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RESEARCH / ARAŞTIRMA

# Association Angiotensin Converting Enzyme-2 with Lung Cancer

## *Akciğer Kanseri ve Anjiotensin Dönüştürücü Enzim-2 İlişkisi*

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**Abstract**

**Objective:** The lung is one of the organs that contain the most angiotensin-converting enzyme 2 (ACE-2) receptor. There are very few studies in the literature showing the association between ACE-2 expression and lung cancer. This study aims to investigate the expression of ACE-2 in lung cancer (adenocarcinoma and squamous cell lung carcinoma) and healthy lung tissue.

**Material and Method:** ACE-2 receptors were studied by immunohistochemistry (IHC) method in 67 patient tissues diagnosed between 2013 and 2014 in the Bioarchive of Dokuz Eylul University Department of Basic Oncology. ACE-2 expressions were evaluated under a light microscope. Expression values were expressed as percentages (%). Clinical findings and ACE-2 expression results were evaluated with the statistical method over $p<0.05$. The Kaplan-Meier test evaluated the effect of ACE-2 expression on lung cancer survival.

**Results:** We showed that ACE-2 expression was increased in lung cancer tissues compared to healthy tissues. 2.24±6.39 (95% CI: 1.85 – 8.39) in healthy tissue, 9.52 ±17.14 (95% CI: 5.90 – 14.07) in tumor tissue (Wilcoxon test; p=0.049). ACE2 was highly expressed in lung squamous cell carcinoma (LUSC) (p=0.002). (Healthy tissue: 2.31%±7.198%, Tumor tissue: 15.06±21.139%). ACE-2 expression was higher in advanced lung adenocarcinoma cases compared to early-stage cases. There was no significant correlation between ACE-2 and EFS (event-free survival) and OS (overall survival).

**Conclusion:** ACE-2 expression was significantly increased in lung cancer tumor tissue compared to healthy tissue. Furthermore, increased ACE-2 expression in squamous cell lung cancer and advanced-stage lung adenocarcinoma compared to early-stage led us to suggest it can potentially be a diagnostic or prognostic lung cancer biomarker. However, more scientific studies are needed.

**Keywords:** ACE-2, lung cancer, lung squamous cell carcinoma, lung adenocarcinoma, immunohistochemistry.

**Öz**

**Amaç:** Akciğer, Anjiotensin konverting enzim 2 (ACE-2) reseptörünü en çok bulunduran organlardan birisidir. Literatürde ACE-2 ekspresyonunakciğer kanseri ile ilişkisini gösteren çok az sayıda çalışma bulunmaktadır. Bu çalışmanın amacı, akciğer kanserinde (adenokarsinom ve skuamöz hücreli akciğer karsinomunda) ve sağlıklı akciğer dokusunda ACE-2 ekspresyon düzeyini araştırmaktır.

**Gereç ve Yöntem:** Dokuz Eylül Üniversitesi Temel Onkoloji Anabilim Dalı Biyoarşivinde bulunan 2013-2014 yılları arasında tanı almış 67 hasta dokusunda immünohistokimya (IHC) yöntemiyle ACE-2 reseptörleri çalışılmıştır. Ekspresyon yüzde (%) olarak ışık mikroskobunda değerlendirilmiştir. Klinik bulgular ile ACE2ekspresyonu sonuçları istatistiksel yöntemle $p<0,05$ üzerinden değerlendirilmiştir.

**Bulgular:** Normal doku ile kıyaslandığı zaman akciğer kanserli dokularda ACE-2 ekspresyonunun arttığını kanıtladık. Sağlıklı akciğer dokusunda ortalama ACE-2 ekspresyon yüzdesi %2.24±6.39 (%95 CI: 0.68 – 3.80), tümör dokusunda ortalama ACE-2 ekspresyon yüzdesi 9.52 ±17.14 (%95 CI: 5.34– 13.70) idi, tümör dokusunda ACE-2 ekspresyonunda anlamlı artış gözlendi (p=0.049). Tümör dokusunda ACE-2 ekspresyonu skuamöz hücreli akciğer kanserinde sağlıklı dokuya göre anlamlı olarak arttı (p=0.002) (Normal dokuda: %2.31 ±7.198, Tümöral dokuda: %15.06±21.139). ACE-2 ekspresyonunun ileri evre akciğer adenokarsinomu olgularında erken evredeki olgulara göre daha yüksek olduğu saptanmıştır. ACE-2 ile EFS (Event-Free Survival; Hastalıksız Sağkalım) ve OS (overall Survival; Genel Sağkalım) arasında anlamlı bir ilişki gösterilememiştir.

**Sonuç:** ACE-2 ekspresyonu akciğer kanseri tümör dokusunda sağlıklı dokuya kıyasla önemli ölçüde artmıştır. Ayrıca, skuamöz hücreli akciğer kanseri ve ileri evre akciğer adenokarsinomunda erken evreye kıyasla artmış ACE-2 ekspresyonu, potansiyel olarak tanısal veya prognostik bir akciğer kanseri biyobelirteci olabileceğini düşünmemize yol açtı. Ancak daha fazla bilimsel çalışmaya ihtiyaç vardır.

**Anahtar Kelimeler:** ACE-2, akciğer kanseri, skuamöz hücreli akciğer kanseri, akciğer adenokarsinom, immunhistokimya.

## 1. Introduction

Lung cancer is one of the most common cancers worldwide and is the leading cause of cancer-related death (1, 2). Angiotensin-converting enzyme-2 (ACE-2) is a zinc-containing metalloprotease and is a single-pass type I glycoprotein enzymatically located in the cell membrane (3). ACE-2 is expressed in many tissues, including the brain, intestines, kidneys, heart, lungs, testes, placenta, thyroid gland, liver, pancreatic adipose tissue, arterial and venous endothelial cells, and arterial smooth muscle cells (3, 5). The lungs are the organs that contain one of the most ACE-2 receptors. In the lungs, ACE-2 is located in the nasal mucosa, bronchi, ciliated epithelial cells, goblet cells, type I and II alveolar pneumocytes, endothelial cells of large and small blood vessels, and smooth muscle cells (4).

The essential function of ACE-2 is a physiological counterbalance to the angiotensin-converting enzyme (ACE), providing homeostatic regulation of circulating angiotensin II (Ang II) levels. It is a negative regulator of the renin-angiotensin system (RAS) (3-5, 9). Besides, ACE-2 facilitates amino acid transport and is an intracellular entry receptor for SARS-CoV and SARS-CoV-2 viruses (4, 7).

ACE-2 is an essential enzyme in angiotensin metabolism. ACE-2 is a crucial regulator within the RAS and counteracts with ACE in two ways: Either it metabolizes the vasoconstrictive and pro-inflammatory Ang 1-8 directly to generate the vasodilatory and anti-proliferative Ang 1-7, a counter-regulatory enzyme to ACE, or it competes for Ang 1-10 with ACE to form Ang 1-9, a precursor of Ang 1-7. ACE-2 promotes vasodilation while reducing the effects of Ang-II. It provides diuresis, natriuresis. In addition, it protects the cell with its anti-inflammatory, anti-oxidant, anti-fibrotic, anti-proliferative, and anti-thrombotic effects (6).

Studies have shown an increase in the number of ACE-2 receptors on the cell surface in smokers. The increase in ACE-2 levels not only paves the way for viral infection but also suppresses immunity with the effect of smoking (10,11). In addition to high ACE-2 expression in smokers, there is an increase in secretory cells and inflammatory signaling (10,11).

Studies indicate that ACE-2 is expressed more in tumor tissue than healthy tissue in lung cancer, but it does not differ in histopathological subtypes. In the study of Zang et al., ACE-2 expression in lung adenocarcinoma and squamous cell lung cancer tissues did not differ significantly. It was also stated that there was no significant relationship with the stage of the disease (8). This study also showed DNA methylation deviation of ACE-2 in lung tumors and stated that it might be one of the primary mechanisms leading to increased ACE-2 expression (8).

Besides DNA methylation, upregulation of different epigenetic factors such as HAT-1 (Histone acetyl transferase-1), HDAC-2 (Histone deacetylase -2), and KDM5B (lysine demethylase 5B) triggers ACE-2 transcription (11,12,13). Furthermore, transcription factors (TF) that bind to the promoter region of the ACE-2 gene induce ACE-2 expression, especially in lung cancer. These TFs are BCL6 (B-cell lymphoma 6), WT1 (Wilms' tumor protein), STAT3, YY1 (Yin Yang-1), AREB6, ERG, GKLF (or KLF4), and GATA2 (13,14,15). All these cancer-promoting transcription factors are highly expressed in lung tumors and can directly stimulate the transcription of ACE-2 during different stages of lung tumor progression (13, 16).

In this study, we aimed to investigate the differences in ACE-2 protein expression in lung cancer patients' healthy and tumor tissue samples by immunohistochemical staining. We also aimed to reveal the differences in ACE-2 protein expression in squamous cell lung cancer and lung adenocarcinoma and to investigate the relationship between ACE-2 expression and disease-free survival and overall survival in lung cancer patients.

## 2. Material and method

### 2.1. Study Population

This study was a cross-sectional descriptive study. The study protocol was reviewed and approved by the Dokuz Eylül University Faculty of Medicine Non-İnterventional Ethic Committee (12/01/2022; 2022/02-15). Sixty-seven (67) case tissues in our bio-archive diagnosed with lung cancer between 2013 and 2014 were included in the study. ACE-2 receptors were stained with DAB (diaminobenzidine) by the immunohistochemistry (IHC) method. Expression was evaluated under the light microscope and expressed as a percentage (%). The clinical findings of the cases were obtained from the Dokuz Eylul University Faculty of Medicine case registry data system. Considering the security of personal data of the cases, age, gender, smoking history, histopathological tissue diagnosis of the disease, stage of the disease, survival of the cases, and the presence of progression, metastasis, or recurrence in the follow-up period were recorded. Death, progression, metastasis, or relapse of the case were considered "events". Diagnosis, stage and "event" were statistically compared with ACE-2 expression.

### 2.2. Immunohistochemistry Protocol

In immunohistochemistry, Fresh tissue or cell smears were first fixed with methanol and formol and then treated with HO. Then, inhibitory CM, primary antibody ACE-2 (Abcam), and secondary antibody (Universal HRB) were inoculated, respectively. It was stained with diaminobenzidine (DAB) and counterstained with hematoxylin. They were dehydrated to rising levels of alcohol (ethanol), cleared in xylol, and coated with Italian, and the positivity rate of over 100 cells was evaluated under the light microscope (Figure 1).

### 2.3. Statistical Analysis

Statistical analyses were performed with SPSS version 22.0 (IBM SPSS Statistics, USA) software. Data are summarized with percent distribution, mean ± standard deviation, and 95% confidence interval. The conformity of the variables to the normal distribution was examined using analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Student-T test, Mann-Whitney U test, and Wilcoxon test were used according to the normal distribution of the variables. Variables indicated by count were compared with the Chi-Square or Fisher Exact test. $p<0.05$ values were accepted as significant. Survival analysis was evaluated with Kaplan Meier.

### 2.4. Ethical Aspect of the Research

This study protocol was reviewed and approved by the Dokuz Eylul University Faculty of Medicine İnterventional Ethic Committee (12/01/2022; 2022/02-15).

The material used in the study is taken from the bio-archive of the Basic Oncology Department. Consent was obtained from the patients and their relatives who could be reached. For patients sampled before 19 August 2011, the samples of deceased patients whose legal representatives could not be reached were considered anonymous, and consent was not obtained.

## 3. Results

Lung cancerous and normal tissues of 67 cases were included in the study. Cancer tissues of the 67 cases included in the study consisted of surgical biopsy material 83.6% (n=56), bronchoscopic biopsy material 11.9% (n=8), and mediastinal lymph tissue samples 4.5% (n=3). The age average was 62.88 ± 8.70 (Min. 47 years, Max. 87 years). 16.4% (n=11) were female, and 81.2% (n=56) were male. 92.5% (n=62) had a history of smoking. 46.3% (n=31) were adenocarcinoma (LUAD), and 53.7% (n=36) were squamous cell carcinomas (LUSC). The cases were re-evaluated according to the 8th TNM. Early and advanced stages were evaluated as two groups starting from stage IIIA. It was classified as the early stage before stage IIIA (55.2% (n=37)) and advanced stage from IIIA and above (44.8% (n=30)) (Table 1). 29.9% (n=20) were alive and 70.1% (n=47) were dead. The oncological treatments of the cases are shown in Table 4. Tissue samples were obtained at the time of diagnosis, prior to treatment.

***Table 1. Demographic Data***

| **Dermographic Data** | | **n** |
|---|---|---|
| Sex | Male | 56 |
| | Female | 11 |
| Age (Mean) | | 62.8 ± 8.7 |
| Smoking History | | 62 |
| Adenocarcinoma | | 31 |
| Squamous Cell Carcinoma | | 36 |
| Early Stage (<III A) | | 37 |
| Advanced Stage | | 30 |

ACE-2 expression in tumor tissue was examined under a light microscope with IHC. ACE-2 expression levels in healthy and tumor tissue are presented as percentage (%) values (Table 2). It was observed that ACE-2 expression increased in lung cancer tissues compared to healthy tissue. The mean value of ACE-2 in healthy lung tissue was 2.24%±6.39% (95% CI: 0.68 – 3.80), while the mean value of ACE-2 in tumor tissue was 9.52 ±17.14 (95% CI: 5.34 – 13.70), a significant increase in ACE-2 expression was observed in tumor tissue (p=0.049). ACE-2 expression in tumoral tissue significantly increased in squamous cell lung cancer compared to healthy tissue (p=0.002). In the lung adenocarcinoma group, we found no statistically significant difference in ACE-2 expressions between tumor and healthy tissue (p=0.461).

***Table 2. ACE-2 Expression in Tumor and Healthy Tissues***

| | **Mean** | **Std. Dev.** | **%95 CI** | **p** |
|---|---|---|---|---|
| ACE-2 eks. % in Healthy Tissue | 2.24 | 6.39 | 0.68 – 3.80 | **p=0.049** |
| ACE-2 eks. % in Tumoral tissue | 9.52 | 17.141 | 5.34 – 13.70 | |
| ACE-2 eks. % in Healthy Tissue; LUSC | 2.31 | 7.198 | 0.13 – 4.74 | **p=0.002** |
| ACE-2 eks. % in Tumoral Tissue; LUSC | 15.06 | 21.139 | 7.9 – 22.21 | |
| ACE-2 eks. % in Healthy Tissue; LUAD | 2.16 | 5.429 | 0.17 – 4.15 | p=0.461 |
| ACE-2 eks. % in Tumoral tissue; LUAD | 3.10 | 6.759 | 0.62 – 5.58 | |

LUSC: squamous cell lung cancer, LUAD: lung adenocarcinoma

There was no difference in ACE-2 expression in healthy tissues of adenocarcinoma and squamous cell lung cancer cases (p=0.09). When tumor tissues were compared, ACE-2 expression was significantly higher in squamous cell lung cancer than in adenocarcinoma (p=0.003) (Table 2). Because the mean ACE-2 expression value in the tumor tissue was 9.52% ± 17.14, the cases were evaluated in two groups with ACE-2 expression <10% and ≥10%. Similarly, the percentage of ACE-2 expressed was significantly higher in squamous cell lung cancer tissue than in adenocarcinoma. In adenocarcinoma, ACE-2 expression in tumor tissue was <10% in 83.9% (n=26) cases, and ACE-2 expression in tumor tissue was ≥10% in 16.1% (n=5) cases. In squamous cell lung cancer, ACE-2 expression in tumor tissue was <10% in 55.6% (n=22) cases, and ACE-2 expression in tumor tissue was ≥10% in 44.4% (n=16) cases (p=0.018). No significant correlation was observed in the tumor tissue between ACE-2 expression and gender (p=0.424) or age (p=0.305). There was no significant association between ACE-2 and overall survival (OS) (p=0.319) in tumor tissue, including adenocarcinoma (p=0.385) and squamous cell lung cancer cases (p=0.451).

According to the Kaplan-Meier test, there was no statistically significant difference between lung adenocarcinoma and lung squamous cell carcinoma regarding EFS (p=0.363) or OS (p=0,443). In lung squamous cell carcinoma, there was no statistically significant association between ACE-2 expressions with OS (p=0.694) (Graphic 1) or EFS (p=0.893) (Graphic 2). In lung adenocarcinoma, there was no statistically significant association between ACE-2 expressions and OS (p=0.597) (Graphic 3) and EFS (p=0.739) (Graphic 4).

The EFS was 51.5±5.5 months (40.6-62.5; 95%CI). The OS was 56.1±5.3 months (45.5-66.6; 95%CI). In adenocarcinoma, OS was 50.06±7.11Months (36.1-64.0 %95CI), and EFS was 43.4±7.6 months (28.4±58.4 %95CI). In squamous cell carcinoma, OS 60.7±7.7 (45.6-75.8 %95CI), EFS 57.9±7.7 (42.7-73.1 %95%CI).

No significant correlation exists between early or advanced stage and ACE-2 expression in lung cancer tissue (p=0.131). When the ACE-2 expression and the stage of the disease were compared in the adenocarcinoma cases in the tumor tissue, we found that ACE-2 expression in the tumor tissue was higher in the advanced-stage than in the early-stage (p=0.027). There was no significant difference between the stage of the disease and ACE-2 expression in squamous cell lung cancer (p=0.911) (Table 3).

***Table 3. ACE-2 Expression Regarding Stages in LUAD and LUSC***

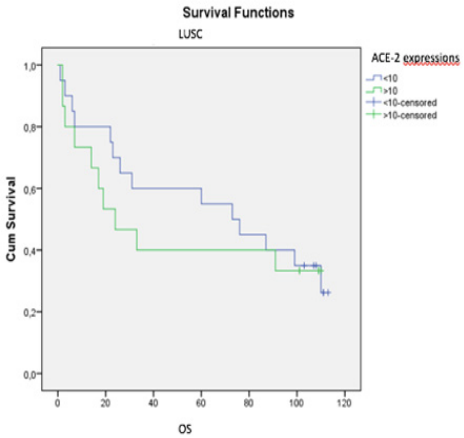
| Histopathological Diagnosis | ACE-2 Eks. in early stage (%) | ACE2 Eks. % in advance stage (%) | p |
|---|---|---|---|
| LUAD | 12.30 | 19.47 | **0.027** |
| LUSC | 18.34 | 18.75 | 0.911 |

Association between the stage of the disease and ACE-2 expression in histopathological diagnosis. LUSC: squamous cell lung cancer, LUAD: lung adenocarcinoma

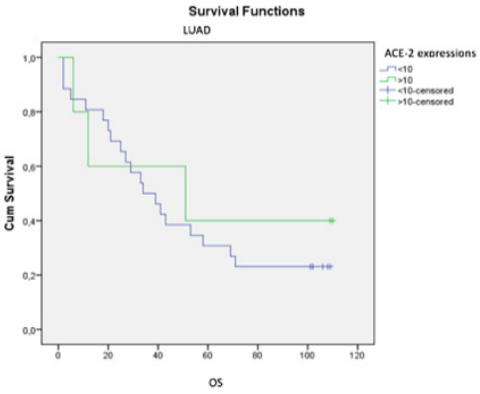
No association existed between the histopathological diagnosis and the event (metastasis, progression, relapse, death) (p=0.788). There was no correlation between histopathological diagnosis and survival (p=0.547). There was a significant association between the early or advanced stage and the event (p=0.016). In the early stage, there were no events in 40.5% (n=15) cases, but the event was observed in 59.5 % (n=22). In the advanced stage, there was no event in 13.3% (n=4) of the cases, while at least one event was observed in 86.7% (n=26). There was a significant association between early or advanced stage and survival (p=0.034). 83.3% (n=25) were dead in the advanced stage.

***Tablo 4. Oncologic Treatment***

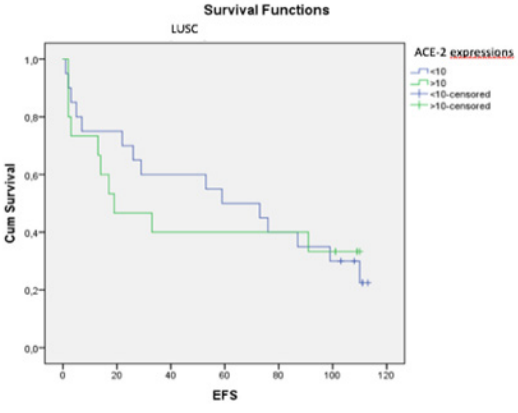
| | Frequency (n) | Percent (%) |
|---|---|---|
| No treatment received | 28 | 41.8 |
| cisplatin-vinorelbine | 22 | 32.8 |
| carboplatin-gemcitabine | 3 | 4.5 |
| paklitaxel | 1 | 1.5 |
| neoadjuvant CRT | 1 | 1.5 |
| vinorelbin | 1 | 1.5 |
| cisplatin-docetaxel | 1 | 1.5 |
| dosetaksel | 1 | 1.5 |
| cisplatin-gemcitabine | 1 | 1.5 |
| gemcitabin | 2 | 3.0 |
| carboplatin-vinorelbine | 1 | 1.5 |
| unknown | 5 | 7.5 |
| **Total** | **67** | **100.0** |



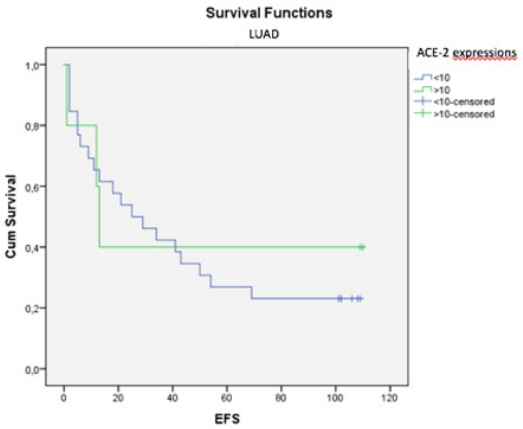

***Graphic 1. Association between overall survival (OS) and ACE-2 expressions in squamous cell lung cancer (LUSC)***




***Graphic 3. Association between overall surviva l(OS) and ACE-2 expressions in lung adenocarcinoma (LUAD).***




***Graphic 2. Association between event free survival (EFS) and ACE-2 expressions in lung squamous cell cancer (LUSC).***




***Graphic 4. Association between event free survival(EFS) and ACE-2 expressions in lung adenocarcinoma (LUAD)***

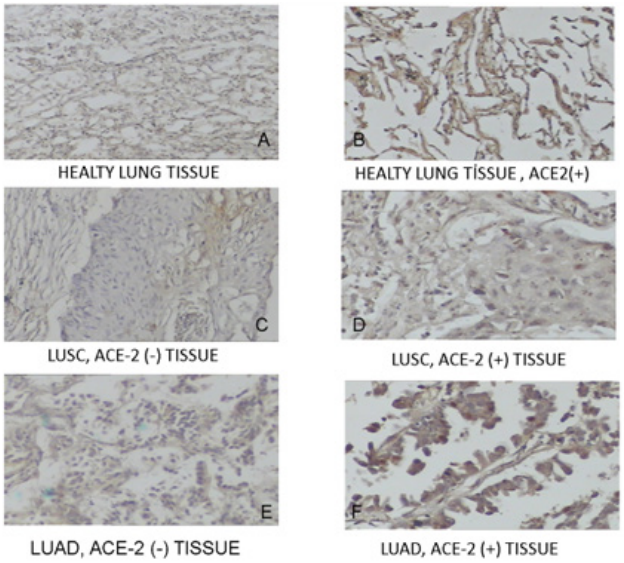



***Figure 1. ACE-2 expression was examined in normal lung tissue and lung cancer tissues by immunohistochemistry staining. A- Healty Lung tissue, B- Healty Lung tissue with ACE-2 (+), C-LUSC, ACE-2 (-) tissue, D- LUSC, ACE-2 (+) tissue, E- LUAD, ACE-2 (-) tissue, F- LUAD, ACE-2 (+) tissue***

## 4. Discussion

In the literature, ACE-2 expression was significantly higher in lung cancer, especially in lung adenocarcinoma and lung squamous cell carcinoma, compared to healthy tissues (6, 7, 12, 17, 18). Samad et al. investigated ACE-2 in various cancer types. The results revealed strong and significant evidence of higher expression of ACE-2 in LUAD ($p=1.36e^{-11}$) and LUSC ($p=0.01$) tissues compared to healthy tissues. Also, they showed that ACE-2 expression increased with patient age and gender in LUAD and LUSC. They investigated survival in lung cancer and found that for both OS ($p=3.5e^{-16}$) and DFS (Disease-Free Survival) ($p=1e^{-06}$), increased ACE-2 mRNA expression was associated with a worsen survival rate (7). In our study, we similarly showed increased ACE-2 expression in tumor tissue compared to healthy tissue. We could not determine the relationship of this increase with gender or age because most of our patients were male and elderly. We reached different results when we examined the association between ACE-2 expression and OS and EFS in our study.

Zhang et al. showed that ACE-2 expression increased in tumor tissue compared to healthy tissue (8). They revealed that increased ACE-2 expression was found in lung adenocarcinoma and lung squamous cell carcinoma independent of gender and age. It also reported that no significant correlation was observed between ACE-2 expression and stage in both types of lung cancer. ACE-2 was not statistically associated with DFS in LUAD, but higher ACE-2 expression tended to show worse OS. Conversely, higher ACE-2 expression was associated with prolonged DFS in LUSC cases, while no statistical difference was found for OS (8). Our study found that the difference in ACE-2 expression in tumor and healthy tissue was higher in squamous cell lung cancer than in adenocarcinoma. Zhang et al. did not show a difference between the stages in their study. However, unlike in our study, ACE-2 expression was higher in advanced stages of lung adenocarcinoma compared to early stages, and OS was worse.

Chai et al. compared ACE-2 expression in many tumor tissues with healthy tissue. They found that ACE-2 expression increased in six cancer tissues, including LUAD. ACE-2 was significantly increased in LUAD, while it remained unchanged in lung squamous cell carcinoma. They investigated the effect of ACE-2 expression on OS and DFS. In lung adenocarcinoma, ACE-2 expression was not statistically significant with patients' prognoses in DFS ($p=0.27$) nor OS ($p=0.19$). However, ACE-2 upregulation and DFS were statistically significant in LUSC ($p<0.05$) (17).

In another study, Kong et al. found that ACE-2 protein was expressed at higher levels in LUAD ($p=4.43e^{-06}$) and LUSC tissue ($p=0.000302$) than in healthy lung tissue (18). They compared ACE-2 gene expression in different stages of LUAD and LUSC and did not find statistically significant values in both LUAD ($p=0.634$) and LUSC ($p=0.589$) (18). Similar to the literature, our study showed that ACE-2 receptors stained by the IHC method were higher in tumoral tissues than in healthy tissues (7, 8, 17, 18). When we compared ACE-2 expression in patients with different histopathologic cancer diagnoses, we observed a significant increase in ACE-2 expression in squamous cell lung cancer. Unlike the literature, we did not find higher expression of ACE-2 in LUAD tissue compared to healthy tissue. However, when the ACE-2 expression in the tumor tissue in LUAD and the stage of the disease were compared, we found that ACE-2 expression in the tumor tissue was higher in the advanced stages of the adenocarcinoma group than in the early-stage cases. We also found that advanced-stage patients had worse overall survival than early-stage patients with lung adenocarcinoma. These results led us to suggest that increased ACE-2 expression may be one of the prognostic markers in advanced lung adenocarcinoma. On the other hand, we did not observe a significant difference between early and advanced stages in squamous cell lung cancer patients. We thought there were two reasons for this. First, our database is limited. In the literature we discussed above, we noticed that these studies were conducted with large databases such as The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx). Due to our limited database, we could

not detect significant association with ACE-2 and EFS or OS. Second, we followed the cases in this study for at least ten years. Thus, the mean overall survival is higher than the other studies. Our work is also valuable in this respect. We could have obtained different results with a more comprehensive database.

## 5. Conclusion

Our study observed that ACE-2 expression increased in lung cancer tissues compared to healthy tissue. When tissue phenotyping is performed according to histological subtypes of lung cancer, we found that the ACE-2 expression increase was significant, especially in squamous cell lung carcinoma. Increased ACE-2 expression in tumor tissue compared to healthy tissue espacially in LUSC suggests that ACE-2 expression may be important as a diagnostic biomarker in lung cancer. Also, increased ACE-2 expression in LUAD at advanced-stage compared to early stage suggests that ACE-2 expression may also be significant as a prognostic biomarker. Studies should be conducted on the effects of ACE-2 on lung cancer.

## 6. Contribution to the Field

Lung cancer continues to be an area where biomarkers are investigated in diagnosis. To meet this need, we investigated the importance of ACE-2 levels, which we studied in tissue subtypes and survival in lung cancer.

## Acknowledgment

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## Ethical Aspect of the Research

This study protocol was reviewed and approved by the Dokuz Eylul University Faculty of Medicine İnterventional Ethic Committee (12/01/2022; 2022/02-15).

The material used in the study is taken from the bio-archive of the Basic Oncology Department. Consent was obtained from the patients and their relatives who could be reached. For patients sampled before 19 August 2011, the samples of deceased patients whose legal representatives could not be reached were considered anonymous, and consent was not obtained.

## Conflict of Interest

There is no conflict of interest regarding any person and/or institution.

## Authorship Contribution

**Concept:** AVE, GÖŞ, SA; **Design:** AVE, GÖŞ, SA; **Supervision:** AVE, GÖŞ, SA; **Funding:** AVE, GÖŞ, SA; **Materials:** AVE, GÖŞ, SA; **Data Collection/Processing:** AVE, GÖŞ, TÇA, MK, SA; **Analysis/Interpretation:** AVE, GÖŞ, TÇA; **Literature Review:** AVE, GÖŞ; **Manuscript Writing:** AVE, GÖŞ; **Critical Review:** AVE, GÖŞ, TÇA, MK, SA.

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