Jurnal Medical Aradean (Arad Medical Journal)
Vol. XIX, issue 3, 2016, pp. 32-35

THE IMPLICATIONS OF LYMPHANGIOGENESIS IN LUNG SQUAMOUS CELL CARCINOMAS

AMALIA NICOLESCU [1], TEODORA OLARIU[2]

[1] Department of Intensive Care, „Vasile Goldis" Western University of Arad, Romania,
[2] Department of Intensive Care, „Vasile Goldis" Western University of Arad, Romania

ABSTRACT. Lung cancer (PBC) frequency increased alarmingly in the last decades, especially in economically developed countries. The lymphatic microvascular density can be one of the important factors for prognosis in patients with lung carcinomas. The aim of this study was to analysed the D2-40 and VEGF-C expression in tumor cells and lymphatic vessels in the lung squamous cell carcinoma. Our study included a total of 27 cases. The immunoexpression of VEGF-C and D2-40 was evaluated according to the following score: 0 (0% positive cells), 1 (<10% positive cells), 2 (10-30% positive cells) and 3 (> 30% positive cells). VEGF-C score ranged from 0 to 3. The score 0 was found in 7.40% of cases, score 1 in 14.81%, score 2 in 25.92% and score 3 in 51.85% of cases. The lung squamous cell lung carcinomas showed a positive D2-40 lymphatic vessel density higher in the peritumoral comparatively with the intratumoral area. They may contribute to a better understanding of lung cancer biology and individualised therapies.
KEYWORDS: lung squamous cell carcinoma, D2-40, VEGF-C

INTRODUCTION

Lung cancer (PBC) is one of the most important and frequent malignancy, accounting more than 90% of lung tumors [1]. PBC has increased alarmingly in recent decades, especially in developed countries economically. It is more common in urban than in rural areas. The average age at diagnosis is 50 years for men and 60 years for women [2]. If 20 years ago scientific papers recorded a male / female ratio of 9/1, nowadays it has changed to 2/1. Lung cancer is a disease which presented the uncontrolled growth of cells in the tissues of the lungs. The majority of lung cancers are primary carcinomas derived from epithelial cells.

Lymphangiogenesis, the process leading to the development new vessels, plays a major role in local growth of the tumor and the development of distant metastasis [3]. Tumor lymphatic vessels formation is dependent on lymphangiogenic growth factors production by tumor cells. The development of tumor vessels is stimulated by growth factors, including: VEGF-C, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF) [4, 5]. VEGF-C has the central role in the regulation of lymphangiogenesis in

D2-40 expression on tumor cells was more frequently seen in squamous cell carcinoma than in adenocarcinoma of the lung. In addition, D2-40 expression on lymphatic vessels in tumor tissues was a statistically significant prognostic factor in squamous cell carcinoma [11].

The aim of this study was to analysed the D2-40 and VEGF-C expression in tumor cells and lymphatic vessels in squamous cell carcinoma type of the lung.

MATERIAL AND METHODS

cancer. The role of VEGF-C in the perivascular lymphatic remodeling and lymph node metastasis was demonstrated in a variety of cancers: thyroid, prostate, gastric, colorectal, lung [6, 7]. Some data showed that the intratumoral lymphatic vessel density represent an important prognostic factor of NSCLC compared with the peritumoral lymphatic vessel density, which is not correlated with the prognosis [8].

In case of pulmonary neoplasia in a study conducted in 2006, Guo et al. [9] have demonstrated that lymphatic vascular microdensity can be one of the important factors for prognosis in patients with non-small cell lung carcinomas. They showed thatVEGF-C and COX-2 may play an important role in tumor progression by stimulating lymphangiogenesis. Inhibition of these pathways may play an important role in the therapy of patients with non-small cell lung carcinomas Lymphangiogenesis rates are significantly higher in lung adenocarcinoma than that in squamous cell carcinoma. VEGF-C expression is an independent prognostic factor of stage IIIa (N2) lung adenocarcinoma and squamous cell carcinoma [10].

Our study included a total of 27 cases. We have investigated pulmonary samples obtained by video assisted thoracoscopy surgery (VATS) from the patients diagnosed with lung squamous cell carcinoma type, evaluated in the Clinical Hospital of Infectious Diseases and Pneumology "V Babes", Timisoara between 2012-2014. Local ethics committee approved the study protocol and informed consent was obtained from all patients in accordance with the World Medical Association Declaration of Helsinki.

The specimens were fixed in 10% buffer formalin for 48 hours and than paraffin embedded, using the conventional histological procedure. The primary processing was completely standardized, using Shandon embedding center. From each paraffin block, there were perfomed serial sections, 3 μm in thickness. Sections from each case were stained with haematoxylin-eosin (HE) method.

The immunohistochemical technique started with dewaxing, rehydration of the sections and heat pretreatment, the last one automatically performed with PT Link (DakoCytomation, Denmark). Target retrieval solution applyied for 30 minutes had pH 6 (DakoCytomation, Denmark). After endogenous peroxidase activity blocking with 3% hydrogen peroxide, the incubation with primary antibody: VEGF-C, H-190 clone, dilution 1: 200, (Labvision / Neomarkers, Fremont, CA, USA) and D2-40 (monoclonal, prediluted, Dako Cytomation) for 30 minutes at room temperature was performed. Visualisation system was represented by EnVision Advance. 3, 3 diaminobenzidine was used as chromogen. For the counterstaining Lille's hematoxylin was used. The entire immunohistochemical technique was performed with DakoCytomation Autostainer (DakoCytomation, Denmark). Immunoexpression of VEGF-C and D2-40 was evaluated according to the following score: 0 (0% positive cells), 1 (<10% positive cells), 2 (10-30% positive cells), 3 (> 30% positive cells). Microscopic images were taken in JPEG format using Nikon Lucia G microscopic image analysis (Nikon, Tokyo, Japan).

RESULTS

Majority of biopsies had the cover ciliated epithelium, under which in lamina propria constantly appeared inflammatory infiltrate and marked proliferation of small blood vessels (figure 1a).

Different proliferation types were found. Thus a compact and diffuse proliferation type, with cells disposed in large tumors area, usually with central necrosis was noticed. The second type was characterized by the presence of short and branched cords invading the stroma. The compact form of proliferation cells had medium and large size, with many anisocharias, anisocytosis and atypical mitoses. They had multiples prominent nucleoli (figure 1b). In most of the cases the cytoplasm of tumor cells was acidophile, variable quantitatively.

In the well-differentiated squamous cell carcinoma forms we noticed a constant presence of keratin and parakeratin pearls (figure 1c). Some of the well differentiated cases mimicked the aspects noticed in the intermediate layer of the epidermis, where the cells had chromophobe aspect.

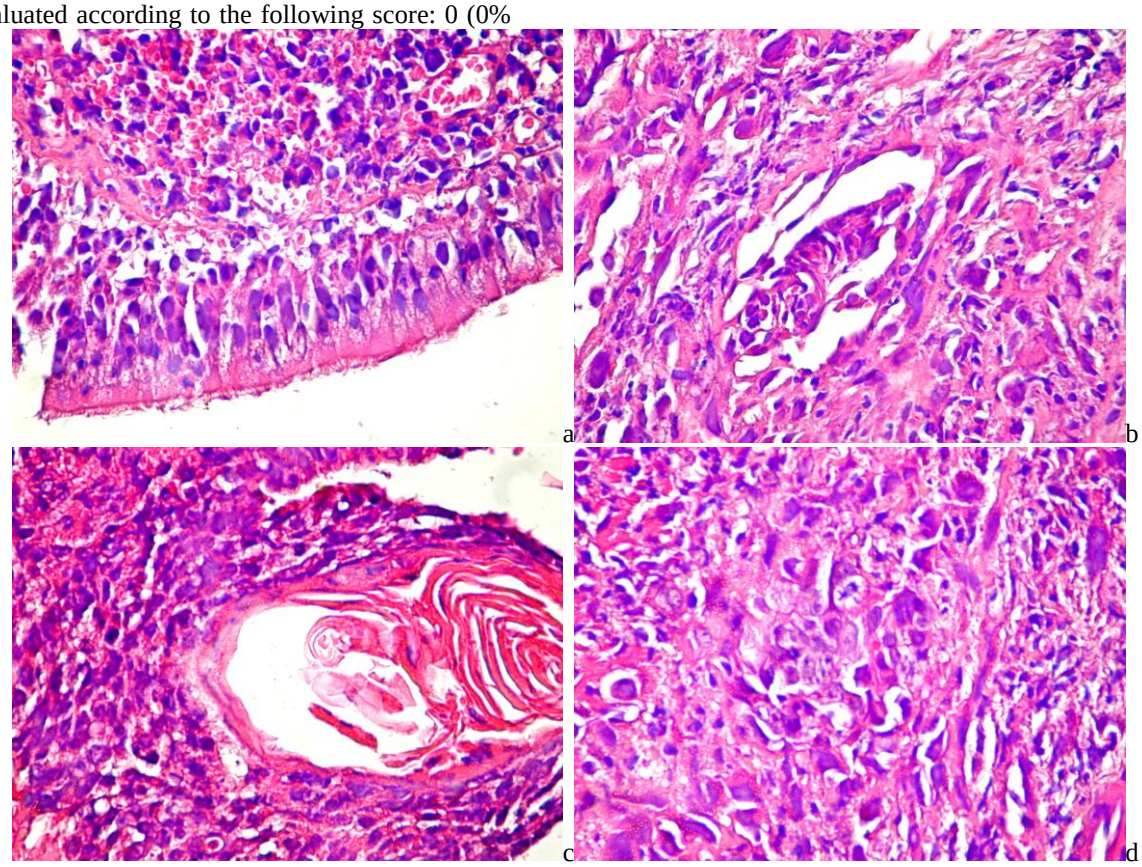

Figure 1a: covering epithelium and lamina propria with inflammatory changes, X200, H&E staining
Figure 1b: details of tumor cells morphology, X400, H&E staining
Figure 1c: well differentiated squamous cell carcinoma with keratin pearl, X200, H&E staining
Figure 1d: poorly differentiated carcinoma, X400, H&E staining

In the the poorly differentiated forms of squamous cell carcinoma- G3 the preveously elements, noticed in the well differentiated type, were not found. The tumor cells showed severe anaplasia (figure 1d). Necrosis was noticed in

Jurnal Medical Aradean (Arad Medical Journal)
Vol. XIX, issue 3, 2016, pp. 25-31

almost all of the cases. It was in this group being present or over large areas of the section, or in the center of the tumor beaches.

For the 27 cases of squamous cell carcinomas, VEGF-C score ranged from 0 to 3. Two cases (7.40%) had a score of 0, four cases (14.81%) - score one, seven cases (25.92%) – 2 and 14 cases (51.85%), score 3. The cytoplasm expression of VEGF C in tumor cells for different values of lead is found in figures (figure 2a, figure 2b).

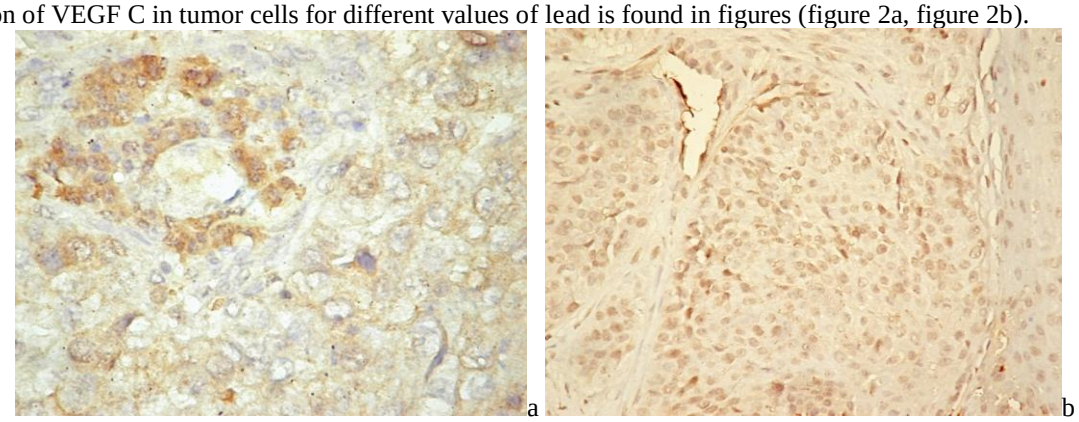

Figure 2a: Immunoexpression of VEGF-C in squamous cell carcinoma, score 2, X400
Figure 2b: Immunoexpression of VEGF-C in squamoous cell carcinoma, score 3, X200

Squamous cell lung carcinomas type showed a positive D2-40 lymphatic vessel density higher in peritumoral (figure 3a) comparatively with the intratumoral area (figure 3b). The values founded for all cases were 9.73 for peritumoral, respectively 3.83 for intratumoral area. The appearance with the highest number of lymphatic vessels disposed peritumoral predominantely was noticed in all cases included in the study.

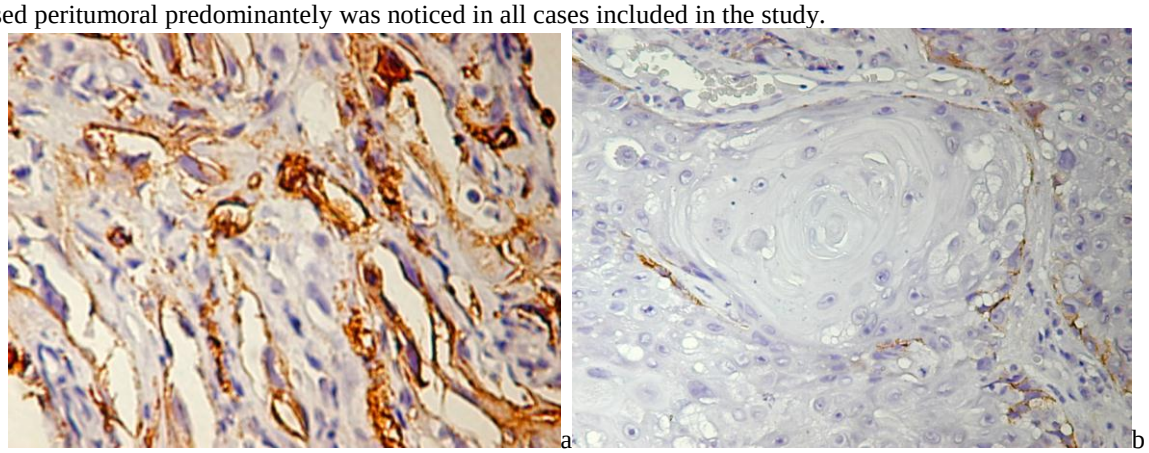

Figura 3a: Squamous cell carcinoma, D2-40 positive limphatic vessels in the peritumoral area, X400
Figura 3b. Squamous cell carcinoma, D2-40 immunoexpression in the intratumoral area, X400

We found D2-40 expression in tumor cells. Thus, from the 27 cases of squamous cell carcinoma, 18 expressed score 0 (0% positive tumor cells), 2 cases score 1 (<10% positive cells), 4 cases, score 2 (10-30% positive cells), 1 case score 3 (> 30% positive cells).

DISCUSSIONS

Although studies using immunohistochemical markers specific nodes have demonstrated intratumoral lymphatic vessels proliferating in several tumor types, the functional significance of intratumoral lymphatic vessels remains controversial [12]. Was given the idea that intratumoral lymphatic vessels are capable of carrying tumor cells because of the high hydrostatic pressure can compress the intratumoral vessels [12, 13]. Intratumoral lymphatic vascular density has been reported as an important factor for poor prognosis in many important human tumors, such as melanoma, mammary carcinoma, endometrial, colon, lung, ovarian, pancreatic, head and neck. Peritumoral lymphatic vessels are vessels immediately surrounding a tumor. It has been suggested that they may be compressed into preexisting vessels peritumoral edge by expanding tumor mass [14].

However, it was observed in lymphatic endothelial cell proliferation in certain tumor types peritumoral lymph, suggesting that these vessels may also occur as a result of lymphangiogenesis [15, 16]. Min et al. [17], using immunohistochemical methods identified D2-40 expression in tumor cells and lymphatic vascular density in lung carcinomas in the peritumoral area. For D2-40 immunoreactive tumor cells was negative adenocarcinomas, but squamous carcinomas 47% of cells were positive. In our study squamous carcinomas noted in a variable proportion of tumor cells D2-40 positive in varying degrees of intensity between 0 and +3. The percentage of cells positive D2-40, quantified score of between + 1 + 3 was 34.61%, compared to tumor cells did not express D2-40 present in an amount of 65.38%.

In 2008, Kadota et al. [18], have reviewed the density of lymphatic vessels in the vascular and

lymphatic invasion using specific antibodies in patients with various types of non-small cell lung carcinomas. At the same patients were followed microdensity intratumoral vascular blood. Lymphatic vascular density in patients with SCC was higher than those with adenocarcinomas. Shorter survival rate was correlated with elevated LVD and MVD. Lymphangiogenesis correlates with increased lymphatic vascular density. These observations indicated that LVD and MVD is independent prognostic factors in patients with non-small cell lung carcinomas.

Type examinations and computed tomography positron emission tomography, enable the discovery of small lung cancer. However, even if the size is less than 1 cm, lymph node metastasis rate is 10% [19]. It is known that lymph node metastasis is one of the most important prognostic factors in small cell lung carcinomas [20]. It is therefore desirable to identify molecular markers for the assessment of metastasis to lymph nodes and determining the prognosis in patients with lung carcinoma.

Data from the literature have shown a correlation between high levels of expression of VEGF-C and VEGFR3 and poor prognosis in non-small cell lung carcinomas, including adenocarcinomas [21, 22]. Other studies contradict these results [23]. A study by Maekawa et al. [24], revealed that the pulmonary adenocarcinomas, VEGF-C expression is not correlated with lymph node metastasis and prognosis. Instead, between reduced levels of VEGF-D, metastasis and prognosis significant correlation was observed. This may occur as a result of a possible inhibition of the VEGF-D to induce the proliferation of tumor factors, such as cytokines.

Cao et al. [25] observed in their study that the level of VEGF-C was increased in bronchoalveolar lavage fluid of patients with squamous cell carcinoma. In our study, we observed an intense expression, score 3 in more than a half of evaluated cases.

CONCLUSIONS

Our study provided evidence that VEGF-C and D2-40 represented risk factors for squamous cell carcinoma of the lung. They may contribute to a better understanding of lung cancer biology and to use therapies in an individual manner.

REFERENCES

[1] Schwarz MI, Epstein PE (1999) Pulmonary medicine and critical care. Knowlegde self – assessment Programe. Atlanta.
[2] Alberg A, Samet J (2003) Epidemiology of Lung Cancer. Chest, 123, pp. 21-49.
[3] Al-Rawi MA, Mansel RE, Jiang WG (2005) Lymphangiogenesis and its role in cancer. Histol Histopathol., 20, pp. 283–298.
[4] Inoue A, Moriya H, Katada N, Tanabe S, Kobayashi N, Watanabe M, Okayasu I (2008) Intratumoral lymphangiogenesis of esophageal squamous cell carcinoma and relationship with regulatory factors and prognosis. Pathol Int., 58, pp. 611–619.
[5] Jackson DG, Prevo R, Clasper S, Banerji S (2001) LYVE-1, the lymphatic system and tumor lymphangiogenesis. Trends Immunol., 22, pp. 317–321.
[6] Skobe M, Hawighorst T, Jackson DG, Prevo R, Janes L, Velasco P, Riccardi L, Alitalo K, Claffey K, Detmar M (2001) Induction of tumor lymphangiogenesis by VEGF-C promotes breast cancer metastasis. Nat Med, 7, pp. 192–198.
[7] Mattila MM, Ruohola JK, Karpanen T, Jackson DG, Alitalo K, Harkonen P, (2002) VEGF-C induced lymphangiogenesis is associated with lymph node metastasis in orthotopic MCF-7 tumors. Int J Cancer., 98, pp. 946–951.
[8] Hu Z, Wang W, Zhang J, Mao F, Shen-Tu Y (2012) Prognostic value of lymphangiogenesis and lymphatic vessel invasion in non-small cell lung cancer. Zhongguo Fei Ai Za Zhi, 15(11), pp. 656-662.
[9] Guo J1, Higashi K, Ueda Y, Oguchi M, Takegami T, Toga H, Sakuma T, Yokota H, Katsuda S, Tonami H, Yamamoto I (2006) Micro vessel density: correlation with 18F-FDG uptake and prognostic impact in lung adenocarcinomas. J Med., 47(3), pp. 419-425.
[10] Dai X, Wang W, Shen-Tu Y, Zhang J (2011) Expression and prognostic value of VEGF-C and lymphangeogenesis in lung adenocarcinoma and squamous cell carcinoma. Zhongguo Fei Ai Za Zhi, 14(10), pp. 774-779.
[11] Iwakiri S, Nagai S, Katakura H, Takenaka K, Date H, Wada H, Tanaka F (2009) D2-40-positive lymphatic vessel density is a poor prognostic factor in squamous cell carcinoma of the lung. Ann Surg Oncol., 16(6), pp. 1678-1685.
[12] Leu AJ, Berk DA, Lymboussaki A, Alitalo K, Jain RK (2000) Absence of functional lymphatics within a murine sarcoma: a molecular and functional evaluation. Cancer Res, 60, pp. 4324-4327.
[13] Dadras SS, Bertoncini PT, Brown LF, Muzikansky A, Jackson DG, Ellwanger U, Garbe C, Mihm MC, Detmar M (2004) Tumor lymphangiogenesis: a novel prognostic indicator for cutaneous melanoma metastasis and survival. Am. J. Pathol., 162, pp. 1951-1960.
[14] Valencak J, Heere-Ress E, Kopp T, Schoppmann SF, Kittler H, Pehamberger H (2004) Selective immunohistochemical staining shows significant prognostic influence of lymphatic and blood vessels in patients with malignant melanoma. Eur. J. Cancer, 40, pp. 358-364.
[15] Kyzas P.A., Geleff S., Batistatou A., Agnantis N.J., Stefanou D. (2005) Evidence for lymphangiogenesis and its prognostic implications in head and neck squamous cell carcinoma. J. Pathol., 206, pp. 170-177.

CORRESPONDENCE: Amalia Nicolescu, Western University of Arad, Romania**,** Department of Intensive Care, Piata Mihai Viteazu, nr. 7-8, tel: (004)0257/257080', e-mail: birauamalia@yahoo.com

Jurnal Medical Aradean (Arad Medical Journal)
Vol. XIX, issue 3, 2016, pp. 25-31