RESEARCH ARTICLE

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Down-Regulation and Clinic-Pathological Correlation of SIK-1 and SIL-1-LNC in Non-Small Cell Lung Cancer Patients

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Abstract

Background: Non-small-cell lung cancer (NSCLC) is currently the leading cause of mortality cancer. Introducing noninvasive approaches to diagnose NSCLC, especially at an early phase, might improve the disease's prognosis. Long noncoding RNAs (lncRNAs), which are important regulators of the expression genes inside the cells, have been linked to a range of biological processes, such as cancer progression and metastasis, including NSCLC. The present work aims to determine the potential involvement of SIK-1-LNC and SIK-1 in NSCLC pathogenesis and the possible use of these molecules as novel biomarkers or therapeutic targets. **Methods:** In this work, the expression levels of SIK-1-LNC and SIK-1 in 50 pairs of NSCLC tumor and tumor marginal tissues were evaluated. So, after total RNA extraction and complementary DNA synthesis, the SIK-1-LNC and SIK-1 expression levels were evaluated by real-time PCR. In the study groups, clinical and pathological characteristics of the NSCLC patients were also examined. **Results:** Our findings showed that tumor samples had much lower levels of SIK-1 and SIK-1-LNC expression than tumor margin samples. SIK-1-LNC expression was correlated with SIK-1 levels in NSCLC samples. Interestingly, both stage and lymph node metastasis features of the tumor were associated significantly with SIK-1 and SIK-1-LNC expression levels. A ROC curve analysis indicated a biomarker index of 0.69 and 0.74 for SIK-1 and SIK-1-LNC, respectively. **Conclusion:** Collectively, our study emphasized the role of SIK-1-LNC and SIK-1 downregulation in NSCLC oncogenesis. Additionally, SIK-1 and SIK-1-LNC, particularly the latter, have shown remarkable potential to be utilized as new NSCLC biomarkers and therapeutic targets.

Keywords: SIK-1- SIK-1-LNC- non-small cell lung cancer- metastasis

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Introduction

Lung cancer (LC) is the most highly prevalent carcinoma and the leading cause of cancer-related mortality globally, with over 2,090,000 new cases, 1,760,000 deaths, and a 5-year survival rate of 19.4%. Notably, the incidence rates of LC have grown up in the Northern and Southern regions of Africa, China, Eastern Europe, and Indonesia (Bade et al., 2020; de Groot et al., 2018). Small cell carcinoma (SCLC) and non-small cell carcinoma (NSCLC), the latter which also encompasses adenocarcinoma and squamous cell carcinoma, are the two subtypes of LC. About 85% of all LCs are diagnosed as NSCLC (Zarredar et al., 2018; Zarredar et al., 2019). The use of tobacco and smoking, familial history, drinking alcohol, chronic inflammation, ionizing radiation, occupational exposures, asbestosis, silica, mixed occupation exposures, and air pollution are the main risk factors for NSCLC (Gholi-Nataj et al., 2022; Malhotra et al., 2016). Surgical removal, chemotherapy, and radiation therapy are the conventional approaches in the treatment of NSCLC. Comparatively, NSCLC is more chemoresistant than SCLC and most patients with NSCLC have attained an advanced stage due to inadequacy of early diagnostic techniques (Arbour et al., 2019; Sharma et al., 2019). So, developing novel biomarkers and therapeutic targets could be of significance in NSCLC.

Long noncoding RNAs (lncRNA) are a subtype of RNA molecules which are larger than 200 nucleotides. LncRNAs are implicated in several biological procedures, particularly in the progression and metastasis of different cancers. Also, the interaction between lncRNAs and protein, RNA, and DNA leads to carcinogenesis of colorectal (Wen et al., 2016), gastric (Chen et al., 2018),

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lung (Yao et al., 2016), osteosarcoma (Aftabi et al., 2021; Li et al., 2018), and esophageal (Congxiu Huang et al., 2016) cancers via targeting specific genes. Therefore, investigation of lncRNAs that are implicated in cancer signaling pathways might provide us with perspicuity into the evolution of neoplasms (Liang et al., 2020; Shuai et al., 2020). SIK1-LNC is identified at chromosome 21q22.3 and it indicated a positive tendency with salt-inducible kinases 1 (SIK1) in various cancerous cells. SIK1 is a serine/threonine kinase that has crucial functions in regulating the proliferation of cells (Yang et al., 2018). Previous studies recommend that SIK1 performs vital roles in several cancers including gastric cancer (Selvik et al., 2014) and epithelial ovarian cancer (Wu et al., 2017). It has been revealed that down-regulation of LKB1-SIK1 signaling increases EMT (epithelial to mesenchymal transition) in NSCLC, which causes metastasis enhancement (Yao et al., 2016). Thus, the low levels of SIK-1 expression in some cancer cells, could be linked to poor prognosis. However, the role and expression of SIK1-LNC and SIK-1 remain unclear in lung NSCLC cells. In the present work, we evaluated the expression level of SIK1-LNC and SIK-1 mRNA in biopsy samples of NSCLC patients and its critical function in the prognosis of this cancer.

Materials and Methods

Research population and sampling method

We obtained tumor and margin healthy tissues from 50 NSCLC patients after surgery between 2019 and 2021. The samples were identified by surgical oncologists and validated by a board-certified pathologist at Imam Reza Hospital, Tabriz, Iran. The clinical and pathological features of the patients are demonstrated in Table 1. Following surgery, tissue samples were promptly placed in RNase inhibitor solution (Qiagen, Hilden, Germany) and transported to the laboratory. All patients signed a written informed consent form prior to sampling. Also, this research has been approved by the Ethical Committee of Tabriz University of Medical Sciences (Grant number: IR.TBZMED.REC.1400.756).

RNA extraction

RNX-PLUS Solution (SinaClon, Tehran, Iran) was used to extract total RNA in accordance with the manufacturer's instructions. A NanoDrop (Thermo Fisher Scientific, USA) spectrophotometer was used to confirm the quality and quantity of total RNA. Gel electrophoresis on 1% agarose was also used to evaluate RNA samples. Then, until cDNA synthesis, the RNA samples were maintained in an -80°C refrigerator.

cDNA synthesis and Real-time PCR

Using cDNA synthesis kits, cDNA synthesis was carried out in a total volume of 20 µl. (Biofact, Seoul, South Korea). The house-keeping gene was GAPDH. The Roche real-time PCR Light Cycler 96 instrument (Roche Diagnostics, Mannheim, Germany) was used to perform RT-PCR using the master mix SYBER Green, and the findings were reported using the $2^{-\Delta\Delta CT}$ method. Primer sequences used for qRT-PCR and respective annealing temperatures are summarized in Table 2.

Statistical analysis

Data evaluation was carried out using GraphPad Prism 6 (San Diego, CA, USA). The normality distribution of variables was determined using the Kolmogorov-Smirnov test. Statistical comparison was made using Paired Student T-test (P-value < 0.05, mean ± SD).

Results

Expression of SIK-1-LNC in NSCLC tissues

We have assessed the expression of SIK-1 and SIK-1-LNC in 50 NSCLC samples compared to the tumor margin samples. The expression of SIK-1 and SIK-1-LNC in tumor and tumor margin tissues was then evaluated. In parallel to RT-PCR analysis, clinical and pathological features of NSCLC patients were assessed in the research groups, including gender and age, and tumor-related attributes such as stage and lymph node metastasis. The results revealed a substantial reduction in SIK-1 and SIK-1-LNC expression in tumor samples as compared to tumor margin samples, with p values of 0.0025 and

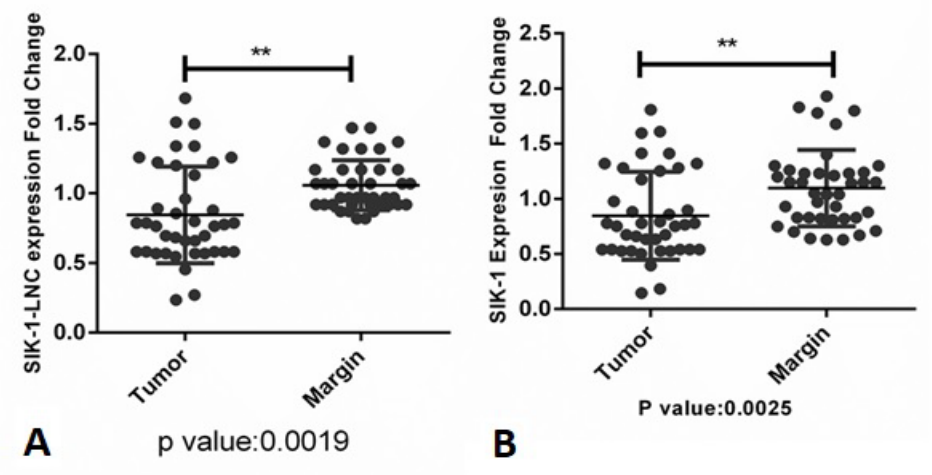

Figure 1. In NSCLC Tissues Relative to the Marginal Zone, SIK1-LNC (A) and SIK (B) expression levels were significantly downregulated.

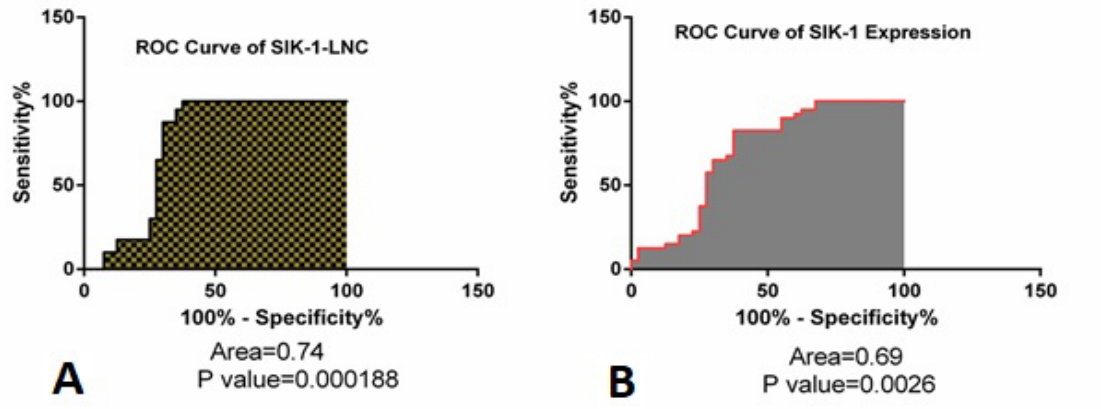

Figure 2. The Correlation between SIK1-LNC and SIK1 Expression Levels

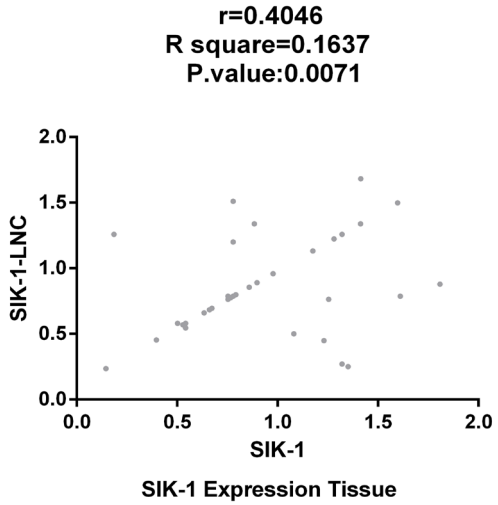

Figure 3. The Correlation between SIK1-LNC and SIK1 Expression Levels in NSCLC Samples. Correlation analysis indicated a significant (p-value = 0.0071) positive correlation between SIK-1-LNC and SIK-1 expression in NSCLC samples (r = 0.4046).

0.0019, respectively (Figure 1). The expression level of SIK-1 and SIK-1-LNC were not significantly associated with the age and sex of the patients. However, both stage and lymph node metastasis features of the tumor were associated significantly with SIK-1 and SIK-1-LNC expression levels. The exact p values of the association are demonstrated in Table 3.

The diagnostic competence SIK-1 and SIK-1-LNC in NSCLC

The sensitivity and specificity of SIK-1 and SIK-1-LNC as potential novel biomarkers for NSCLC were determined using the Receiver operating characteristic (ROC) curve.

Table 1. Clinicopathological Features of NSCLC Patients

Number of Samples	Total:50	
Age	< 55	23
	> 55	27
Sex	Male	24
	Female	26
Stage	Stage 2	23
	Stage 3	25
	Stage 4	2
Lymphnod metastasis	Negative	21
	Positive	29

In NSCLC patients, the result indicated a ROC area of 0.69 and 0.74 biomarker index for SIK-1 and SIK-1-LNC, respectively. ROC curve data are demonstrated in Figure 2.

SIL-1-LNC expression was correlated with SIK-1 expression levels in NSCLC samples

We also evaluated the relationship between SIK-1-LNC with SIK-1 expression levels. According to Fig. 3, Pearson's correlation analysis indicated a significant (p-value = 0.0071) positive correlation between SIK-1-LNC and SIK-1 expression in NSCLC samples (r = 0.4046), indicating that SIK-1-LNC may play a role in NSCLC pathogenesis via modifying SIK-1 expression.

Discussion

Considering the satisfactory outcome of therapeutic strategies used in the treatment of NSCLC patients in the clinic, understanding the molecular mechanisms and recognition of new diagnostic biomarkers and therapeutic targets implicated in NSCLC is of significant importance.

Table 2. List of Primer Sequences Used for qRT-PCR Analysis

Genes	Forward (5′-3′)	Reverse (5′-3′)	Annealing temp. (°C)	Product Size
SIK-1	5′-CTCCGGGTGGGTTTTTACGAC-3′	5′-CTGCGTTTTGGTGACTCGATG-3′	59	93bp
SIK-1-LNC	5′-GAATGGGAGGGGAAGGAAGAAGC-3′	5′-TCAGCAGAGCAAGAAGAGGTCAG-3′	60	283bp
GAPDH	5′-CAAGATCATCAGCAATGCCTCC-3′	5′-GCCATCACGCCACAGTTTCC-3′	59	166bp

Table 3 a. Relation of the Clinical and Demographic Features of the Studied NSCLC Patients with the Expression of SIK-1 and SIK-1-LNC

Features			Age	Sex	Stage	Lymph node metastasis
Genes	Change in expression level	p-value				
SIK-1	Downregulated	0	None-significant	None-significant	Significant (p-value = 0.0031)	Significant (p-value = 0.001)
SIK-1-LNC	Downregulated	0.05	None-significant	None-significant	Significant (p-value = 0.0015)	Significant (p-value = 0.029)

Table 3 b. Clinicopathological Features of Patients Coloration with Genes

	SIK-1	SIK-1-LNC
Age	P value:0.42	P value:0.23
Sex	P value:0.18	P value:0.29
Stage	P value:0.0031	P value:0.0015
Lymphnod metastasis	P value:0.001	P value:0.029

Previous studies revealed that lncRNAs have crucial functions in the proliferation, metastasis, apoptosis, and cell differentiation of cancerous cells. Changing cytoplasmic localization of proteins, and modulating the action of corresponding proteins are some biological functions of lncRNAs (Wang et al., 2011; Wilusz et al., 2009). SIK-1 as a member of the AMP-activated protein kinase-related kinases has main roles in the cell metabolism and distal metastasis of cancers (Chen et al., 2016). In this work, we demonstrated that the expression levels of SIK1-LNC and SIK-1 were significantly reduced in NSCLC tissues in comparison with adjacent normal tissues. SIK-1-LNC expression was correlated with SIK-1 levels in NSCLC samples and SIK-1-LNC through modulating SIK-1 is involved in NSCLC pathogenesis. Furthermore, SIK-1-LNC was shown to be a more efficient potential biomarker of NSCLC than SIK-1 with a biomarker index of 0.74 versus 0.69.

Similar to our study, but in a different population, Yang et al., (2018) showed that SIK-1 and SIK-1-LNC are downregulated in NSCLC and also induction of SIK-1-LNC expression could suppress proliferative and invasive abilities of cancerous cells. Consequently, SIK-1-LNC may be used in NSCLC as both a prognostic marker and a therapeutic target. Also, recent studies reported that down-regulation of SIK1-LNC and SIK-1 enhances proliferative characteristics of various other malignancies. Wang et al. showed that the expression level of SIK1-LNC was attenuated in AML patients and the down-regulation of SIK1-LNC leads to leukemogenesis. They indicated that SIK1-LNC improves the anti-cancer effects of retinoic acid in AML. Moreover, SIK1-LNC induces autophagy by targeting E2F1 in NB4 AML cells (Wang et al., 2022). Chen et al., (2016) demonstrated that miR-141 acts as an oncomir and enhances the cell growth of ovarian cancer cells. They also showed that down-regulation of miR-141 causes the elevation of SIK-1 expression in ovarian cancer. In another study, Ren et al., (2016) revealed that the expression of SIK-1 was reduced in pancreatic cancerous cells and this down-regulation of SIK-1 is correlated with gemcitabine chemoresistance in pancreatic cancerous cells. Qu et al., (2016) showed that the expression level of SIK-1 was down-regulated in hepatocellular carcinoma (HCC) and the over-expression of SIK-1 causes EMT suppression and cell proliferation inhibition in HCC. They also showed that Twist-1 negatively regulates the promotor activity of SIK-1 and the expression levels of SIK-1 are inversely associated with Twist-1 in HCC. Huang et al., (2019) established that SIK-1 expression was attenuated in colorectal cancer cells. They ascertained that the expression level of miR-17 was enhanced in colorectal cancer and the up-regulation of miR-17 induced the cell growth by targeting SIK-1. Ponnusami et al., (2021) demonstrated that the expression level of SIK-1 was reduced in human breast cancer. They also demonstrated that SIK-1 interacted with p53 and facilitates oxidative phosphorylation in breast carcinoma and leads to proliferation and invasion in breast cancer.

Collectively, our study in parallel with previous studies emphasized the role of SIK-1-LNC and SIK-1 downregulation in NSCLC oncogenesis. It has been shown that SIK-1 and SIK-1-LNC, particularly the latter, have the potential to be utilized as both new biomarkers and therapeutic targets in NSCLC.

Author Contribution Statement

MR, HV, and HZ took responsibility for design of the study, data acquisition, statistical analysis and wrote the paper; AA, SH, MAV, and RP participated in the data acquisition and drafted the manuscript; MA made substantial contributions to the conception or design of the work and English edit. All authors read and approved the final manuscript.

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Study Approval

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Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Availability of Data

On reasonable request, the associated author will release the datasets used in this work.

Conflict of Interest

None.

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