124

[55] Lai TY, Chiang TC, Lee CY, Kuo TC, Wu CH, Chen YI, Hu CM, Maskey M, Tang SC, Jeng YM, Tien YW, Lee EYP, Lee WH. Unraveling the impact of cancer-associated fibroblasts on hypovascular pancreatic neuroendocrine tumors. Br J Cancer. 2024 Apr; 130(7): 1096-1108. https://doi.org/10.1038/s41416-023-02565-8

[56] Wang B, Han Y, Liu J, Zhang X, Deng Y, Jiang Y. Intratumoral high endothelial venules in solid tumors: a pooled study. Front Immunol. 2024 Jul 8; 15: 1401118. https://doi.org/10.3389/fimmu.2024.1401118

[57] Mamilos A, Lein A, Winter L, Haas M, Reichert TE, Ettl T, Künzel J, Spanier G, Brochhausen C. Immunohistochemical Assessment of Microvessel Density in OSCC: Spatial Heterogeneity of Angiogenesis and Its Impact on Survival. Biomedicines. 2023 Oct 8; 11(10): 2724. https://doi.org/10.3390/biomedicines11102724

[58] Randall LM, Monk BJ, Darcy KM, Tian C, Burger RA, Liao SY, Peters WA, Stock RJ, Fruehauf JP. Markers of angiogenesis in high-risk, early-stage cervical cancer: A Gynecologic Oncology Group study. Gynecol Oncol. 2009 Mar; 112(3): 583-9. https://doi.org/10.1016/j.ygyno.2008.11.013

[59] Tong YH, He Y, Hu LY, Liu YJ, Zheng XW, Sun J, Song Y, Zhang YW, Fang L, Huang P. Elevated proportion of collapsed microvessels indicate poor survival outcome in patients with non-small cell lung cancer. Tumori. 2019 Dec; 105(6): 494-500. https://doi.org/10.1177/0300891619871103

[60] Ding H, Sun J, Song Y, Xin W, Zhu J, Zhong L, Chen Y, Zhang Y, Tong Y, Fang L. Long Distance From Microvessel to Cancer Cell Predicts Poor Prognosis in Non-Small Cell Lung Cancer Patients. Front Oncol. 2021 Jun 11; 11: 632352. https://doi.org/10.3389/fonc.2021.632352

[61] Ferician AM, Ferician OC, Nesiu A, Cosma AA, Caplar BD, Melnic E, Cimpean AM. The Mutually Mediated Chloride Intracellular Channel Protein 1 (CLIC1) Relationship between Malignant Cells and Tumor Blood Vessel Endothelium Exhibits a Significant Impact on Tumor Angiogenesis, Progression, and Metastasis in Clear Cell Renal Cell Carcinoma (ccRCC). Cancers (Basel). 2022 Dec 3; 14(23): 5981. https://doi.org/10.3390/cancers14235981

[62] Zhang X, Ji J, Zhang G, Fang C, Jiang F, Ma S, Hou J. Expression and significance of B7-H3 and Tie-2 in the tumor vasculature of clear cell renal carcinoma. Onco Targets Ther. 2017 Nov 13; 10: 5417-5424. https://doi.org/10.2147/OTT.S147041

[63] Lu M, Tian H, Yue W, Li L, Li S, Qi L, Hu W, Gao C, Si L. TFIIB-related factor 2 over expression is a prognosis marker for early-stage non-small cell lung cancer correlated with tumor angiogenesis. PLoS One. 2014 Feb 11; 9(2): e88032. https://doi.org/10.1371/journal.pone.0088032

[64] Acs G, Dumoff KL, Solin LJ, Pasha T, Xu X, Zhang PJ. Extensive retraction artifact correlates with lymphatic invasion and nodal metastasis and predicts poor outcome in early stage breast carcinoma. Am J Surg Pathol. 2007 Jan; 31(1): 129-40. https://doi.org/10.1097/01.pas.0000213316.59176.9b

[65] Huang L, Li Y, Du J, Li H, Lu M, Wang Y, Zhou W, Wang W, Wu H. The Prognostic Value of Retraction Clefts in Chinese Invasive Breast Cancer Patients. Pathol Oncol Res. 2021 Apr 21; 27: 1609743. https://doi.org/10.3389/pore.2021.1609743

[66] Acs G, Khakpour N, Kiluk J, Lee MC, Laronga C. The presence of extensive retraction clefts in invasive breast carcinomas correlates with lymphatic invasion and nodal metastasis and predicts poor outcome: a prospective validation study of 2742 consecutive cases. Am J Surg Pathol. 2015 Mar; 39(3): 325-37. https://doi.org/10.1097/PAS.0000000000000339

[67] Li W, Jia H, Wang S, Guo X, Zhang X, Zhang L, Wen HY, Fu L. The presence of retraction clefts correlates with lymphovascular invasion and lymph node metastasis and predicts poor outcome: Analysis of 2497 breast cancer patients. Ann Diagn Pathol. 2022 Dec; 61: 152047. https://doi.org/10.1016/j.anndiagpath.2022.152047

Research Field

Marina Senchukova: Tumor angiogenesis, Tumor microvessels, Gastric cancer, Breast cancer, Cervical squamous cell carcinoma, Lung squamous cell carcinoma