

# A comparative analysis of angiotensin 2 immunohistochemical staining in various stages of lung cancer

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## Abstract

**Objective:** The role of Angiotensin 2 (Ang 2), which is necessary for tumor growth, extension, and metastasis is not fully elucidated. The presented study aimed to investigate the relationship between Ang 2 staining intensity, expression rate in tumor tissue, and the stage of lung cancer.

**Materials and Methods:** Fifty cases of lung cancer (34 non-small and 16 small cell cases) were included in the study. Immunohistochemistry was done to evaluate Ang 2 staining intensity and expression rate in tumor and stromal cells of lung cancer tissue.

**Results:** Ang 2 was positive for 45 (90%) cases and negative for five (10%) cases ( $P = 0.04$ ). There was a significant correlation between Ang 2 expression rate of expression and the histologic type of lung cancer ( $P = 0.033$ ). Ang 2 expression rate in tumor cells of cancer tissues diagnosed with adenocarcinoma was low. There was a significant correlation between Ang 2 expression rate in stromal cells of cancer tissue and the type of lung cancer ( $P = 0.021$ ). Stromal cell expression rate of Ang 2 in adenocarcinoma was found to be low.

**Conclusions:** As a result, the relationship between lung cancer stage and Ang 2 was documented with this study and the expression rate was found to be lower in adenocarcinomas. By this analysis, we can suggest that angiotensins may be used as an option for targeted treatment in lung cancer.

**Key words:** lung cancer, angiotensin 2, biopsy, stage, immunohistochemical staining

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## Introduction

Lung cancer remains as one of the leading causes of cancer deaths worldwide. It is important to gain knowledge of different biological behaviors of lung cancer to provide additional tools in fighting such a deadly disease.

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Chemotherapy remains as an essential treatment modality in managing lung cancer, however, at the cost of systemic side effects. Studies have been on progress in the field of specific to target therapy especially in relation to tumor growth and angiogenesis.<sup>[1]</sup>

Angiopoietins are angiogenic regulators that are released from the endothelium under the control of the vascular endothelial growth factor (VEGF) and play an essential role in the angiogenesis pathway.<sup>[2]</sup> For instance, Angiopoietin I (Ang I) plays a role in vessel wall stabilization and maturation, whereas Angiopoietin 2 (Ang 2) is its competitive inhibitor. Both of these proteins are at equilibrium in normal tissue but this equilibrium tilts toward Ang 2 in direction in tumor tissue. VEGF stimulates the release of Ang 2 from tumoral and nearby tissues. Although Ang 2 may inhibit angiogenesis the combination of Ang 2 and VEGF may lead to vessels destabilization followed by tumor budding through the vascular wall.<sup>[3]</sup>

## Materials and Methods

This study was conducted as a single center retrospective study at Gulhane Askeri Tip Akademisi (GATA) Haydarpasa Training Hospital, Istanbul, Turkey. The study was approved by the Institutional Ethical Board. All pathological specimens of patients diagnosed with lung cancer between January 2007 and December 2010 have been evaluated for Ang 2 immunohistochemical staining characteristics.

In the present study, lung cancer samples were collected through bronchoscopic biopsy, transthoracic needle aspiration biopsy, trucut biopsy, or thoracic surgery. Tissue specimens were evaluated in the Pathology Department.

TNM Classification of Malignant Tumors (T: tumor, N: lymph nodes, M: metastasis) was utilized for staging of the non-small-cell lung cancer (NSCLC) (International Lung Cancer Study Group in 2010), whereas small-cell lung cancer (SCLC) was classified according to the Veterans Administration Lung Cancer Group. Tumor size (T) and metastasis (M) were evaluated through Thorax CT, Thorax MRI, and bronchoscopy, whereas regional lymph node invasion was evaluated by Thorax CT, PET/CT, and mediastinoscopy. Distant metastasis (M) was evaluated with Ultrasonography, CT, scintigraphy, and PET/CT.

Pathological specimens were stained with Hematoxylin/eosine and saved in paraffin blocks. Specimens of 4 $\mu$ m section thickness were prepared from paraffin blocks and stained with Ang 2 (F-1): sc-74403 (Santa Cruz Biotechnology Inc., California, USA) antibody diluted with 1:50 ratio. Automated immunohistochemical device was used for staining Benchmark XT (Ventana Medical Systems, Inc., Arizona, USA).

Immunohistochemical staining of specimen was evaluated with light microscope (Olympus BX53). Staining intensity and expression rate of Tumor cells and stromal elements were evaluated by two experienced pathologists. The results were given when double agreement was reached.

Classification of Ang 2 staining intensity was defined as (0=absent, 1=low, 2=intermediate, 3=high), whereas classification of Ang 2 staining density was defined as (0=absent, 1=<1-2%, 2=between 2 and 10%, 3=>10% of the specimen area). Cytoplasmic staining of tumor cells and other cells in the stroma was scored as low, intermediate, or high. Expression rate in tumoral and stromal tissue was calculated as an arithmetical mean of staining intensity and density for tumor and stromal tissue. Ang 2 expression rate of at least 2.5 was considered as high expression.<sup>[4-6]</sup>

## Statistical analysis

Statistical analysis was performed with SPSS version 14 (SPSS Inc., Chicago, Illinois, USA). Descriptive analyses were defined as mean and standard deviation. Categorical variations (type and subtype of lung cancer, stage, T,N,M, diameter of tumor, staining intensity, Ang 2 expression rate in tumor, or stromal tissue) were defined as real numbers and percent in crosstabs. Spearman correlation was calculated between staining intensity and stage of tumor. Chi-square and Fisher test was used to analyze difference between groups.  $P=0.05$  was used as a cut-off value.

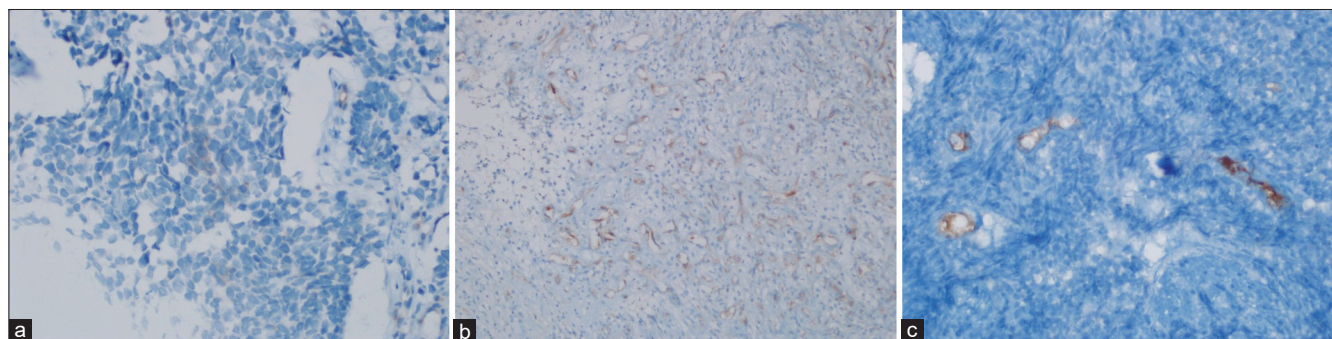
## Results

### Demographics

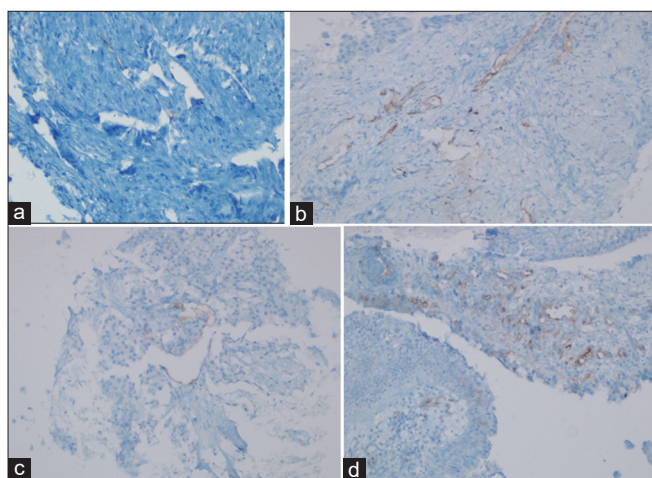
Fifty lung cancer patients were included in the study and 43 (86%) were men, whereas seven (14%) were women in sex. The mean age was 66.3 $\pm$ 9.9 years (age range 38–86 years).

**Table 1: Angiopoietin 2 immunohistochemical staining intensity and stage of tumor**

	No staining	Low staining	Intermediate staining	High staining	Total
Stage 1	[2] (50%)	-	[2] (50%)	-	[4] (100%)
Stage 2	-	[2] (33,3%)	[2] (33,3%)	[2] (33,3%)	[6] (100%)
Stage 3	[2] (13%)	[4] (27%)	[9] (60%)	-	[15] (100%)
Stage 4	[1] (4%)	[4] (16%)	[11] (44%)	[9] (36%)	[25] (100%)
Total	5	10	24	11	50



**Figure 1:** (a) Small cell lung cancer (SCLC), Low Angiopoietin 2 (Ang 2) immunohistochemical staining. (b) Squamous cell carcinoma, Intermediate Ang 2 immunohistochemical staining. (c) SCLC, High Ang 2 immunohistochemical staining.



**Figure 2:** (a) Squamous cell carcinoma, low stromal Angiopoietin 2 (Ang 2) expression. (b) Squamous cell carcinoma, high stromal Ang 2 expression. (c) Squamous cell carcinoma, low tumoral Ang 2 expression. (d) Squamous cell carcinoma, high tumoral Ang 2 expression.

Out of 50 cases 23 were squamous, 11 were adenocarcinoma, and 16 were SCLC.

### Histologic type and stage of tumor

Thirty-four of patients (68%) had NSCLC and 16 had (32%) SCLC. Out of 34 patients diagnosed with NSCLC, 23 (46%) had squamous, 11 (22%) had adenocarcinoma type. Out of 16 patients diagnosed with SCLC, 11 had (22%) advanced and five had (10%) limited disease.

Of patients diagnosed with lung cancer, 25 were (50%) at stage IV, 15 were (30%) at Stage III, six were (12%) at Stage II and four were (8%) at Stage I. Of patients with SCLC, five were (31%) at limited and 11 were (69%) at advanced stage of the disease.

### Characteristics of metastasis

Twenty six of patients (52%) had metastasis in which three had (11%) opposite lung, six had (21%) liver, six had (21%) brain, seven had bone, three had (11%) pleura, and three (11%) had renal involvement.

**Table 2: Stromal expression rate of Angiopoietin 2 immunohistochemical staining and histologic type of tumor**

	Low Stromal Expression	High Stromal Expression	Total
Adenocarcinoma	6 (%75)	2 (%25)	8 (%100)
SCLC	4 (%100)	0 (%0)	4 (%100)
Limited disease	3 (%27)	8 (%73)	11 (%100)
Advanced disease	8 (%36)	14 (%64)	22 (%100)

**Table 3: Tumoral expression rate of Angiopoietin 2 immunohistochemical staining and histologic type of tumor**

	Low Tumoral Expression	High Tumoral Expression	Total
Adenocarcinoma	8 (100%)	0 (0%)	8 (100%)
SCLC	4 (100%)	0 (0%)	4 (100%)
Limited disease	5 (45%)	6 (55%)	11 (100%)
Advanced disease	16 (73%)	6 (27%)	22 (100%)

SCLC, small cell lung cancer.

TNM characteristics of lung cancer cases were as follows: six patients (12%) had T1, 18 had (36%) T2, 16 had (32%) T3, 10 had (20%) T4, 12 had (24%) N0, six had (12%) N1, 25 had (50%) N2, seven had (14%) N3, 24 had (48%) M0, and 26 had (52%) M1.

### Ang 2 immunohistochemical staining intensity and correlation with stage, T,N, and M:

Five cases were negative, ten had (20%) low, 24 cases showed (48%) had intermediate, and 11 had (22%) high Ang 2 staining intensity. Ang 2 immunohistochemical staining intensity and Stage, T, N, and M were evaluated



and only Stage was found to be correlated ( $P=0.004$ ) as shown at [Table 1].

Examples of lung cancer tissue specimens of Ang 2 staining intensity are shown at [Figure 1].

### **Tumoral and stromal expression rate of Ang 2 immunohistochemical staining and the correlation between stage, T, N, M, and histologic type of tumor**

Examples of tumoral and stromal expression rate of Ang 2 immunohistochemical staining is shown at [Figure 2]. Both stromal and tumoral expression rate of Ang 2 immunohistochemical staining and type of tumor were insignificant ( $P=0.623$  and  $P=0.142$ ).

NSCLC subtypes were also analyzed. Stromal expression rate of Ang 2 immunohistochemical staining and subtype of tumor was correlated ( $P=0.021$ ).

Tumoral expression rate of Ang 2 immunohistochemical staining was also correlated with tumor subtype ( $P=0.033$ ).

Stromal and tumoral expression rates of Ang 2 staining in terms of lung cancer type were summarized in [Table 2] and [Table 3].

Spearman correlation were not significant between staining intensity and stage of tumor ( $P=0.57$ ).

## **Discussion**

Angiopoietins are growth factors that have effects on vascular maturation and stabilization.<sup>[4]</sup>

In this study, we investigated the cohort of lung cancer patients diagnosed in a tertiary level teaching hospital. Ang 2 expression rates at specimens of lung cancer tissue were studied with immunohistochemical staining. Correlation between tumor subtype, tumor stage, and the Ang 2 expression rate was also evaluated.

In the present study, we observed a correlation between stage and Ang 2 staining intensity ( $P=0.04$ ) in NSCLC lung cancer. However, analysis of T, N, and M separately with immunohistochemical staining was not significant. Interestingly, we observed low tumoral and stromal expression of Ang 2 in adenocarcinomas of lung cancer.

In a study by Hsueh C *et al.*,<sup>[7]</sup> VEGF and angiopoietins level were analyzed in papillary thyroid carcinoma and Ang 1, Ang 2, VEGF ve Tek/Tie-2 receptor expression rates were found significantly higher. Also in this study, the investigators found that Ang 1 expression was higher in patients with metastasis.

In a study by Lin Z *et al.*<sup>[8]</sup> angiogenetic regulators such as Ets-1, Ang 2, protease inhibitor maspinin, which play a role in angiogenesis were investigated in epithelial ovarian tumor tissue. No correlation was found between Ang 2 expression and pathologic parameters of ovarian cancer. Ang 2 expression was observed higher at stroma of tumor but no significant difference was observed between expression patterns of benign and malignant tissue.

Tomic TT *et al.*<sup>[9]</sup> studied effects of angiogenic modulators on vessel stabilization at tissue samples of patients with prostate cancer. Tumoral expression of Ang 2 and VEGF were investigated and were found to be elevated in patients with castration resistant prostate cancer.

Andersen S *et al.*<sup>[10]</sup> studied tumoral and stromal expression of Ang 2 levels of NSCLC patients and prognosis. Low stromal expression of Ang 2 was found to be correlated with poor prognosis. The demographics of our study group were similar to this study, however, our study included SCLC group, which was not studied by Andersen *et al.*

As a conclusion, angiogenesis is a necessary step for tumor progression to an advanced stage. Ang 2 as an angiogenic marker may not show tumoral growth alone but this is a complex and multifactorial process. Higher levels of Ang 2 expression is observed in advanced stages of lung cancer. We believe angiopoietins may be used as an option for targeted treatment in lung cancer. Studies are needed to investigate the role of Ang 2 in lung cancer, type, and subtype expression in addition to staging and prognosis.

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Nil

### **Conflicts of interest**

There are no conflicts of interest to declare.

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