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Evaluation of Fis-1 and miR-484 Expression Levels in Tumor Tissue Samples and Healthy Tumor Margins in Lung Cancer

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Abstract

Background: MicroRNAs (miRNAs) regulate gene expression and various cellular activities. They also hold significant importance in the progression and development of human malignancies. Among these, miRNA-484 and the Fis-1 gene have been identified as having substantial roles in lung cancer. This study aims to ascertain miRNA-484 and Fis-1 gene expression levels in non-small cell lung cancer (NSCLC) patients.

Method: In this case-control study, 45 pairs of tumor tissues and their corresponding healthy margin tissues were surgically obtained from NSCLC patients and promptly preserved in liquid nitrogen after excision. Total RNA extraction was performed using TRIzol, followed by cDNA synthesis using a designated kit. Afterward, we used quantitative reverse transcription polymerase chain reaction (qRT-PCR) to measure the expression levels of miRNA-484 and the Fis-1 gene. Furthermore, the clinico-pathological characteristics of the NSCLC patients were assessed.

Results: Our findings revealed an upregulation of miRNA-484 expression and downregulation of Fis-1 gene expression in NSCLC tissues compared with non-tumor tissues. Additionally, significant correlations were observed between miRNA-484 and Fis-1 gene expression levels and clinicopathological features of the patients, including factors such as lymph node involvement and distant metastasis.

Conclusion: These findings suggest the potential utility of Fis-1 and miR-484 as prognostic and diagnostic markers in NSCLC.

Keywords: miRNA-486, Fis-1 protein, Carcinoma, Non-small-cell lung, Malignancy

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Introduction

Lung cancer poses a significant global health concern due to its escalating mortality and prevalence rates among men and women. Based on their pathological characteristics, lung tumors can be categorized into small-cell lung cancer (SCLC), accounting for 15%, and non-small cell lung cancer (NSCLC), constituting 85%. Gaining insight into the molecular attributes of a patient's tumor tissues holds value in guiding therapeutic decisions. Recent therapeutic strategies encompass targeted therapy, immunotherapy, chemotherapy, radiotherapy, or synergistic utilization. Unfortunately, a considerable number of patients receive diagnoses at advanced malignancy stages, underscoring the ongoing pursuit of biomarker development for early detection and prognosis.[1, 2]

Mitochondria undergo regular fission and fusion, essential processes for organelle division and inheritance maintenance. Given their pivotal role in apoptosis, aberrant mitochondrial fission contributes to the pathogenesis of several diseases, although its mechanism remains elusive.[3, 4] Mitochondrial fission 1 protein (Fis-1) is acknowledged in yeast as pivotal to mitochondrial fission or division; however, its precise functions in humans, particularly relating to mitochondrial fission, remain enigmatic. Furthermore, Fis-1 assumes a critical role in mitophagic and apoptotic pathways, implying its multifaceted significance. The apoptotic functions of Fis-1 remain relatively ambiguous, as it exhibits pro-apoptotic functions in specific tissues and anti-apoptotic effects in others.[5] Earlier investigations have demonstrated frequent disruptions in the regular expression of Fis-1 in cancers.[6] Diminished Fis-1 expression can prompt cells to acquire oncogenic attributes through uncontrolled cell cycle progression at the G2/M checkpoint.[7]

MicroRNAs (miRNAs), ranging from 20 to 24 nucleotides in length, are non-coding RNAs pivotal in regulating gene expression at the post-transcriptional level by binding to the 3'-UTR of target mRNAs. MiRNAs serve as oncogenes or tumor suppressors in diverse cancers.[8, 9] The expression levels miR-484 exhibit variations across various malignancies, including cervical and breast tumors.[10] The expression levels miR-484 exhibit variations across various malignancies, including cervical and breast tumors.[10] Within this study, the expression levels of miR-484 and Fis-1 genes within NSCLC tissues and their adjacent margins were scrutinized to elucidate any down- or up-regulation within this cancer type.[11] Consequently, this investigation delved into the expression levels of miR-484 and Fis-1 genes within NSCLC tissues and their neighboring margins to elucidate potential dysregulation within this cancer subtype.

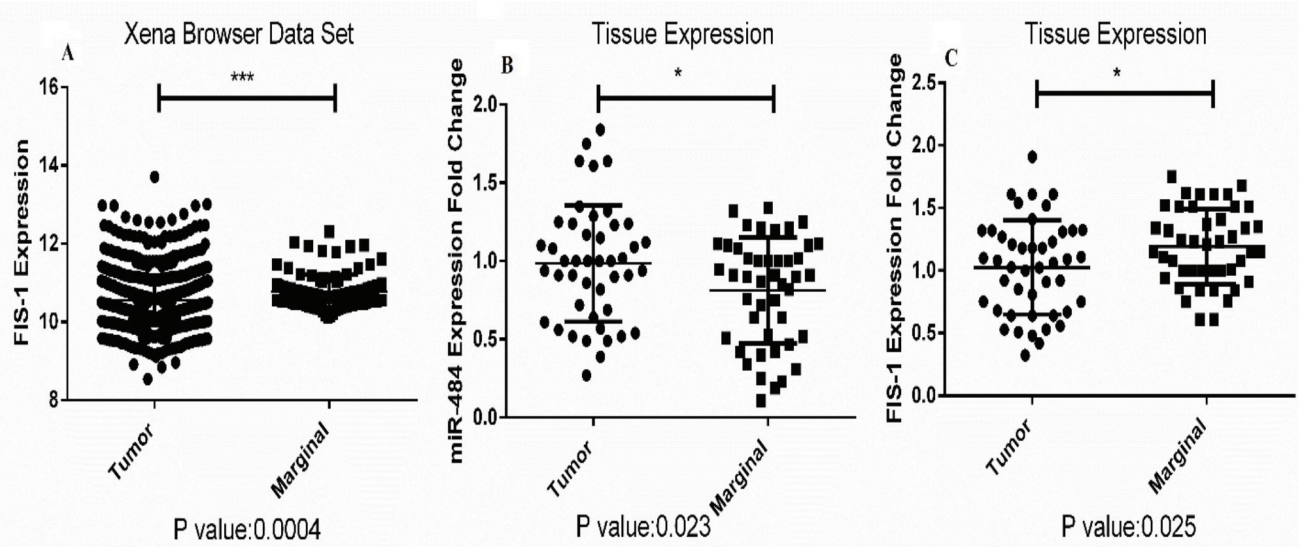

Figure 1. This illustration depicts the expression levels of Fis-1 and miR-484 within lung cancer groups. Panels A and B compare clinical samples (cancer tissues vs. margins), while Panel C represents analysis using TCGA datasets through the Xena Browser.
*$P < 0.1$, ** $P < 0.01$, *** $P < 0.001$

Methods and Materials

Bioinformatics analysis

In assessing Fis-1 expression levels in NSCLC, we employed datasets from the Cancer Genome Atlas (TCGA). The retrieved dataset underwent bioinformatic analysis using the Xena Functional Genomics Explorer, accessible at https://xena.ucsc.edu/ (Figure 1-A).

Ethics approval and consent to participate

This study adhered to the amended Declaration of Helsinki. The institutional review board approved the study, and all participants provided written informed consent. Every patient completed the requisite forms, and all personal information was confidential. The Academic Committee on Research Ethics (Studies in Human Subjects) of Tabriz University of Medical Sciences evaluated and endorsed this study as a research project (trial Registration Number: IR.TBZMED.REC.1400.572).

Study population and sampling method

Registered with the Academic Committee on Research Ethics (Human Studies Subjects) at Tabriz University of Medical Sciences (IR.TBZMED.REC.1400.572), this case-control study spanned two years (2020 to 2021) at Imam Reza Hospital in Tabriz, Iran. The trial encompassed 45 NSCLC patients (22 men and 23 women) diagnosed based on paraclinical examinations and clinical signs by pathologists. Tumor and margin biopsies were acquired during surgery, placed in RNAase inhibitor solution (Biocompare), and stored at -80°C until RNA extraction.

RNA extraction and cDNA synthesis

We performed total RNA extraction using TRIzol (Thermo Fisher Scientific), following the supplier's recommendations. The NanoDrop spectrophotometer (Thermo Fisher Scientific) was used to assess the quantity and quality of the extracted RNA (at 260/280). The extracted RNAs were stored at -80°C for subsequent cDNA synthesis. CDNA synthesis kits (Biofact, Korea) were employed in a final volume of 20 µl. The internal control gene used was GAPDH. Quantitative real-time polymerase chain reaction (qRT-PCR) was executed using SYBER Green master mix on a Roche Real-time PCR Light Cycler 96 (Germany), and results were interpreted using the 2-ΔΔCT method. The PCR program and primer sequences are detailed in table 1.

Description of Fis-1 and miR-484 analysis as tumor markers

The relative expression analysis of Fis-1 was conducted through REST (randomization test employing the Relative Expression Software Tool). To predict NSCLC from normal tissues, we assessed the sensitivity and specificity of Fis-1 and miR-484 expression levels via a receiver operating characteristic (ROC) curve plot, utilizing Sigma Plot 12.5 software. This graphical representation depicts the false-positive rate against the true-positive rate for varying descriptor thresholds.[12] The ROC curve's horizontal axis signifies the false-positive rate, while the vertical axis indicates the true-positive rate.

Statistical analysis

The statistical analysis was conducted using GraphPad Prism 6 software (San Diego, CA, USA). The unpaired Student's T-test was employed to assess the statistical significance of the differences among variables ($P < 0.05$, mean ± SD).

Results

Expression levels of miR-484 and Fis-1 in NSCLC tissues

We examined the expression levels of miR-484 and Fis-1 in 45 NSCLC tumors and their

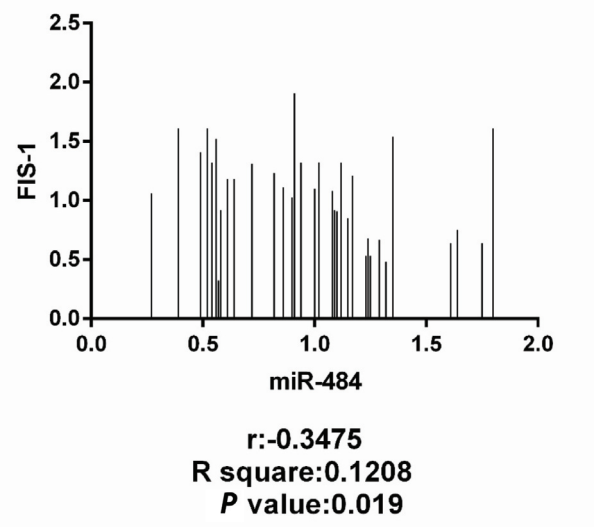

Figure 2. This graphical representation highlights the inverse relationship between Fis-1 and miR-484, illustrating the role of miR-484 as a regulator of Fis-1.

Table 1. Primer sequence and characteristics utilized for quantifying target genes

Gene	Primer sequence	Annealing temperature (°C)
miR-484	Forward: 5'-TACCCTTCAGGCTCAGTC-3' Reverse: 5'- CCAGTGCAGGGTCCGAGGTA-3'	59
Reverse: 5'- CCAGTGCAGGGTCCGAGGTA -3'	Forward: 5'-CAAGATCATCAGCAATGCCTCC-3' Reverse 5'-GCCATCACGCCACAGTTTCC-3'	59.5
GAPDH	Forward: 5'- GCTTCGGCAGCACATATACTAAAAT -3' Reverse: 5'- CGCTTCACGAATTTGCGTGTCAT -3'	59
Reverse 5'-GCCATCACGCCACAGTTTCC-3'	Forward: 5'- TGGAGACTGTGGCACAGTAGA -3' Reverse: 5'- CTTCAGCAGGTCCTCCACAGA -3'	59

corresponding non-tumor peripheral tissues. Additionally, we documented the clinicopathological characteristics of the patients, including age, sex, and tumor-related factors such as distant metastasis. The results revealed a notable upregulation of miR-484 (fold change = 1.213, $P = 0.0231$) (Figure 1-B). Furthermore, our findings indicated that Fis-1 expression was downregulated in cancerous tissues compared with peripheral non-tumor tissues (fold change: 0.857, $P = 0.0225$) (Figure 1-C). Additionally, we identified an inverse relationship between Fis-1 and miR-484 expression levels in NSCLC tissues (Figure 2).

Association between miR-484 and Fis-1 expression levels and clinicopathological characteristics of NSCLC patients

The overexpression of miR-484 and the reduced expression of Fis-1 exhibited significant correlations with clinicopathological characteristics such as distant metastasis, lymph node metastasis, and cancer stage. However, no substantial associations were found between miR-484 and Fis-1 expression levels and the variables of sex and age (Table 2).

miR-484 and Fis-1 as diagnostic markers in NSCLC

We employed ROC curves to ascertain the potential of miR-484 and Fis-1 as new biomarkers for NSCLC. The outcomes demonstrated ROC area under the curve values of 0.6306 and 0.6190 for Fis-1 and miR-484, respectively, in NSCLC patients (Figure 3). This finding highlights a substantial diagnostic potential.

Discussion

This study investigated the expression levels of miR-484 and Fis-1 in NSCLC tissues compared with non-cancerous marginal tissues. MiR-484 exhibited overexpression, while Fis-1 displayed lower expression in NSCLC tissues compared with non-cancerous counterparts. Furthermore, these regulatory patterns exhibited significant correlations with clinicopathological characteristics, including distant metastasis, lymph node metastasis, and cancer stage.

Additionally, we assessed Fis-1 expression data within the Cancer Genome Atlas (TCGA) datasets using the Xena Functional Genomics Explorer. The results indicated decreased Fis-1 expression in NSCLC tissues compared with non-cancerous tissues. Furthermore, we identified an inverse relationship between miR-484 expression and Fis-1 expression.

Lung cancer ranks as the second most prevalent cancer globally.[13] The therapeutic approaches for NSCLC encompass surgery, immunotherapy, chemotherapy, and radiotherapy. However, these methods exhibit limited efficacy in advanced cancer stages, resulting in a persistently high mortality rate.[14] Accumulating evidence underscores the significant role of miR-484 in various tumor types.

In a pioneering study, Zhuang et al. reported an elevated miR-484 expression level in NSCLC serum compared with healthy controls. Their findings demonstrated a definitive association between serum miR-484 and lymph node metastasis, distant metastasis, clinical stage, and

Table 2. Clinicopathological characteristics of lung cancer patients

	Number	Fis-1 (*P* value)	miR-484 (*P* value)
Sex			
Female	23	0.511	0.328
Male	22		
Age			
<50	27	0.241	0.411
>50	18		
Stage			
II	6	0.018	0.012
III	27		
IV	12		
Distant metastasis			
Positive	12	0.0041	0.032
Negative	33		
Lymph node metastasis			
Positive	39	0.032	0.074
Negative	6		

histological grade. Additionally, ROC analysis indicated the potential of serum miR-484 expression to differentiate healthy controls from NSCLC patients.[15]

A study by Yang et al. unveiled miR-484's oncogenic role in liver malignancies by suppressing SAMD9, thereby initiating carcinogenesis and cellular transformation through the induced interferon pathway.[16] Similarly, Mei et al. uncovered miR-484's tumor-suppressive function in the colon by inhibiting CD137L. This inhibition led to reduced IL-8 expression levels and decreased cell viability in tumor cells.[17] Hence, miR-484's role in tumor biology seems closely linked to cancer type and tissue.

Pashaei's meta-analysis in prostate malignancies suggested high miR-484 expression, indicating its potential as a negative prognostic biomarker.[18] Likewise, Lee et al. identified miR-484's oncogenic role in prostate malignancies, exerting control over PSMG1 and promoting cell mobility. This study also proposed miR-484 as a potential adverse prognostic biomarker.[19] In NSCLC, Li et al. demonstrated miR-484's

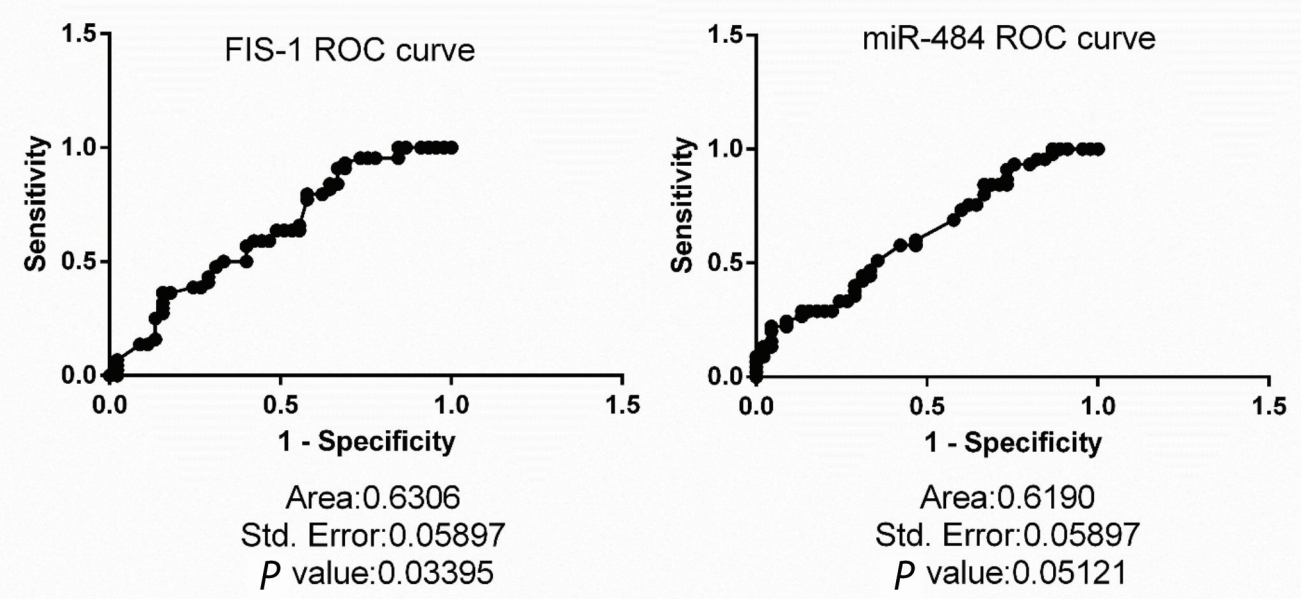

Figure 3. Schematic representation of the ROC curve, evaluating the diagnostic potential through AUC analysis for both miR-484 and Fis-1.

ROC: Receiver operating characteristics; AUC: Area under the curve; std.: Standard

promotion of cancer development by suppressing Apaf-1, leading to suppressed apoptosis.[20] Furthermore, miR-484 expression levels were significantly lower in gastric tumor cell lines compared with standard cell lines. Mir-484's independent prognostic significance for 5-year overall survival in gastric tumor cases was established, with decreased expression correlating with poor survival. Additionally, miR-484's expression in gastric malignancies hindered cell metastasis, progression, and invasion.[21]

Lin et al. identified low Fis-1 expression in tamoxifen-resistant breast tumor cell lines compared with chemo-sensitive parental cell lines. They proposed that reduced Fis-1 expression contributed to enhanced survival of breast cancer cells.[22] Yamamori et al. linked ionizing radiation to mitochondrial fragmentation via Drp1 expression, and suppressing Fis-1 or Drp-1 mitigated cellular radiosensitivity.[23] Jin et al. illustrated that inhibiting Fis-1 or Drp-1 expression under high-dose radiation bolstered cell progression and survival.[24] After prostate tumor radiation therapy, Hsiao et al. observed increased Fis-1 expression in patients' whole blood.[25]

Wang et al. uncovered miR-484's inhibition of Fis-1 expression, preventing Fis-1-mediated fission and apoptosis in cardiomyocytes and adrenocortical cancer cells. They established the necessity of Fis-1 upregulation for apoptosis and mitochondrial fission, with overexpression during anoxia, whereas miR-484 expression declined.[26] Karimi et al. reported significantly elevated Fis-1 expression in gastric malignancies compared with healthy marginal tissues, implicating Fis-1 in gastric carcinogenesis. Further analysis linked high Fis-1 expression to tumor cell migration in patients.[27]

In conclusion, this research highlights miR-484's heightened expression and Fis-1's diminished expression in NSCLC. Their dysregulation is closely associated with NSCLC's pathogenesis and tumorigenesis. The limited efficacy of advanced-stage treatments emphasizes the need for innovative therapeutic strategies. Furthermore, the potential utilization of Fis-1 and miR-484 as prognostic and diagnostic markers in NSCLC warrants exploration. To achieve more precise outcomes, further studies involving a larger patient cohort and genes associated with Fis-1 and miR-484 are necessary for their potential application as prognostic and diagnostic factors in melanoma malignancies.

Conclusion

Our study reveals that miR-484 is significantly overexpressed, while Fis-1 is notably under-expressed in cancerous lung tissues compared with peripheral non-tumor tissues. Furthermore, these findings suggest the potential utility of Fis-1 and miR-484 as both prognostic and diagnostic markers in NSCLC.

Acknowledgments

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Conflict of Interest

None declared.

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