Research Article

Nurhilal Yusufoğlu, Melis Kant*, Merve Akış, Aydınlı Şanlı, Nezih Özdemir and Hüray İşlekel

Evaluation of angiogenesis with serum and tissue vascular endothelial growth factor, angiopoietin-1 and angiopoietin-2 levels in relation to clinicopathological features in lung cancer patients

Akciğer kanserli hastalarda serum ve doku vasküller endotelyal Büyüme Faktörü, anjiyopoietin-1 ve anjiyopoietin-2 düzeyleri ile anjiyogenezizin klinikopatolojik özelliklere göre değerlendirilmesi

DOI 10.1515/tjb-2016-0174
Received September 29, 2016; accepted March 27, 2017; previously published online May 20, 2017

Abstract

Objective: The aim of this study was to investigate serum and tissue levels of vascular endothelial growth factor (VEGF), angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) and to evaluate correlations between serum and tissue parameters with respect to clinicopathological features in patients with lung cancer. 

Methods: The study was conducted on 34 patients with stage I-IV primary lung cancer and 32 healthy controls. Preoperative serum, tumor and matched normal tissue VEGF, Ang-1 and Ang-2 levels were determined with ELISA. 

Results: Serum Ang-2 levels were significantly increased in lung cancer patients (p < 0.001). VEGF levels were significantly higher in tumor tissue than in matched normal tissue (p < 0.001). Ang-1 and Ang-2 levels were significantly higher in normal tissue than in tumor tissue (p < 0.001). A significant negative correlation was found between normal lung tissue Ang-2 and serum VEGF levels (r = −0.400, p = 0.019). A significant correlation was observed between serum and lung tissue Ang-2 levels (r = 0.397, p = 0.020).

Conclusion: This study clearly demonstrated that VEGF, Ang-1 and Ang-2 are all involved in lung cancer process. This was the first study to show a correlation between serum and tissue levels of Ang-2 in lung cancer. This finding might be the basis for therapeutic strategies against lung cancer.

Keywords: Lung carcinoma; Angiogenesis; VEGF; Angiopoietin-1; Angiopoietin-2; ELISA.

*Corresponding author: Melis Kant, Institute of Health Sciences, Dokuz Eylül University, Department of Medical Biochemistry, İzmir, Turkey, e-mail: melissdinc@gmail.com. http://orcid.org/0000-0002-1556-4920

Nurhilal Yusufoğlu: Bayındır Hospital, Department of Medical Biochemistry, İzmir, Turkey
Merve Akış: Institute of Health Sciences, Dokuz Eylül University, Department of Medical Biochemistry, İzmir, Turkey
Aydınlı Şanlı: Institute of Health Sciences, Dokuz Eylül University, Department of Medical Biochemistry, İzmir, Turkey
Nezih Özdemir: Faculty of Medicine, Dokuz Eylül University, Department of Thoracic Surgery, İzmir, Turkey
Hüray İşlekel: Faculty of Medicine, Dokuz Eylül University, Department of Medical Biochemistry and Department of Molecular Medicine, İzmir, Turkey

Özet

Amaç: Bu çalışmanın amacı akciğer kanserli hastaların serum ve dokularında VEGF, anjiyopoietin-1 (Ang-1) and
anjiyopoietin-2 (Ang-2) düzeylerinin belirlenmesi ve bu faktörlerin klinikopatolojik parametreler ile ilişkilerinin araştırılmasıdır.

Yöntem: Bu çalışma tümör evresi I-IV arasında olan 34 primer akciğer kanseri hastası ve 32 sağlıklı gönlü ile gerçekleştirdi. Hastalardan operasyon öncesi alınan venöz kan, operasyon sırasında alınan tümör ve eşlenik normal akciğer dokusunda VEGF, Ang-1 ve Ang-2 düzeyleri ELISA yöntemi ile ölçüldü.

Bulgular: Serum Ang-2 düzeyleri akciğer hastalarında anlamlı olarak yüksek bulundu (p<0.001). VEGF düzeylerinin tümör dokuda eşlenik normal dokuya göre anlamlı olarak yüksek olduğunu (p<0.001) Ang-1 ve Ang-2 düzeyleri eşlenik normal dokuda tümör dokuya göre anlamlı yüksek bulundu (p<0.001). Normal akciğer dokusunda Ang-2 düzeyleri ile serum VEGF düzeyleri arasında anlamlı korelasyon olduğu belirlendi (r = −0.400, p = 0.019). Serum Ang-2 ile akciğer dokusunda Ang-2 düzeyleri arasında negatif korelasyon olduğu gözlandı (r = 0.397, p = 0.020).

Sonuç: Bu çalışma VEGF, Ang-1 and Ang-2’nin akciğer kanserinde rol oynadığını açıkça ortaya koymaktadır. Serum ve doku Ang-2 düzeyleri arasındaki anlamlı korelasyon ilk kez bu çalışma ile gösterilmiştir. Bu bulgu akciğer kanseri için tedavi statejilerinin belirlenmesinde temel oluşturabilir.

Anahtar Kelimeler: Akciğer kanseri; Anjiyogenez; VEGF; Anjiyopoietin-1; Anjiyopoietin-2; ELISA.

Introduction

Lung cancer is the most frequent cause of deaths from cancer and accounts for 25% of all cancer deaths [1]. Diagnosis of lung cancer is usually delayed and long-term survival rates of lung cancer patients are low in that it has a high incidence, high mortality rates and an asymptomatic course at onset [2]. Therefore, new methods are needed to diagnose, prevent and treat this condition.

Angiogenesis is an essential process in tumorigenesis and pathogenesis of human malignancy [3]. When solid tumors become larger than 2–3 mm³, they cannot fulfill their needs of oxygen and nutrients through diffusion and need angiogenesis [4]. Angiogenesis is controlled by a balance of stimulating and inhibiting regulators involved in multiple pathways which result in endothelial cell proliferation, differentiation and organization into a functional network of vascular channels [4]. When the balance between stimulating and inhibiting factors in angiogenesis is disrupted in favor of stimuli, neovascularization occurs in the tumor tissue [5, 6].

There is some evidence that vascular endothelial growth factor (VEGF) and angiopoietins (angiopoietin-1 and angiopoietin-2) collaboratively and coordinately regulate vascular formation and maturation during angiogenesis [7–9].

VEGF is one of the most important angiogenic factors and effective in all stages of angiogenesis [10]. It is overexpressed by a majority of solid tumors, which increases vascular permeability and promotes endothelial cell proliferation, survival and migration in both physiological and pathological angiogenesis [11].

Other important factors in angiogenesis are angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2). The former stimulates interaction between endothelial cells and extracellular matrix around it and stabilizes vessels. The latter antagonizes the stabilizing effect of Ang-1 and causes destabilization in vessels [12, 13]. The destabilization created by Ang-2 as an anti-angiogenic factor goes through angiogenic change in the presence of VEGF [9].

Most of the studies have investigated VEGF, Ang-1 and Ang-2 in serum or tissue, whereas we used both serum and tumor tissue and matched normal tissue in lung cancer patients. Thus, the aim of this study was to determine VEGF, Ang-1 and Ang-2 levels in patients with lung cancer and to evaluate relations between these angiogenic factors in terms of clinicopathological parameters in these patients.

Materials and methods

Subjects

The study was approved by Clinical Research Ethics Committee of Dokuz Eylül University School of Medicine. It included 34 patients with stage I–IV primary lung cancer and 32 healthy controls. Thirty two of the patients were diagnosed with non-small cell lung cancer (NSCLC) including 18 squamous cell carcinoma, nine adenocarcinoma and five mixed adenocarcinoma and two were small cell lung cancer (SCLC). The patients were selected at the Department of Thoracic Surgery at Dokuz Eylül University Hospital and informed consent was obtained from all the cases. Exclusion criteria were malignancies other than those of the lungs, lung carcinoid tumors, and positive history of chemotherapy and/or radiotherapy. Healthy controls who had already
undergone routine and clinical analyses were selected from Smoking Cessation Outpatient Clinic and assigned into the control group. Demographic and anthropometric data and medical history of the subjects and the healthy controls were recorded. Routine biochemical and clinical analyses were performed in the Central Laboratory of Dokuz Eylul University Hospital directly after sample collection. Preoperative blood specimens were collected and stored at −40°C until their analyses were made.

In addition, tumor tissues (300–400 mg) and matched normal lung tissues 5 cm away from tumor were obtained from the fields of the surgical specimens approved by the pathologist. These specimens were rinsed with 0.9% NaCl and kept at −80°C until they were analyzed. Clinico-pathological features including age, gender, tumor size, histological types of tumors, histological differentiation degree, tumor node metastasis (TNM) on pathological examinations and invasions into veins, arteries, lymph vessels and nerve tissue were evaluated. Tumor stage was determined according to 2004 WHO classification of lung tumors [14].

**Measurement of VEGF, Ang-1 and Ang-2 levels**

Blood samples were centrifuged at 4000 rpm for 10 min and aliquots of supernatants were stored at −80°C until testing. Frozen serum samples were mixed thoroughly after thawing and centrifuged before analysis. Samples with more than a trace of hemolysis were discarded.

Frozen tissue samples were homogenized with Tissue Lyser (Qiagen, Hilden, Germany) in lysis buffer (20 mM Tris HCl pH 7.4, 5 mM MgCl₂, 20 μM Pepstatin A, 20 μM Leupeptin, 50 μM PMSF) [12]. Protein concentrations of serum and tissue lysates were determined by using BCA Protein Assay Kit (Pierce, USA).

VEGF, Ang-1 and Ang-2 protein levels were measured by ELISA according to the manufacturer’s instructions (R&D Systems, Abingdon, Oxfordshire, UK). The absorbance was measured at 450 nm using a microplate reader (BioTek KC4, USA). Quantifications were achieved by construction of standard curves using known concentrations. The results were expressed as picogram per milliliter (pg/mL) for serum and picogram per milligram of protein (pg/mg protein) for tissue samples. Assay reproducibility was assessed by precision of intra-day measurements (n = 10). The intra-day CVs for VEGF, Ang-1 and Ang-2 were 8.4%, 6.9% and 7.3%, respectively.

**Statistical analysis**

Statistical analyses were performed using SPSS 15.0. All measurements were done in triplicate. Each variable was checked for normality of distribution by Shapiro-Wilk normality test. Student’s t-test was used for normally distributed variables in the analysis of continuous variables. Variables with non-Gaussian distribution were compared by using Mann-Whitney U test and Kruskal-Wallis test. The data were expressed as mean ± standard deviation for normally distributed variables and median and range for non-normally distributed variables. Pearson and Spearman correlation coefficients were used for the analysis of correlations for continuous normally and non-normally distributed variables, respectively. Values of p < 0.05 were considered to indicate a statistically significant difference.

**Results**

A total of 34 patients with primary lung cancer presenting to respiratory surgery department and 32 healthy controls referring to smoking cessation outpatient clinic of Dokuz Eylul University Hospital were included into the study. Out of 34 patients, 18 (53%) had squamous cell carcinoma, nine (26%) had adenocarcinoma, five (15%) had mixed adenocarcinoma and two (6%) had small cell carcinoma. Diagnoses in pathological examinations revealed that tumors were well-differentiated in 12 patients, moderately differentiated in 10 patients and poorly-differentiated in 12 patients. Thirty-two individuals (26 males and six females) referring to smoking cessation outpatient clinic were found to be healthy based on routine investigations. There was no significant difference in age between the patients and the healthy controls (p > 0.97).

Serum VEGF, Ang-1 and Ang-2 levels were compared between the patients with lung cancer and the healthy controls. While the patients and the controls did not have a significant difference in terms of serum VEGF and Ang-1, the patients had significantly higher levels of Ang-2 levels (p < 0.001) (Table 1). VEGF, Ang-1 and Ang-2 levels in tumor tissues and matched normal tissues in the patients were determined. VEGF levels were significantly higher in tumor than in normal matched normal tissues (p < 0.001) (Table 2). Ang-1 and Ang-2 levels were significantly lower in tumor than in matched normal tissues (p < 0.001) (Table 2).

VEGF, Ang-1 and Ang-2 levels in serum and tumor tissues were evaluated with respect to clinico-pathological parameters of lung cancer patients. Serum VEGF
levels were significantly higher in the patients with tumor stages T3 and T4 than in those with tumor stages T1 and T2 (p = 0.042) (Table 3). Ang-1 levels in tumor tissues were also significantly higher in the patients aged over 65 years than in those younger than 65 years old and in the patients with moderately and poorly differentiated tumors than in those with well-differentiated tumors (p = 0.038 and p = 0.044, respectively) (Table 3). No significant differences were found in sex, tumor size, histological type, lymph node, perineural invasion, artery invasion, lymphovascular invasion and vein invasion between VEGF, Ang-1 and Ang-2 levels in serum and tumor tissues (data not shown).

In the patient group, there was a significant positive correlation between serum VEGF and Ang-1 levels (r = 0.446, p = 0.000), a significant negative correlation between serum VEGF and normal tissue Ang-2 levels (r = −0.400, p = 0.019) and a significant positive correlation between serum Ang-2 levels and normal tissue Ang-2 levels (r = 0.397, p = 0.020).

Discussion

Lung cancer is the leading cause of cancer related deaths with a survival rate of 10% [15]. The reason for the low survival rate is the lack of early diagnosis methods. Therefore, early diagnosis and effective treatment of this malignant disease become more important [16]. Just as all malignant solid tumors, tumors in lung cancer need new vascularization in their primary foci and metastases so that they can fulfill their needs of oxygen and nutrients for growth [4]. There is a widespread agreement that angiogenic activation of a tumor results from disruption of a balance between positive and negative factors of angiogenesis [5, 6]. It is important to use stimulating and inhibiting factors in angiogenesis as early markers of monitoring tumor progression, to decide strategies to be used in treatment and to develop new treatment methods. Several studies have revealed that VEGF and angiopoietins play a critical role in tumor angiogenesis [7–9].

There have been few studies directed towards examination of serum VEGF and angiopoietins together in lung cancer patients [17–22]. In studies on pulmonary tumor tissues, either VEGF and angiopoietin levels were examined with semi-quantitative immunohistochemical methods or mRNA expression was determined with Reverse Transcription Polymerase Chain Reaction (RT-PCR) [20, 23–27]. There have been no studies quantitatively evaluating both VEGF, Ang-1 and Ang-2 in serum, tumor tissues and matched normal tissues in lung cancer patients.

In the present study, VEGF, Ang-1 and Ang-2 levels in serum, tumor tissues and matched normal tissue supernatants from lung cancer patients and healthy controls were determined and obtained results were evaluated together with clinicopathological parameters. Furthermore, the patients were divided into groups according to clinicopathological parameters including gender, age, tumor differentiation, histological type, lymph node (N), tumor invasion (perineural, venous and lymphatic), and VEGF, Ang-1 and Ang-2 levels were compared between these groups.

Regarding VEGF, despite lack of a significant difference between serum levels in the patients and healthy controls, the higher values found in tumor tissues than in matched normal lung tissues seem to support the critical
role of VEGF in angiogenesis. It has also been reported before that although VEGF levels in tumor cells can be increased, serum VEGF levels may not be sufficiently high to measure [22]. On the other hand, as the patients were subdivided into groups, serum VEGF were significantly higher in the patients with tumor stages T3 and T4 than in those with tumor stages T1 and T2. This suggests that VEGF can be associated with tumor size and confirms the idea that it is one of the most important angiogenic factors in angiogenesis mediated tumor growth. Most of the studies have reported no statistically significant difference for tumor stage and histological type in terms of VEGF [22, 28, 29]. Similar to our findings, increased serum VEGF levels were found in advanced tumor stages compared to those in early tumor stages [30–32].

Having an angiogenic effect in normal and developing tissues, Ang-1 levels were significantly lower in tumor tissues than in normal lung tissues, which is consistent with the results of a study by Park et al. [28]. Conflicting findings about Ang-1 from other studies suggest Ang-1 expression is heterogeneous and does not always reflect the whole tissue [27 –30]. Due to its angiogenic effect, Ang-1 has a crucial role in development and growth of tumors. In the present study, higher levels of Ang-1 found in moderately and poorly differentiated tumors compared to highly differentiated ones show more aggressive growth.

Serum Ang-2 levels were found to be significantly higher in the lung cancer patients than in the healthy controls. It has been proposed that increased Ang-2 levels are a sign of survival and prevent tumor growth. However, VEGF upregulation accompanied by increased Ang-2 levels stimulates angiogenesis at tumor margins, which causes tumor growth. Consistent with the present study, other studies found significantly higher Ang-2 levels in lung cancer patients than in healthy controls [22, 28, 33, 34]. In addition, Canadas et al. measured Ang-2 levels in lung cancer patients with a method different from the one used in the present study (Luminex Technology) and reported significantly higher Ang-2 levels [35]. When angiopoietins were evaluated according to the tumor stage and histologic type, supporting our findings most of the studies have found no significant relation [3, 12, 22, 29]. On the other hand, Park et al. have shown that serum Ang-2 levels were increased in stage II than those in stage I [28].

There have been few studies on the relation between clinicopathological features and VEGF, Ang-1 and Ang-2 in lung cancer patients, with conflicting results. These studies have been directed towards evaluation of either one or two of the parameters serum VEGF, Ang-1 or Ang-2 and their results have been presented semi-quantitatively.

Table 3: Serum and tumor tissue VEGF, Ang-1, Ang-2 levels compared in terms of clinicopathological parameters of lung cancer.

| Clinicopathological parameters of lung cancer | Serum VEGF | Tissue VEGF | Serum Ang-1 | Tissue Ang-1 | Serum Ang-2 | Tissue Ang-2 |
|---------------------------------------------|------------|-------------|-------------|--------------|-------------|--------------|
| Differentiation of tumor                    |            |             |             |              |             |              |
| Well                                        | 12         | 302.78      | (235.72–725.56) | 6283         | (4795.27–6920.00) | 0.971        | 0.042        |
| Moderate-poor                               | 22         | 348.33      | (1925.04–495.56) | 5003         | (44282.72–1825.03) | 0.249        | 0.042        |
| Tumor stage                                 |            |             |             |              |             |              |
| T1–T2                                       | 20         | 286.31      | (235.84–429.44) | 5053         | (41615.26–9658.85) | 0.971        | 0.042        |
| T2–T4                                       | 14         | 457.32      | (30558.92–492.50) | 5053         | (48116.50–70199.75) | 0.37         | 0.17         |
| p-Value                                     |            |             |             |              |             |              |
| a                                          | 0.137      | 0.861       | 0.242       | 0.042        | 0.576        |              |

Results were expressed as median (interquartile range); (pg/mL for serum, pg/mg protein for tumor tissue); patients with moderately and poorly differentiated tumors versus well-differentiated tumors; patients with tumor stages T3 and T4 versus tumor stages T1 and T2.
In the present study, the significant negative correlation found between serum VEGF and lung tissue Ang-2 reveals clearly the opposing roles of these factors. On the other hand, a moderately significant positive correlation between serum VEGF and Ang-1 was found. This positive correlation was supported by the fact that both VEGF and Ang-1 are angiogenic factors. Park et al. reported that serum VEGF was correlated with Ang-1 and Ang-2, but there was no correlation between Ang-1 and Ang-2 [28]. However, in the present study, there was not a significant correlation between serum VEGF and serum Ang-2 levels. One of the most striking findings of the present study was presence of a significant positive correlation between normal tissue Ang-2 and serum Ang-2, reflecting a parallel rise in both tissue and serum. This finding suggests that serum levels of these parameters can be a potential surrogate marker.

The limitation of this study is that the number of patients in the subgroups based on histological tumor types was relatively small (nine patients with adenocarcinoma and 18 patients with squamous cell carcinoma). Therefore, further studies with larger samples are needed.

The results of this study are important in that it increases our understanding of the role of different angiogenic parameters in lung cancer. To conclude, this study formed a basis for further studies on angiogenic and anti-angiogenic factors in lung cancer, especially Ang-2. The finding that Ang-2 levels were increased in both normal lung tissue and serum will provide guidance for follow-up and evaluation of patients and creation of new treatment strategies.

Acknowledgements: This research was supported by a grant from Dokuz Eylül University Scientific Research Projects Coordination Unit (Project number: 2009, KB.SAG.054). Some of the data given in this article were used in a poster presentation at 23rd National Congress of Biochemistry of the Turkish Biochemical Society-Turkey.

Conflict of interest statement: There are no conflicts of interest among the authors.

References

1. American Cancer Society. Cancer facts & figures 2014. Cancer Facts Fig 2014:1–72.
2. Kanoda NM, Silvestri GA, Tanner NT. Screening and early detection efforts in lung cancer. Cancer 2015;121:1347–56.
3. Naumnik W, Naumnik B, Niewiarowska K, Ossolinska M, Chyczewska E. Angiogenic axis angiopoietin-1 and angiopoietin-2/Tie-2 in non-small cell lung cancer: a bronchoalveolar lavage and serum study. Adv Exp Med Biol 2013;788:341–8.
4. Risau W. Mechanisms of angiogenesis. Nature 1997;386:671–4.
5. Cox G, Jones JL, Walker RA, Steward WP, O’Byrne KJ. Angiogenesis and non-small cell lung cancer. Lung Cancer 2000;27:81–100.
6. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 1996;86:353–64.
7. Holash J, Maisonnierie PC, Compton D, Boland P, Alexander CR, Zagzag D, et al. Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. Science 1999;284:1994–8.
8. Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J. Vascular-specific growth factors and blood vessel formation. Nature 2000;407:242–8.
9. Maisonnierie PC, Suri C, Jones PF, Bartunkova S, Wiegand SJ, Radziejewski C, et al. Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. Science 1997;277:55–60.
10. Kaiser PK. Antivascular endothelial growth factor agents and their development: therapeutic implications in ocular diseases. Am J Ophthalmol 2006;142:660–8.
11. Ferrara N. Vascular endothelial growth factor as a target for anticancer therapy. Oncologist 2004;9(Suppl 1):2–10.
12. Tanaka F, Ishikawa S, Yanagihara K, Miyahara R, Kawano Y, Li M, et al. Expression of angiopoietins and its clinical significance in non-small cell lung cancer. Cancer Res 2002;62:7124–9.
13. Fagiani E, Christofori G. Angiopoietins in angiogenesis. Cancer Lett 2013;328:18–26.
14. Travis WD, Brambilla E, Müller-hermelink HK, Harris CC. Pathology & genetics tumours of the lung, pleura, thymus and heart. In: W.D. Travis, Ed. World Health Organization Classification of Tumours WHO Classification. Lyon: IARC/Press, 2004:9–122.
15. Siegel R, Miller K, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015;65:5–29.
16. Ettinger DS, Akerley W, Bepler G, Blum MG, Chang A, Cheney RT, et al. Non-small cell lung cancer. J Natl Compr Canc Netw 2010;8:740–801.
17. Takigawa N, Segawa Y, Fujimoto N, Hotta K, Eguchi K. Elevated vascular endothelial growth factor levels in sera of patients with lung cancer. Anticancer Res 1998;18:1251–4.
18. Joo HP, Kwang JP, Young SK, Keu SL, Hyoung NL, et al. Serum angiopoietin-2 as a clinical marker for lung cancer. Chest 2007;132:200–6.
19. Shimamuky, Takahashi K, Cui R, Hori S, Takahashi F, Miyamoto H, et al. Role of serum vascular endothelial growth factor in the prediction of angiogenesis and prognosis for non-small cell lung cancer. Lung 2005;183:29–42.
20. Bailey-Wilson JE, Amos CI, Pinney SM, Petersen GM, de Andrade M, Wiest JS, et al. A major lung cancer susceptibility locus maps to chromosome 6q23-25. Am J Hum Genet 2004;75:460–74.
21. Ilhan N, Ilhan N, Deveci F. Functional significance of vascular endothelial growth factor and its receptor (receptor-1) in various lung cancer types. Clin Biochem 2004;37:840–5.
22. Akin Kabalak P, Çiledağ A, Demir N, Çelik G, Yüksek C, Köşüst G, et al. Prognostic significance of serum vascular endothelial growth factor and Angiopoietin-2 in patients with lung cancer. Tuberk Toraks 2015;63:71–7.
23. Yuan HT, Khankin EV, Karumanchi SA, Parikh SM. Angiopoietin 2 is a partial agonist/antagonist of Tie2 signaling in the endothelium. Mol Cell Biol 2009;29:2011–22.

24. Yano T, Tanikawa S, Fujie T, Masutani M, Horie T. Vascular endothelial growth factor expression and neovascularisation in non-small cell lung cancer. Eur J Cancer 2000;36:601–9.

25. Tait CR, Jones PF. Angiopoietins in tumours: the angiogenic switch. J Pathol 2004;204:1–10.

26. Stefanou D, Batistatou A, Arkoumani E, Ntzani E, Agnantis NJ. Expression of vascular endothelial growth factor (VEGF) and association with microvessel density in small-cell and non-small-cell lung carcinomas. Histol Histopathol 2004;19:37–42.

27. Wong MP, Chan SY, Fu KH, Leung SY, Cheung N, Yuen ST, et al. The angiopoietins, tie2 and vascular endothelial growth factor are differentially expressed in the transformation of normal lung to non-small cell lung carcinomas. Lung Cancer 2000;29:11–22.

28. Park JH, Choi H, Kim YB, Kim YS, Sheen SS, Choi JH, et al. Serum angiopoietin-1 as a prognostic marker in resected early stage lung cancer. Lung Cancer 2009;66:359–64.

29. Takahama M, Tsutsumi M, Tsujiuchi T, Nezu K, Kushiba K, Taniguchi S, et al. Enhanced expression of Tie2, its ligand angiopoietin-1, vascular endothelial growth factor, and CD31 in human non-small cell lung carcinomas. Clin Cancer Res 1999;5:2506–10.

30. Laack E, Scheffler A, Burkleholder I, Boeters I, Andritzky B, Schuch G, et al. Pretreatment vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9) serum levels in patients with metastatic non-small cell lung cancer (NSCLC). Lung Cancer 2005;50:51–8.

31. Carrillo-de Santa Pau E, Carrillo Arias F, Caso Pelaez E, Muguruza Trueba I, Sanchez Hernandez I, Munoz Molina GM, et al. Vascular endothelial growth factor (VEGF) serum levels are associated with survival in early stages of lung cancer patients. Cancer Invest 2010;28:393–8.

32. Dalaveris E, Kerenidi T, Katsabekis-Katsafli A, Kirooulos T, Tanou K, Gourgoulianis K, et al. VEGF, TNF-alpha and 8-isoprostane levels in exhaled breath condensate and serum of patients with lung cancer. Lung Cancer 2009;64:219–25.

33. Takanami I. Overexpression of Ang-2 mRNA in non-small cell lung cancer: association with angiogenesis and poor prognosis. Oncol Rep 2004;12:849–53.

34. Coelho AL, Araújo AM, Gomes MP, Catarino RJ, Andrade EB, Lopes AM, et al. Combined Ang-2 and VEGF serum levels: Holding hands as a new integral biomarker in non-small-cell lung cancers. Futur Oncol 2015;11:3233–42.

35. Cañadas I, Taus Â, Villanueva X, Arpí Q, Pijuan L, Rodriguez Y, et al. Angiopoietin-2 is a negative prognostic marker in small cell lung cancer. Lung Cancer 2015;90:302–6.