MicroRNA-486-5p Suppresses Lung Cancer via Downregulating mTOR Signaling *In Vitro* and *In Vivo*

Lei Ding1,2†, Wu Tian3†, Hui Zhang1†, Wanqiu Li1, Chunyu Ji4*, Yuanyuan Wang5* and Yanli Li1*

1 Lab for Noncoding RNA & Cancer, School of Life Sciences, Shanghai University, Shanghai, China, 2 Department of Pancreatic Surgery, Shanghai Cancer Centre, Fudan University, Shanghai, China, 3 Department of General Surgery, Orthopedics Hospital of Guizhou Province, Guiyang, China, 4 Department of Thoracic Surgery, Shanghai Chest Hospital, Jiaotong University Medical School, Shanghai, China, 5 Department of Respiratory and Critical Care Medicine, East Hospital, Tongji University School of Medicine, Shanghai, China

Lung cancer is one of the central causes of tumor-related deaths globally, of which non-small cell lung cancer (NSCLC) takes up about 85%. As key regulators of various biological processes, microRNAs (miRNAs) have been verified as crucial factors in NSCLC. To elucidate the role of miR-486-5p in the mTOR pathway, we investigated its role in NSCLC and related signaling. Our results confirmed that miR-486-5p was downregulated in most of human NSCLC tissue samples and cell lines. Further study confirmed that it inhibited NSCLC through repression of the mTOR pathway via targeting both ribosomal proteins S6 kinase A1 (RPS6KA1, RSK) and ribosomal proteins S6 kinase B1 (RPS6KB1, p70S6K), which are critical components of the mTOR signaling. Additionally, miR-486-5p impeded tumor growth *in vivo* and inhibited tumor metastasis through repression of the epithelial-mesenchymal transition (EMT). Taken together, our study verified the role that miR-486-5p exerts in NSCLC, and its expression pattern in the different stages and morphologies of NSCLC makes it a promising biomarker in the early diagnosis of the disease.

**Keywords:** NSCLC, miR-486-5p, mTOR signaling, RSK, p70S6K

**INTRODUCTION**

Lung cancer is known for its high occurrence and mortality rate both domestically and abroad (1), in which non-small cell lung cancer (NSCLC) accounts for about 85%. As a result, the prognosis of the disease remains poor (2, 3). Further categorization of it comes to lung adenocarcinoma (LUAD, 40–50% of all cases), lung squamous cell carcinoma (LUSC, 20–30% of all cases) and large cell lung cancer (1).

microRNAs (miRNAs) are noncoding RNAs with single strand of ~22 nucleotides by which about 60% of genes are regulated post-transcriptionally through either translational repression or mRNA degradation (4). MiRNAs are reported to play critical roles in diverse biological processes...
including cell proliferation (5, 6), cell cycle (7–9), cell migration (10–12), cell apoptosis (13, 14) immune response (15) and tumorigenesis (16, 17), suggesting their crucial roles in the development of various malignancies. To date, numerous miRNAs have been studied in NSCLC, revealing their crucial roles in the tumorigenesis and development of NSCLC (18–21), shedding some bright light in the diagnosis and treatment of lung cancer.

Our previous work has demonstrated the downregulation of miR-486-5p in NSCLC cell lines and patients’ tissue samples and it suppressed NSCLC cell growth and promoted apoptosis by direct repression of cyclin-dependent kinase 4 (CDK4) (22). However, it was also reported to play an oncogenic role by directly repressing cyclin-dependent kinase 4 (CDK4) (23). Here, we identified and subsequently activating the phosphatidylinositol 3-kinase (PI3K)/AKT signaling (23). These pGL3-derived vectors and miR-486-5p mimics were cotransfected into 293T cells, and the Dual-Luciferase Reporter Assay (Promega, Madison, WI, USA) was applied for luciferase activity measurement as previously described (25).

The sequences of RSK and p70S6K were constructed into the pcDNA3.1 plasmid. siRNAs for RSK (siRSK #1-3) and p70S6K (sip70S6K #1-3) were synthesized by RIBOBIO (Guangzhou, China). All siRNA sequences were listed in Table S2. Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) was used for transient transfection. All the constructed vectors were sequenced (Sangon Biotech, Shanghai, China) and the primers are listed in Table S3.

qRT-PCR and Western Blot
Total RNA was extracted, and reverse transcribed according to manufactures’ guide. The RNA level was quantified by qRT-PCR using an SYBR Green PCR master mix (TaKaRa, Dalian, China). The endogenous controls for mRNA and miRNA were 18S RNA and U6 snRNA, respectively. The relative quantification (2ΔΔCT) method was used for results analyzing.

Total protein was extracted from the cells using RIPA lysis buffer (CWBio, Beijing, China) and quantified with a Bradford Kit (Bio-Rad, Hercules, California, USA). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was applied to separate proteins, which were then transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore Corporation, Billerica, MA, USA). After blocking with non-fat powdered milk at room temperature for 1 h following an incubation with rabbit anti-RSK, anti-p70S6K, anti-p-p70S6K, anti-mTOR, anti-p-mTOR, anti-E-Cad, and anti-N-Cad antibodies overnight at 4°C. After incubation with a goat-anti-rabbit secondary antibody conjugated to horseradish peroxidase (HRP) (1:10000, Transgene Biotech, Beijing, China), protein bands were photographed with a chemiluminescent HRP substrate (Millipore, Billerica, MA, USA) and imaged with an E-Gen Imager (Biotanon, Shanghai, China). All experiments were repeated for three times and the representative images of the protein bands are shown in the figures. The number indicates the grayscale value of the protein bands relative to GAPDH. All the antibodies used in this study are listed in Table S4.

CCK-8 and Colony Formation Assay
CCK-8 (Dojindo, Japan) and colony formation assays were performed according to a formerly described protocol (24). For CCK-8 assay, 2500 cells per well were seeded in 96-well plates and incubated with 5% CCK-8 for 2.5 h every 24 h, and then the absorbance of the supernatant at 450 nm was measured with a plate reader. For colony formation assay, 500 cells were seeded in
Flow Cytometry Assay
Flow cytometry (BD Biosciences, San Jose, CA, USA) was used for cell sorting, cell cycle analysis apoptosis detection. For apoptotic rate assessment, pLenti/pLenti-miR-486 stably transfected cells were treated with actinomycin D (AMD, 5 μg/mL) for 4 h and then stained for Annexin-V APC/7AAD (Keygentec, Jiangsu, China) prior to analysis by flow cytometry. Transient transfected cells were stained for Annexin-V FITC/PI (BD Biosciences, San Jose, CA, USA) following treatment with actinomycin D and analyzed by flow cytometry.

Wound Healing Assay
Cells were scratched with 20 μL tips and washed with PBS twice, and the position was recorded by using a 10× lens on a light microscope and a digital camera. After 24 h (H1299) or 48 h (A549), cell positions were recorded again.

Mouse Xenograft Model
Six-week-old female SCID mice were purchased from the Shanghai Laboratory Animal Center (SLAC, Shanghai, China) and maintained under specific-pathogen-free (SPF) conditions with a healthy state test by SLAC. After random assignment to one of 2 groups, with 5 mice in each group, each mouse was injected subcutaneously in the right flank with 5 × 10^6 H1299 cells and intravenously with 2.5 × 10^6 cells transfected with pLenti or pLenti-miR-486. Tumor diameters were measured with calipers weekly, and the formula: volume = length × width^2/2 was applied to determine the tumor size. Eight weeks after the injection, the mice were sacrificed with CO2 without using any anesthesia, with the xenografts excised and weighed. The results were analyzed between the groups. All experimental protocols were approved under the institutional Animal Care and Use Committee of Shanghai University (Shanghai, China).

IHC Analysis
The xenografts were embedded in paraffin, then deparaffinized and rehydrated, followed by antigen retrieval. The sections were incubated with primary antibodies against Ki67, RSK, p70S6K, p-p70S6K, mTOR, p-mTOR, E-cad, and N-cad followed by incubation with secondary antibodies. The sections were then stained with 3, 3′-diaminobenzidine reaction solution and photographed using a digitalized microscope camera. All the antibodies used in this study are listed in Table S4.

Bioinformatics
miRWalk, together with miRTarBase and TargetScan, was used for targets prediction. UALCAN and Kaplan-Meier Plotter were used to analyze the data from TCGA. All the databases are listed in Table S5.

Statistical Analysis
Data were analyzed with GraphPad Prism software 8.0 (GraphPad Software, San Diego, CA, USA) and IBM SPSS v25.0 software (Abbott Laboratories, Chicago, IL, USA). Results were presented as the mean ± SD. Statistical analyses were performed using the Student’s t-test and one-way ANOVA. Pearson’s chi-square test was used to analyze the associations between miR-486-5p and RSK and p70S6K expression in tissue samples. All the experiments were repeated at least three times independently. Statistical significance was established at p < 0.05.

RESULTS
miR-486-5p Is Downregulated in NSCLC
To study the relationship between the expression of miR-486-5p and NSCLC, quantitative real-time polymerase chain reaction (qRT-PCR) results showed the marked downregulation of miR-486-5p in the 68 human NSCLC samples than in their paired adjacent non-tumor tissues (Figure 1A), the expression of miR-486-5p based on the pathological type, namely LUAD and LUSC samples, the tumor size, and the TNM neoplasm staging were also compared. It turned out that miR-486-5p was mainly downregulated in LUAD (Figure 1B) and samples with diameters <3 cm (Figure 1C) and the stage I and II samples (Figure 1D), revealing that miR-486-5p might play different roles in the tumorigenesis of LUAD and LUSC, and is positively regulated in advanced NSCLC.

miR-486-5p was also downregulated in most of our NSCLC cell lines, comparing to the non-transformed human lung epithelial cell line HBE (Figure 1E). A549 and H1299 cell lines were chosen for further study, for miR-486-5p expression in these two cell lines was relatively the lowest. Moreover, analyzing The Cancer Genome Atlas (TCGA) database also confirmed the downregulation of miR-486-5p in both LUAD and LUSC tissues (Figure S1) (26), and we can also conclude from TCGA database that higher miR-486-5p level is positively correlated with higher probability of survival both in LUAD (Figure 1F) and LUSC (Figure 1G) patients (27). To sum up, our results indicated that miR-486-5p might function as cancer suppressor in NSCLC.

miR-486-5p Inhibits NSCLC Proliferation
miR-486-5p (pLenti-miR-486) or an empty vector (pLenti) was forced expressed with lentivirus to assess the roles of miR-486-5p on NSCLC cell phenotypes. The successful transfection of the vectors can be indicated from the fluorescence intensity of green fluorescence protein (GFP) (Figure S2). miR-486-5p expression was upregulated remarkably in the pLenti-miR-486-expressing A549 and H1299 cells relative to the pLenti-expressing ones, with nearly 8- and 18-fold increases respectively (Figure 2A). CCK-8 assay revealed that miR-486-5p overexpression inhibited H1299 and A549 cell proliferation (Figures 2B, C). In search of the underlying mechanism of the suppressing role that miR-486-5p played in NSCLC, we analyzed its function on cell cycle, colony formation, cell migration and cell apoptosis. It turned out that the cell cycle progress of A549 and H1299 was retarded by miR-486-5p (Figure 2D), as well as the colony formation (Figure 2E) and migration rate (Figure 2F), with the latter determined by wound healing assay. On the contrary, the cell
apoptosis was considerably facilitated by miR-486-5p (Figure 2G). In conclusion, these results verified that miR-486-5p acted as a suppressor in the NSCLC.

RSK and p70S6K Are Targets of miR-486-5p

To elucidate the mechanism by which miR-486-5p hinders NSCLC progress, we predicted its potential targets using miRWalk 3.0 (28). Four candidates stood out after overlapping the predicted targets by miRWalk with the mTOR-, apoptosis- and cell migration-related proteins (Figure 3A). Given the functions of miR-486-5p in NSCLC cell lines, we ultimately chose the target genes RSK and p70S6K. The wild type and mutated binding sites for miR-486-5p with RSK and p70S6K are demonstrated in Figure 3B, and their binding relationship was determined by dual-luciferase reporter assay. Significant decrease of the relative luciferase activity in HEK293T cells cotransfected with the 3’ untranslated region (3’UTR) of RSK and p70S6K (pGL3-RSK/pGL3-p70S6K WT 3’UTR), pRL vector, and miR-486-5p mimic compared to the control (cotransfected with RSK and p70S6K 3’UTR, pRL vector and NC mimic) was observed, while no significant change in luciferase activity was found in cotransfection of miR-486-5p mimic with the RSK and p70S6K 3’mutUTR (pGL3-RSK/pGL3-p70S6K Mut 3’UTR), indicating that miR-486-5p has direct binding sites in RSK and p70S6K mRNA 3’UTRs (Figures 3C, D).

To determine how miR-486-5p affected the expression of RSK and p70S6K, we investigated the expression of RSK and p70S6K in pLenti-miR-486 A549 and H1299 cells compared to that in pLenti cells by qRT-PCR and western blot. RSK and p70S6K expression was decreased by miR-486-5p on both the mRNA (Figures 3E, F) and protein (Figure 3G) levels in A549 and H1299 cells. Also, RSK and p70S6K expression showed to be negatively correlated with that of miR-486-5p in the NSCLC tissue samples (Figures 3H, I), with their expression being significantly upregulated in most of NSCLC cell lines (Figures 3N, S). In accordance with the expression pattern of miR-486-5p in NSCLC tissue samples, both RSK and p70S6K showed to be upregulated in most LUAD tissues, tissues with diameters >= 3 cm and the stage III/IV tissues (Figures 3J–M, O–R). Taken together, RSK and p70S6K are confirmed to be targets of miR-486-5p.

To further study the effect of RSK and p70S6K on NSCLC, RSK and p70S6K siRNAs (siRSK 1-3 and sip70S6K 1-3) were transfected into A549 and H1299 cells, resulting in a significant reduction of mRNA (Figure 4E, F). As the siRSK-2 and sip70S6K-3 downregulated the mRNA of their respective target most significantly, we chose them for subsequent research (simplified as siRSK and sip70S6K in the main Figures). The siRNAs we chose significantly downregulated the mRNA (Figures 4A, B) and protein (Figure 4C) expression of RSK and p70S6K. Similar to the effect of the overexpression of miR-486-5p, siRSK and
sip70S6K dramatically inhibited the proliferation (Figures 4D, E), cell cycle (Figure 4F), colony formation (Figure 4G) and cell migration (Figure 4H) of A549 and H1299 cells, whereas the cell apoptotic rate was upregulated (Figure 4I) by the siRNAs. These results showed that the downregulation of RSK and p70S6K could mimic the effects of miR-486-5p in NSCLC cells, indicating that the function of miR-486-5p on NSCLC cell phenotype was exerted at least partly via downregulation of RSK and p70S6K.

**Ectopic Express of RSK and p70S6K Rescues the Effect of miR-486-5p**

For determination of whether miR-486-5p exerted its function in NSCLC by targeting RSK and p70S6K, we constructed RSK and p70S6K overexpression vectors pcDNA3.1-RSK/pcDNA3.1-p70S6K to find out if restoration of RSK and p70S6K expression could rescue the effects of miR-486-5p.

Transfection of pcDNA3.1-RSK/p70S6K in pLenti/pLenti-miR-486 stably transfected A549 and H1299 cells resulted in a marked upregulation of RSK/p70S6K (Figures 5A, B). Notably, forced expression of RSK/p70S6K promoted cell proliferation (Figures 5C–F) and migration (Figure 5G), reversing the inhibition by miR-486-5p. These findings demonstrated that the upregulation of RSK/p70S6K could rescue the effects of miR-486-5p overexpression in NSCLC cells, further verifying the function mechanism of miR-486-5p in NSCLC.

**miR-486-5p Suppresses mTOR Pathway In Vivo**

To explore the effect of miR-486-5p on tumor growth and migration in vivo, 5 × 10^6 H1299 cells expressing either pLenti or pLenti-miR-486 were injected subcutaneously at the left flank and 2.5 × 10^6 cells intravenously into SCID mice. Upon implantation, tumor volume was measured every week, and mice were sacrificed at week 8. The tumors are displayed in Figure 6A. Suppression of tumor growth by miR-486-5p became remarkable at week five and got less significant later (Figure 6B). One possible reason is that the tumor growth space is confined as the tumor grows, and nutrients and oxygen are insufficient for the tumor development, ultimately leading to festering. A noted induction in tumor weight was also observed (Figure 6C).
Moreover, miR-486-5p expression was significantly upregulated, and RSK and p70S6K were downregulated at the RNA level in the pLenti-miR-486 stably transfected xenografts (Figures 6D–F). Protein expression of RSK and p70S6K were also downregulated in the pLenti-miR–486 group, together with a marked reduction on the protein level of phosphorylated p70S6K, as well as the downstream mTOR and phosphorylated mTOR level (Figure 6G), indicating the inactivation of the mTOR pathway by miR-486-5p. H&E staining of the tumor tissues was displayed (Figure 6H, left panel), and that of the lung specimens showed that miR-486-5p significantly reduced the number and size of metastatic nodes (Figure 6H, right panel), indicating miR–486-5p suppressed tumor migration in vivo. Immunohistochemistry (IHC) of tumor specimens showed a visible decrease in the expression of Ki67, RSK, p70S6K, mTOR, p-mTOR and N-cadherin (N-Cad), and the E-cadherin (E-Cad)
expression was increased compared with control (Figure 6H). In accordance with the phenotypic results, the protein expression of E-Cad was significantly upregulated by miR-486-5p, of which the N-Cad showed the opposite results (Figure 6G). As E-Cad and N-Cad are two critical cadherins marking the occurrence of the EMT, the IHC results suggested that miR-486-5p suppressed the migration of NSCLC by inhibition of the EMT process. To sum up, these results revealed that miR-486-5p impeded xenograft growth by targeting RSK and p70S6K and further leading to inactivation and inhibition of the mTOR signaling, and the migration of tumor was also hindered partly via the inhibition of the EMT process.

**DISCUSSION**

The mammalian or mechanistic target of rapamycin (mTOR) is a serine/threonine kinase composed of two protein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) that differ in structure and function (29), which has been broadly studied and found to play fundamental roles in cell growth, metabolism and the EMT process (30–32). The overactivation of mTOR is observed in more than 70% of cancers (33, 34), indicating its critical functions in tumorigenesis. RSK and p70S6K are members of RPS6K family (35). Plenty of studies have demonstrated that the mTOR signaling is tightly regulated by miRNAs and long noncoding RNAs (lncRNAs) in various kinds of tumors (36–42), whereas how RSK and p70S6K are regulated is barely understood.

Our work revealed that miR-486-5p could hinder lung cancer tumorigenesis through inhibition and inactivation of the mTOR pathway by targeting RSK and p70S6K, and the miR-486-5p/RSK/mTORC1/p70S6K axis (Figure 7) could be active.

miR-486-5p is broadly studied in an array of tumor types, including NSCLC (22, 23, 43–45), leukemia (46–48), colorectal cancer (49, 50), renal cell carcinoma (RCC) (51), prostate cancer (52, 53), ovarian cancer (54), hepatocellular carcinomas (55), and breast cancer (56, 57). While most of the studies demonstrated that miR-486-5p played tumor-suppressing function in different...
malignancies, there are also several studies claiming that it played a tumor-facilitating role in leukemia (46), NSCLC (23), and prostate cancer (52). Thus, we decided that it’s important for us to figure out its function in NSCLC.

Moreover, the downregulation of miR-486-5p in NSCLC is found to be caused by the hypermethylation of the miR-486-5p gene promoter region, which is also confirmed by others’ work (58). To make it clear whether miR-486-5p plays a suppressive or promoting role in NSCLC development, we decided to perform more in-depth research into its function in NSCLC and figure out the underlying mechanisms of this function.

The overexpression of miR-486-5p not only suppressed the expression of RSK and p70S6K both in vitro and in vivo but also inhibited p-p70S6K, mTOR and p-mTOR. Also, the expression of CDK4 in the pLenti-miR-486 expressing xenografts was significantly downregulated (Figure S5), consisting with our former study (22). These findings suggested that miR-486-5p inhibited NSCLC by inactivating the mTOR signaling.

The expression pattern of miR-486-5p in NSCLC tissue samples has aroused much interest in its function in NSCLC tumorigenesis. Specifically, the miR-486-5p expression is mainly downregulated in the LUAD and early-stage samples, and the expression of RSK and p70S6K showed the reversed tendency. There have been several studies exploring the differentially expressed noncoding RNAs between the LUAD and LUSC patients, thereafter, some IncRNAs, miRNAs, circular RNAs and molecules like interleukin-6R (IL-6R) and IL-1β are considered as diagnostic markers in distinguishing LUAD and LUSC. Our study may establish miR-486-5p as a biomarker for the early diagnosis of NSCLC, especially for the LUAD cases.

**CONCLUSION**

Taken together, our work further confirmed that miR-486-5p acts as a suppressor in NSCLC at least partly by targeting the mTOR signaling and CDK4. With more exploitation of its expression pattern and function mechanism in the in vivo model, including exploration of its effective delivery onto the tumor site, we believe it’s a promising target in the clinical treatment of NSCLC.
miR-486-5p inhibits tumor growth and metastasis in vivo. (A) The xenografts images of H1299 cells transfected with pLenti or pLenti-miR-486. (B) The growth curve of xenografts from one-week post-injection until sacrifice at week 8. *p < 0.05; **p < 0.01; ***p < 0.001. (C) The tumor weight of xenografts at week 8. *p < 0.05. (D–F) The RNA expression of miR-486-5p (D), RSK (E) and p70S6K (F) in pLenti-miR-486 transfected xenografts compared with pLenti transfected ones. *p < 0.05; **p < 0.01. (G) The protein expression of E-Cad, N-Cad, mTOR, p-mTOR, RSK, p70S6K and p-p70S6K in xenografts. (H) The H&E staining (Left first) and IHC results of Ki67, RSK, p70S6K, mTOR, p-mTOR, E-Cad and N-Cad of the xenografts and the H&E staining (Right first) of the Lung of the tumor-bearing mice.

A diagram of the biogenesis and function of miR-486-5p in NSCLC. Locating downstream of ANK1, the biogenesis of miR-486-5p is often inhibited by hypermethylation of its promoter. By targeting CDK4, RSK, p70S6K and other genes, miR-486-5p can inhibit NSCLC by hindering cell proliferation and metastasis and promoting cell apoptosis.
The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

The animal study was reviewed and approved by the institutional Animal Care and Use Committee of Shanghai University. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

YL and CJ designed the study and supervised the experiments. YW and CJ collected the NSCLC specimens and evaluated medical information. LD, WT, HZ and WL carried out the experiments and analyzed the results. LD and YL prepared the manuscript and revised with WT. All authors contributed to the article and approved the submitted version.

AUTHOR CONTRIBUTIONS

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. CA Cancer J Clin (2021) 71:7–33. doi: 10.3322/caac.21654
2. Ettinger DS, Akerley W, Borden EC, Curley SA, et al. Non-Small-Cell Lung Cancer. J Natl Compr Canc Netw JNCCN (2012) 10:1236–71. doi: 10.6004/jnccn.2012.0029
3. O’Donnell L, Askin FF, Gabrielson E, Li QK. Current WHO Guidelines and the Classification of Non-Small Cell Lung Carcinoma (NSCLC). Moving From Targeted Therapy to Immunotherapy. Semin Cancer Biol (2017) 52:103–9. doi: 10.1016/j.semcancer.2017.11.019
4. Mohr AM, Mott JL. Overview of MicroRNA Biology. Semin Liver Dis (2015) 35:3–11. doi: 10.1055/s-0034-1397344
5. Han D, Li J, Wang H, Su X, Hou J, Gu Y, et al. Circular RNA Circmtor1 Acts as the Sponge of MicroRNA-9 to Suppress Hepatocarcinoma Progression. Hepatology (2017) 66:1151–64. doi: 10.1002/hep.29270
6. Fang F, Chang R, Yu L, Lei X, Xiao S, Yang H, et al. MicroRNA-188-5p Suppresses Tumor Cell Proliferation and Metastasis by Directly Targeting FGFR5 in Hepatocarcinoma. J Hepatol (2015) 63:874–85. doi: 10.1016/j.jhep.2015.05.008
7. Zhou J, Liu J, Zheng C, Lin W, Wang G, Liao S, et al. MicroRNA-202 Maintains Spermatogonial Stem Cells by Inhibiting Cell Cycle Regulators and RNA Binding Proteins. Nucleic Acids Res (2017) 45:4142–57. doi: 10.1093/nar/gkx1287
8. Hyödynmäki P, Wang Y, Fassl A, Li X, Matia V, Otto T, et al. Cell-Cycle-Targeting Micrornas as Therapeutic Tools Against Refractory Cancers. Cancer Cell (2017) 31:575–90.e8. doi: 10.1016/j.ccell.2017.03.004
9. Otto T, Candido SV, Pilarz MS, Sicinska E, Bronson RT, Brown M, et al. Cell Cycle-Targeting Micrornas Promote Differentiation by Enforcing Cell-Cycle Exit. Proc Natl Acad Sci USA (2017) 114:10660–5. doi: 10.1073/pnas.1702914114
10. Zhang Y, Liu D, Chen X, Li J, Li L, Bian Z, et al. Secreted Monocytic Mir-150 Enhances Targeted Endothelial Cell Migration. Mol Cell (2010) 39:133–44. doi: 10.1016/j.molcel.2010.06.010
11. Cui Y-X, Bradbury R, Flamini V, Wu B, Jordan N, Jiang WG. Microrna-7 Suppresses the Homing and Migration Potential of Human Endothelial Cells to Highly Metastatic Human Breast Cancer Cells. Br J Cancer (2017) 117:89–101. doi: 10.1038/bjc.2017.156
12. Wang J, Song W, Shen W, Yang X, Sun W, Qu S, et al. MicroRNA-200a Suppresses Cell Invasion and Migration by Directly Targeting GAB1 in Hepatocellular Carcinoma. Oncol Res (2017) 25:1–10. doi: 10.3727/096504016X14685034103798
13. Park H, Huang X, Lu C, Cairo MS, Zhou X. MicroRNA-146a and MicroRNA-146b Regulate Human Dendritic Cell Apoptosis and Cytokine Production by Targeting TRAF6 and IRAK1 Proteins. J Biol Chem (2015) 290:2831–41. doi: 10.1074/jbc.M114.591420
14. Du B, Dai X-M, Li S, Qi G-L, Cao G-X, Zhong Y, et al. Mir-30c Regulates Caspinatin-Induced Apoptosis of Renal Tubular Epithelial Cells by Targeting Bnip3L and Hspa5. Cell Death Dis (2017) 8:e2987. doi: 10.1038/cddis.2017.377
15. Liu G, Abraham E. Micrornas in Immune Response and Macrophage Polarization. Arterioscler Thromb Vasc Biol (2013) 33:170–7. doi: 10.1161/ATVBAHA.112.300068
16. Chen CZ. Micrornas as Oncogenes and Tumor Suppressors. New Engl J Med (2005) 353:1768–71. doi: 10.1056/NEJMmp058190
17. Hayes JD, Peruzzi PP, Lawler S. Micrornas in Cancer: Biomarkers, Functions and Therapy. Trends Mol Med (2014) 20:460–9. doi: 10.1016/j.molmed.2014.06.005
18. Wu Z, Yuan Q, Yang C, Zhang X, Qi P, Huang H, et al. Downregulation of Oncogenic Gene Tgfβ2 by Mirna-170 Suppresses Non-Small Cell Lung Cancer. Pathol Res Pract (2020) 216:0344–38. doi: 10.1016/j.prp.2019.152690
19. Qi P, Li Y, Liu X, Jafari FA, Zhang X, Sun Q, et al. Cryptotanshinone Suppresses Non-Small Cell Lung Cancer Via MicroRNA-146a-5p/EGFR Axis. Int J Biol Sci (2019) 15:1072–9. doi: 10.7150/ijbs.31277
20. Zhang X, Zhang X, Liu X, Qi P, Wang H, Ma Z, et al. MicroRNA-296, a Suppressor Non-Coding RNA, Downregulates SGLT2 Expression in Lung Cancer. Int J Oncol (2019) 54:199–208. doi: 10.3892/ijo.2018.4599
21. Shukuya T, Ghai V, Amann JM, Okimoto T, Shilo K, Kim T-K, et al. Circular Micrornas and Extracellular Vesicle-Containing Micrornas as Response Biomarkers of Anti-Programmed Cell Death Protein 1 or Programmed Death-Ligand 1 Therapy in NSCLC. J Thorac Oncol (2020) 15:1773–81. doi: 10.1016/j.jtho.2020.05.022
22. Shao Y, Shen Y-Q, Li Y-L, Liang C, Zhang B-J, Lu S-D, et al. Direct Repression of the Oncogene CDK4 by the Tumor Suppressor Mir-486-5p in Non-Small Cell Lung Cancer. Oncotarget (2016) 7:34011–21. doi: 10.18632/oncotarget.8514
23. Gao Z-J, Yuan W-D, Yuan J-Q, Yuan K, Wang Y. Mir-486-5p Functions as an Oncogene by Targeting PTEN in Non-Small Cell Lung Cancer. Pathol Res Pract (2018) 214:700–5. doi: 10.1016/j.prp.2018.03.013
24. Li Y-L, Liu X-M, Zhang C-Y, Zhou J-B, Shao Y, Liang C, et al. MicroRNA-34a/EGFR Axis Plays Pivotal Roles in Lung Tumorigenesis. Oncogenesis (2017) 6:e372. doi: 10.1038/oncosci.2017.50

25. Ma Z-L, Hou P-P, Li Y-L, Wang D-T, Yuan T-W, Wei J-L, et al. MicroRNA-34a Inhibits the Proliferation and Promotes the Apoptosis of Non-Small Cell Lung Cancer H1299 Cell Line by Targeting Tgfbr2. Tumour Biol (2015) 36:2481–90. doi: 10.1007/s13277-014-2861-5

26. Chandrashekar DS, Bashel B, Balasubramanya SA, Creighton CJ, Ponce-Rodriguez I, Chakravarti BV, et al. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. Neoplasia (2017) 19:649–58. doi: 10.1016/j.neo.2017.05.002

27. Gyorffy B, Surowiak P, Budczies J, Lenczky A. Online Survival Analysis Software to Assess the Prognostic Value of Biomarkers Using Transscriptomic Data in Non-Small Cell Lung Cancer. Plos One (2013) 8:e28224. doi: 10.1371/journal.pone.0082241

28. Sticht C, Lo Torre C de, Parveen A, Gretz N, Mirwak: An Online Resource for Prediction of Microrna Binding Sites. Plos One (2018) 13:e0206239. doi: 10.1371/journal.pone.0206239

29. Ciuffreda L, Di Sanza C, Incani UC, Milella M. The Mtor Pathway: A New Frontier in Oncology | www.frontiersin.org May 2021 | Volume 11 | Article 65523611

30. Li Y, Wang T, Sun Y, Huang T, Li C, Fu Y, et al. P53-Mediated PI3K/AKT/Mtor Pathway Played a Role in Phoxd4-Induced EMT Inhibition in Liver Cancer Cell Lines. Oxid Med Cell Longev (2016) 2016:6141902. doi: 10.1155/2016/6141902

31. Zhou J, Cheng H, Wang Z, Chen H, Suo C, Zhang H, et al. Bortezomib Regulating the EMT Induced by TNF-α Attenuates Renal Interstitial Fibrosis in Kidney Transplantation Via Targeting the EMT Induced by TNF-α. J Cell Mol Med (2019) 23:5390–402. doi: 10.1111/jcmm.14420

32. Cheng K, Hao M. Metformin Inhibits TGF-β1-Induced Epithelial-to-Mesenchymal Transition Via PKM2 Relative-Myt/or70s6k Signaling Pathway in Cervical Carcinoma Cells. Int J Mol Sci (2016) 17:20000. doi: 10.3390/ijms17122000

33. Forbes SA, Bindal N, Bamford S, Cole C, Kok CY, Beare D, et al. COSMIC: Mining Complete Cancer Genomes in the Catalogue of Somatic Mutations in Cancer. Nucleic Acids Res (2013) 41:39245–50. doi: 10.1093/nar/gkq929

34. Tian T, Li X, Zhang J. Mtor Signaling in Cancer and Mtor Inhibitors in Solid Tumor Targeting Therapy. Int J Mol Sci (2019) 20(3):755. doi: 10.3390/ijms20030755

35. Slattery ML, Lindgren A, Herrick JS, Wolff RK. Genetic Variation in RPS6KA1, RPS6KA2, RPS6KB1, RPS6KB2, and PDK1 and Risk of Colon or Rectal Cancer. Mutat Res (2019) 830:61–9. doi: 10.1016/j.mrfmmm.2010.10.005

36. Shi H, Pu J, Zhou X-L, Ning Y-Y, Bai C. Silencing Long Non-Coding RNA UCA1 Promotes Glycolysis by Upregulating Hexo kinase 2 Through the Mtor-STAT3/Mtor/P70s6k Signaling Pathway Played a Role in Ptoxdpt-Induced EMT Inhibition in Liver Cancer Cell Line Bel-7404. Oncotarget (2017) 8:6909–706. doi: 10.18632/oncotarget.7236

37. Slattery ML, Lundgreen A, Herrick JS, Wolff RK. Genetic Variation in RPS6KA1, RPS6KA2, RPS6KB1, RPS6KB2, and PDK1 and Risk of Colon or Rectal Cancer. Mutat Res (2019) 830:61–9. doi: 10.1016/j.mrfmmm.2010.10.005

38. Tian T, Li X, Zhang J. Mtor Signaling in Cancer and Mtor Inhibitors in Solid Tumor Targeting Therapy. Int J Mol Sci (2019) 20(3):755. doi: 10.3390/ijms20030755

39. Shui X, Zhou C, Lin W, Yu Y, Feng Y, Kong J. Long Non-Coding RNA UCA1 Promotes Glycolysis by Upregulating Hexokinase 2 Through the Mtor-STAT3/Mtor/P70s6k Signaling Pathway Played a Role in Ptoxdpt-Induced EMT Inhibition in Liver Cancer Cell Line Bel-7404. Oncotarget (2017) 8:6909–706. doi: 10.18632/oncotarget.7236

40. Shibahara N, Chuma Y, Shiraishi K, Nakajima T, Suzuki M, Takayama T. Estrogen Receptor-Related Mir-486-5p Inhibits Cell Proliferation and Invasion of Colorectal Cancer Cells Through Targeting PIK3R1. Oncol Lett (2018) 15:2473–8. doi: 10.3892/ol.2018.8233

41. Shui X, Zhou C, Lin W, Yu Y, Feng Y, Kong J. Long Non-Coding RNA UCA1 Promotes Glycolysis by Upregulating Hexokinase 2 Through the Mtor-STAT3/Mtor/P70s6k Signaling Pathway Played a Role in Ptoxdpt-Induced EMT Inhibition in Liver Cancer Cell Line Bel-7404. Oncotarget (2017) 8:6909–706. doi: 10.18632/oncotarget.7236

42. Shibahara N, Chuma Y, Shiraishi K, Nakajima T, Suzuki M, Takayama T. Estrogen Receptor-Related Mir-486-5p Inhibits Cell Proliferation and Invasion of Colorectal Cancer Cells Through Targeting PIK3R1. Oncol Lett (2018) 15:2473–8. doi: 10.3892/ol.2018.8233

43. Shibahara N, Chuma Y, Shiraishi K, Nakajima T, Suzuki M, Takayama T. Estrogen Receptor-Related Mir-486-5p Inhibits Cell Proliferation and Invasion of Colorectal Cancer Cells Through Targeting PIK3R1. Oncol Lett (2018) 15:2473–8. doi: 10.3892/ol.2018.8233

44. Shibahara N, Chuma Y, Shiraishi K, Nakajima T, Suzuki M, Takayama T. Estrogen Receptor-Related Mir-486-5p Inhibits Cell Proliferation and Invasion of Colorectal Cancer Cells Through Targeting PIK3R1. Oncol Lett (2018) 15:2473–8. doi: 10.3892/ol.2018.8233

45. Shibahara N, Chuma Y, Shiraishi K, Nakajima T, Suzuki M, Takayama T. Estrogen Receptor-Related Mir-486-5p Inhibits Cell Proliferation and Invasion of Colorectal Cancer Cells Through Targeting PIK3R1. Oncol Lett (2018) 15:2473–8. doi: 10.3892/ol.2018.8233

46. Shibahara N, Chuma Y, Shiraishi K, Nakajima T, Suzuki M, Takayama T. Estrogen Receptor-Related Mir-486-5p Inhibits Cell Proliferation and Invasion of Colorectal Cancer Cells Through Targeting PIK3R1. Oncol Lett (2018) 15:2473–8. doi: 10.3892/ol.2018.8233

47. Shibahara N, Chuma Y, Shiraishi K, Nakajima T, Suzuki M, Takayama T. Estrogen Receptor-Related Mir-486-5p Inhibits Cell Proliferation and Invasion of Colorectal Cancer Cells Through Targeting PIK3R1. Oncol Lett (2018) 15:2473–8. doi: 10.3892/ol.2018.8233

48. Shibahara N, Chuma Y, Shiraishi K, Nakajima T, Suzuki M, Takayama T. Estrogen Receptor-Related Mir-486-5p Inhibits Cell Proliferation and Invasion of Colorectal Cancer Cells Through Targeting PIK3R1. Oncol Lett (2018) 15:2473–8. doi: 10.3892/ol.2018.8233

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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