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Molecular Role of SGK3 Gene in Promoting the Progression of Non-small Cell Lung Cancer

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Abstract: Objective: To investigate the role of SGK3 in the progression of non-small cell lung cancer and investigate its effect on cell growth using lung cancer cell lines. Method: A retrospective analysis was conducted on 134 non-small cell lung cancer patients diagnosed in our hospital from January 2014 to December 2018, including 83 cases of adenocarcinoma and 51 cases of squamous cell carcinoma. Immunohistochemical staining was used to divide lung cancer specimens from patients into SGK3 negative and positive groups, and the correlation between SGK3 expression and clinical data was analyzed. Subsequently, cellular level experimental validation was carried out using the A549 lung cancer cell line. Result: Clinical data confirmed that SGK3 was significantly overexpressed in lung cancer tissue ($P<0.05$), and the difference in its expression level was not related to factors such as age and gender of SGK3 patients, but only showed a significant correlation with TNM staging and lymph node metastasis ($P<0.05$). In addition, through CCK-8 cell growth rate measurement experiments and cell scratch and invasion experiments, it is speculated that the above clinical findings may be due to the pro cancer effect of SGK3. Conclusion: SGK3 is highly expressed in cancer tissues of non-small cell lung cancer patients. SGK3 promotes lung cancer cell growth and division, suggesting that this regulatory factor may be involved in the occurrence and development of non-small cell lung cancer.

Keywords: Non-small Cell Lung Cancer; SGK3; Immunohistochemistry; Lung Cancer.

1. Introduction

In recent years, with the growth of smokers and changes in lifestyle, the incidence rate and mortality of lung cancer have increased significantly, which has become the main cause of death affecting human life [1]. Given that over 80% of clinical cases are diagnosed with non-small cell lung cancer (NSCLC) [2], conducting molecular mechanism research on it is of great significance for understanding cancer regulatory signaling pathways and the development and clinical application of targeted anticancer drugs. Screening and research on clinical NSCLC lung cancer specimens have shown that SGK3 (Serum and Glucocorticoid Kinase-3) is highly expressed in lung cancer tissues of NSCLC patients. As SGK3 is a downstream serine/threonine kinase regulated by PI3K (Phosphatidylinositol 3-Kinase), it is widely involved in ion channels [4], glucose homeostasis [5], and regulation of cell activities such as proliferation, survival, and migration [6]. Therefore, studying the mechanism of SGK3 molecular regulatory function in NSCLC patients can help people to gain a deeper understanding of the molecular regulatory signaling pathway of NSCLC, and can also provide insights for medical researchers. Provide certain reference and inspiration for biological theory research.

2. Materials and Methods

2.1. Clinical Specimens

The preliminary research of this project collected lung cancer (adjacent) tissue specimens from 134 NSCLC patients diagnosed in our hospital from January 2014 to December 2018. The research results of this part have been published in the Journal of Shenyang Medical College [7]. The clinical characteristics of patients such as gender and age are shown in Table 1. The criteria for inclusion in the study are as follows: 1) Confirmed by postoperative pathology; 2) Did not receive radiotherapy, chemotherapy, or any targeted drug treatment before surgery. To ensure that the statistical results accurately reflect the case situation, the patients included in the statistics should not have the following medical history: 1) lung or other infectious diseases; 2) Other malignant tumors; 3) Severe liver and kidney diseases; 4) Autoimmune system diseases and metabolic diseases. This clinical project has been approved by the hospital ethics committee and the patient has informed consent.

2.2. Experimental Materials and Instruments

The rabbit anti human SGK3 antibody (ab218251) used for immunohistochemistry and the antibody (ab153981) used for Western blot experiments were both purchased from Abcam. The lung cancer specimens were sliced and stained by Nanjing Novozan Biotechnology Co., Ltd. The inhibitor of SGK3 (PROTAC SGK3 grader-1, Catalog S967201) was purchased from Selleck Corporation in the United States. The qPCR SYBR Green Premium and CCK-8 reagents were purchased from Shanghai Yisheng, and the conventional consumables for Western Blot experiments (secondary antibodies, developer, SDS-PAGE reagent kit, etc.) were all purchased from Kangwei Century. The low-temperature centrifuge, imaging equipment, multifunctional enzyme-linked immunosorbent assay (ELISA) reader, well plate, and fluorescence quantitative PCR reactor are all Thermo Fisher products ® Company.

2.3. Methods

Lung cancer tissue samples were taken from patients with clinically diagnosed NSCLC, stained with HE immunohistochemistry, and scored for each sample by counting the average proportion of brown positive cells in 5 fields. The scores shall be based on the following standards,

and the sum of the total scores of the two sets of standards, 0-2 points, shall be considered negative, and 3-7 points shall be considered positive:

(1) Standard 1:

0 points: No positive cells in the field of vision;

1 point: The average proportion of positive cells is ≤ 25%;

2 points: The average proportion of positive cells is 26% to 50%;

3 points: The average proportion of positive cells is 51% to 75%;

4 points: The average proportion of positive cells is>75%.

(2) Standard 2:

0 points: No brownish yellow staining in the field of vision;

1 point: The field of view is light yellow;

2 points: The field of view is brownish yellow;

3 points: The field of view is brownish brown.

2.4. Determination of Growth Activity of Lung Cancer Cells

To verify the effect of SGK3 on the growth activity of A549 cells, we applied SGK3 inhibitors to A549 cells (dissolved in DMSO and treated with a 0.1% final concentration cell suspension as a positive control). After testing, the concentration of SGK3 inhibitor was 0.15 μ mol/L (which can inhibit about 50% of cell growth).

This study first analyzed the effect of SGK3 on the growth rate of lung cancer cells by measuring the 5-day growth curve of lung cancer cell A549 using CCK-8 reagent. Subsequently, cell scratch and invasion experiments were conducted on A549 lung cancer to confirm the role of SGK3 in promoting lung cancer cell growth.

2.5. Statistical Processing

SPSS software (version 29.0.1.0) and GraphPad (version 9.5.0) were used for statistical analysis in this study, and different testing methods were used to demonstrate inter group differences in different experiments. $P<0.05$ is considered a statistically significant difference.

3. Results

3.1. Statistical Analysis of NSCLC Patients

Analysis of the case characteristics of clinical patients included in the statistics revealed that the difference in positive expression rate of SGK3 was only related to the TNM stage and lymph node metastasis of NSCLC patients ($P<0.05$), and was not related to other factors listed in Table 1, including age ($P>0.05$).

Table 1. Correlation analysis between SGK3 gene expression and NSCLC cases

Feature	Group	Total number of cases	Positive rate% (+)	χ2	P
Gender	Male	ninety	73.3% (66)	3.699	0.054
	Female	forty-four	56.8% (25)		
Age	≤ 60 years old	fifty-six	69.6% (39)	0.132	0.716
	>60 years old	seventy-eight	66.7% (52)		
Tumor type	Squamous cell carcinoma	fifty-one	70.6% (36)	0.271	0.603
	Adenocarcinoma	eighty-three	66.3% (55)		
Tumor diameter	≤ 3 cm	sixty-five	64.6% (42)	0.629	0.428
	>3 cm	sixty-nine	710% (49)		
Differentiation degree	Low	thirty-seven	70.3% (26)	0.384	0.825
	in	fifty-two	69.2% (36)		
	High	forty-five	64.4% (29)		
TNM staging	I&II	one hundred and five	71.3% (64)	10.779	0.001
	III&IV	twenty-nine	93.1% (27)		
Lymph node metastasis	nothing	ninety-four	59.6% (56)	10.041	0.002
	have	forty	87.5% (35)		

3.2. Regulatory Effect of SGK3 on the Growth of Lung Cancer Cells

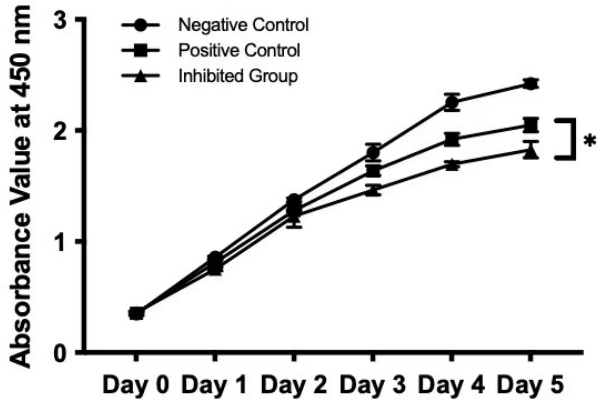

Figure 1. The growth rate of A549 cells significantly slowed down with the addition of SGK3 inhibitors

Applying SGK3 inhibitors to A549 lung cancer cells, as shown in Figure 1, demonstrated that the inhibition of SGK3 expression significantly inhibited cell growth rate ($P<0.05$), indicating that SGK3 has a promoting effect on cell proliferation.

3.3. A549 Cell Viability Test

To further determine the impact of SGK3 on cell viability, combined with previous literature, we validated the changes in A549 cell viability under in vitro conditions of SGK3 deficiency through scratch and cell invasion experiments. As shown in Figure 2, compared to the positive control, the SGK3 inhibition group showed a significantly slower scratch healing rate within 48 hours ($P<0.05$), and also showed a significant decrease in the number of invasive cells ($P<0.05$). The data for the scratch experiment is obtained by measuring the ratio of the scratch width at a specified time to the initial (0 h) width. The invasion experiment is calculated by counting the number of invading cells in any three fields of

view of the group of cells.

The results confirmed that after inhibition of SGK3, the cellular activity of A549 cells was significantly inhibited, including scratch healing ability and invasion ability.

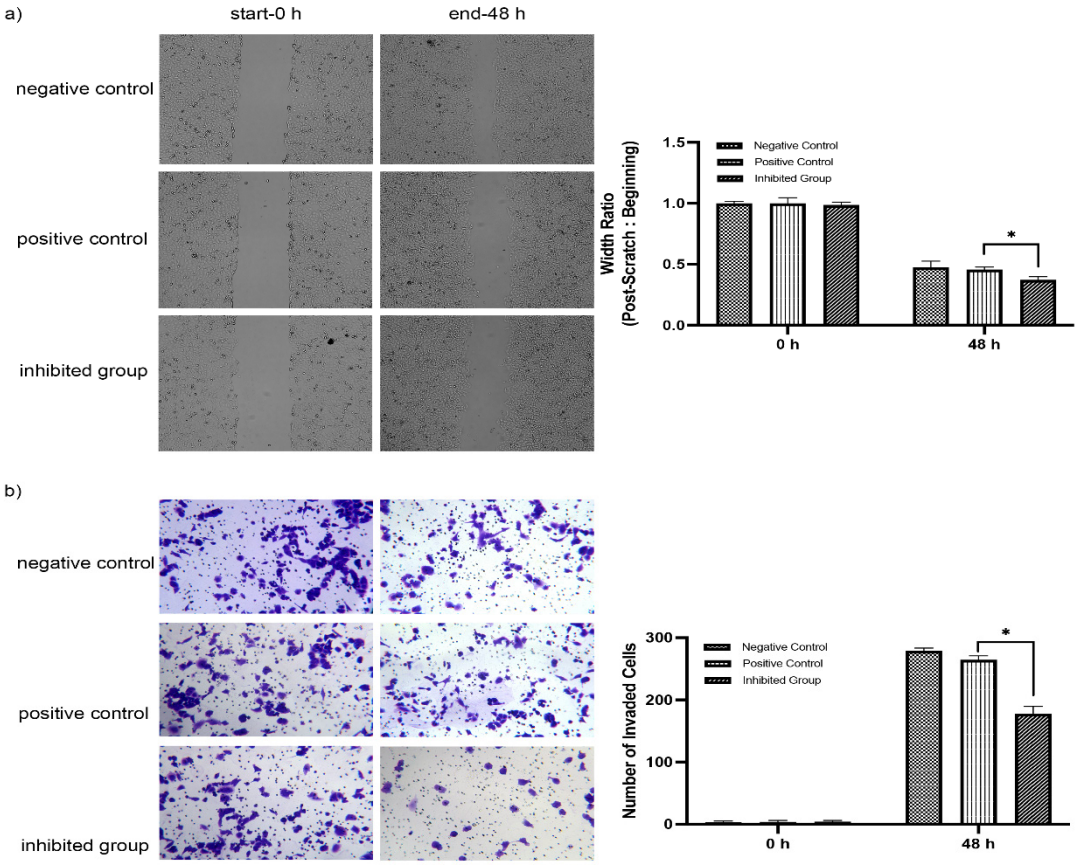

Figure 2. Detection of A549 cell growth ability: (a) scratch growth experiment and (b) cell invasion experiment

4. Discussion

With the gradual deepening of research on the carcinogenesis mechanism of NSCLC in recent years, a large amount of literature has reported the molecular regulatory mechanisms involved, which control various cell activities, such as apoptosis, cell proliferation, angiogenesis, cell migration, and cell invasion [8]. However, all of the above cell activities are regulated by activating the epidermal growth factor receptor (EGFR). Due to the close correlation between cell apoptosis and proliferation processes and the PI3K Akt signaling pathway, abnormal regulation of this signaling pathway can lead to cancer occurrence [9]. In recent years, research on this signaling pathway has found that SGK3 factor is a downstream action factor that is independent of Akt and directly regulated by PI3K [3], and also plays a key role in carcinogenesis. Further research on SGK3 has reference significance for targeted treatment of NSCLC lung cancer [10].

This study demonstrates the relationship between SGK3 and NSCLC from multiple levels. At the individual level, we analyzed the correlation between SGK3 and clinical data of NSCLC patients and found that the expression of SGK3 is positively correlated with the clinical stage and lymph node metastasis of NSCLC patients. That is, the later the TNM stage (stage III+stage IV) of NSCLC patients, the higher the expression of SGK3, the presence of lymph node metastasis, and the higher the expression of SGK3. At the cellular level, results 2 and 3 also confirmed that SGK3 can promote the growth and proliferation of NSCLC cells.

The regulatory process of SGK3 on NSCLC may be based on its molecular structure. The PX characteristic domain of SGK3 protein is located at its N-terminus, and the PX domain is necessary for the normal functioning of SGK3. It can regulate the phosphorylation of Thr320 and Ser486 sites in SGK3, thereby activating the protein function of SGK3 and forming the PI3K/SGK/mTOR regulatory axis, thereby controlling cell migration, cell proliferation, and other cell growth related activities [11-12]. The mechanism by which SGK3 exerts its pro cancer effect may be that overexpression of SGK3 causes phosphorylation and inactivation of TSC2, leading to overexpression of mTOR factors negatively regulated by TSC2 [13], causing cell proliferation and growth [14].

SGK3, as a potential cancer therapeutic target, plays a pro cancer role in various cancers through different signal transduction pathways. For example, in breast cancer, SGK3 is induced by estrogen [15] and promotes the growth and migration of breast cancer cells through intermediate factors [16-17]. In prostate cancer, SGK3 regulates cancer cell growth by promoting cell cycle G1-S transition [15]. In addition, tumor diseases such as renal cell carcinoma [18] and leukemia [19] are regulated by SGK3 factor. This study validated the promoting effect of SGK3 factor in the carcinogenesis process of non-small cell lung cancer through multiple levels of experiments and analysis. However, there are still some shortcomings, such as: (1) lack of animal level validation, which can be verified through SGK3 gene deficient mouse induced lung cancer models in the future; (2) The research results lack evidence of upstream and downstream regulatory factor networks. In the later stage, we can conduct bioinformatics analysis on the aforementioned

animal models, such as metabolomics and transcriptomics analysis, to study the signal network regulated by SGK3.

In summary, through the study of clinical specimens of NSCLC patients, SGK3 has been proven to have a pro cancer effect, and this effect may be achieved by promoting the growth of lung cancer cells.

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