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Original Article / Özgün Makale

Importance of TAK-1 levels in patients with non-small cell lung carcinoma

Küçük hücre dışı akciğer kanserli hastalarda TAK-1 düzeylerinin önemi

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ABSTRACT

Background: In this study, we aimed to investigate the relationship between survival, tumor dimension, grade and stage in respect to transforming growth factor-β-activating kinase (TAK-1) extensity, severity and total score in patients undergoing resection for Stage 1B-2B non-small cell lung cancer.

Methods: Between January 2000 and December 2014, a total of 70 patients (64 males, 6 females; mean age: 63.4+9.6 years; range, 32 to 78 years) who underwent surgery with resectable non-small cell lung cancer in Stage 1-2b were included. The patients were divided into two groups as Group 1 (n=35) consisting of patients with squamous cell carcinoma and Group 2 (n=35) consisting of patients with adenocarcinoma. The control group consisted of 20 patients (Group 3) who underwent surgery due to non-cancer causes. The relationship between TAK-1 staining (extensity, severity, total scores) and grade, survival time, T factor, N factor, and chemotherapy administration was examined. Pathology specimens of the patients were evaluated for the degree of staining with TAK-1 primary antibody.

Results: There was a strong correlation between the tumor grade and TAK-1 primary antibody staining level, independently from histopathological type. A significant correlation was found between dimension, stage, and TAK-1 staining in patients with squamous cell carcinoma. No statistically significant difference was found in the other factors, except for grade factor, in patients with adenocarcinoma.

Conclusion: The current study provides precious information about the effects of TAK-1, in clinicopathological behavior and survival of malignant cells, particularly in common histopathological types of lung cancer. We believe that our data can be useful, particularly in evaluating the response to targeted therapies and the prognosis of the disease.

Keywords: Extensity, lung cancer, severity, TAK-1, total scores.

ÖZ

Amaç: Bu çalışmada, Evre 1B-2B küçük hücreli dışı akciğer kanseri nedeniyle rezeksiyon yapılan hastalarda dönüştürücü büyüme faktörü-beta aktive edici kinaz (TAK-1) yaygınlığı, şiddeti ve total skoru açısından sağkalım, tümör boyutu, gradı ve evresi arasındaki ilişki incelendi.

Çalışma planı: Ocak 2000 - Aralık 2014 tarihleri arasında Evre 1-2b rezektabl küçük hücreli dışı akciğer kanseri nedeniyle ameliyat edilen toplam 70 hasta (64 erkek, 6 kadın; ort. yaş: 63.4+9.6 yıl; dağılım, 32-78 yıl) çalışmaya alındı. Hastalar, skuamöz hücreli kanserli olanlar Grup 1 (n=35) ve adenokarsinomlu olanlar Grup 2 (n=35) olmak üzere iki gruba ayrıldı. Kontrol grubu (Grup 3), kanser dışı nedenlerle ameliyat edilen 20 hastadan oluşuyordu. TAK-1 boyanma dereceleri, (şiddet, yaygınlık, toplam skor) ile grade, sağkalım süresi, T faktörü, N faktörü ve kemoterapi uygulaması arasındaki ilişki incelendi. Hastaların patoloji örnekleri, TAK-1 primer antikorunun boyanma derecesi açısından değerlendirildi.

Bulgular: Histopatolojik tipten bağımsız olarak, tümör gradı ile TAK-1 primer antikoru boyanma düzeyleri arasında güçlü bir ilişki bulundu. Skuamöz hücreli karsinomlu hastalarda tümör boyutu, evresi ve TAK-1 primer antikoru boyanma düzeyleri arasında anlamlı bir ilişki vardı. Adenokarsinomlu hastalarda ise, tümör gradı dışında diğer faktörler arasında istatistiksel olarak anlamlı bir fark saptanmadı.

Sonuç: Bu çalışma, TAK-1'in, özellikle yaygın histopatolojik akciğer kanseri tiplerinde, malign hücrelerin klinikopatolojik davranışı ve sağkalımı üzerindeki etkileri hakkında değerli bilgiler vermektedir. Verilerimizin, özellikle hedefe yönelik tedavilere yanıtın ve hastalığın prognozunun değerlendirilmesinde kullanışlı olabileceğini düşünmekteyiz.

Anahtar sözcükler: Yaygınlık, akciğer kanseri, şiddet, TAK-1, total skor.

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Angiogenesis plays an important role in the pathogenesis of non-small cell lung cancer (NSCLC). Increased lung tumor microvessel density is associated with the increased metastatic potential and decreased survival.[1-3]

Transforming growth factor (TGF)-β-activated kinase 1 (TAK-1), which is also found in angiogenesis, is one of the most recent molecules. It is a mitogen-activated protein kinase that is activated by the formation of a protein complex by various cytokines. It is a serine/threonine kinase and is associated with the progression of human cancer cells.[4] However, although there are many studies on other molecular markers in patients with lung cancer, very few studies have shown the relationship between TAK-1 positivity and prognosis.[5] In addition, in this study, the relationship between tumor development, progression and degree of invasion and TAK-1 in NSCLC patients was evaluated. However, the subtypes were not compared and difference of stage were not considered.[5]

In the present study, we aimed to investigate the relationship between histopathological grade, tumor size, pathological stage, and survival in terms of TAK-1 distribution, intensity, and total score in the pathology slides of the patients undergoing resection for Stage 1-2B squamous cell carcinoma (SCC) and adenocarcinoma (ADC).

PATIENTS AND METHODS

This single-center, retrospective study was conducted at the Department of Thoracic Surgery, Faculty of Medicine of Ondokuz Mayıs University between January 2000 and December 2014. A total of 610 patients who underwent surgical resection for NSCLC were evaluated. Thoracic computed tomography (CT), positron emission tomography (PET)-CT, cranial magnetic resonance imaging (MRI), and bronchoscopy were performed in all of patients for preoperative diagnosis and staging. If necessary, transthoracic needle biopsy was also performed for the diagnosis. If the patient had mediastinopathic pathological lymph node, mediastinoscopy was performed before resection. Finally, a total of 70 patients (64 males, 6 females; mean age: 63.4+9.6 years; range, 32 to 78 years) who were evaluated as Stage I a,b-II a,b NSCLC and underwent anatomic resection were included. Twenty patients who underwent wedge resection for non-cancer reasons (spontaneous pneumothorax) were included in the control group. Exclusion criteria were as follows: patients who received any specific treatment such as chemotherapy and radiotherapy before surgery, synchronous or metachronous tumors, positive mediastinal lymphadenopathy, could not be performed anatomical resection, Stage IIIa and above disease, having any lung disease such as pneumonia and abscess, or having any life-limiting disease such as chronic liver disease, renal failure, severe arrhythmia, ischemic heart disease, and collagen tissue disease.

The patients were divided into three groups. Group 1 consisted of 35 patients diagnosed with SCC, Group 2 consisted of 35 patients diagnosed with ADC, and Group 3 (control group) consisted of 20 patients who underwent surgery due to non-cancer causes. All patients in Group 3 underwent wedge resection for recurrent spontaneous pneumothorax. Staging was performed both via radiographical examinations preoperatively and via histopathological examinations postoperatively. Pre- and postoperative tumor stage was determined according to 7th Lung Cancer Tumor, Node, Metastasis (TNM) classification and staging system.[6] Histopathological evaluations were performed based on the diagnostic criteria proposed by the 2015 edition of the World Health Organization (WHO) classification system.[7]

All of patients in Groups 1 and 2 were followed with thoracic CT, cranial MRI, and PET-CT at 3, 6 and 12 months annually for five years. Follow-up data were obtained using the electronic medical records of our hospital and by phone calls.

Immunohistochemical staining

All samples were fixed in 10% buffered formalin for 24 h. All sections obtained from blocks belonging to these specimens were examined and, then, blocks

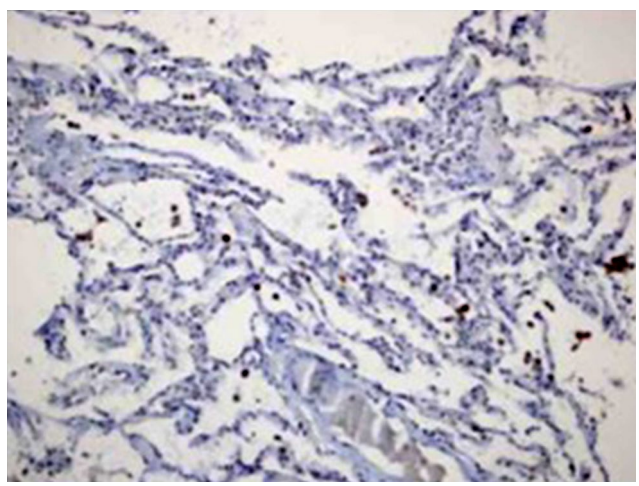


Figure 1. No staining with TAK-1 on control patients (×400).
TAK-1: Transforming growth factor (TGF)-β-activated kinase 1.

that best represented the invasive tumor were selected. Totally, 4-micron thick sections were obtained from these blocks, and these sections were studied immunohistochemically with TAK-1 primary antibody (anti-TAK 1 antibody, rabbit monoclonal antibody, ab196955, abcam) on the Ventana Benchmark XT2 (Roche Diagnostic, Solna, Sweden) automated staining instrument. The best dilution ratio was determined as 1/50 after various tests. As a positive control, the breast cancer specimen proposed by the company

Table 1. Overall characteristics of patients

	Adenocarcinoma (n=35)				Squamous cell carcinoma (n=35)			
	n	%	Median	Range	n	%	Median	Range
Age (year)			62.3	32-78			64.5	45-78
Sex								
Female	6	17.1			-	-		
Male	29	82.9			35	100.0		
Tobacco use (Pocket)								
0-20	13	37.1			6	17.1		
20-40	12	34.3			10	28.6		
40	10	28.6			19	54.3		
Surgical type								
Upper left lbt	4	11.4			11	31.4		
Upper right lbt	9	25.7			3	8.6		
Lower left lbt	6	17.1			6	17.1		
Lower right lbt	6	17.1			5	14.3		
Bilobectomy (LR)	1	2.9			3	8.6		
Bilobectomy (UR)	3	8.6			5	8.6		
Left pnt	2	5.7			1	2.9		
Right pnt	1	2.9			1	2.9		
Right middle lbt	3	8.6			0	0		
Grade								
High	8	22.9			11	31.4		
Medium	20	57.1			13	37.1		
Low	7	20.0			11	31.4		
Pathological stage								
1A	10	28.6			9	25.7		
1B	7	20.0			6	17.1		
2A	15	42.9			17	48.6		
2B	3	8.6			3	8.6		
T Factor, size								
T1A	4	11.4			2	5.7		
T1B	7	20.0			8	22.9		
T2A	15	42.9			16	45.7		
T2B	9	25.7			9	25.7		
N Factor								
N0	23	65.7			21	60.0		
N1	12	34.3			14	40.0		
Chemotherapy								
No	23	65.7			21	60.0		
Yes	12	34.3			14	40.0		
Survival								
Less than a year	3	8.6			2	5.7		
1-5 years	9	25.7			11	31.4		
More than 5 years	23	65.7			22	62.9		

lbt: Lobectomy; pnt: Pneumonectomy; LR: Lower right; UR: Upper right.

Table 2. TAK-1 distribution, intensity, overall score and grade for patients with ADC

	TAK-1															
	Distribution						**Intensity**						**Overall score**			
Test statistical value	χ^2= 12.863 Cramer's V=0.477						χ^2=11.487 Cramer's V=0.471						χ^2=13.256 Cramer's V=0.479			
Significance	p=0.005, p=0.002						p=0.012, p=0.003						p=0.003, p=0.002			
	11-25%		26-50%		>51%		Slight yellow		Dark yellow		Brown		No staining (0-2)		Mild (3-4)		Severe (5-6)	
Grade	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Low (n=8)	6	75.0	2	25.0	0	0.0	5	62.5	2	25.0	1	12.5	3	37.5	5	62.5	0	0.0
Medium (n=20)	3	15.0	13	65.0	4	20.0	1	5.0	9	45.0	10	50.0	0	0.0	10	50.0	10	50.0
High (n=7)	1	14.3	2	28.6	4	57.1	0	0.0	4	57.1	3	42.9	0	0.0	2	28.6	5	1.4

TAK-1: Transforming growth factor (TGF)-β-activated kinase 1; ADC: Adenocarcinoma.

was used. Findings were evaluated using the Leica HMLB45 (Leica, Germany, 2000) light microscope.[8] The distribution and intensity of staining were scored semi-quantitatively by taking cytoplasmic staining into account. The staining distribution was classified as follows: 0= between 0 and 10%, 1= between 11 and 25%, 2= between 26 and 50%, and 3= more than 50%. The staining intensity was classified as follows: 0= no staining (Figure 1), 1= slight yellowish staining, 2= dark yellowish staining, and 3= brown staining. The scores for the distribution and intensity of staining were collected, and they were classified as follows: negative (0) = between 0 and 2, mild (1) = between 3 and 4, and severe (2) = between 5 and 6. All pathological scorings were performed by two different pathologists.

Statistical analysis

Statistical analysis was performed using the SPSS version 11.0 software (SPSS Inc., Chicago, IL, USA). Continuous data were expressed in mean ± standard deviation (SD) or median (min-max), while categorical data were expressed in number and frequency. The assumptions of normality and of homogeneity of variances were checked by the Shapiro-Wilk test and the Levene's test. Then, the differences among the age and T measurements of the patients were assessed using the Student t-test according to the type of disease (SCC and ADC). The relationship between qualitative data presented in the cross-table and TAK-1 distribution, intensity and total score was assessed using the chi-square test and Cramer's V coefficient. A p value of <0.05 was considered statistically significant.

RESULTS

Baseline features of the patients are shown in Table 1.

Adenocarcinoma patient data for TAK-1

Pathological results for ADC patients showed that TAK-1 prevalence, intensity, and overall score were significantly different according to cancer grade (p=0.005). As the grade became higher, TAK-1 prevalence staining intensity and overall score of the tumor also increased (Table 2, Figure 2, 3a, b).

We found no correlation between survival and all variables of TAK-1 in ADC patients (p>0.05).

In addition, relationship between tobacco use, clinical and pathological stage, tumor size, mediastinal involvement, chemotherapy indication and TAK-1 staining properties were statistically non-significant (p>0.05).

Figure 2. Adenocarcinoma hematoxylin and eosin staining (H&E, ×400).

Figure 3. (a) Score 3 staining with ADC-TAK-1 primary antibody (×400). **(b)** Score 6 staining with ADC-TAK-1 primary antibody (×400).

ADC: Adenocarcinoma; TAK-1: Transforming growth factor (TGF)-β-activated kinase 1.

Table 3. TAK-1 distribution, intensity, overall score and grade for patients with SCC

	TAK-1															
	Distribution						**Intensity**						**Overall score**					
Test statistical value	χ^2= 21.815 Cramer's V=0.564 p=0.001, p=0.001						χ^2=19.465 Cramer's V=0.552 p=0.001, p=0.001						χ^2=22.098 Cramer's V=0.571 p=0.001, p=0.001					
Significance	11-25%		26-50%		>51%		Slight yellow		Dark yellow		Brown		No staining (0-2)		Mild (3-4)		Severe (5-6)	
Grade	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Low (n=11)	11	100	0	0,0	0	0,0	6	54.5	4	36.4	1	9.1	6	54.5	5	45.5	0	0.0
Medium (n=13)	2	15,4	9	69.2	2	15.4	0	0.0	2	15.4	11	84.6	0	0.0	4	30.8	9	69.2
High (n=11)	2	18,2	6	54.5	3	27.3	0	0.0	2	18.2	9	81.8	0	0.0	2	18.2	9	81.8

TAK-1: Transforming growth factor (TGF)-β-activated kinase 1; ADC: Adenocarcinoma.

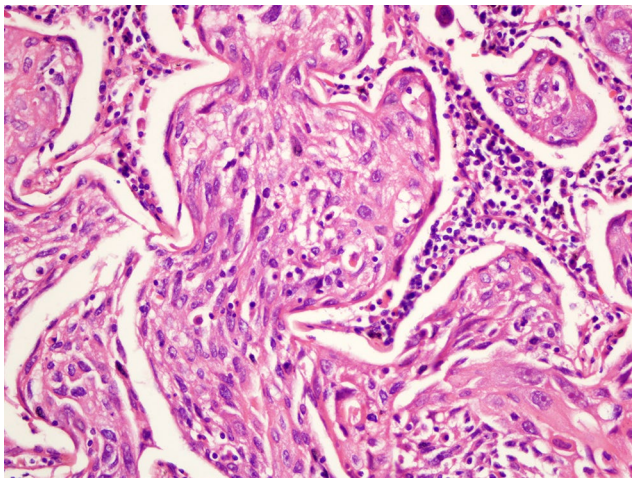


Figure 4. Squamous cell carcinoma hematoxylin and eosin staining (H&E, ×400).

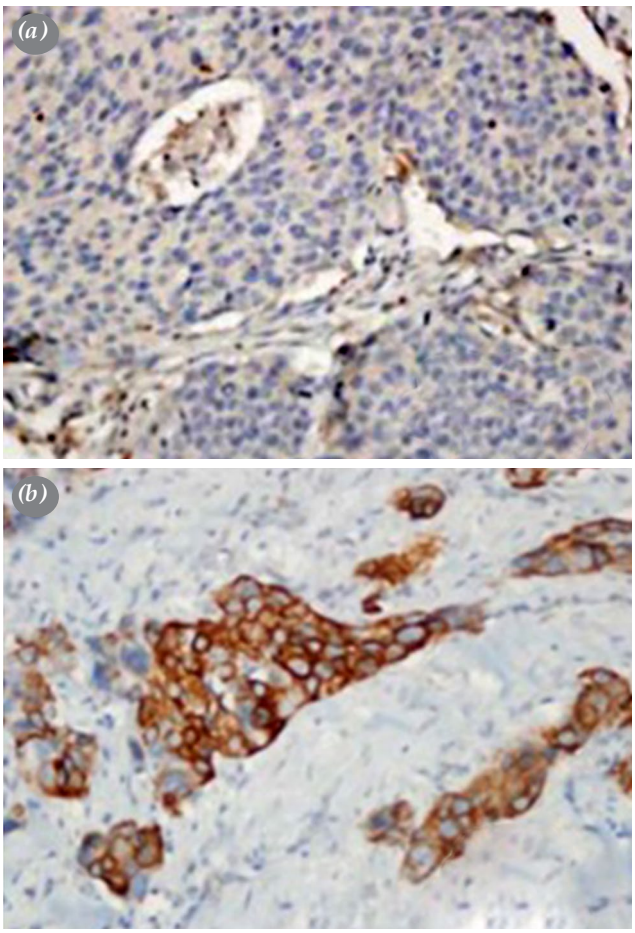


Figure 5. (a) Score 3 staining with SCC-TAK-1 primary antibody (×400). **(b)** Score 6 staining with SCC-TAK-1 primary antibody (×400).

SCC: Squamous cell carcinoma; TAK-1: Transforming growth factor (TGF)-β-activated kinase 1.

Squamous cell carcinoma patient data for TAK-1

The results for SCC patients showed that TAK-1 distribution, intensity, and overall score were significantly different according to tumor grade. As the grade became higher, TAK-1 prevalence staining intensity and overall score of the tumor increased (Table 3, Figure 4, 5a, b).

The TAK-1 distribution, intensity, and overall score values changed based on pathological stage subgroups (1A,1A,2A,2B). As the pathological stage increased, TAK-1 distribution, TAK-1 intensity and overall score increased (Table 4).

The relationship between TAK-1 distribution values and T factor subgroups (T1A, T1B, T2A and T2B) were statistically non-significant, while TAK-1 intensity and overall score showed a correlation with an increasing T factor ($p>0.05$) (Table 5).

Relationship between tobacco use, N factor subgroups, chemotherapy indication and TAK-1 staining properties were statistically non-significant ($p>0.05$). While we found no correlation with survival and TAK-1 an increase in T subgroup and grade resulted in statistically significantly less survival years (Table 6).

DISCUSSION

The TAK-1 is a serine/threonine kinase that contains ligands of TNF-alpha, interleukin (IL)-1 and tall-like receptor (TLR) and is a key role in proinflammatory cytokine release.[9-12]

Tumor growth and metastasis development depend on the presence of the appropriate vascular source (angiogenesis). The mechanism of angiogenesis involves multiple, sequential or mutually independent pathways. The TAK-1, a molecule that is investigated to make any contribution to cancer development and progression, has been reported to play multiple roles in angiogenesis and cancer progression. There are studies in the literature showing that TAK-1 can play different roles in various cancers by acting with a tumor suppressor effect.[13] Despite all these studies, there are few studies in the literature investigating the relationship between TAK-1 and lung cancer.[5] Zhu et al.[5] found that TAK-1 expression in patients with NSCLC significantly increased tumor stages, metastasis rates and lymph node positivity, and survival was shorter in these patients compared to other patients. However, in this study, patients with NSCLC were evaluated in general, and subtype and stage were not specified specifically. In our study, TAK-1 levels were evaluated in patients who had

Table 4. TAK-1 Distribution, intensity, overall score and pathological stage for patients with SCC

	TAK-1																	
	Distribution						**Intensity**						**Overall score**					
Test statistical value	χ^2= 14.008 Cramer's V=0.458						χ^2=16.724 Cramer's V=0.510						χ^2=16.799 Cramer's V=0.497					
Significance	p=0.009, p=0.017						p=0.002, p=0.005						p=0.002, p=0.007					
Pathological stage	11-25%		26-50%		>51%		Slight yellow		Dark yellow		Brown		No staining (0-2)		Mild (3-4)		Severe (5-6)	
Grade	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
1A (n=9)	8	88.9	1	11.1	0	0.0	4	44.4	4	44.4	1	11.2	4	44.4	5	55.6	0	0.0
1B (n=6)	1	16.7	4	66.6	1	16.7	0	0.0	1	16.7	5	83.3	0	0.0	1	16.7	5	83.3
2A (n=17)	4	23.5	10	58.8	3	17.6	2	11.8	1	5.9	14	82.4	2	11.8	3	17.6	12	70.6
2B (n=3)	2	66.7	0	0.0	1	33.3	0	0.0	2	66.7	1	33.3	0	0.0	2	66.7	1	33.3

TAK-1: Transforming growth factor (TGF)-β-activated kinase 1; SCC: Squamous cell carcinoma.

Table 5. Distribution, intensity, overall score of TAK-1 and T factor subgroups on patients with SCC

	TAK-1																	
	Distribution						**Intensity**						**Overall score**					
Test statistical value	χ^2= 9.264 Cramer's V=0.388						χ^2=24.815 Cramer's V=0.643						χ^2=22.099 Cramer's V=0.609					
Significance	p=0.099, p=0.099						p<0.001, p<0.001						p<0.001, p<0.001					
T factor	11-25%		26-50%		>51%		Slight yellow		Dark yellow		Brown		No staining (0-2)		Mild (3-4)		Severe (5-6)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
1A (n=2)	2	100.0	0	0.0	0	0.0	2	100.0	0	0.0	0	0.0	2	100.0	0	0.0	0	0.0
1B (n=8)	6	75.0	2	25.0	0	0.0	3	37.5	5	62.5	0	0.0	3	37.5	5	62.5	0	0.0
2A (n=16)	3	18.8	9	56.3	4	25.0	1	6.3	1	6.3	14	87.4	1	6.3	2	12.4	13	81.3
2B (n=9)	4	18.8	4	56.3	1	25.0	0	0.0	2	22.2	7	77.8	0	0.0	4	44.4	5	55.6

TAK-1: Transforming growth factor (TGF)-β-activated kinase 1; SCC: Squamous cell carcinoma.

Table 6. Distribution of grade, T factor and survival on patients with SCC

		Less then a year		1-5 years		More than 5 years	
Grade	n	n	%	n	%	n	%
Test statistical value χ^2=10.339							
Cramer's V=0.365							
Significance p=0.012, p=0.034							
Low	11	1	9.1	0	0.0	10	90.9
Medium	13	0	0.0	5	38.5	8	61.5
High	11	1	9.1	6	54.5	4	36.4
T Factor							
Test statistical value χ^2=13.044							
Cramer's V=0.471							
Significance p=0.012, p=0.012							
T1a	4	0	0.0	2	0.0	2	100.0
T1b	8	0	0.0	0	0.0	8	100.0
T2a	9	0	0.0	9	56.3	7	43.8
T2b	16	2	22.2	2	22.2	5	55.6

SCC: Squamous cell carcinoma.

SCC and ADC, separately. Our results support the literature. In addition, TAK-1 levels were found to be correlated with the tumor grade, pathological stage and T factor in SCC patients, whereas it was found to be positively correlated with tumor grade in patients with ADC. Furthermore, in our study, TAK-1 levels were not considered in only one parameter. The TAK-1 levels were evaluated histopathologically in terms of distribution, density, and overall score.

Activation of the TAK-1 signaling pathway enhances tumor development, invasion, and metastatic activities.[5,12,13] The TAK-1 has played an important role in the development of metastatic lesions in patients with breast, renal cell, and lung carcinomas. When TAK-1 was suppressed, there was a decrease in the bone-invading capacity and osteolysis of tumor cells in patients with breast cancer.[13,14] In the study of Zhu et al.,[5] prolonged TAK-1 activation in patients with lung cancer was effective in cancer progression, tumor development, and metastasis.[5] In our study, the slides were positively stained with TAK-1 in all patients with tumors regardless of grade, tumor size, and histopathological type. When the pathology slides of 20 patients, who were considered as the control group, undergoing surgery for benign lung diseases were examined, the TAK-1 was poorly stained at all variables. Both results of the literature and our study indicate that TAK-1 is strongly associated with tumorigenesis.

Futrhermore, TAK-1 has a major role in cigarette smoke-induced IL-8 release in airway smooth muscle cells and contributes to the development and progression of chronic obstructive lung disease and its associated lung cancer.[15,16] We found that there was no statistically significant relationship between smoking status and TAK-1 distribution, intensity, and total score in patients with lung cancer. However, considering all scores related to TAK-1, almost all parameters were closely associated with TAK-1 in patients with SCC. This suggests that smoking may potentiate the effects of TAK-1 positivity on tumorigenesis.

The TAK-1 positivity has been shown to have a positive effect on tumor growth rate. Cai et al.[17] reported that tumor growth rate was two-fold greater in TAK-1 positive ovarian cancer cells than in the control group. We found that there was no significant relationship between tumor size and TAK-1 distribution, intensity, and total score in patients with ADC. However, TAK-1 staining intensity increased, as the tumor size increased in patients with SCC. It was also observed that the mean survival time

decreased as tumor size increased in patients with SCC. As a result, it may be possible to conclude that survival decreased as TAK-1 staining intensity increases, considering that TAK-1 staining intensity increases as the tumor size increases in patients with SCC. When TAK-1 staining distribution was assessed in patients with ADC, there was a decrease in the number of patients who lived longer than five years as TAK-1 staining distribution and intensity increased. Although this result was not statistically significant, we believe that the fact that the number of patients was small and advanced cancer patients were not included in this study may have led to a non-significant relationship between TAK-1 staining distribution and survival.

Histopathological evaluation of tumor cells in the literature showed that TAK-1 staining distribution and intensity of tumor cells increased progressively from low-grade ovarian cyst ADC to high-grade ovarian cyst ADC.[17] In our study, TAK-1 distribution, intensity, and total score were found to increase significantly with increasing tumor grade regardless of histopathological type. Moreover, while comparing tumor grade with survival time, no significant difference was observed in patients with ADC; however, survival time decreased significantly, as the tumor grade increased in patients with SCC. In addition, there was no statistically significant increase in patients with ADC, when the pathological stages of the patients were compared in terms of all TAK-1 variables. However, TAK-1 staining distribution and intensity increased, as the tumor stage progressed from Stage 1A to Stage 2B in patients with SCC.

The study of Carbone and Melis[18] showed that the tumor burden was reduced, the effect of chemotherapy was potentiated, and the survival rate increased thanks to genetic suppression of TAK-1 expression. While we found no correlation with survival and TAK-1, increase in T subgroup and grade resulted in statistically significantly less survival years. Although no significant difference between survival and TAK-1 was found in our study, it is thought that TAK -1 affects indirectly survivor through variables such as grade and T factor based on the relationship between T-factor and grade and TAK-1. In addition, it is believed that our results depend on the fact that our study was performed on early stage patients who had high survival rates.

In the literature, TAK-1 kinase selective inhibitors have been shown to increase anti-tumor activity of chemotherapeutics. Gene-targeted cancer therapy has given promising results in improving survival and quality of life in advanced and high-grade tumors. In recent years, there is a significant increase in the number of studies on targeted therapies in NSCLC and colon cancer.[18-20] Our study supports the data in the literature. Moreover, it is planned to examine both TAK-1 activity and the effects of chemotherapeutics on TAK-1 activity in further studies to be performed on advanced cancer patients who require postoperative adjuvant CT in our clinic. However, there was no significant difference in terms of TAK-1 distribution, intensity and total score in patients with N1 node-positive NSCLC who received chemotherapy after resection. We believe that this may be due to the fact that the cancer stages of the patients included in the study were low and they had no metastatic cancer.

While considering that an increase in the stage of lung cancer has a negative effect on five-year survival, TAK-1 staining distribution and intensity negatively affected survival rate in patients with SCC as in ovarian cancer and pancreatic cancer in the literature. Lam et al.[21] reported that TAK-1 caused an increase in the transition from normal cells to invasive cells by increasing free oxygen radicals in the SCC cells in the skin after subcutaneous injection. In our study, according to histopathological types of the tumor, no relationship was established in terms of TAK-1 distribution, intensity, and total score. Although there was a strong correlation with the levels of TAK-1 in patients with SCC, there was a significant relationship with only tumor grade in patients with ADC. Considering that TAK-1 is effective on angiogenesis of the tumor, this situation may be explained by the fact that ADCs usually use the lymphatic pathways for metastasis.

In conclusion, the TAK-1 has been one of the most popular targeted therapies in recent years. There are many *in vitro* studies on TAK-1. These studies have provided valuable information on the immunohistochemical effects of TAK-1. Therefore, we believe that our study would provide valuable information about the effects of TAK-1 on pathological clinical behavior and survival in cancer cells, particularly in the most common types of lung cancer. We consider that valuable information on targeted therapies, particularly in lung cancer, would be obtained thanks to future studies to be performed on this target molecule. Moreover, it is thought that it can provide very important information in the arrangement of the treatment plan, the identification of chemotheraphy, radiotherapy, chemoradiotherapy and targeted therapies, and the evaluation of the prognosis of the disease.

Ethics Committee Approval: The study protocol was approved by the Ondokuz Mayıs University Faculty of Medicine Clinical Research Ethics Committee (date: 28.11.2014, no: 2014/875PYO.TIP.1904.15.0.16). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient Consent for Publication: A written informed consent was obtained from each patient.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Alberg AJ, Brock MV, Ford JG, Samet JM, Spivack SD. Epidemiology of lung cancer: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 2013;143(5 Suppl):e1S-e29S.
2. Nowak-Sliwinska P, Alitalo K, Allen E, Anisimov A, Aplin AC, Auerbach R, et al. Consensus guidelines for the use and interpretation of angiogenesis assays. Angiogenesis 2018;21:425-532.
3. Viallard C, Larrivée B. Tumor angiogenesis and vascular normalization: Alternative therapeutic targets. Angiogenesis 2017;20:409-26.
4. Taniguchi F, Harada T, Miyakoda H, Iwabe T, Deura I, Tagashira Y, et al. TAK1 activation for cytokine synthesis and proliferation of endometriotic cells. Mol Cell Endocrinol 2009;307:196-204.
5. Zhu J, Li Q, He JT, Liu GY. Expression of TAK1/TAB1 expression in non-small cell lung carcinoma and adjacent normal tissues and their clinical significance. Int J Clin Exp Pathol 2015;8:15801-7.
6. Mirsadraee S, Oswal D, Alizadeh Y, Caulo A, van Beek E Jr. The 7th lung cancer TNM classification and staging system: Review of the changes and implications. World J Radiol 2012;4:128-34.
7. Travis WD, Brambilla E, Nicholson AG, Yatabe Y, Austin JHM, Beasley MB, et al. WHO Panel. The 2015 World Health Organization Classification of Lung Tumors: Impact of Genetic, Clinical and Radiologic Advances Since the 2004 Classification. J Thorac Oncol 2015;10:1243-60.
8. Sullu Y, Demirag GG, Yildirim A, Karagoz F, Kandemir B. Matrix metalloproteinase-2 (MMP-2) and MMP-9 expression in invasive ductal carcinoma of the breast. Pathology, Research and Practice 2011;207:747-53.
9. Yamaguchi K, Shirakabe K, Shibuya H, Irie K, Oishi I, Ueno N, et al. Identification of a member of the MAPKKK family as a potential mediator of TGF-beta signal transduction. Science 1995;270:2008-11.
10. Shirakabe K, Yamaguchi K, Shibuya H, Irie K, Matsuda S, Moriguchi T, et al. TAK1 mediates the ceramide signaling to stress-activated protein kinase/c-Jun N-terminal kinase. J Biol Chem 1997;272:8141-4.
11. Sakurai H, Miyoshi H, Toriumi W, Sugita T. Functional interactions of transforming growth factor beta-activated kinase 1 with IkappaB kinases to stimulate NF-kappaB activation. J Biol Chem 1999;274:10641-8.
12. Santoro R, Carbone C, Piro G, Chiao PJ, Melisi D. TAK-ing aim at chemoresistance: The emerging role of MAP3K7 as a target for cancer therapy. Drug Resist Updat 2017;33-35:36-42.
13. Muraoka-Cook RS, Dumont N, Arteaga CL. Dual role of transforming growth factor beta in mammary tumorigenesis and metastatic progression. Clin Cancer Res 2005;11:937s-43s.
14. Shin EM, Hay HS, Lee MH, Goh JN, Tan TZ, Sen YP, et al. DEAD-box helicase DP103 defines metastatic potential of human breast cancers. J Clin Invest 2014;124:3807-24.
15. Safina A, Sotomayor P, Limoge M, Morrison C, Bakin AV. TAK1-TAB2 signaling contributes to bone destruction by breast carcinoma cells. Mol Cancer Res 2011;9:1042-53.
16. Ahmed N, Zeng M, Sinha I, Polin L, Wei WZ, Rathinam C, et al. The E3 ligase Itch and deubiquitinase Cyld act together to regulate Tak1 and inflammation. Nat Immunol 2011;12:1176-83.
17. Cai PC, Shi L, Liu VW, Tang HW, Liu IJ, Leung TH, et al. Elevated TAK1 augments tumor growth and metastatic capacities of ovarian cancer cells through activation of NF-κB signaling. Oncotarget 2014;5:7549-62.
18. Carbone C, Melisi D. NF-κB as a target for pancreatic cancer therapy. Expert Opin Ther Targets 2012;16 Suppl 2:S1-10.
19. Zhang Y, Mi X, Song Z, Li Y, YingShi, Niu J. Cripto-1 promotes resistance to drug-induced apoptosis by activating the TAK-1/NF-κB/survivin signaling pathway. Biomed Pharmacother 2018;104:729-37.
20. Vucur M, Roderburg C, Bettermann K, Tacke F, Heikenwalder M, Trautwein C, et al. Mouse models of hepatocarcinogenesis: What can we learn for the prevention of human hepatocellular carcinoma? Oncotarget 2010;1:373-8.
21. Lam CR, Tan C, Teo Z, Tay CY, Phua T, Wu YL, et al. Loss of TAK1 increases cell traction force in a ROS-dependent manner to drive epithelial-mesenchymal transition of cancer cells. Cell Death Dis 2013;4:e848.