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Evaluation of tumor angiogenesis in patients with non-small cell lung carcinoma

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ABSTRACT

The aim of this study is to evaluate tumor angiogenesis in patients with non-small cell lung carcinoma. A total of 20 patients with pulmonary adenocarinoma have been included in the study. In order to evaluate tumor angiogenesis we studied the importance of CD34 expression. Evaluation of vascular density was performed with a semiautomated method using the dedicated software called ImageJ. We introduced in our study 20 patients with lung adenocarcinoma. We were able to identify tumor angiogenesis in 19 cases (95%). Immunolabeling of CD34 positive endothelial cells provided a good overview of tumor vascularization. Immunohistochemical staining of CD34 positive endothelium cells provided a good basis for tumor vascularity assessment, and also an excellent contrast for computer assisted morphometric measurements. Also we studied the intensity of the immunohistochemical staining of CD34 in the tumoral cells. We obtained the following results: a minor expression in 4 cases (20%), a moderate expression in 9 cases (45%) and an

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Department of Anatomy, University of Medicine and Pharmacy Târgu Mureş Gheorghe Marinescu Str., No. 50 Târgu Mureş, România klara_branzaniuc@yahoo.com intense expression in 6 cases (30%). The histological type of adenocarcinomas influences the architecture and branching of the vessels. The density of newly developed vessels is higher in patients with papillary pulmonary adenocarcinomas, which may indicate a possible relationship between the histological type and development of vascular supply.

Keywords: lung cancer, adenocarcinoma, angiogenesis

Introduction

Lung cancer continues to be the main cause of mortality by cancer in spite of progress seen in molecular biology and cytostatic therapy [1]. The importance of angiogenesis in the etiology of lung cancer has been established by numerous research studies in the field. The significance of circulating VEGF levels and vascular microdensity of the tumor has been established as prognostic factors in patients with non-small cell lung carcinoma [2]. Certain authors have shown that approximately 30% to 40% of patients with non-small cell lung carcinoma demonstrate extensive tumor angiogenetic processes. In this context it has been demonstrated that the intensity of vascular microdensity of the tumor is a prognostic factor of a poor outcome for the cancer patient [3].

Several markers are used to demonstrate intratumoral angiogenesis for vascular microdensity studies in patients with non-small cell lung carcinoma, like CD34 or CD105 [4]. The aim of this study is the evaluation of angiogenesis in a group of patients with non-small cell lung carcinoma, using CD34 as marker of tumor angiogenesis.

Material and method

Tumor angiogenesis in patients with nonsmall cell lung carcinoma was evaluated on a group of 20 lung cancer patients hospitalized and operated at the Surgery Clinic no. 1 of the Mures County Clinical Emergency Hospital, all of them having the histopathological diagnosis of lung adenocarcinoma. The Ethics Committee of the University of Medicine and Pharmacy Tg. Mureş has authorized the study. We used patient data obtained from the patient charts, and data from the histopathology reports.

Vascular patterns of the tumors were evaluated by immunohistochemistry. We used the Novolink Polymer Detection System (Leica Biosystems, Newcastle, UK) method, with the endothelium marker CD34, clone QVEnd/10 (Leica Biosystems, Newcastle, UK), dilution 1:100 and 30 minutes incubation time at room temperature. Antigen retrieval was performed using microwave cooking. Developing was performed using the peroxidase method with DAB chromogen, followed by hematoxylin staining.

The endogenous control was the endothelium of the alveolar capillaries. Evaluation of vascular density was performed using a semiautomated method using the dedicated software named ImageJ (version 1.48v, Wayne Rasband, National Institutes of Health, Bethesda, USA). The method consisted of a digital morphometric evaluation, by measuring the DAB positive areas and comparing the obtained values between the cases. The immunohistochemical slides were microphotographed, capturing five representative fields from each case. These fields were subjected to digital morphometric evaluation. The areas with the most vascular activity were chosen, and those areas with extensive necrosis or significant stroma component were avoided.

Results

Age < 50 years 5 cases (25%) > 50 years 15 cases (75%) Gender Male 14 cases (70%) Female 6 cases (30%) **Smoking history** Non-smokers 7 cases (35%) Smokers 13 cases (65%) Tumor size < 3 cm5 cases (25%) > 3 cm 15 cases (75%) Tumor grade of differentiation - Well differentiated (G1) 3 cases (15%) - Moderately differentiated (G2) 10 cases (50%) - Poorly differentiated (G3) 7 cases (35%) Histological type of lung adenocarcinoma - Acinar 9 cases (45%) - Papillary 4 cases (20%) - Mixed 4 cases (20%) - Solid 2 cases (10%) 1 case (5%) - Mucinous

The data obtained from the patient charts are presented in Table I.

We used the TNM classification of lung cancer for staging, with the following results: Stage IA - 1 case (5%), Stage IB - 2 cases (10%), IIA - 4 cases (20%), IIB - 6 cases (30%), IIIA - 6 cases (30%), IIIB - 1 case (5%) (Figure 1).



Figure 1 - Patient staging

We were able to identify tumor angiogenesis 19 cases (95%). Immunohistochemical staining of CD34 positive endothelium cells provided a good basis for tumor vascularity assessment, and also an excellent contrast for computer assisted morphometric measurements (Figures 2, 3 and 4).



Figure 2 - Lung adenocarcinoma after immunohistochemical staining for vascularity (CD34 antibody, clone QBEend/1, DAB, objective 10)



Figure 3 - Lung adenocarcinoma, DAB filter applied after immunohistochemistry



Figure 4 - Aspect of the microscopic image following elimination of tissue fragments, other than those stained by CD34 (this image was subjected to morphometric assessment of vascularity).

In addition, we studied the intensity of the immunohistochemical staining of CD34 in the tumoral cells. We obtained the following results: a minor expression in 4 cases (20%), a moderate expression in 9 cases (45%) and an intense expression in 6 cases (30%) (Figure 5).



Figure 5 - The intensity of the immunohistochemical staining of CD34 in the tumoral cells

We did not find a significant correlation between vascular supply and histological grade of the tumor. Nevertheless, we noted a relatively poor vascular supply of tumor with cells showing a high discohesive potential. We observed that the histological type of adenocarcinomas influences the architecture and branching of the vessels. We also noted that the density of newly developed vessels is higher in patients with papillary adenocarcinomas, which may indicate a possible relationship between the histological type and development of vascular supply.

Discussions

Development of new vessels through angiogenesis in lung cancer patients has an important role in the disease course of cancer patients, affecting tumor growth, tumor invasion, and the metastatic capabilities of the tumor [5]. It has been demonstrated that neoangiogenesis is required for tumor growth over 1-2 mm to provide nutritional supply to the tumor tissue [6]. Also, the metastatic nature of the tumor in influenced by tumor angiogenesis as newly developing capillaries display increased permeability that may lead to mobilization of tumor cells [7].

The first study to evaluate the significance of

neoangiogenesis in patients with non-small cell lung carcinoma was published in 1992 by Macchiarini [8]. Since then, several studies have been published about the importance of angiogenesis in these patients, and there are still ongoing controversies regarding the significance as prognostic factors of pro-angiogenic factors in patients with non-small cell lung carcinoma. Still, most of the published studies demonstrated that the existence of areas with high density of vessels developed as a result of neoangiogenesis represents a negative prognostic factor for these patients [3].

The most widely used method for angiogenesis evaluation in cancer patients is immunohistochemistry with monoclonal antibodies. Current studies about the importance of angiogenesis in patients with nonsmall cell lung carcinoma are based especially on evaluation of the following markers of endothelial proliferation: factor VIII, CD31, CD34 and CD105. CD34 is an antigen found in endothelium cells. It consists of glycoproteins, which render it resistant to formalin fixation of tumor tissues [9]. Anti-CD34 antibodies are sensitive markers of endothelial cell proliferation and tumor angiogenesis. Currently only a small number of publications are found about the significance of CD34 as angiogenetic factor in patients with non-small cell lung carcinoma [10].

There are controversies regarding the evaluation of vascular microdensity using paraffin blocks of the tumor tissue. Certain authors consider that for maximum accuracy vascular microdensity should be studied using several paraffin blocks obtained from the same tumor [11]. Another dispute is about choosing the area of the histological slide to perform vascular microdensity evaluation. It is known that there may be areas with very large differences in neoangiogenic vessel density within the tumor. Consequently, certain authors recommend that the evaluation be performed in areas with the highest vascular density (hotspots) [12]. However, it was observed that areas with the highest vascular density are found at the periphery of the tumors, usually in areas without intratumoral necrosis or tissue sclerosis [13].

There may be differences in microvascular density evaluation regarding the employed technical modalities (type of microscope, degree of image amplification). The vast majority of authors recommend that counting of neoangiogenesis vessels should be correlated to a surface area of 1 mm2 [14-16]. Currently, certain authors consider that one of the possible limitations of the importance of microvascular density in these patients is that immunohistochemistry is a semiquantitative method prone to subjective factors occurring during the course of evaluation of the patients [17].

Numerous authors recommend the standardization of neoangiogenetic vessel density evaluation methods in patients with non-small cell lung carcinoma. This is due to the fact that current studies, evaluating the significance of angiogenesis in these patients, use different specific antigens, the criteria used for identification of areas with increased neoangiogenetic vessel density ("hotspots") are subjective, and there are differences in the counting methods used for the counting of neoangiogenetic vessels identified in the field of view. The existence of several evaluation methods of the importance of angiogenesis in these patients, often leads to contradicting results [3].

Aside the significance of angiogenesis as prognostic factor in patients with non-small cell lung carcinoma, tumor angiogenesis in these patients is also especially important for a correct therapeutic approach. It has been demonstrated that initiation of anti-angiogenic (Bevacizumab) therapy administered concomitantly with platinum compounds to patients with non-small cell lung carcinoma improved survival of these patients with an average duration of 3-4 months [18]. Also, it has been shown that bevacizumab therapy has maximum effects in patients that display high levels of angiogenesis in the tumor [19].

Conclusions

The study of tumor angiogenesis in patients with non-small cell lung carcinoma is especially important in establishing the prognosis for these patients, and finding the correct therapeutic approach. The histological type of adenocarcinomas influences the architecture and branching of the vessels. The density of neoangiogenetic vessels is higher in patients with papillary pulmonary adenocarcinomas, which may indicate a possible relationship between the histological type and development of vascular supply.

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