RESEARCH ARTICLE

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Spexin Adipokinin and *TRPM2* ION Channel in Lung Cancer Use as a Bıomarker in Hıstopathologic Diagnosis

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Abstract

Background: Lung cancer is the most common cancer with the highest mortality rate. Spexin peptide acts as a potential regulatory factor in glucose and energy metabolism in this cancer type. It has been reported that TRPM2 ion channel is formed by oxidative stress and stimulates Ca2++ influx in cancer patients. **Objective:** In our study, we aimed to immunohistochemically determine the activity levels of Spexin peptide and *TRPM2* ion channels in lung cancer patients and to differentiate their levels according to cancer type. **Methods:** To determine the effects of these peptides and ion channels in lung cancer, 30 cases with lung cancer and 30 randomised control groups were formed. The activity levels of Spexin peptide and *TRPM2* ion channels in different types of lung cancer and non-cancerous groups were compared. **Results:** The study examined lung cancer subtypes (Adeno Ca, SCC, and Small Cell Ca) and a control group. Spexin and *TRPM2* levels were significantly higher in Adeno Ca and SCC compared to controls and Small Cell Ca. Small Cell Ca showed no significant difference from controls. No differences were found between Adeno Ca and SCC.**Conclusion:** Immunoreactivity levels of these markers were found to be higher in adenocarcinoma and squamous cell lung cancer tissues than in small cell cancer and non-cancerous tissues. In conclusion, spexin and *TRPM2* ion channel levels can be evaluated as a marker in adenocarcinoma and squamous cell lung cancer.

Keywords: Lung cancer- Spexin peptide and *TRPM2* ion channel iminoreactivity

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Introduction

Lung cancers are divided into two groups: small cell (SC) and non-small cell (NSC). NSCLC lung cancers are also divided into several subtypes. Each type of lung cancer grows and spreads differently and is treated in different ways. In order to establish treatment protocols correctly, each subtype needs to be precisely defined. This identification is done by histopathologic examination of tissue samples obtained by various methods. Many biological markers are used for accurate pathologic diagnoses [1].

Although the mechanism of action of spexin is unknown, it has been shown to be directly involved in the regulation of adrenocortical cell proliferation and it has been proven that it is also secreted from the pancreas [2]. It is a newly defined peptide with a length of fourteen amino acids and is widely secreted in many central and peripheral tissues such as pancreas, brain, heart, lung, liver and kidney. It is found in the systemic circulation and also functions as a neuroendocrine signal [3].

TRPM2 is a member of the TRPM family and carries out ion flows between the segments in the 5th and 6th cation channels [4]. These ion channels are found mostly in the brain but also in the lung, liver, spleen, bone marrow and other tissues.It has been reported in many literatures that ion channels belonging to the TRPM family are involved in cancerous cases and also the effect of spexin and *TRPM2* ion channels on histopathologic diagnosis [5, 6]. In our study, we have tried to show that spexin and *TRPM2* ion channel may be effective in histopathologic diagnosis as biomarkers in lung cancer and may be one of the histopathologic diagnostic methods. we aimed to immunohistochemically determine the activity levels of Spexin peptide and *TRPM2* ion channels in lung cancer patients and to differentiate their levels according to cancer type.

Materials and Methods

The pathologic materials of a total of 30 patients who were diagnosed with lung cancer in the preoperative evaluation, diagnosed with cancer by intraoperative frozen section and underwent wedge resection or lobectomy were included in the study and the ages and genders of the patients were recorded.

To determine the effects of these peptides and ion channels in lung cancer, 30 cases with lung cancer

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and 30 randomised control groups were formed. The histopathologic values of Spexin and *TRPM2* ion channels were statistically compared.

Immunohistochemical study method

Sections taken 4-6 mm thick from paraffin blocks were transferred to polylysine slides and deparaffinized. The sections were then passed through a graded alcohol series and boiled in citrate buffer solution at pH: 6 in a microwave oven (750W) for 12 minutes. After boiling, the tissues were kept at room temperature for cooling and washed with PBS (Phosphate Buffered Saline) and hydrogen peroxide solution was applied for 6 minutes to inhibit endogenous peroxidase activity. After the tissues were washed with PBS for 3x5 minutes, block solution was applied for 5 minutes and incubated with 1/250 diluted spexin and *TRPM2* primary antibodies (Anti-Neuropeptide Q/NPQ SPX Antibody, A04088-1, Boster Biological technology, USA / Rabbit Anti-*TRPM2* antibody, ab101738, Abcam, Cambridge, UK) for 60 minutes in a humid environment at room temperature.

After primary antibody application, the tissues were washed with PBS for 3x5 minutes and incubated with secondary antibody compatible with the primary antibody for 30 minutes in a humidified environment at room temperature. After secondary antibody application, the tissues were washed with PBS for 3x5 minutes and incubated with Streptavidin Peroxidase (TS-125-HR, Lab Vision Corporation, USA) for 30 minutes at room temperature in a humidified environment.

After 3-amino-9-ethylcarbazole (AEC) Substrate+AEC Chromogen solution was added to the tissues and the image signal was obtained under the light microscope, all groups were simultaneously washed with PBS. The tissues counterstained with Mayer's hematoxylin were washed with PBS and distilled water and covered with the appropriate closing solution (Large Volume Vision Mount, TA-125-UG, Lab Vision Corporation, USA). The slides were examined, evaluated and photographed under a Leica DM500 microscope (Leica DFC295).

A histoscore was created based on the prevalence (0.1: <25%, 0.4: 26-50%, 0.6: 51-75%, 0.9: 76-100%) and severity (0: none, +0.5: very little, +1: little, +2: moderate, +3: severe) of immunoreactivity in staining. Histoscore= prevalence x severity

Statistical Analysis

The data were statistically analyzed using SPSS 22.0 (IBM Corporation, Armonk, NY, USA) package program. In data analysis, the distribution of continuous variables was determined by Shapiro-Wilk normality test. Numerical data that did not fit the normal distribution were expressed as median (Minimum-Maximum) and qualitative data as percentage. Kruskal-Wallis test was used to compare more than two groups. After the Kruskal-Wallis test, pairwise comparisons were made with Post Hoc Dunn's test. Pearson chi-square test was used to compare categorical data. P<0.05 was considered significant.

Results

Twenty-two (69%) of the patients were male, 8 (31%) were female and the mean age was 66 (50-80) years. 10 patients had adenocarcinoma, 10 had squamous cell carcinoma and 10 had small cell carcinoma. In addition, 30 randomized control groups without cancer cells in the lung were formed.

The median (min-max) spectin level in lung tissue containing cancer cells was 0.90 (0.60-1.20) for Adeno Ca, 0.85 (0.45-1.20) for SCC, 0.30 (0.20-0.60) for Small Cell Ca and 0.30 (0.10-0.60) for the control group. The median value of the spectrum of patients with lung tissue containing cancer cells was statistically more significant than that of the control group, while the median value of Small Cell Ca was the same as the median value of the spectrum of the control group. Adeno Ca and SCC were more significant than Small Cell Ca (p<0.001) (Table 1).

The median (min-max) *TRPM2* level in lung tissues containing cancer cells was 0.90 (0.60-1.80) for Adeno Ca, 0.60 (0.45-1.80) for SCC, 0.40 (0.20-0.60) for small cell Ca and 0.20 (0.10-0.60) for the control group. Statistically, lung patients with cancer cells had a more significant median *TRPM2* value than the control group. Adeno Ca was more significant than SCC small cell Ca (p<0.001) (Table 1).

Immunohistochemical Findings

Spexin immunoreactivity

As a result of examination of immunohistochemical staining under light microscopy; Spexin immunoreactivity of all groups was found to be significantly different when compared by Kruskal-Wallis test. As a result of the pairwise comparison of the groups with Dunn's test after the Kruskal-Wallis test; Compared to the control group (Figure 1a), Spexin immunoreactivity was similar in the Small cell carcinoma group (Figure 1b) (p=0.679), while Spexin immunoreactivity was significantly increased in the Adeno cancer (Figure 1c) (p<0.001) and Squamous cell carcinoma (Figure 1d) (p<0.001) groups.

Compared to the small cell carcinoma group, Spexin immunoreactivity was significantly increased in the Adeno cancer (p<0.001) and Squamous cell carcinoma (p<0.001) groups. However, no statistically significant difference was observed between Spexin immunoreactivity in Adeno cancer and Squamous cancer groups (p = 0.712) (Table 1).

Table 1. Spexin and TRPM2 Immunoreactivity histoscore

	Spexin Median (min-max)	TRPM2 Median (min-max)
Control	0.30 (0.10-0.60)	0.20 (0.10-0.60)
Small Cell Ca	0.30 (0.20-0.60)	0.40 (0.20-0.60)
Adeno Ca	0.90 (0.60-1.20)[ab]	0.90 (0.60-1.80)[ab]
SCC	0.85 (0.45-1.20)[ab]	0.60 (0.45-1.80)[ab]
P*	<0.001	<0.001

Values are given as Median (min-max). [a], Compared to the control group; [b], Compared with the Small Cell ca group, (p<0.05); *, Kruskal-Wallis Test

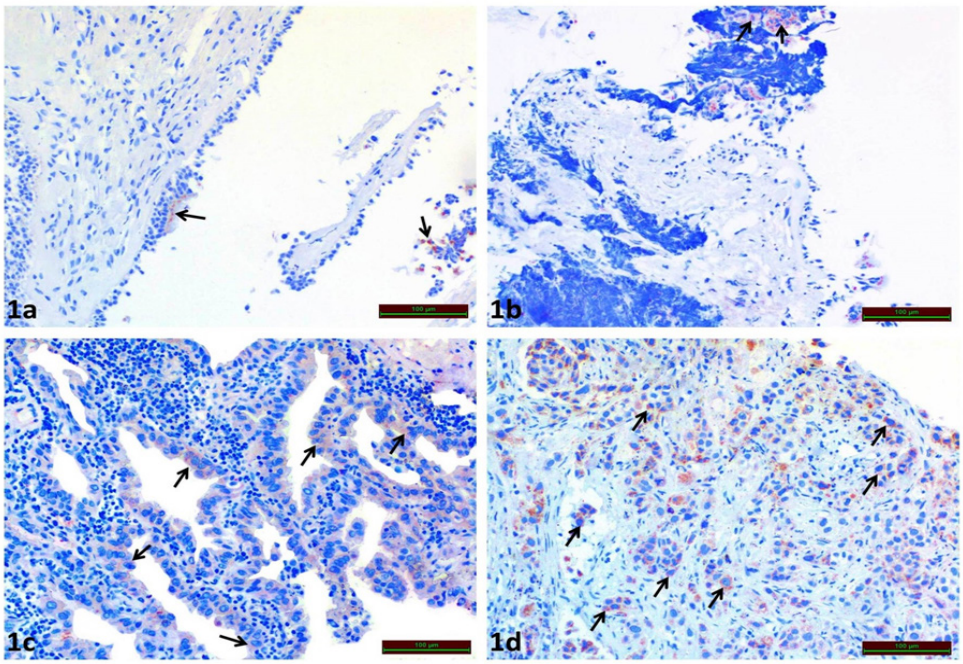

Figure 1. Spexin Expression in Lung Tissues of Cancer and Control Groups. The arrow indicates the immunoreactivity of the spexin.

TRPM2 immunoreactivity

As a result of the examination of immunohistochemical staining for *TRPM2* immunoreactivity under light microscopy; a significant difference was found when *TRPM2* immunoreactivity of all groups was compared by Kruskal-Wallis test. As a result of the pairwise comparison of the groups with Dunn's test after the Kruskal-Wallis test; Compared to the control group (Figure 2a), *TRPM2* immunoreactivity was similar in the Small cell carcinoma group (Figure 2b) ($p = 0.147$), whereas *TRPM2* immunoreactivity was significantly increased in the Adeno cancer (Figure 2c) ($p<0.001$) and Squamous cell carcinoma (Figure 2d) ($p<0.001$) groups.

TRPM2 immunoreactivity was significantly increased in Adeno cancer ($p<0.001$) and Squamous cell carcinoma ($p<0.001$) compared to small cell carcinoma. However, there was no statistically significant difference in *TRPM2* immunoreactivity between Adeno cancer and Squamous cell carcinoma groups ($p=0.149$) (Table 1).

Discussion

Lung cancer ranks first in men and third in women among cancers seen worldwide. Late diagnosis is the

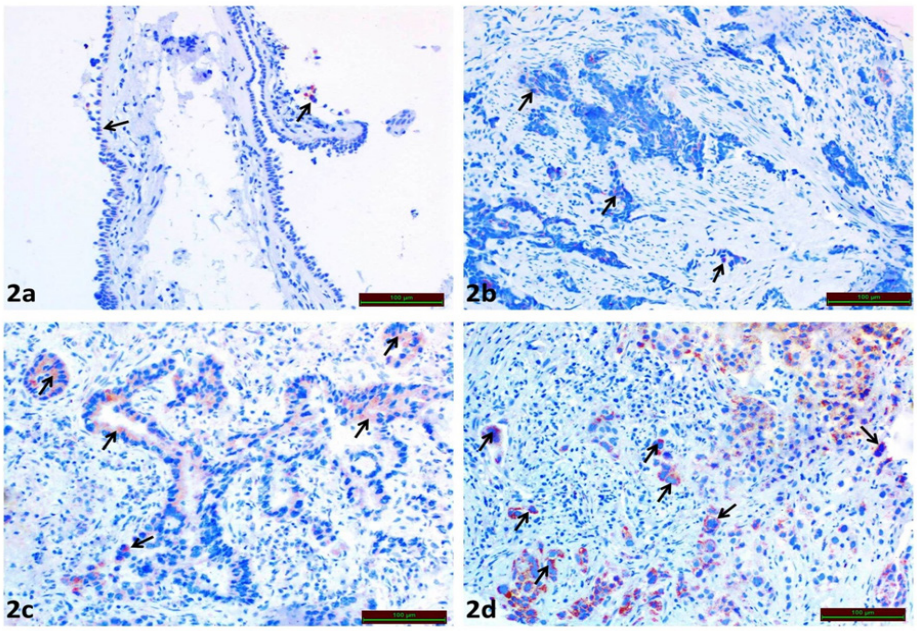

Figure 2. *TRPM2* Expression in Lung Tissues of Cancer and Control Groups. The arrow indicates the immunoreactivity of the *TRPM2*.

most important factor affecting survival in lung cancer. Due to the low long-term survival rates of patients with lung cancer, new methods are needed for earlier diagnosis and treatment of this disease. These biomarkers can be used in lung cancer screening, diagnosis and monitoring response to treatment [1, 7].

Many immunohistochemical techniques are used in the pathologic typing of lung cancers. Chromogranin A, synaptophysin, CD56, CD57, thyroid transcription factor-1 (TTF-1), p63, p16(INK4A) (p16) are some of them [8-11]. In our study, we found that spexin and *TRPM2* values could be used in histopathologic diagnosis.

The effect of Spexin adipokinin and *TRPM2* ion channel on histopathologic diagnosis has been reported in many literatures. Spexin immunoreaction is mainly cytoplasmic and has been demonstrated in tissues of the skin, respiratory, digestive, urinary and reproductive systems despite different staining intensities [5]. In this study, we demonstrated that spexin adipokinin and *TRPM2* ion channels were found at higher levels in cancerous lung tissues compared to normal lung tissues.

Spexin levels were reported to be associated with diabetes and spexin levels were compared in blood and ocular aqueous fluid of type 2 diabetic patients with and without diabetic retinopathy and cataract. It was reported that spexin levels decreased in the cataract and diabetic retinopathy group compared to the control group and that spexin has an important role in the pathophysiology of diabetic retinopathy and cataract [5]. In our study, we found that spexin levels were high in patients with lung cancer.

Spexin is a newly identified peptide that is widely secreted in many organs, including the pancreas, brain, heart, lung and liver. Spexin can function both through the blood and as a neuroendocrine signal. It has been shown to be a molecule that causes a feeling of satiety, which is lower in obese compared to lean adults, and is directly involved in the regulation of adrenocortical cell proliferation [5, 6, 12].

TRPM2 ion channel has two regions, N and C. While the N-terminal region acts as a regulator, the C region contains the enzyme ADPR pyrophosphatase and this enzyme catalyzes the formation of amp and ribose 5-phosphate from ADPR [4, 13, 14]. It has been reported that oxidative stress increases ADPR production in mitochondria and ADPR stimulates Ca2+ influx and opens *TRPM2* channels [15, 16]. It has been reported that activation of *TRPM2* is related to the sensitivity of the effects of hydrogen peroxide or TNFα or both to calcium entry and cell death. Antisense structures or negative inhibition of *TRPM2*-associated dominant factors can stop the activity of *TRPM2*. Inhibition of *TRPM2* functions to protect potential target cells against oxidant stress and possibly other adverse stimuli provides favorable results in therapy [17, 18].

As a result, the immunoreactivity of the Speksin peptide and *TRPM2* ion channel is significantly increased in Adeno Ca and Squamous Ca tissues compared to cancer cell-free lung tissues and lung small cell Ca ($p<0,001$, Table 1). Since many peptides and ion channels are increased in the presence of tumours, tumour markers are useful in cancer diagnosis, determination of treatment and prognosis prediction. Spexin and *TRPM2* are peptides and ion channels that have been recently identified but not sufficiently investigated. Spexin peptide and *TRPM2* ion channel expression is higher in lung cancer types Adeno Ca and Squamous Ca tissues compared to lung tissues without cancer cells and lung small cell Ca tissues ($p<0,001$, Table 1). Increased spexin peptide and *TRPM2* ion channel immunoreactivities can be used as biomarkers for Adeno Ca and Squamous Ca tissues.

Author Contribution Statement

Author contributed to the design, conception, data collection, data analysis, interpretation, manuscript writing, approved the final version of the study.

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Approval

The study was approved by Firat University Scientific Research and Publication Ethics Board (2021/06-08).

Availability of Data

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare no conflicts of interest.

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