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Differences in Expression and Prognostic Values of p16(Ink4) and VEGF in Squamous and Adenocarcinoma of the Lung

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ABSTRACT

Introduction: Growing body of evidence suggests that molecular markers could have important prognostic value in non-small cell lung carcinoma (NSCLC) patients. Using targeted therapy based on these markers leads to improved outcomes in lung adenocarcinoma. However, progress of targeted therapy in squamous lung carcinoma is still modest. p16(ink4) protein acts as tumor suppressor.

Aim: Purpose of this study was to evaluate the difference in p16(ink4) expression between squamous and adenocarcinoma of the lung; to evaluate the relationship of p16(ink4) expression to survival outcomes in NSCLC patients, and the difference of their prognostic values between squamous and adenocarcinoma subtypes.

Material and Methods: 100 NSCLC patients (50 squamous and 50 adenocarcinoma) and 80 healthy individuals were included. p16(ink4) was immunohistochemicaly detected on formalin-fixed tissues. One- and 2-year progression-free survival (PFS) and overall survival (OS) were observed.

Results: p16(ink4) expression was significantly lower in squamous type compared to adenocarcinoma. In both squamous and adenocarcinoma, low p16(ink4) expression correlated with worse 1- and 2-year PFS,

as well as OS. In squamous lung carcinoma p16(ink4) expression was an independent negative prognostic marker.

Conclusion: Our study confirms the difference of p16(ink4) protein expression in squamous and adenocarcinoma of the lung. Besides anti-VEGF therapy, the regulation of p16(ink4) expression could have a therapeutic potential in NSCLC, especially in squamous lung carcinoma.

Keywords: Non-Small Lung Carcinoma; p16(ink4); VEGF; Expression; Survival

Abbreviations: OS: Overall Survival; PFS: Progression-Free Survival; NSCLC: Non–Small–Cell Lung Carcinoma; VEGF: Vascular Endothelial Growth Factor; MCLC: Macrocellular Lung Carcinoma; ADC: Adenocarcinoma; SCC: Squamous Cell Carcinoma

Introduction

Lung carcinoma is usually diagnosed in an advanced stage [1]. Clinically it is divided into small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC) compromising 80-85% patients. Histopathologically NSCLC is divided into adenocarcinoma (ADC), squamous cell carcinoma (SCC) and macrocellular lung carcinoma (MCLC) [2]. Therapy based on this classification showed modest improvement in survival benefits [1,2]. In the pathogenesis of lung carcinoma essential place have numerous biological processes of which tumor suppression and disorders in angiogenesis seems to be crucial. Growing body of evidence suggests that lung carcinoma is even more heterogenous pathological entity rather than 4 basic types [3]. So far, a large number of molecular markers have been found, but further research is needed to determine their clinical significance. In this content, latest improvement in therapy based on molecular changes (targeted therapy) showed significant clinical benefit in advanced adenocarcinoma of lung [4]. On the other side, there is still no targeted therapy of squamous lung carcinoma [5], which is implication from poorly understood molecular profile of this disease. p16 protein, which is product of INK4a gene, similar to p53, plays important role in the control of cell cycle by inhibition of cyclin-depended kinase 4 (Cdk4) [6,7]. p16(ink4) inactivation could be found in many solid tumours [7]. At the same time, loss of p16(ink4) suppression could be one of the first initiator in promotion of carcinogenesis [7,8]. Clinical value of p16(ink4) in lung carcinoma is still poorly understood and results from several studies are quite contradictory. So, p16(ink4) methylation did not correlated significantly to overall survival [9] and expression of p16 protein was not significant prognostic factor in resectabile non-small lung carcinoma [10]. On the other side, low or aberrant p16(ink4) expression was strong and independed poor prognostic factor in NSCLC [11-13].

Vascular endothelial growth factor (VEGF) is a signaling protein that is an essential part of the process of vasculogenesis and angiogenesis [14]. Overexpression of VEGF is important in the growth of the tumor mass, malignant invasion and metastasis, and is caused primarily by molecular mechanisms related to hypoxia [14-17]. Similar to p16(ink4), results of studies regarding prognostic value of VEGF in lung carcinoma are inconclusive. Some authors report that sera VEGF expression does not correlate significantly to histological type or clinical stage [16], and tissue VEGF expression does not correlate to survival rates [18]. Meanwhile, another authors concluded that combination of VEGF-A and VEGFR-2 is independent prognostic factor in advanced lung carcinoma [19], and sera VEGF expression could be negative prognostic factor [17,20]. To our knowledge, our study is the first to compare p16(ink4) and VEGF expression between tumour and tumour surroundings, as well as between ADC and SCC. The aim of this study was to evaluate the difference in p16(ink4) and VEGF expression between tumour tissue and tumour surroundings in NSCLC patients; between squamous and adenocarcinoma of the lung; to evaluate the relationship of p16(ink4) and VEGF expression to survival outcomes in NSCLC patients, and the difference of their prognostic values between squamous and adenocarcinoma subtypes. The results of this study could improve the molecular mapping of lung carcinoma and may offer valuable insights into identifying potential anti-angiogenic targeted therapy in lung carcinoma.

Materials and Methods

Subjects

This retrospective-prospective, longitudinal and consecutive study was begun after it was approved by the Human Research Ethics Committee of Bosnia and Herzegovina and all patients gave their informed written consent. Collection and subsequent preservation (formalin fixation, paraffin embedding) of tissue samples from the participating patients and controls as well as immunohistochemical evaluation were performed at General Hospital Tesanj (Bosnia and Herzegovina).

100 patients with non–small–cell lung carcinoma (NSCLC), including SCC (n=50) and ADC (n=50) (Table 1), and 80 age-, sex- and smoke-habits-matched individuals with no clinical evidence of a malignant disease (control group) were included (Table 2). For pathological examination and immunohistochemistry, forceps biopsies of tumor tissue and surrounding tissue (2 cm radius around primary tumor tissue without malignant cells) were taken from all patients during routine bronchoscopy examination. The diagnosis of SCC or ADC in all tumor tissue samples was confirmed by two experienced pathologist before enrolment into the study. Tissue samples were immediately formalin fixed and then paraffin embedded using standard procedures.

Table 1: Demographic data and clinical characteristics of SCC and ADC patients.

Characteristic		All patients	Adenocarcinoma	Squamous carcinoma
Number of patients		100 (100)	50(50)	50(50)
Age in years: median (range)		61 (40-82)	61 (40-82)	61 (46-78)
Sex	Female	40 (40)	21 (42)	19 (38)
	Male	60 (60)	29 (58)	31 (62)
Active smokers		80 (80)	24 (48)	42 (84)
Clinical stage of disease	II	8 (8)	4 (8)	4 (8)
	III	69 (69)	36 (72)	33 (66)
	IV	23 (23)	10 (20)	13 (26)

Table 2: Demographic data and clinical characteristics of control group.

Characteristic		N (%) 80	(100)
Age in years: median (range)	60 (46-76)		
Sex	Female	40	(50)
	Male	40	(50)
Active smokers		64	(80)
	No pathological finding	49	(61)
	Acute inflammation	12	(15)
	Chronic inflammation	19	(24)

Tissue Sampling and Immunohistochemistry

Sections of 5 μm were cut from paraffin-embedded tissue blocks. From each tissue specimen, sections stained with haematoxylin-eosin were used for morphological examination. For the purposes of the p16 immunohistochemical analysis, sampled tissue was deparaffinised with xylene solution, then rehydrated, washed with alcohol, and washed twice with water. In order to antigen unmasking, all samples were incubated in 0.01 mol/l of citrated buffer (pH 6.0) for 25 minutes. In the next 15 minutes, the tissue material was alternately cooled and rinsed with distilled water, and treated with 3% H202 deluded in methanol. Thereafter the samples were rinsed with water again, then with phosphate saline buffer, and conjugated with 10% horse serum during 30 minutes. Unmasking of p16 antigen (pre-deluded mouse monoclonal antibody) was performed by heating at a temperature of 95°C in 0.001 M EDTA solution (pH 8.0) during 25 minutes, and then been cooled for 15 minutes and rinse in 0.05M Tris-saline buffer with Tween 20.

For the purposes of immunohistochemical analysis of VEGF, sampled tissue was deparfinizirano and rehydrated routinely. Antigen unmasking was performed in 0.01 mol/L citrate buffer with 10 minutes of heating and 30 minutes of cooling. The activity of endogenous peroxidase has been inhibited after incubation with 3% hydrogen peroxide in methanol during 20 minutes. Nonspecific binding was inhibited with 5% bovine serum in phosphate buffered saline at room temperature. After washing, the samples were left overnight at 4°C with anti-human monoclonal anti-VEGF antibody (Clone JH121, NeoMarkers, Lab Vision Corporation, Fremont, CA, USA, 2011). After incubation, the samples were treated with 0.05 M Tris-buffered saline (pH 7.6) in combination with H202. Lung and breast tissue were used as positive control. p16 and VEGF expression on each sample were determined with semiquantitative value of 0 to 3 (0 - no expression; 1 - low deregulated; 2 - moderate expression; 3 - high expression). Pathohistopathological analysis of immunohistochemically stained samples preparations were performed by two experienced cytopathologists independently. Mean result of both analyses was calculated. p16(ink4) and VEGF expression were compared between SCC, ADC and control, as well as in relation to one- and two-year progression-free survival (PFS) and overall survival (OS).

Statistical Analyses

The distribution of data was determined using the D'Agostino and Pearson omnibus normality test. The strength of association between p16 and VEGF expression levels were analyzed with the Mann-Whitney U-test, unpaired t test or Fisher's exact test as appropriate. All patients included in this study were observed for 1 and 2 year; after this time survival probabilities (PFS and OS) were calculated from the day of diagnosis to the time of event (progress or death, respectively) or loss to follow-up using the Kaplan-Meier method and the log-rank test was used to compare different categories. Statistical analyses were performed using GraphPad Prism 5 software (San Diego, California, USA) and probability values of $p<0.05$ were accepted as significant according to Bonferroni correction for 5 tests. Statistically significant differences are presented as: *$p<0.05$; **$p<0.01$; ***$p<0.001$.

Results

p16(ink4) and VEGF Expression in Adenocarcinoma and Squamous Carcinoma of Lung and Corresponded Surroundings

Tumor and tumor surrounding showed similar p16(ink4) and VEGF expression in both SCC and ADC patients ($p>0.05$ for both groups). In both SCC and ADC, p16(ink4) expression was significantly lower in tumor tissue, as well as in tumor surrounding tissue compared to the control (SCC tumor tissue vs. control: $p<0.0001$; SCC tumor surrounding tissue vs. control: $p<0.0001$; ADC tumor tissue vs. control: $p<0.05$; ADC tumor surrounding tissue vs. control: $p<0.05$).

On the other side, VEGF expression, in SCC as well as in ADC patients, was significantly higher in tumor tissue and also in tumor surrounding tissue compared to the control (SCC tumor tissue vs. control: p<0.01; SCC tumor surrounding tissue vs. control: p<0.05; ADC tumor tissue vs. control: p<0.001; ADC tumor surrounding tissue vs. control: p<0.01) (Figure 1).

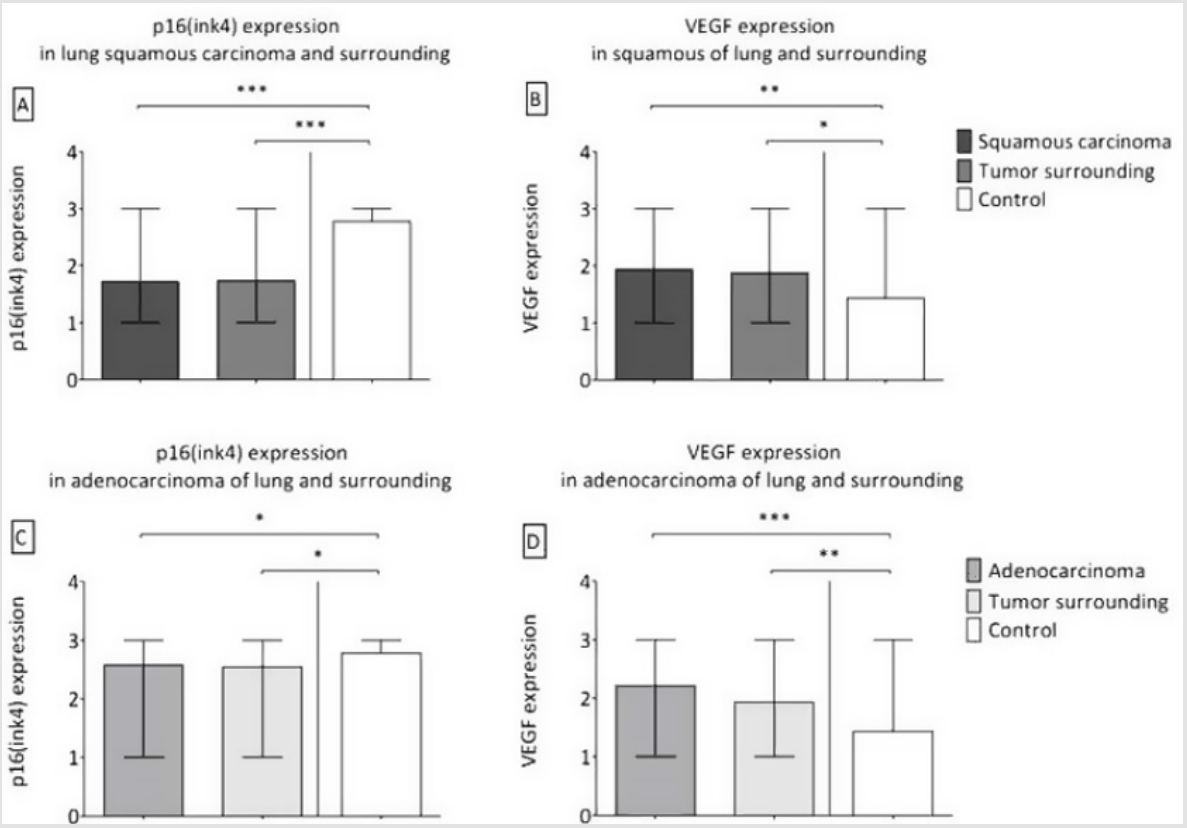

Figure 1: (A, B, C and D). p16(ink4) (A and C) and VEGF (B and D) expression in SCC (A and B) and ADC (C and D) and their surroundings.

p16(ink4) and VEGF Expression Between Adenocarcinoma and Squamous Carcinoma of Lung

p16(ink4) expression in SCC patients was significantly lower compared to ADC (p<0.0001). On the contrary, VEGF expression was similar in SCC and ADC (p>0.05) (Figure 2).

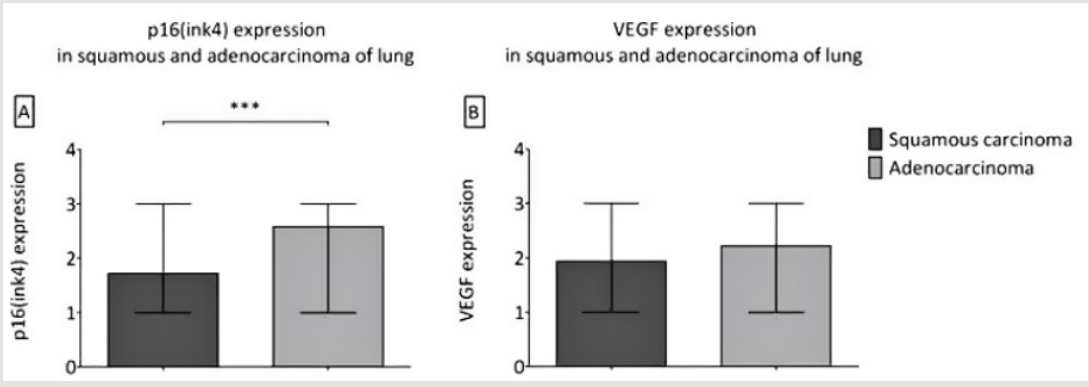

Figure 2: (A and B). p16(ink4) (A) and VEGF (B) expression between squamous and adenocarcinoma of lung.

Survival Analyses

Median PFS for the entire group of patients was 86 days (one year) and 453 (two years), while median OS was 178.5 days (one year) and 542 (two years). No significant differences in PFS or OS for the entire group of patients were observed when patients were stratified according to histological type of lung carcinoma or disease stages (data not shown). In both SCC and ADC, higher p16(ink4) expression was significantly associated with longer one-year PFS, as well as with longer OS ($p<0.001$ for all measurement) (Figure 3). Also, higher p16(ink4) expression was significantly associated with longer two-year PFS and OS in both SCC and ADC ($p<0.0001$ for all measurement) (Figure 4). Similarly, higher VEGF expression was significantly associated with longer one-year PFS and OS in SCC and ADC ($p<0.001$ for all measurements) (Figure 5). However, higher VEGF expression was significantly associated with longer two-year PFS ($p<0.001$) and OS ($p<0.001$) in ADC, but not in SCC ($p>0.05$ for both PFS and OS) (Figure 6).

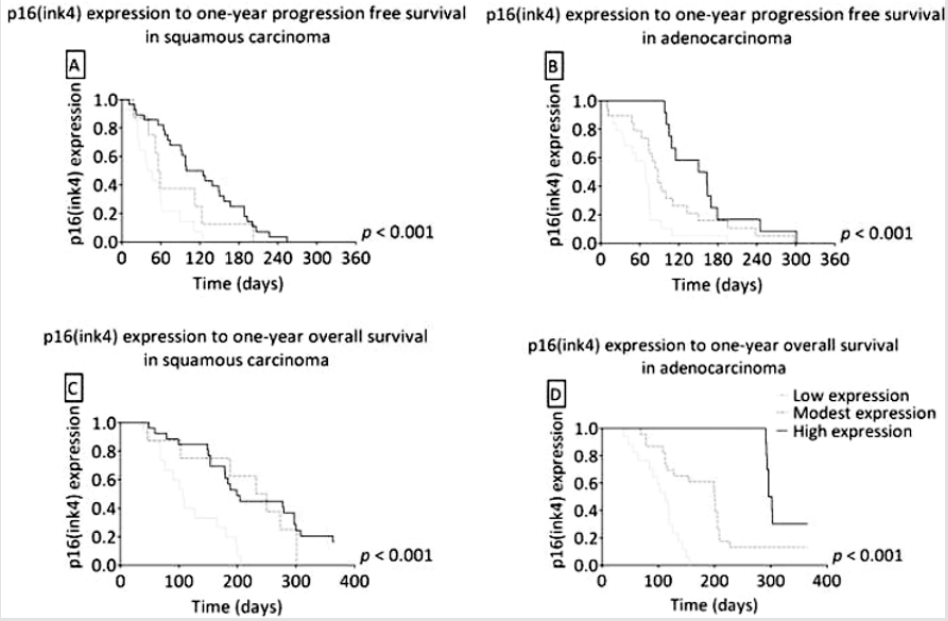

Figure 3: (A, B, C, D). p16(ink4) expression to one-year progression free survival (A and B) and overall survival (C and D) in squamous carcinoma (A and D) and adenocarcinoma of lung (B and D).

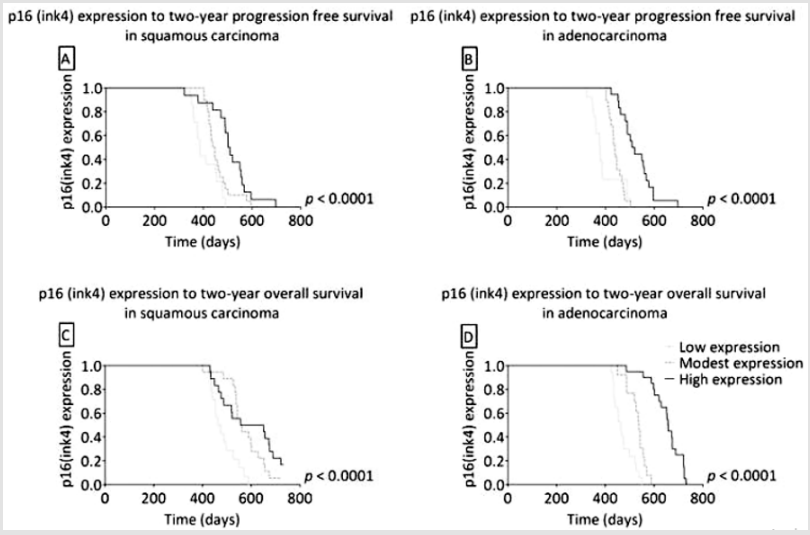

Figure 4: (A, B, C, D). p16(ink4) expression to two-year progression free survival (A and B) and overall survival (C and D) in squamous carcinoma (A and D) and adenocarcinoma of lung (B and D).

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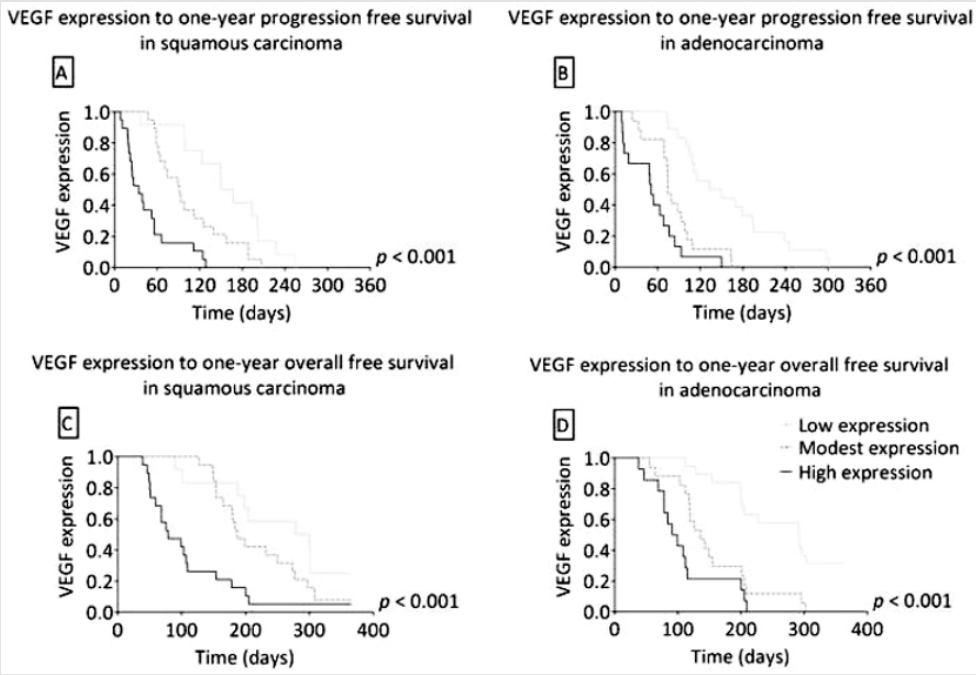

Figure 5: (A, B, C, D). VEGF expression to one-year progression free survival (A and B) and overall survival (C and D) in squamous carcinoma (A and D) and adenocarcinoma of lung (B and D).

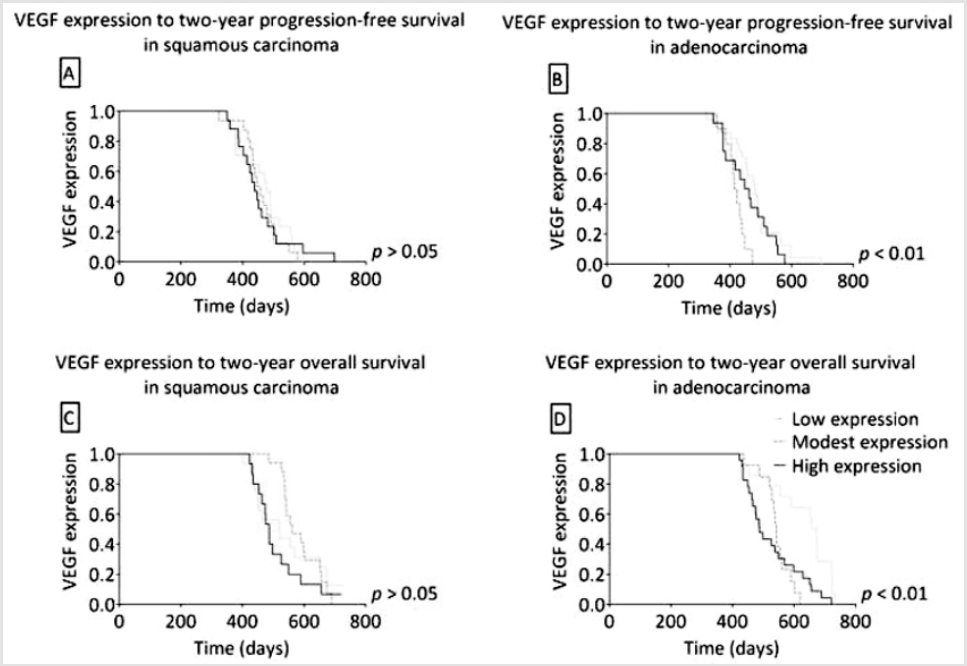

Figure 6: (A, B, C, D). VEGF expression to two-year progression free survival (A and B) and overall survival (C and D) in squamous carcinoma (A and D) and adenocarcinoma of lung (B and D).

Discussion

Molecular mechanisms that underlie the complex regulation of tumor initiation and angiogenesis are still poorly understood, as well as molecular processes in tumor surrounding. Recently many molecular markers were recognized in adenocarcinoma of lung, which has resulted in the creation of targeted therapies, often based on the inhibition of angiogenesis [1,2]. However, the molecular profile of squamous cell carcinoma of lung is still largely unknown, and treatment of this disease results in a low rate of survival [5]. We demonstrated significantly decreased p16(ink4) expression and increased VEGF expression in tumor tissue and surrounding tissue, but no differences were observed in expression levels of these two markers between tumor tissue and surrounding tissue from NSCLC patients. Also, we demonstrated significant difference of p16(ink4), but not of VEGF expression between SCC and ADC of lung. Furthermore, we observed significant correlation of p16(ink4) expression to all survival rates in both ADC and SCC of lung, while VEGF expression was limited in SCC. These observations strongly suggest more potent p16(ink4) tumor suppressive role in SCC of lung carcinoma compared to ADC, but also significant prognostic value of p16(ink4) expression in SCC. Moreover, the regulation of p16(ink4) expression could have therapeutic potential in NSCLC patients in regard to suppression of tumor growth. Our study showed significantly lower levels of p16(ink4) expression, and significantly higher level of VEGF expression in tumor tissue compared to the control. These results are consistent to the results of other published studies in regard to p16(ink4) supressive role and overexpression of VEGF in tumor tissue [21]. So, the loss of p16(ink4) expression is described in a number of malignancies of different organs [22-24]. This protein stops the cell cycle after inactivation of Rb gene, which is one of the main controllers [25-27]. Besides low expression of tumor suppressor p16(ink4), tumor tissue is characterized with a high expression of pro-angiogenic factor VEGF. This results in the creation of multiple blood vessels within the tumor tissue [28,29] and the very process of induced tumor angiogenesis is very complex and still insufficiently understood [21,30].

In our study, we observed a great similarity in the level of p16(ink4) and VEGF expression between the tumor and surrounding tissue. Several studies report multifocal and extensive molecular changes in non-malignant respiratory epithelium of lung carcinoma patients (genetic instability, deletions and mutations), which are particularly pronounced in the fields around clinically manifest tumors [31-33]. These abnormalities result in reduced expression of tumor suppressors and increased expression of pro-oncogenic and pro-angiogenic factors [33-35], and also in significantly higher molecular pro-oncogenic potential compared to other cells of healthy mucosa broncho-pulmonary system [36]. Besides this, the presence of inflammation and its effect on the angiogenic processes in these fields must be considered. Inflamed and reactive cells abundantly secrete NF-kβ, numerous matrix metalloproteinases, COX-2 and nitrite oxide synthase, and a large number of chemokines and inflammatory cytokines, whose significance was also proven in tumor invasion and angiogenesis [35]. Moreover, stromal cells that secrete a large number of pro-inflammatory cytokines, chemokines, growth factors and proteases contribute to the process of inflammation [37]. All of these changes promote the growth of the tumor [38,39]. In available and reviewed literature, we found only one comparative study of molecular genetic changes in lung carcinoma and its surrounding [40]. These authors report about similar expression of proangiogenic miRNAs (let-7b and miR-126) in lung carcinoma tissue and its surrounding. However, in the reviewed literature, we could not find any study that describes the p16(ink4) and VEGF expression in lung carcinoma surrounding. Consequently, our study, from the point of p16(ink4) and VEGF, again indicate the great importance of the tumor microenvironment in carcinoma development, progression and its involvement in tumor angiogenesis. We observed singnificantly lower p16(ink4) expression, but similar VEGF expression in SCC patients compared to ADC patients. In the available and reviewed literature, we could find only a few studies describing p16(ink4) and VEGF expression between SCC and ADC patients, but the results were opposite. So, Ann, et al. [41] report no significant difference in loss of heterozygosity frequencies of p16 between SCC and ADC. On the other side, Huang, et al. [42] found that p16-negative tumours in SCC were significantly more than those in ADC, whereas Leversha, et al. [43] observed that SCC had substantially increased p16 loss (SCC 75%, ADC 53%). Also, only several studies report about differences of VEGF expression between SCC and ADC, and their results are opposite too. So, some authors reported significantly higher VEGF expression in ADC than in SCC [44,45], and another authors observed no significant difference between SCC and ADC [46,47]. Clinical data suggest a distinct biologic role of the VEGF pathway in the different histologic subtypes of lung carcinoma [44]. Moreover, cigarette smoking seems to correlate positively to p16[INK4α] gene hypermethylation in NSCLC patients [48]. So, our and other observations suggest that some other factors, like histological subtype, differentiation, grade, clinical stage, age or smoking habits of SCC and ACD patients, could distinct influence on p16(ink4), but particularly on VEGF expression [49]. Nevertheless, larger studies are warranted to determine differences of p16(ink4) and VEGF expression between SCC and ADC, since only a limited number of studies in lung carcinoma have been published so far.

Our study showed significant correlation of p16(ink4) expression to all survival rates in both ADC and SCC, while VEGF expression was limited in two year PFS, as well as OS in SCC. The literature data regarding relationship of p16(ink4) expression and survival rates are mutually contradictory also. Specifically, a number of studies defined p16(ink4) as an important prognostic factor in patients with lung carcinoma [11-13,50,51,53-55], while the authors of other studies observed no significant relationship of p16(ink4) and survival rates [9,10]. Similar, relationship of VEGF expression and survival rates

in NSCLC patients is still controversial. According to some authors height of VEGF expression was significantly correlated with longer survival [4,15], [16,18,56], but results from other authors deny this relationship [19,20]. Furthermore, some authors observed significant correlation of p16(ink4) expression and survival rates only in younger NSCLC patients [53], but also in patients with non-advanced stage of SCC [55]. According our and the results from other authors one may conclude that new studies of p16(ink4) and VEGF expression relationship to survival rates are mandatory regarding subtype, differentation, grade and clinical stage of the diseases as well as patients age and smoking habits. In conclusion, our results indicate that significantly decreased p16(ink4) has an impact on prognosis in SCC patients. These findings could provide us an important step toward understanding the complex pathways necessary for development and progression of SCC. Moreover, besides anti-VEGF therapy, p16(ink4) could be proposed as an attractive target for developing treatment strategies that would achieve better clinical outcomes in NSCLC patients, particularly in SCC. However, the present results provide only for conclusions based on correlative analysis and further validation through mechanistic studies seems mandatory.

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References

1. Blumenthal GM, Karuri SW, Zhang H, Zhang L, Khozin S, et al. (2015) Overall Response Rate, Progression-Free Survival, and Overall Survival With Targeted and Standard Therapies in Advanced Non-Small-Cell Lung Cancer: US Food and Drug Administration Trial-Level and Patient-Level Analyses. J Clin Oncol 33(9): 1008-1014.
2. Lonardo F, Li X, Kaplun A, Soubani A, Sethi S, et al. (2010) The natural tumor suppressor protein maspin and potential application in non small cell lung cancer. Curr Pharm Des 16(16): 1877-1881.
3. Chen Z, Fillmore CM, Hammerman PS, Kim CF, Wong KK, et al. (2014) Non-small-cell lung cancers: a heterogeneous set of diseases. Nat Rev Cancer 14(8): 535-546.
4. Takeda M, Okamoto I, Yamanaka T, Nakagawa K, Nakanishi Y, et al. (2012) Impact of treatment with bevacizumab beyond disease progression: a randomized phase II study of docetaxel with or without bevacizumab after platinum-based chemotherapy plus bevacizumab in patients with advanced nonsquamous non-small cell lung cancer (WJOG 5910L). BMC Cancer 12(327): 1471-2407.
5. Gollard R, Garcia D, Natale R (2014) Pulmonary squamous cell carcinoma and sorafenib. Clin Case Rep 2(5): 206-208.
6. Zhu J, Chen M, Chen N, Ma A, Zhu C, et al. (2015) Glycyrrhetinic acid induces G1-phase cell cycle arrest in human non-small cell lung cancer cells through endoplasmic reticulum stress pathway. Int J Oncol 46(3): 981-988.
7. Shiraz OB, Galehdari H, Yavarian M, Geramizadeh B (2011) Possible down regulation of the p16 gene promoter in individuals with hepatocellular carcinoma. Hepat Mon 11(9): 719-723.
8. Liu Y, Johnson SM, Fedoriw Y, Rogers AB, Yuan H, et al. (2011) Expression of p16(INK4a) prevents cancer and promotes aging in lymphocytes. Blood 117(12): 3257-3267.
9. Kurakawa E, Shimamoto T, Utsumi K, Hirano T, Kato H, et al. (2001) Hypermethylation of p16(INK4a) and p15(INK4b) genes in non-small cell lung cancer. Int J Oncol 19(2): 277-281.
10. Hommura F, Dosaka Akita H, Kinoshita I, Mishina T, Hiroumi H, et al. (1999) Predictive value of expression of p16INK4A, retinoblastoma and p53 proteins for the prognosis of non-small-cell lung cancers. British Journal of Cancer 81(4): 696-701.
11. Gessner C, Liebers U, Kuhn H, Stiehl P, Witt C, et al. (2002) BAX and p16INK4A are independent positive prognostic markers for advanced tumour stage of nonsmall cell lung cancer. Eur Respir J 19(1): 134-140.
12. Cheng YL, Lee SC, Harn HJ, Chen CJ, Chang YC, et al. (2003) Prognostic prediction of the immunohistochemical expression of p53 and p16 in resected nonsmall cell lung cancer. Eur J Cardiothorac Surg 23(2): 221-228.
13. Zhao W, Huang CC, Otterson GA, Leon ME, Tang Y, et al. (2012) Altered p16(INK4) and RB1 Expressions Are Associated with Poor Prognosis in Patients with Nonsmall Cell Lung Cancer. J Oncol.
14. Lakshmikanthan S, Sobczak M, Chun C, Henschel A, Dargatz J, et al. (2011) Rap1 promotes VEGFR2 activation and angiogenesis by a mechanism involving integrin $\alpha v\beta_3$.Blood 118(7): 2015-2026.
15. Mroz RM, Korniluk M, Panek B, Ossolinska M, Chyczewska E, et al. (2013) sVEGF R1 and Tie-2 levels during chemotherapy of lung cancer patients. Adv Exp Med Biol 756: 313-319.
16. Park SH, Lee SS (2003) The relationship between serum VEGF concentration and prognosis of lung cancer. Korean J Intern Med 18(4): 207-211.
17. Shingyoji M, Ando S, Nishimura H, Nakajima T, Ishikawa A, et al. (2009) VEGF in patients with non-small cell lung cancer during combination chemotherapy of carboplatin and paclitaxel. Anticancer Res 29(7): 2635-2639.
18. Ucvet A, Kul C, Gursoy S, Erbaycu AE, Kaya SO, et al. (2011) Prognostic value of epithelial growth factor receptor, vascular endothelial growth factor, E-cadherin, and p120 catenin in resected non-small cell lung carcinoma. Arch Bronconeumol 47(8): 397-402.
19. Jantus Lewintre E, Sanmartín E, Sirera R, Blasco A, Sanchez JJ, et al. (2011) Camps C. Combined VEGF-A and VEGFR-2 concentrations in plasma: Diagnostic and prognostic implications in patients with advanced NSCLC. Lung Cancer 74(2): 326-331.
20. Kumar S, Guleria R, Singh V, Bharti AC, Mohan A, et al. (2009) Efficacy of plasma vascular endothelial growth factor in monitoring first-line chemotherapy in patients with advanced non-small cell lung cancer. BMC Cancer 3(9): 421-427.
21. Weinberg RA (2012) Bengt Westermark and our current understanding of tumor pathogenesis. Ups J Med Sci 117(2): 81-82.
22. Sterlacci W, Tzankov A, Veits L, Zelger B, Bihl MP, et al. (2011) A comprehensive analysis of p16 expression, gene status, and promoter hypermethylation in surgically resected non-small cell lung carcinomas. J Thorac Oncol 6(10): 1649-1657.
23. Kim DH, Nelson HH, Wiencke JK, Zheng S, Christiani DC (2001) p16(INK4a) and histology-specific methylation of CpG islands by exposure to tobacco smoke in non-small cell lung cancer. Cancer Res 61(8): 3419-3424.
24. Rocco JW, Sidransky D (2001) p16(MTS-1/CDKN2/INK4a) in cancer progression. Exp Cell Res 264(1): 42-55.

25. Paradiso A, Ranieri G, Stea B, Zito A, Zehbe I, et al. (2004) Altered p16INK4a and Fhit expression in carcinogenesis and progression of human oral cancer. Int J Oncol 24(2): 249-255.

26. Yanagawa N, Wang A, Kohler D, Santos Gda C, Sykes J, et al. (2012) Human papilloma virus genome is rare in North American non-small cell lung carcinoma patients. Lung Cancer 79(3): 215-220.

27. Zahra SN, Khattak NA, Mir A (2013) Comparative modeling and docking studies of p16ink4/Cyclin D1/Rb pathway genes in lung cancer revealed functionally interactive residue of RB1 and its functional partner E2F1. Theor Biol Med Model 10(1).

28. Kerbel RS (2008) Tumor angiogenesis. N Engl J Med 358: 2039-2049.

29. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144(5): 646-674.

30. He J, Qian X, Carpenter R, Xu Q, Wang L, et al. (2013) Repression of miR-143 mediates Cr (VI)-induced tumor angiogenesis via IGF-IR/IRS1/ERK/IL-8 pathway. Toxicol Sci 134(1): 26-38.

31. Wistuba II, Behrens C, Milchgrub S, Bryant D, Hung J, et al. (1999) Sequential molecular abnormalities are involved in the multistage development of squamous cell lung carcinoma. Oncogene 18(3): 643-650.

32. Wistuba II, Berry J, Behrens C, Maitra A, Shivapurkar N, et al. (2000) Molecular changes in the bronchial epithelium of patients with small cell lung cancer. Clin Cancer Res 6(7): 2604-2610.

33. Stenvold H, Donnem T, Andersen S, Al Saad S, Al Shibli K, et al. (2012) Overexpression of matrix metalloproteinase-7 and -9 in NSCLC tumor and stromal cells: correlation with a favorable clinical outcome. Lung Cancer 75(2): 235-241.

34. Peng D, Wang H, Cai C (2013) Tracheal adenoid cystic carcinoma followed by tracheal squamous cell carcinoma: Caa case report. Zhongguo Fei Ai Za Zhi 16(1): 58-60.

35. Wistuba II, Gazdar AF (2006) Lung cancer preneoplasia. Annu Rev Pathol 1: 331-348.

36. Merrick DT (2013) GRP78, intronic polymorphisms, and pharmacogenomics in non-small cell lung cancer. Chest 141(6): 1377-1378.

37. Trinchieri G (2012) Cancer and inflammation: an old intuition with rapidly evolving new concepts. Annu Rev Immunol 30: 677-706.

38. Egeblad M, Littlepage LE, Werb Z (2005) The fibroblastic coconspirator in cancer progression. Cold Spring Harb Symp Quant Biol 70: 383-388.

39. Lewis CE, Pollard JW (2006) Distinct role of macrophages in different tumor microenvironments. Cancer Res 66(2): 605-612.

40. Jusufovic E, Rijavec M, Keser D, Korosec P, Sodja E, et al. (2012) let-7b and miR-126 are down-regulated in tumor tissue and correlate with microvessel density and survival outcomes in non-small-cell lung cancer. PLoS One 7(9): 1-10.

41. An Q, Liu Y, Huang J (2001) Comparison of tumor suppressor gene deletion between squamous cell carcinoma and adenocarcinoma lung cancer in Chinese 23(6): 470-472.

42. Huang CI, Taki T, Higashiyama M, Kohno N, Miyak M, et al. (2000) p16 protein expression is associated with a poor prognosis in squamous cell carcinoma of the lung. British Journal of Cancer 82(2): 374-380.

43. Leversha MA, Fielding P, Watson S, Gosney JR, Field JK et al. (2003) Expression of p53, pRB, and p16 in lung tumours: a validation study on tissue microarrays. J Pathol 200(5): 610-619.

44. Pajares MJ, Agorreta J, Larrayoz M, Vesin A, Ezponda T, et al. (2012) Expression of tumor-derived vascular endothelial growth factor and its receptors is associated with outcome in early squamous cell carcinoma of the lung. J Clin Oncol 30(10): 1129-1136.

45. 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): 774-779.

46. Shijubo N, Uede T, Kon S, Maeda M, Segawa T, et al. (1999) Vascular endothelial growth factor and osteopontin in stage I lung adenocarcinoma. Am J Respir Crit Care Med 160(4): 1269-1273.

47. Imada A, Shijubo N, Kojima H, Abe S (2000) Mast cells correlate with angiogenesis and poor outcome in stage I lung adenocarcinoma. Eur Respir 15(6): 1087-1093.

48. Zhang B, Zhu W, Yang P, Liu T, Jiang M, et al. (2011) Cigarette smoking and p16INK4α gene promoter hypermethylation in non-small cell lung carcinoma patients: a meta-analysis. PLoS One 6(12).

49. Han H, Silverman JF, Santucci TS, Macherey RS, d Amato TA, et al. (2001) Vascular endothelial growth factor expression in stage I non-small cell lung cancer correlates with neoangiogenesis and a poor prognosis. Ann Surg Oncol 8(1): 72-79.

50. Quentin T, Henke C, Korabiowska M, Schlott T, Zimmerman B, et al. (2004) Altered mRNA expression of the Rb and p16 tumor suppressor genes and of CDK4 in transitional cell carcinomas of the urinary bladder associated with tumor progression. Anticancer Res 24(2B): 1011-1023.

51. Ghazizadeh M, Jin E, Shimizu H, Fujiwara M, Arai S, et al. (2005) Role of cdk4, p16INK4, and Rb expression in the prognosis of bronchioloalveolar carcinomas. Respiration 72(1): 68-73.

52. Buckingham L, Penfield Faber L, Kim A, Liptay M, Barger C, et al. (2010) PTEN, RASSF1 and DAPK site-specific hypermethylation and outcome in surgically treated stage I and II nonsmall cell lung cancer patients. Int J Cancer 126(7): 1630-1639.

53. Bradly DP, Gattuso P, Pool M, Basu S, Liptay M, et al. (2012) CDKN2A (p16) promoter hypermethylation influences the outcome in young lung cancer patients. Diagn Mol Pathol 21(4): 207-213.

54. Jarmalaite S, Kannio A, Anttila S, Lazutka JR, Husgafvel Pursiainen K, et al. (2003) Aberrant p16 promoter methylation in smokers and former smokers with nonsmall cell lung cancer. Int J Cancer 106(6): 913-918.

55. Tong J, Sun X, Cheng H, Zhao D, Ma J, et al. (2011) Expression of p16 in non-small cell lung cancer and its prognostic significance: a meta-analysis of published literatures. Lung Cancer 74(2): 155-163.

56. Ramlau R, Gorbunova V, Ciuleanu TE, Novello S, Ozguroglu M, et al. (2012) Aflibercept and Docetaxel versus Docetaxel alone after platinum failure in patients with advanced or metastatic non-small-cell lung cancer: a randomized, controlled phase III trial. J Clin Oncol 30(29): 3640-3647.

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