Research Article

miR-598 Represses Cell Migration and Invasion of Non-Small-Cell Lung Cancer by Inhibiting MSI2

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Non-small-cell lung cancer (NSCLC) is one of the most frequent solid tumors and regarded as a significant threat to individual health around the world. MicroRNAs (miRs) are recognized as critical governors of gene expression during carcinogenesis, while their clinical significance and mechanism in NSCLC occurrence and development are required for further investigation. In this report, we characterized the functional role of miR-598 and its regulation mechanism in NSCLC. The expression level of miR-598 in NSCLC tissues and cell lines was detected by qRT-PCR. A549 cells were transiently transfected with miR-598 mimics or miR-598 inhibitors. Scratch assay and Transwell assay were used to detect cell transfection, migration, and invasion. Possible binding sites of miR-598 in MSI2 mRNA were predicted by bioinformatics and validated by dual-luciferase reporter gene system. The ability of migration and invasion was examined on cells transfected with MSI2 alone or cotransfected A549 cells with miR-598. The expression of miR-598 in NSCLC tissues was significantly lower than that in adjacent tissues, and the expression of miR-598 in NSCLC cell lines (A549, H1650, and H1299) was also significantly lower than that of normal lung epithelial cell line BEAS-2B. A549 cells were significantly inhibited in migration and invasion after transfection with miR-598 mimics, while miR-598 inhibitors were significantly enhanced in migration and invasion. MSI2 was a direct target gene of miR-598. MSI2 can promote the migration and invasion of A549 cells, but the ability to promote cell migration and invasion was reversed when miR-598 was introduced. In conclusion, miR-598 inhibits the migration and invasion of NSCLC by downregulating the target gene MSI2.

1. Introduction

Lung cancer is a common malignant tumor of the respiratory system, and the incidence and mortality rates of lung cancer have significantly increased in recent years in China [1]. Due to diversity in lifestyles and socioeconomic development, lung cancer presents geographic and gender differences in China [2]. Non-small-cell lung cancer (NSCLC) is the most common type of lung cancer, accounting for about 80% to 85% [3]. In the recent three decades, the 5-year survival of patients with lung cancer remains only 19% with minimal improvement [4]. Although the design of nanoparticle-based carriers for targeted drug delivery has shown promise in treating human cancers [5, 6], therapeutic strategies of NSCLC are still needed to be further explored. Thus, it is imperative and meaningful to explore the underlying molec-

ular mechanisms and identify novel prognostic biomarkers and potential therapeutic targets for lung cancer [7].

As an endogenous class of noncoding small-molecule RNAs with a length of 18 to 22 nucleotides, miRNAs specifically bind to the 3'UTR end of the target gene mRNA molecules, thereby regulating the expression of the target gene and participating in proliferation, migration, invasion, apoptosis, and epithelial-mesenchymal transition of a variety of tumor cells [8]. An in-depth study of miRNA-related molecular mechanisms is of great significance for the diagnosis and treatment of NSCLC [9]. Studies have shown that miR-598 is significantly suppressed in colorectal cancer [10], gastric cancer [11], osteosarcoma [12], retinoblastoma [13], and ovarian cancer [14], suggesting that miRNA-598 may be a potential tumor suppressor miRNA. In recent years, miRNA-598 has been

FIGURE 1: Expressions of miR-598 in NSCLC tissues and cell lines. (a) Relative expression levels of miR-598 in NSCLC tissues ($n = 37$) and corresponding adjacent tissues ($n = 37$) were determined by qRT-PCR. (b). Relative expression levels of miR-598 in the three NSCLC cell lines and normal lung epithelial cell line BEAS-2B were determined by qRT-PCR. a indicates $P < 0.01$ compared with corresponding adjacent tissues and BEAS-2B, respectively.

widely studied in inhibiting tumor cell growth, migration, and invasion, but its role in the occurrence and development of NSCLC is unclear. Musashi 2 (MSI2) protein is a member of the Musashi protein family. MSI2 is not only an important molecular marker of stem cells and early progenitor cells but also closely related to the prognosis and metastasis of various tumors [15]. MSI2 is highly expressed in the context of pancreatic cancer [16], liver cancer [17], and glioma [18], and its expression level is closely related to the invasion and metastasis of tumor cells.

This study investigated the expression of miR-598 in non-small-cell lung cancer tissues and the effect of miRNA-598 targeting MSI2 NSCLC cell migration and invasion in order to provide new theoretical basis for the diagnosis and treatment of NSCLC.

2. Materials and Methods

2.1. Sample Collection. Thirty-seven cases of primary NSCLC tissues resected by thoracic surgery from August 2017 to March 2019 in the First People's Hospital of Wen Ling were collected. At the same time, normal lung tissues from the above cases (distance from the lesion > 5 cm and pathologically confirmed no tumor cell infiltration) were set as a control. Of the 37 patients, 18 were males and 19 were females, with an average age of 56.7 ± 8.9 years. Among them, 23 were having squamous cell carcinomas and 14 were having adenocarcinomas. All patients were not treated with chemotherapy and radiation before surgery. After the specimens were cut, they were quickly frozen in liquid nitrogen and then transferred to a -80°C refrigerator for storage. Patients in this study have signed an informed consent, and this study was approved by the hospital medical ethics committee.

2.2. Cell Culture. Human lung cancer cell lines A549, H1650, and H1299 and human normal lung epithelial cells BEAS-2B were purchased from the Cell Resource Center of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. All cells were routinely cultured in RPMI-1640

medium (including 10% fetal calf serum + 100 U/mL penicillin + 100 $\mu\text{g/mL}$ streptomycin), placed in a 37°C , 5% CO_2 saturated humidity incubator.

2.3. qRT-PCR Experiment. The total RNA of tissues and cells was extracted by a Trizol method. TaqMan MicroRNA Reverse Transcription kit was used for reverse transcription reaction. The reaction conditions were 16°C for 30 min, 42°C for 30 min, and 85°C for 5 min. TaqMan MicroRNA Assay Real-time PCR kit was used for quantitative amplification reaction; the reaction conditions were 95°C for 5 min, 95°C for 15 s, 62°C for 30 s, and a total of 40 cycles. The reaction uses U6 as an internal reference, and the sequence of the primers is as follows: miR-598 is 5'-AGCTACGTCATCGT TGTCATC-3'; U6 is 5'-CTCGCTTCGGCAGCACA-3'. Three replicates were made for each test index, and the expression level of miR-598 was relatively quantified by the $2^{-\Delta\Delta\text{Ct}}$ method.

2.4. Cell Transfection. When A549 cells grow to about 80%, Lipofectamine 2000 reagents (Invitrogen, Carlsbad, CA, USA) were used to transfect miRNA control (miR-NC), miR-598 mimics, and miR-598 inhibitors (GenePharma, Shanghai, China) into A549 cells. The fresh medium was changed after 8 h, and cells were collected for subsequent experiments after 48 h.

2.5. Scratch Healing Assay. A549 cells were seeded into a 6-well plate. After the cells were covered with the bottom of the well, a "1"-shaped scratch was made on the bottom of the culture plate with a $10\ \mu\text{L}$ sterile pipette tip. Under the inverted microscope, observe the migration of cells in the scratches after 36 h and take pictures. Measure the width of the scratch at three locations along the edge of the scratch at an equal interval, and take the average value (scratch healing rate (%) = $(0\ \text{h scratch width} - 36\ \text{h scratch width}) / 0\ \text{h scratch width} \times 100\%$).

2.6. Transwell Invasion Assay. Transwell experiments were performed using an $8\ \mu\text{m}$ polycarbonate filter culture

FIGURE 2: An inhibitory effect of miR-598 on migration of A549 cells: (a) representative image of the scratch healing from 0 to 36 h after miR-598 mimic or inhibitor transfection, examined by scratch test; (b) A549 cell migration rate after 36 h transfection of miR-598 mimic or inhibitor transfection. a indicates $P < 0.01$, and b indicates $P < 0.05$ compared with miR-NC, respectively.

FIGURE 3: An inhibitory effect of miR-598 on invasion of A549 cells: (a) A549 cell invasion rate after 36 h transfection of miR-598 mimic or inhibitor transfection; (b) representative image of cells invading from the upper Transwell chambers to the lower chamber. a indicates $P < 0.01$, and b indicates $P < 0.05$ compared with miR-NC, respectively.

chamber preplated with Matrigel. A549 cells in the logarithmic growth phase were made into 1×10^5 cell suspension at a density of 1 mL/mL, and 200 μ L of the cell suspension was inoculated into the upper chamber of the Transwell chamber. 500 μ L of RPMI-1640 culture medium containing 10% fetal bovine serum (Gibco, Grand Island, NY, USA) was added to the lower chamber, and three replicates were set in each group. After 36 hours of incubation at 37°C and 5% CO₂, gently wipe away the Matrigel and the upper nonmembrane cells with a cotton swab, fix the cells with 4% paraformaldehyde for 10 minutes, and stain with 0.1% crystal violet for 10 minutes. The number of cells in 5 fields of view was

counted randomly under a 100x microscope, and the average was taken as the number of cells passing through each chamber.

2.7. Double-Luciferase Reporter Gene Detection. 293FT cells (ATCC, USA) were seeded into a 24-well plate (1.5×10^5 /well), and when the cells grew to about 80%, the plasmid miR-NC + pGL3-MSI2-wt, miR-598 mimics + pGL3-MSI2-wt, miR-NC + pGL3-MSI2-mut, and miR-598 mimics + pGL3-MSI2-mut were cotransfected into 293FT cells, and fresh DMEM medium was replaced after 6 h. 48 hours after transfection, the cells in each group were

FIGURE 4: MSI2 is the target gene of miR-598: (a) sequence alignment of miR-598 and its conserved target site in RTKN-3' -UTR, predicted in the ENCORI database (<http://starbase.sysu.edu.cn/>); (b) relative luciferase activity in 293FT cells cotransfected with pGL3-MSI2-wt or pGL3-MSI2-mut reporter plasmids containing miR-598 potential binding sites or control plasmids; (c) the MSI2 mRNA expression level in A549 cells upon miR-598 mimic transfection. a indicates $P < 0.01$ compared with miR-NC.

lysed, and the fluorescence intensity of the cells in each group was expressed by the ratio of firefly luciferase activity to Renilla luciferase activity according to the instructions of the dual-luciferase reporter assay system.

2.8. Western Blotting. Cells were lysed on ice for 30 min with RIPA lysate, and protein concentration was quantified by bicinchoninic acid (BCA) assay. 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used for gel electrophoresis separation, and the protein loading amount of each lane was 20 μ g. Concentrated gel was electrophoresed at 60 V for 1 h, and separated gel was electrophoresed at 100 V for 2 h. The membrane was transferred by conventional wet transfer method for 90 min, blocked with 5% bovine serum albumin (BSA) in Tris-buffered saline Tween (TBST) buffer for 1 h, and added with primary antibody to MSI2 (ab76148, Abcam, Cambridge, UK) or β -actin at 4°C for overnight incubation. After two times, the corresponding secondary antibody was added and incubated at

room temperature for 2 h, and the membrane was washed 3 times with TBST. The ECL light-emitting kit was illuminated and developed and analyzed after fixing.

2.9. Statistical Processing. All experiments were repeated three times, and the experimental data were expressed as mean $\pm$ standard deviation. SPSS 20 software was used for statistical analysis. One-way analysis of variance was used for data comparison between multiple groups, and q test was used for pairwise comparison. $P < 0.05$ was considered statistically significant.

3. Results

3.1. miR-598 Was Downregulated in NSCLC Tissues and Cell Lines. To study expression pattern of miR-598 in the context of lung cancer, the expression of miR-598 in 37 cases of lung cancer and adjacent tissues was detected by qRT-PCR. The results showed that compared with normal tissues, the

FIGURE 5: miR-598 represses NSCLC cell migration and invasion by regulating MSI2: (a) Western blots of MSI2 protein in A549 cells transfected with miR-598 mimic and MSI2 expression vector; (b) migration of A549 cells examined by Transwell test after with transfection with miR-598 mimic and MSI2 expression vector; (c) invasion of A549 cells examined by Transwell test after transfection with miR-598 mimic and MSI2 expression vector; (d) expression of miR-598 was negatively correlated with the expression of MSI2 in NSCLC tissues. a indicates $P < 0.01$ compared with A549 cells transfected with MSI2 expression vector.

expression of miR-598 in lung cancer tissues was significantly downregulated ($P < 0.001$, Figure 1(a)). In lung cancer cell line compared to human normal lung skin cells BEAS-2B, the expression of miR-598 in A549, H1650, and H1299 was also downregulated ($P < 0.001$, Figure 1(b)).

3.2. miR-598 Inhibited NSCLC Cell Migration. The results of scratch test showed that compared with the negative control group, the migration ability of A549 cells in the miR-598 mimic group was significantly inhibited ($P < 0.01$), while the miR-598 inhibitor group had enhanced migration ability ($P < 0.01$, Figures 2(a) and 2(b)).

3.3. miR-598 Inhibited NSCLC Cell Invasion. Transwell invasion assays showed that compared with the negative control group, the invasion ability of A549 cells in the miR-598 mimic group was significantly reduced ($P < 0.01$), while the invasion ability of the miR-598 inhibitor group was enhanced ($P < 0.05$, Figures 3(a) and 3(b)).

3.4. MSI2 Was a Target Gene of miR-598. Bioinformatics prediction in the ENCORI database (<http://starbase.sysu.edu.cn/>) showed that miR-598 and the 3'UTR of the oncogene MSI2 have multiple base binding sites, which may be a good target gene for miR-598 (Figure 4(a)). Subsequently, the dual-luciferase reporter gene system was used to detect the correlation of MSI2 and miR-598. The MSI2 gene wild-type (MSI2-wt) and MSI2-mutant (MSI2-mut) luciferase reporter vectors were constructed and cotransfected in 293FT cells with miR-598 mimics.

The results showed that miR-598 mimics significantly inhibited MSI2 wild-type luciferase activity ($P < 0.01$) without affecting MSI2-mutant luciferase activity (Figure 4(b)). Subsequently, the MSI2 mRNA expression level in A549 cells overexpressing miR-598 mimics was detected, and the expression level was significantly reduced ($P < 0.01$, Figure 4(c)).

3.5. miR-598 Inhibited NSCLC Cell Migration and Invasion by Regulating MSI2. In order to prove that miR-598 inhibited

the migration and invasion of lung cancer cells by regulating MSI2, we transfected the MSI2 gene alone or cotransfected with miR-598 mimics in A549 cells and found that the level of MSI2 protein increased after transfection alone. Protein levels returned to normal after cotransfection with miR-598 mimics (Figure 5(a)). The subsequent scratch test results showed that MSI2 could significantly enhance the migration ability of A549 cells, but when miR-598 mimics were introduced, the cell migration ability was significantly reversed (Figure 5(b)). Transwell invasion experiment results showed that MSI2 can significantly enhance the invasion ability of A549 cells, but the cell invasion ability was also significantly reversed when miR-598 was introduced (Figure 5(c)). At last, we performed Spearman correlation analysis and found that the expression of miR-598 was negatively correlated with the expression of MSI2 in NSCLC tissues (Figure 5(d)).

4. Discussion

Current research suggested that miRNAs were closely related to the occurrence and development of diseases such as differentiation, proliferation, invasion, and apoptosis of lung cancer cells [19]. miRNAs can downregulate the activity of oncogenes to inhibit tumorigenesis and cancer development [20]. Therefore, finding and studying miRNA molecules closely related to the occurrence and development of lung cancer are of great significance for exploring the initiation mechanism of lung cancer, preventing the occurrence of lung cancer metastasis, and selecting appropriate targets for intervention and treatment.

miR-598 has been well-studied as a tumor suppressor in several human cancers. For example, miR-598 was reported previously to inhibit epithelial mesenchymal transition through targeted regulation of the JAG1 gene, thereby effectively inhibiting colorectal cancer cell migration and invasion [10]. However, another study claimed that miR-598 functioned as an oncomiR that its overexpression promoted colorectal cancer cell proliferation and cell cycle progression [21]. The controversial role of miR-598 merits further investigation in human cancers. In our study, we upregulated or inhibited the expression of miR-598 in A549 cells through miR-598 mimics and miR-598 inhibitors and verified that miR-598 may play a role in suppressing the migration and invasion of tumor cells in lung cancer cells through scratch and Transwell experiments. Concurring with our results, Yang and his team found that miR-598 suppressed the EMT process to suppress the invasion and migration in NSCLC [22]. Tong et al. demonstrated that ectopic expression of miR-598 reduced NSCLC cell proliferation and invasion *in vitro* [23]. Differently, our double-luciferase experiments verified that MSI2 was the downstream target gene, responsible for the inhibitory role of miR-598 in migration and metastasis of lung cancer cells.

MSI2 protein is a member of the Musashi protein family. Although accumulating evidence showed a role for the MSI2 paralogue MSI1 as oncogenic in some cancer types [24, 25], MSI2 has attracted much less attention. However, MSI2 has been shown to be oncogenic in a mouse model of colon cancer [26]. Besides, myofibroblast-specific MSI2 knockout

inhibits hepatocellular carcinoma progression in a mouse model [27]. Elevated MSI2 expression is associated with poor survival in leukemia [28], and MSI2 knockdown or genetic deletion reduced engraftment and caused a defect in hematopoietic stem cell maintenance *in vivo* as a result of decreased proliferation [29]. In addition, in our experiments, increasing the expression level of MSI2 significantly enhanced the migration and invasion of lung cancer cells. Consistent with our results, MSI2 is an important regulator of the occurrence and metastasis of NSCLC, which may be closely related to the activation of the TGF- β signaling pathway as a result of MSI2 upregulation [30]. In the study reported by Cheng et al., they supported that an increased expression of MSI2 could promote lung cancer cell proliferation and invasion [31]. Although we do not know whether MSI2 regulates the migration and invasion of lung cancer cells and whether it is related to the above signaling pathways, the reason for the migration and invasion of non-small-cell lung cancer is still explained from a new direction through this experiment, and it provides a new target for cancer treatment.

In conclusion, our study provides evidence that miR-598 represses cell migration and invasion in NSCLC through targeted inhibition of MSI2. However, we must acknowledge there are limitations or further investigations in the present study. First, miR-598 acting as oncomiR should be validated in a mouse model of NSCLC. Second, *in vitro* data should be strengthened in more than a single NSCLC cell line.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

All authors declare that they have no conflict of interest.

References

- [1] M. Cao and W. Chen, "Epidemiology of lung cancer in China," *Thoracic Cancer*, vol. 10, no. 1, pp. 3–7, 2019.
- [2] X. Lin, M. S. Bloom, Z. Du, and Y. Hao, "Trends in disability-adjusted life years of lung cancer among women from 2004 to 2030 in Guangzhou, China: A population-based study," *Cancer Epidemiology*, vol. 63, article 101586, 2019.
- [3] R. S. Herbst, D. Morgensztern, and C. Boshoff, "The biology and management of non-small cell lung cancer," *Nature*, vol. 553, no. 7689, pp. 446–454, 2018.
- [4] F. Sun, L. Li, P. Yan et al., "Causative role of PDLIM2 epigenetic repression in lung cancer and therapeutic resistance," *Nature Communications*, vol. 10, no. 1, p. 5324, 2019.
- [5] X. Yu, I. Trase, M. Ren, K. Duval, X. Guo, and Z. Chen, "Design of nanoparticle-based carriers for targeted drug delivery," *Journal of Nanomaterials*, vol. 2016, 15 pages, 2016.
- [6] C. D. Fahrenholtz, J. Swanner, M. Ramirez-Perez, and R. N. Singh, "Heterogeneous responses of ovarian cancer cells to silver nanoparticles as a single agent and in combination with cisplatin," *Journal of Nanomaterials*, vol. 2017, 11 pages, 2017.

- [7] F. Oberndorfer and L. Mullauer, "Molecular pathology of lung cancer: current status and perspectives," *Current Opinion in Oncology*, vol. 30, no. 2, pp. 69–76, 2018.
- [8] G. Di Leva, M. Garofalo, and C. M. Croce, "MicroRNAs in cancer," *Annual Review of Pathology*, vol. 9, no. 1, pp. 287–314, 2014.
- [9] K. L. Wu, Y. M. Tsai, C. T. Lien, P. L. Kuo, and A. J. Hung, "The roles of microRNA in lung cancer," *International Journal of Molecular Sciences*, vol. 20, no. 7, p. 1611, 2019.
- [10] J. Chen, H. Zhang, Y. Chen et al., "miR-598 inhibits metastasis in colorectal cancer by suppressing JAG1/Notch2 pathway stimulating EMT," *Experimental Cell Research*, vol. 352, no. 1, pp. 104–112, 2017.
- [11] N. Liu, H. Yang, and H. Wang, "miR-598 acts as a tumor suppressor in human gastric cancer by targeting IGF-1R," *Oncotargets and Therapy*, vol. Volume 11, pp. 2911–2923, 2018.
- [12] K. Liu, X. Sun, Y. Zhang, L. Liu, and Q. Yuan, "MiR-598: a tumor suppressor with biomarker significance in osteosarcoma," *Life Sciences*, vol. 188, pp. 141–148, 2017.
- [13] F. Liu, Q. Zhang, and Y. Liang, "MicroRNA-598 acts as an inhibitor in retinoblastoma through targeting E2F1 and regulating AKT pathway," *Journal of Cellular Biochemistry*, vol. 121, no. 3, pp. 2294–2302, 2020.
- [14] F. Xing, S. Wang, and J. Zhou, "The expression of microRNA-598 inhibits ovarian cancer cell proliferation and metastasis by targeting URI," *Molecular Therapy - Oncolytics*, vol. 12, pp. 9–15, 2019.
- [15] K. J. Hope and G. Sauvageau, "Roles for MSI2 and PROX1 in hematopoietic stem cell activity," *Current Opinion in Hematology*, vol. 18, no. 4, pp. 203–207, 2011.
- [16] W. Sheng, M. Dong, C. Chen, Y. Li, Q. Liu, and Q. Dong, "Musashi2 promotes the development and progression of pancreatic cancer by down-regulating Numb protein," *Oncotarget*, vol. 8, no. 9, pp. 14359–14373, 2017.
- [17] M. H. Wang, S. Y. Qin, S. G. Zhang et al., "Musashi-2 promotes hepatitis Bvirus related hepatocellular carcinoma progression via the Wnt/ β -catenin pathway," *American Journal of Cancer Research*, vol. 5, no. 3, pp. 1089–1100, 2015.
- [18] J. L. Cox, P. J. Wilder, J. M. Gilmore, E. L. Wuebben, M. P. Washburn, and A. Rizzino, "The SOX2-interactome in brain cancer cells identifies the requirement of MSI2 and USP9X for the growth of brain tumor cells," *PLoS One*, vol. 8, no. 5, article e62857, 2013.
- [19] Z. S. Hashemi, S. Khalili, M. Forouzandeh Moghadam, and E. Sadroddiny, "Lung cancer and miRNAs: a possible remedy for anti-metastatic, therapeutic and diagnostic applications," *Expert Review of Respiratory Medicine*, vol. 11, no. 2, pp. 147–157, 2017.
- [20] A. A. Svoronos, D. M. Engelman, and F. J. Slack, "OncomiR or tumor suppressor? The duplicity of microRNAs in cancer," *Cancer Research*, vol. 76, no. 13, pp. 3666–3670, 2016.
- [21] K. P. Li, Y. P. Fang, J. Q. Liao et al., "Upregulation of miR-598 promotes cell proliferation and cell cycle progression in human colorectal carcinoma by suppressing INPP5E expression," *Molecular Medicine Reports*, vol. 17, no. 2, pp. 2991–2997, 2018.
- [22] F. Yang, K. Wei, Z. Qin et al., "MiR-598 suppresses invasion and migration by negative regulation of Derlin-1 and epithelial-mesenchymal transition in non-small cell lung cancer," *Cellular Physiology and Biochemistry*, vol. 47, no. 1, pp. 245–256, 2018.
- [23] X. Tong, P. Su, H. Yang et al., "MicroRNA-598 inhibits the proliferation and invasion of non-small cell lung cancer cells by directly targeting ZEB2," *Experimental and Therapeutic Medicine*, vol. 16, no. 6, pp. 5417–5423, 2018.
- [24] C. F. Wang, H. C. Zhang, X. M. Feng, X. M. Song, and Y. N. Wu, "Knockdown of MSI1 inhibits the proliferation of human oral squamous cell carcinoma by inactivating STAT3 signaling," *International Journal of Molecular Medicine*, vol. 44, no. 1, pp. 115–124, 2019.
- [25] P. Gong, Y. Wang, Y. Gao et al., "Msi1 promotes tumor progression by epithelial-to-mesenchymal transition in cervical cancer," *Human Pathology*, vol. 65, pp. 53–61, 2017.
- [26] S. Wang, N. Li, M. Yousefi et al., "Transformation of the intestinal epithelium by the MSI2 RNA-binding protein," *Nature Communications*, vol. 6, no. 1, article 6517, 2015.
- [27] C. Qu, L. He, N. Yao et al., "Myofibroblast-specific Msi2 knockout inhibits hepatocellular carcinoma progression in a mouse model," *Hepatology*, 2021.
- [28] R. J. Byers, T. Currie, E. Tholouli, S. J. Rodig, and J. L. Kutok, "MSI2 protein expression predicts unfavorable outcome in acute myeloid leukemia," *Blood*, vol. 118, no. 10, pp. 2857–2867, 2011.
- [29] M. G. Kharas, C. J. Lengner, F. Al-Shahrour et al., "Musashi-2 regulates normal hematopoiesis and promotes aggressive myeloid leukemia," *Nature Medicine*, vol. 16, no. 8, pp. 903–908, 2010.
- [30] A. E. Kudinov, A. Deneka, A. S. Nikonova et al., "Musashi-2 (MSI2) supports TGF- β signaling and inhibits claudins to promote non-small cell lung cancer (NSCLC) metastasis," *Proceedings of the National Academy of Sciences*, vol. 113, no. 25, pp. 6955–6960, 2016.
- [31] T. Cheng, Z. Zhang, Y. Cheng et al., "ETV4 promotes proliferation and invasion of lung adenocarcinoma by transcriptionally upregulating MSI2," *Biochemical and Biophysical Research Communications*, vol. 516, no. 1, pp. 278–284, 2019.