Celal Bayar University Journal of Science

Diagnostic Potential of miR-551b-3p in Lung Cancer: In Vitro and In Silico Experiments

Esen Çakmak[1*], İbrahim Seyfettin Çelik[2]

[1*] Department of Bioengineering and Sciences, Graduate School of Natural and Applied Sciences, Kahramanmaraş Sütçü Imam University, Kahramanmaraş, Türkiye
[2] Vocational School of Health Services, Department of Medical Services and Techniques, Kahramanmaras Sütcü Imam University, Kahramanmaras, Türkiye
* esencakmak@ksu.edu.tr
* Orcid No: 0000-0001-8805-3315

Received: 11 November, 2024
Accepted: 5 February, 2025
DOI: 10.18466/cbayarfbe.1582834

Abstract

Lung cancer is a leading cause of cancer deaths worldwide. miRNAs have attracted attention as promising biomarkers in lung cancer diagnosis and prognosis. This study investigated the molecular mechanism of miR-551b-3p in lung cancer cells. The gene expression level of miR-551b-3p was investigated using qRT-PCR in healthy and cancerous lung cell lines. The target genes of miR-551b-3p and its function in cancer pathogenesis were also determined by in silico analyses. miR-551b-3p expression was higher in cancer cells compared to healthy lung cells ($p<0.01$). The expression level of miR-551b-3p was confirmed in silico in cancerous lung tissue. ERBB4 was identified as a target gene of miR-551b-3p and was down-regulated in cancer cells in comparison to healthy cells. Overexpression of miR-551b-3p and under expression of the ERBB4 gene decreased overall survival in cancer patients. A negative correlation was observed between miR-551b-3p and ERBB4 gene. miR-551b-3p expression was found to be closely associated with clinicopathological factors such as distant metastasis status, lymph node metastasis status and gender. miR-551b-3p target genes were enriched in cancer-related cellular processes. In conclusion, miR-551b-3p may be a potential alternative in treatment strategies as a therapeutic target in lung cancers.

Keywords: ERBB4, Lung cancer, miR-551b-3p, qRT-PCR

1. Introduction

Lung cancer is a high mortality cancer and is the first cancer diagnosed in men and the second cancer diagnosed in women [1]. Lung cancer is histologically classified into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), accounting for 15% and 85% of patients, respectively. NSCLC is subdivided into squamous cell carcinoma of the lung (SCLC), lung adenocarcinoma (LAC) and large cell carcinoma (LBC) [2-3]. Although surgery, chemotherapy and targeted therapies are commonly used to treat the disease, survival rates remain critical. The low survival rate may be due to conditions such as diagnosis at an advanced stage, metastases and high recurrence rates [4]. The lack of effective tools and treatment methods is a major problem for the early diagnosis and treatment in lung cancer [2]. Therefore, there is an urgent need to investigate new potential molecular markers and methods to understand the molecular mechanisms underlying lung cancer.

miRNAs play an important role in cancer biology. They can have oncogenic or tumor suppressor properties by affecting physiological processes such as growth, development and cell cycle of cancer cells through the genes they target [5]. Different miRNA markers are defined for different types of cancer, and while a miRNA may act as an oncogene in one type of cancer, it may act as a tumor suppressor in another [6]. In lung cancer, miR-138-5p and miR-200c were reported to be highly effective in preventing tumor development and progression by targeting PD-L1 [7]. miR-142-3p promoted invasion and metastasis of NSCLC cells by activating MAPK/ERK and NF-κB pathways [8]. miR-210 may lead to epidermal mesenchymal transition (EMT) by targeting the UPF1 gene and inducing the PTEN/PI3K/AKT pathway. This promotes migration and invasion of NSCLC [9].

There are few studies in the literature on the role of miR-551b-3p in cancer. In breast cancer, miR-551b-3p, which is upregulated by microRNA 551b-3p and transported to the nucleus by importin-8 (IPO8), activated STAT3 transcription. miR-551b-3p was associated with poor prognosis in breast cancer patients [10]. In lung cancer, downstream expression of miR-551b-3p causes overexpression lncRNA PVT1 and promotes cell viability, proliferation, migration and invasion [11]. LncRNA SMARCC2/miR-551b-3p/TMPRSS4 axis and miR-551b-3p have been reported to act as a tumor suppressor gene in gastric cancer [12]. In head and neck squamous cell carcinoma, miR-551a, miR-551b-3p and GLIPR2 gene triad partially regulate autophagy and is active in tumor growth, development and invasion [13].

This study aimed to investigate the molecular pathogenesis of miR-551b-3p in lung cancer. There are limited studies on the potential mechanism of miR-551b-3p in different types of cancer. Based on this, the gene expression levels of miR-551b-3p were investigated in a healthy lung cell line (BEAS-2B) and lung cancer cell lines (A549 and Calu-1), and its molecular mechanism was further investigated by in silico analyses.

2. Materials and Methods

2.1 Cell culture

A healthy human lung cell line (BEAS-2B) and cancer cell lines (A549 and Calu-1) were obtained from the ÜSKİM Health Laboratory (Kahramanmaraş, Turkey). Cells were cultivated at 37°C and 5% CO_2 in DMEM containing high glucose concentration (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% FBS (Gibco), 1% penicillin (Gibco) and 100 µg/mL streptomycin (Gibco).

2.2 Transfection

miR-551b-3p mimic, miR-551b-3p inhibitor (anti-miR-551b-3p), miR-551b-3p mimic negative control and miR-551b-3p inhibitor negative control (anti-miR-NC) (Invitrogen, San Diego, CA) were purchased. One day before transfection, they were grown to 80% confluence in a 24-well plate at a density of 5×10^5 per well. For miR-551b-3p mimic, inhibitors and negative controls, transfection was performed using reagent Lipofectamine 3000 (Invitrogen, San Diego, CA) according to the protocol of the manufacturer. The biological behavior of the cells was determined 24 hours after transfection.

2.3 RNA isolations and qRT-PCR

Total RNA was isolated using Trizol reagent (Invitrogen). The isolation was performed according to the manufacturer's protocols. Total RNA concentration and purity were measured by Thermo NanoDrop 2000 device. Complementary DNA (cDNA) was then synthesized using QuantiTect Reverse Transcription Kit. qRT-PCR analyses were performed with ABI 7500 Fast Real Time PCR (Applied Biosystems, Foster City, CA, USA) and SYBR Green PCR kit (Takara, Shiga, Japan). miRNA isolation, RNA isolation, cDNA synthesis and PCR reaction were performed according to the manufacturer's protocol. PCR mixtures were made in a 10 µL volume consisting of 2 µL SYBR Green real-time PCR Master Mix, 1 µL cDNA, 0.5 µL Forward Primer (10 pmol), 0.5 µL Reverse Primer (10 pmol) and 6 µL. The PCR reaction was performed as pre-denaturation at 95°C for 10 min, denaturation at 95°C for 10 s, adhesion at 60°C for 20 s and amplification at 72°C for 10 s for 40 cycles. U6 for miRNA was used as endogenous controls. Negative controls were added during the experiment to control for contamination, and the experiments will be repeated 3 times independently. In addition, the expression of miR-551b-3p after the reaction was calculated by $2^{-\Delta\Delta Ct}$ method. The primers used in this study are as follows: miRNA forward 5'-GCGACCCATACTTGGTTTCAG-3', miRNA reverse 5'-TCGTGAGATGAAGCACTGTAG-3', U6 forward 5'-CTCGCTTCGGCAGCACA-3' and U6 reverse 5'-AACGCTTCACGAATTTGCGT-3'.

2.4 Identification of target genes of miR-551b-3p

The mirWALK (http://mirwalk.umm.uni-heidelberg.de/), miRDB (https://mirdb.org/mirdb/index.html) and Target Scan HUMAN (https://www.targetscan.org/vert_80/) databases were used to predict target genes. A Venn diagram was used to show commonalities among target genes (https://bioinfogp.cnb.csic.es/tools/venny/index.html).

2.5 Enrichment analysis

The PANTHER server (Protein Analysis Through Evolutionary Relationships, v 19.0) was used to identify biological processes, protein classes, cellular components, signalling pathways and molecular functions associated with the target genes of miR-551b-3p (https://pantherdb.org/). The PANTHER classification system is a comprehensive library describing the function, pathway and protein properties of genes and proteins and consists of a bioinformatics algorithm designed to facilitate high-throughput analyses [14].

2.6 KM-plotter survival analysis

Kaplan-Meier Plotter (http://www.kmplot.com) was used to analyze survival curves of miR-551b-3p. Gene expression was classified into high and low expression according to the median value. P value <0.05 indicates significance.

2.7 Association of miR-551b-3p with cancer pathogenesis

ULCAN (https://ualcan.path.uab.edu/index.html), an online platform based on the TCGA datasets, was used to determine the expression levels of miR-551b-3p and its target gene ERBB4 in lung cancer patients. At the same time, the expression level of miR-551b-3p and its cancer-pathogenic effects such as tumor development, stage, grade and survival were investigated using the Oncomir database (https://oncomir.org/oncomir/index.html).

2.8 Statistical analysis

Real-time PCR experiments were performed at least three times. Prism 8 (GraphPad) software was used for statistical data were analyzed by Student's t-test between two groups. $P<0.05$ was considered statistically significant.

3. Results

3.1 miR-551b-3p was more highly expressed in lung cancer cells compare to healthy lung cells

Expression levels were compared between healthy lung cell lines and cancer cell lines to determine the role of miR-551b-3p in lung cancer. miR-551b-3p was more highly expressed in the lung cancer cell line A549 and the Calu-1 healthy cell line BEAS-2B (Fig. 1a,1b,1c). According to the results of the analysis, no significant increase in miR-551b-3p was observed in healthy lung cells. In A549 cell lines, the gene expression level of miR-551b-3p mimic increased approximately 6-fold, while the gene expression level of miR-551b-3p inhibitor did not increase much. The gene expression level of miR-551b-3p mimic increased approximately 4-fold in Calu-1 cell lines. Very little increase was observed in the gene expression level of miR-551b-3p inhibitor.

The miRNA mimics and miRNA inhibitors were statistically compared between healthy and cancerous lung cell lines. As shown in Fig. 2a and Fig. 2b, as a result of statistical analysis, miR-551b-3p mimic showed a significant difference compared to healthy cells ($p<0.01$). The expression levels of the miRNA inhibitors in the cell lines did not show a significant increase.

3.2 miR-551b-3p targets the ERBB4 gene

To further investigate the molecular mechanism of miR-551b-3p, target genes were predicted by three different bioinformatics platforms. The number of target genes predicted in silico was determined to be 44 genes in total (Fig. 3). Among the target genes screened, the ERBB4 gene was found to be the overlapping gene in all platforms. The overlapping gene in miRDB and TargetSanHuman databases is GALNTL6, and the matching genes in miRDB and miRWalk databases are CRB2, PDE4DIP, CDK17, SPATA31D3 and PGAM5. CTIF, PDE4C and ANKRD50 are overlapping genes in TargetSanHuman and miRWalk databases.

3.3 Enrichment analysis of miR-551b-3p target genes

The biological functions, pathway enrichment analyses and protein classifications of common genes targeted by miR-551b-3p were examined by Panther algorithm. According to the results of the analysis, it is seen that common target genes are concentrated in cases such as 'cellular process' (GALNTL6, ERBB4, PDE4DIP, PDE4C, CDK17, CRB2), 'biological regulation' (ERBB4, PDE4DIP, PDE4C, CTIF), 'metabolic processes' (GALNTL6, CDK17). In cellular components 'cellular anatomical formations' (GALNTL6, ERBB4, PDE4DIP, CTIF, CDK17, CDK17, CRB2) and in molecular properties 'catalytic activity' come to the forefront in processes in which genes (GALNTL6, ERBB4, CDK17, PDE4C) are enriched. In KEGG pathway analyses, the ERBB4 gene is concentrated in the 'cadherin signalling pathway' and 'EGF receptor pathway', and in protein classification, it is concentrated in protein subgroups such as 'metabolic interconversion enzymes', 'RNA metabolism proteins', 'protein modifying enzymes' (ERBB4, GALNTL6, PGAM5, PDE4C) (Fig. 4).

3.4 miR-551b-3p expression reduces overall survival in lung cancer patients

Kaplan-Meier (KM) plot, KM online tool was used to determine the prognostic significance of miR-551b-3p gene. The association between the expression levels of miR-551b-3p gene and overall survival rate (OS) in a total of 513 Lung adenocarcinoma patients was calculated by KM curve and log-rank test. The Kaplan-Meier survival analysis demonstrated that the overall survival rate was significantly reduced in lung cancer patients exhibiting expression of miR-551b-3p (HR 0.63, P: 0.0027) expression (Fig. 5).

3.5 Overexpression of miR-551b-3p and low expression of ERBB4 are associated with tumor development

The expression level of miR-551b-3p was validated by analyzing its association with tumor development, correlations with tumor stage, tumor grade and clinical characteristics of patients. According to TCGA datasets in the ULCAN database, miR-551b-3p was significantly more expressed in lung cancer patients compared to normal lung tissues ($p=9.1e-11$). Conversely, the ERBB4 gene was low expressed ($p=1.6e-12$) in cancerous tissues (Fig. 6). When the correlation of miRNAs with clinical parameters is determined, it increases the potential of miRNA as a therapeutic target and biomarker. For this purpose, the relationship between miR-551b-3p and

clinical parameters was determined. The expression of miR-551b-3p was exhibited a significant correlation with pathological M status ($p=4.64e^{-01}$), pathological N status ($p=5.26e^{-01}$) and sex ($p=2.59e^{-01}$) among clinical parameters (Table 1).

Table 1. Association of miR-551b-3p with clinical parameters in lung adenocarcinoma

Clinical Parameter	ANOVA P-value	ANOVA FDR	Multivariate Log Rank P-value	Multivariate Log Rank FDR
Pathologic M Status	$4.64e^{-01}$	$7.79e^{-01}$	$4.03e^{-02}$	$3.33e^{-01}$
Pathologic N Status	$5.26e^{-01}$	$9.99e^{-01}$	$3.71e^{-02}$	$3.40e^{-01}$
Sex	$2.59e^{-01}$	$5.84e^{-01}$	$3.74e^{-02}$	$3.03e^{-01}$

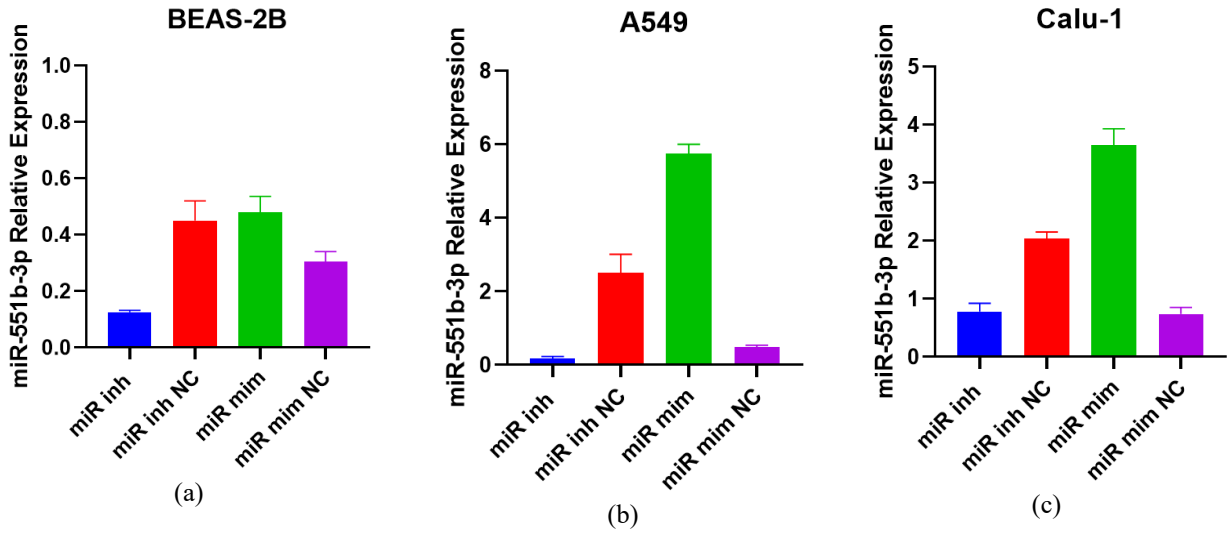

Figure 1. Gene expression levels of miR-551b-3p in cell lines. (a): miR-551b-3p expression level in BEAS-2B cell lines, (b): miR-551b-3p expression level in A549 cell lines, (c): miR-551b-3p expression level in Calu-1 cell lines. miR inh: miR-551-3p inhibitor, miR inh NC: miR-551-3p inhibitor negative control, miR mim: miR-551-3p mimic, miR mim NC: miR-551-3p mimic negative control.

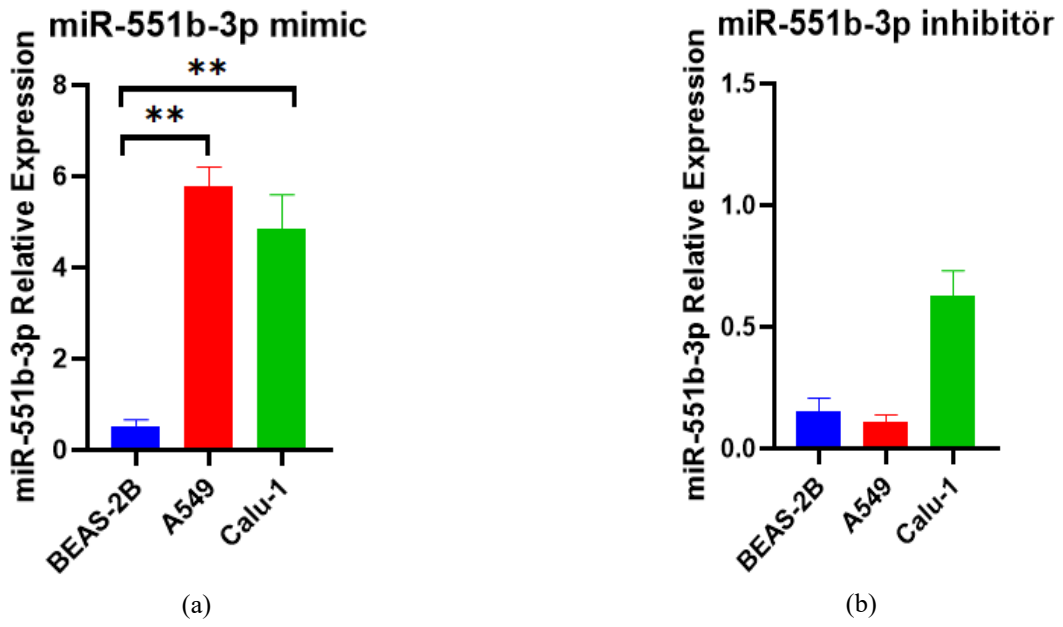

Figure 2. Expression levels of miR-551b-3p in healthy and cancerous lung cells in the presence of miRNA mimic and miRNA inhibitor

Celal Bayar University Journal of Science
Volume 21, Issue 2, 2025, p 125-133
Doi: 10.18466/cbayarfbe.1582834

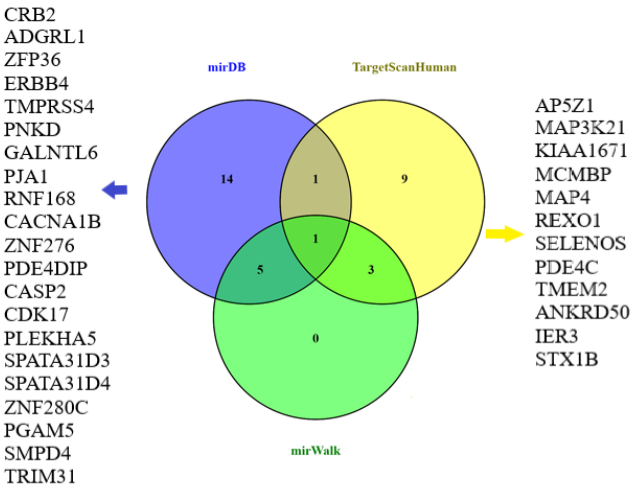

Figure 3. Venn diagram of overlapping target genes of miR-551b-3p in three separate in silico databases

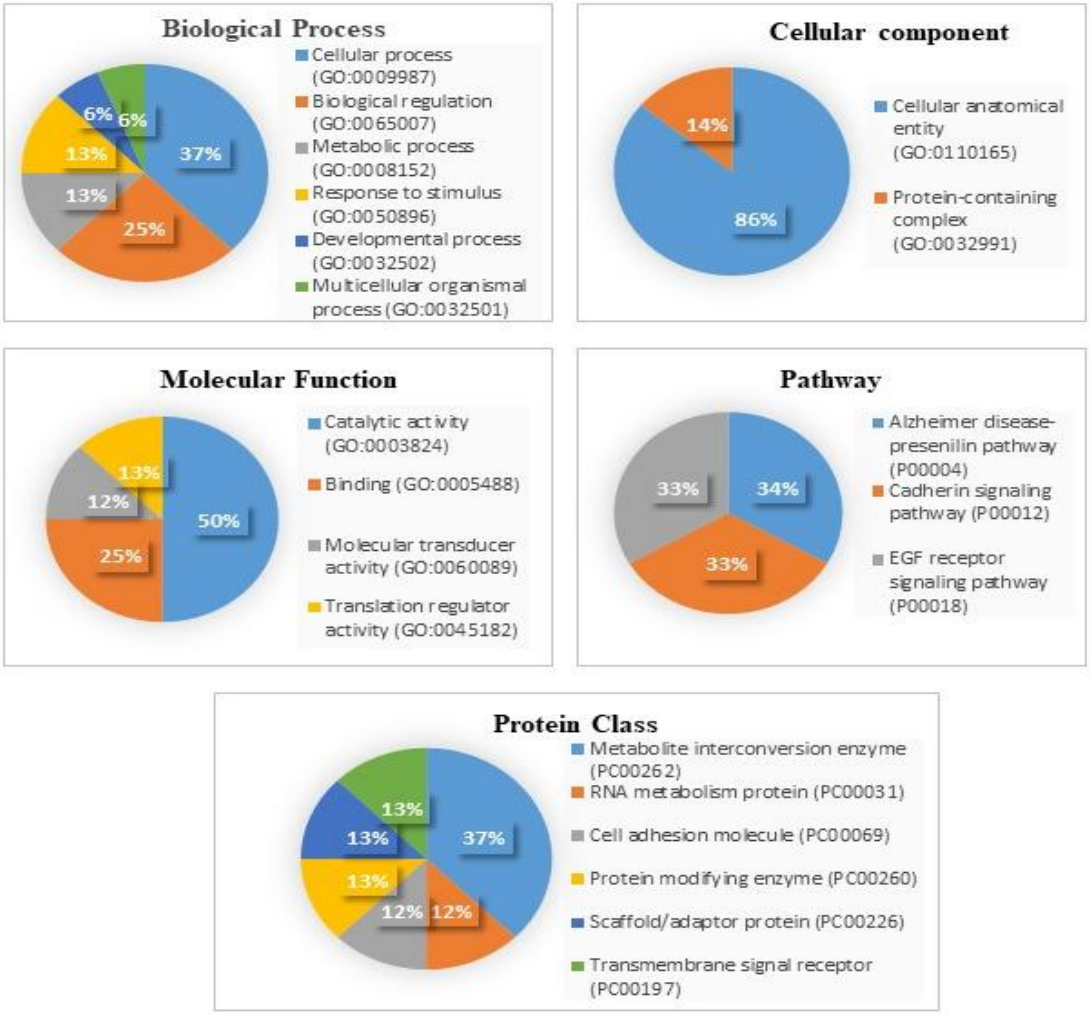

Figure 4. Grouping of miR-551b-3p target genes in biological function, pathway enrichment analysis and protein classification by in silico analysis

Celal Bayar University Journal of Science
Volume 21, Issue 2, 2025, p 125-133
Doi: 10.18466/cbayarfbe.1582834

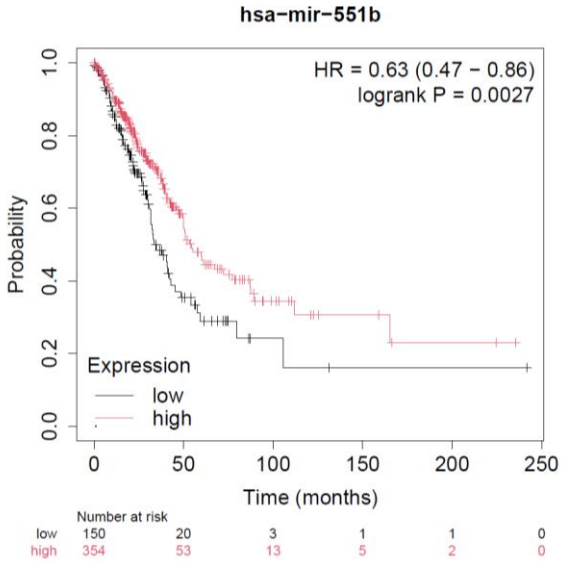

Figure 5. Survival analysis in lung cancer patients

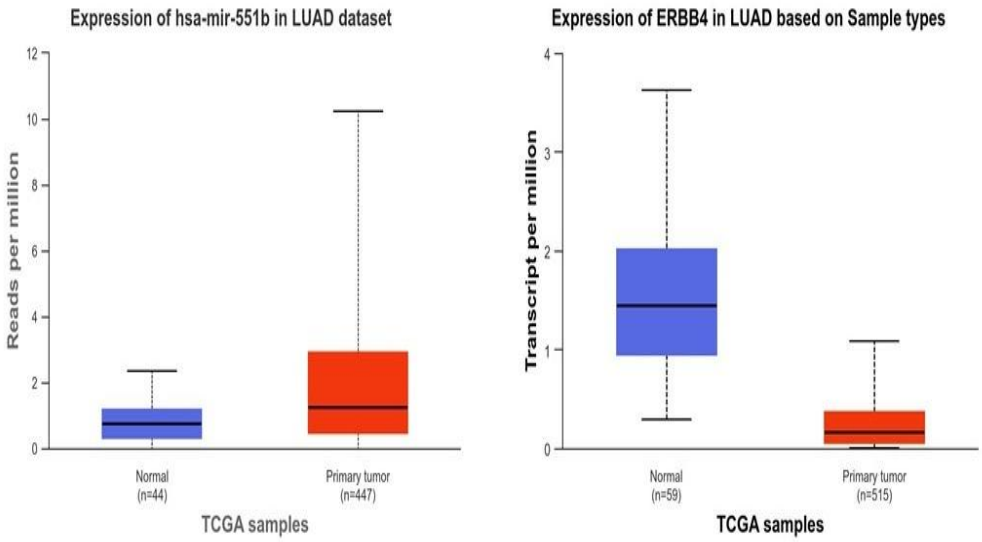

Figure 6. Expression level of miR-551b-3p and ERBB4 genes in lung cancer patients

4. Discussion

miRNAs are known to be involved in many physiological processes, including cell growth and differentiation, apoptosis and tumor progression [15,16]. Dysregulation of miRNA gene expression is closely associated with pathological conditions of these processes. Many miRNAs play a crucial role in carcinoma and have even been proposed as biomarkers for diagnosis and treatment [17-19]. Whether a miRNA is an oncogene or a tumor suppressor, which genes it targets, in which pathways it is effective in cellular processes and in which pathways it causes dysregulation are important for determining the therapeutic role of miRNA.

The aim of this study was to define the biological role of miR-551b-3p in lung cancer cells. miR-551b-3p has been studied in very few cancer cells. According to the available evidence, miR-551b-3p is down-regulated in colorectal cancer [20], gastric cancer [21], breast cancer [22] and cholangiocarcinoma [23], whereas it is up-regulated in ovarian cancer [24] and head and neck cancer [13]. Bioinformatic analysis identified differentially expressed genes in thyroid cancer, and miR-551b-3p was found to be upregulated in cancer cells [25]. The expression level of miR-551b-3p was investigated in colorectal cancer. miR-551b-3p levels were significantly downregulated in CRC cell lines compared to healthy cell lines [20]. A study by Parashar et al. reported that microRNA 551b-3p was up-regulated in triple negative breast cancer [10]. miRNAs can be

over-expressed in some cancer cells and down-regulated in others. These differences in miRNA expression suggest that the mechanisms of miRNAs in tumor formation and development are different.

In lung cancer, only a few studies on miR-551b-3p are available in the literature and these are usually in silico analyses. miR-551b-3p has been reported to be upregulated in lung cancer in silico [26]. Using the TCGA datasets, the expression profiles of microRNA of 418 patients with lung adenocarcinoma (LUAD) were analyzed by bioinformatic analysis. Among the differentially expressed miRNAs, mir-551b-3p was demonstrated to be a prognostic marker for overall survival [27]. Charkiewicz et al. carried out miRNA profiling using next-generation sequencing on serum samples from patients with NSCLC and patients with non-cancerous lung disease. As a result of the analysis, they identified 28 upregulated miRNAs, including miR-551b-3p [28]. miR-551b-3p expression was investigated in lung cancer and was downregulated in tumor cell lines by comparison with normal cell lines [11]. In this study, RT-PCR analyses showed overexpression of the related miRNA in two different lung cancer cell lines compared to normal lung cell lines, and the results were confirmed by bioinformatic analysis. miR-551b-3p expression significantly decreased overall survival in lung cancer patients.

The majority of lung cancer patients are diagnosed at the metastatic stage due to limitations in diagnosis and treatment. However, traditional methods such as surgery and chemotherapy are not very effective in advanced stages of the disease due to disease recurrence and significantly reduce the survival rate of the disease [29]. Different expression patterns of miRNAs in processes such as cell migration, proliferation and metastasis may affect the survival rate of the disease. In particular, evaluating the clinicopathological conditions of patients and developing targeted therapies specific to the histological type of patients can provide significant advances in treatment [30]. For example, miR-106a has been shown to suppress the expression of tumor protein 53-induced nuclear protein 1 (TP53INP1) and is overexpressed in lung adenocarcinoma tissue with bone metastases. miR-106a silencing may offer a novel treatment for bone metastases in lung adenocarcinoma [31]. Expression of miR-551b-3p significantly reduced overall survival in lung cancer patients. Furthermore, miR-551b-3p expression was closely associated with clinicopathological factors such as distant metastasis status, lymph node metastasis status and gender. Decreasing the expression level of miR-551b-3p or silencing the gene may be an alternative treatment strategy and a potential molecular marker.

It is important to characterize miRNAs, which can alter the ability of cells to develop tumors, progress and respond to treatment [32]. In gallbladder cancer, in vivo, in vitro and in silico analyses have shown that miR-551b-3p overexpression inactivates H6PD target gene expression and inhibits cell migration, invasion and EMT [33]. Overexpression of ERBB4, the target of miR-551b, was associated with poor prognosis in patients diagnosed with gastric cancer. miR-551b leads to inhibition of EMT and metastasis through downregulation of ERBB4 expression [34]. In patients with hepatocellular carcinoma, ERBB4 expression was shown to be downregulated and related to cellular differentiation and poorer prognosis [35]. In this study, ERBB4 was identified as a target gene of miR-551b-3p and found to have low levels of expression in lung cancer in comparison to normal tissue. ERBB4 belongs to the family of the ERBB receptor kinase. In cancer, the ERBB4 gene is generally known as a tumor suppressor, but there are also studies suggesting that it promotes tumor growth [34]. The ERBB4 gene is enriched in critical cancer-related processes such as cellular processes, translational regulatory activity, the cadherin signalling pathway and the EGF receptor signalling pathway.

5. Conclusion

In conclusion, the role of miR-551b-3p in lung cancer has been defined both in vitro and in silico. According to qRT PCR analyses, miR-551b-3p expression was higher in tumor cells from healthy cells. The expression level of miR-551b-3p was confirmed in silico in cancerous lung tissue compared to healthy tissues. The target gene of mir-551b-3p was identified as ERBB4 and showed a negative correlation with mir-551b-3p. mir-551b-3p expression decreased overall survival and was markedly related to distant metastasis, lymph node metastasis and gender. The results obtained suggest that miR-551b-3p may be a potential therapeutic target in lung cancer diagnosis and treatment. However, more detailed studies are needed to better understand mir-551b-3p tumor biology.

Acknowledgement

This research was funded by Kahramanmaraş Sütçü İmam University Scientific Research Projects Coordination Unit for the research, grant number 2021/6-19 M.

Author's Contributions

Esen ÇAKMAK: Drafted and wrote the manuscript, performed the experiment and result analysis.

İbrahim Seyfettin ÇELİK: Assisted in analytical analysis on the structure, supervised the experiment's progress, result interpretation and helped in manuscript preparation.

Ethics

There are no ethical issues after the publication of this manuscript.

References

[1]. Leiter, A., Veluswamy, R.R., Wisnivesky, J. 2023. The global burden of lung cancer: current status and future trends. *Nature Reviews Clinical Oncology*; 20(9):624-639.

[2]. Çakmak, E. 2022. A bioinformatics approach to identify potential biomarkers in non-small cell lung cancer. *Cumhuriyet Science Journal*; 43(1):6-13.

[3]. Yang, Q., Wang, W., Cheng, D., Wang, Y., Han, Y., Huang, J. 2024. Non-coding RNA in exosomes: Regulating bone metastasis of lung cancer and its clinical application prospect. *Translational Oncology*; 46:102002.

[4]. Lahiri, A., Maji, A., Potdar, P.D., Singh, N., Parikh, P. 2023. Lung cancer immunotherapy: progress, pitfalls, and promises. *Molecular Cancer*; 22(1):40.

[5]. Chakrabortty, A., Patton, D., Smith, B.F. 2023. miRNAs: potential as biomarkers and therapeutic targets for cancer. *Genes*; 14(7):1375.

[6]. Çakmak, E. 2020. Computational and experimental tools of miRNAs in cancer. *Middle East Journal of Cancer*; 11(4):381-389.

[7]. Zhang, Q., Pan, J., Xiong, D., Zheng, J., McPherson, K.N., Lee, S., You, M. 2023. Aerosolized miR-138-5p and miR-200c targets PD-L1 for lung cancer prevention. *Frontiers in Immunology*; 14:1166951.

[8]. Cui, Y., Wu, X., Jin, J., Man, W. et al. 2023. CircHERC1 promotes non-small cell lung cancer cell progression by sequestering FOXO1 in the cytoplasm and regulating the miR-142-3p-HMGB1 axis. *Molecular Cancer*; 22(1):179.

[9]. Yang, F., Yan, Y., Yang, Y., Hong, X., Wang, M., Yang, Z. et al. 2020. MiR-210 in exosomes derived from CAFs promotes non-small cell lung cancer migration and invasion through PTEN/PI3K/AKT pathway. *Cell signal*; 73:109675.

[10]. Parashar, D., Geethadevi, A., Aure, M.R., Mishra, J., George, J. et al. 2019. miRNA551b-3p activates an oncostatin signaling module for the progression of triple-negative breast cancer. *Cell reports*; 29(13), 4389-4406.

[11]. Wang, X., Cheng, Z., Dai, L., Jiang, T., Li, P., Jia, L. et al. 2021. LncRNA PVT1 facilitates proliferation, migration and invasion of NSCLC cells via miR-551b/FGFR1 axis. *OncoTargets and therapy*; 3555-3565.

[12]. Yuan, H., Chen, Z., Bai, S., Wei, H., Wang, Y. et al. 2018. Molecular mechanisms of lncRNA SMARCC2/miR-551b-3p/TMPRSS4 axis in gastric cancer. *Cancer Letters*; 418, 84-96.

[13]. Karanam, N.K., Ding, L., Vo, D.T., Giri, U., Yordy, J.S. et al.2023. miR-551a and miR-551b-3p target GLIPR2 and promote tumor growth in high-risk head and neck cancer by modulating autophagy. *Advances in Cancer Biology-Metastasis*; 7:100085.

[14]. Thomas, P.D., Ebert, D., Muruganujan, A., Mushayahama, T. et al. 2022. PANTHER: Making genome-scale phylogenetics accessible to all. *Protein Science*; 31(1):8-22.

[15]. An, J., Zhang, M., Fu, Y., Zhang, D. et al. 2024. Emerging electrochemical biosensors for lung cancer-associated protein biomarker and miRNA detection. *International Journal of Biological Macromolecules*; 280(3):135972.

[16]. Singh, S., Saxena, S., Sharma, H., Paudel, K.R., Chakraborty, A. et al. 2024. Emerging role of tumor suppressing microRNAs as therapeutics in managing Non-small cell lung cancer. *Pathology - Research and Practice*; 155222.

[17]. Dong, Z.R., Cai, J.B., Shi, G.M., Yang, Y.F. et al. 2023. Oncogenic miR-93-5p/Gal-9 axis drives CD8 (+) T-cell inactivation and is a therapeutic target for hepatocellular carcinoma immunotherapy. *Cancer Letter*; 564:216186.

[18]. Hsu, C.Y., Allela, O.Q.B., Mahdi, S.A.H., Doshi, O.P., Adil, M. et al. 2023. miR-136-5p: A key player in human cancers with diagnostic, prognostic and therapeutic implications. *Pathology - Research and Practice*; 54794.

[19]. Liu, S., Ruan, Y., Chen, X., He, B., Chen, Q. 2024. miR-137: a potential therapeutic target for lung cancer. *Frontiers in Cell and Developmental Biology*; 12:1427724.

[20]. Kim, K.S., Jeong, D., Sari, I.N., Wijaya, Y.T. et al. 2019. miR551b regulates colorectal cancer progression by targeting the ZEB1 signaling axis. *Cancers*; 11(5):735.

[21]. Bai, S.Y., Ji, R., Wei, H., Guo, Q.H., Yuan H. et al. 2019. Serum miR-551b-3p is a potential diagnostic biomarker for gastric cancer. *Turkish Journal of Gastroenterology*; 30(5):415.

[22]. Yang, Z., Xu, B., Wu, S., Yang, W., Luo, R., Geng, S. et al. 2022. Exosomal microRNA-551b-3p from bone marrow-derived mesenchymal stromal cells inhibits breast cancer progression via regulating TRIM31/Akt signaling. *Human Cell*; 235(6):1797-1812.

[23]. Chang, W., Wang, Y., Li, W., Shi, L., Geng, Z. 2019. Micro RNA-551b-3p inhibits tumor growth of human cholangiocarcinoma by targeting Cyclin D1. *Journal of Cellular and Molecular Medicine*; 23(8):4945-4954.

[24]. Chaluvally-Raghavan, P., Jeong, K.J., Pradeep, S., Silva, A.M., et al. 2016. Direct upregulation of STAT3 by MicroRNA-551b-3p deregulates growth and metastasis of ovarian cancer. *Cell Reports*; 15(7):1493-1504.

[25]. Çaglar, H.O., Aytatli, A., Karatas, O.F. 2024. In silico identification of differentially expressed microRNAs in thyroid cancer. *Human Gene*; 201306.

[26]. Yu, H., Pang, Z., Li, G., Gu, T. 2021. Bioinformatics analysis of differentially expressed miRNAs in non-small cell lung cancer. *Journal of Clinical Laboratory Analysis*; 35(2):e23588.

[27]. Lin, K., Xu, T., He, B.S., Pan, Y.Q., Sun, H.L., Peng, H.X. et al. 2016. MicroRNA expression profiles predict progression and clinical outcome in lung adenocarcinoma. *OncoTargets and therapy*; 5679-5692.

[28]. Charkiewicz, R., Sulewska, A., Mroz, R., Charkiewicz, A., Naumnik, W. et al. 2023. Serum Insights: Leveraging the Power of miRNA Profiling as an Early Diagnostic Tool for Non-Small Cell Lung Cancer. *Cancers*; 15(20), 4910.

[29]. Pandey, M., Mukhopadhyay, A., Sharawat, S. K., & Kumar, S.2021. Role of microRNAs in regulating cell proliferation, metastasis and chemoresistance and their applications as cancer biomarkers in small cell lung cancer. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*; 1876(1), 188552.

[30]. Schegoleva, A. A., Khozyainova, A. A., Fedorov, A. A., Gerashchenko, T. S., Rodionov, E. O., Topolnitsky, E. B., ... & Denisov, E. V. 2021. Prognosis of different types of non-small cell lung cancer progression: current state and perspectives. *Cell Physiol Biochem*; 55(S2), 29-48.

[31]. Han, L., Huang, Z., Liu, Y., Ye, L., Li, D., Yao, Z., ... & Yang, Z. 2021. MicroRNA-106a regulates autophagy-related cell death and EMT by targeting TP53INP1 in lung cancer with bone metastasis. *Cell death & disease*; 12(11), 1037.

[32]. Pourdavoud, P., Pakzad, B., Mosallaei, M., Saadatian, Z., Esmaeilzadeh, E. et al. 2020. MiR-196: emerging of a new potential therapeutic target and biomarker in colorectal cancer. *Molecular Biology Reports*; 47:9913-9920.

[33]. Ji, T., Gao, L., Yu, Z. 2021. Tumor-suppressive microRNA-551b-3p targets H6PD to inhibit gallbladder cancer progression. *Cancer Gene Therapy*; 28(6), 693-705.

[34]. Song, G., Zhang, H., Chen, C., Gong, L., Chen, B., Zhao, S. et al. 2017. miR-551b regulates epithelial-mesenchymal transition and metastasis of gastric cancer by inhibiting ERBB4 expression. *Oncotarget*; 8(28):45725.

[35]. Liu, Y., Song, L., Ni, H., Sun, L., Jiao, W., Chen, L. 2017. ERBB4 acts as a suppressor in the development of hepatocellular carcinoma. *Carcinogenesis*; 38(4):465-473.