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MiRNA-200a-3p suppresses the proliferation, migration and invasion of non-small cell lung cancer through targeting IRS2

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Abstract. – OBJECTIVE: To uncover the biological role of microRNA-200a-3p (miRNA-200a-3p) in the progression of non-small cell lung cancer (NSCLC) and the underlying mechanism.

PATIENTS AND METHODS: The expression levels of miRNA-200a-3p and IRS2 in NSCLC tissues and cell lines were examined through quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). The correlation between the miRNA-200a-3p level and pathological characteristics of NSCLC patients was analyzed. The prognostic value of miRNA-200a-3p in NSCLC was assessed through the Kaplan-Meier method. The potential interaction between miRNA-200a-3p and IRS2 was explored through Dual-Luciferase Reporter Gene Assay and Spearman correlation test. The regulatory effects of miRNA-200a-3p/IRS2 on the proliferative, migratory, and invasive abilities of NSCLC were evaluated by Cell Counting Kit-8 (CCK-8) and the transwell assay. The protein levels of the epithelial-mesenchymal transition (EMT)-related genes in NSCLC cells influenced by miRNA-200a-3p were detected by Western blot.

RESULTS: MiRNA-200a-3p was downregulated in NSCLC tissues and cell lines. The expression level of miRNA-200a-3p was related to tumor size, TNM staging, and lymphatic metastasis of NSCLC. The low level of miRNA-200a-3p predicted worse prognosis in NSCLC patients. The overexpression of miRNA-200a-3p inhibited A549 cells from proliferating, migrating, and invading. The protein levels of E-cadherin were upregulated, while N-cadherin and Vimentin were downregulated in A549 cells overexpressing miRNA-200a-3p. The Dual-Luciferase Reporter Gene Assay verified the binding between miRNA-200a-3p and IRS2. The level of IRS2 was negatively regulated by miRNA-200a-3p. Moreover, the overexpression of IRS2 could reverse the regulatory role of miRNA-200a-3p in the cellular behaviors of A549 cells.

CONCLUSIONS: MiRNA-200a-3p suppresses the proliferative, migratory, and invasive abilities of NSCLC by targeting IRS2, thus alleviating the progression of NSCLC.

Key Words:
MiRNA-200a-3p, IRS2, Non-small cell lung cancer (NSCLC).

Introduction

Lung carcinoma is a highly prevalent malignancy throughout the world. Non-small cell lung cancer (NSCLC) is the most common subtype, accounting for 85% of all lung carcinoma cases[1]. NSCLC is manifested as high mortality and poor prognosis, and its 5-year survival is lower than 16%[2]. Owing to the insufficient screening and diagnosis approaches in the early phase, the detective rate of the early-stage NSCLC is very low. The prognosis of the advanced NSCLC is extremely poor. The effective inhibition of the proliferation and metastasis of NSCLC contributes to improve the clinical outcomes of the affected patients[3].

MicroRNAs (MiRNAs) are non-coding, single-strand RNAs containing 18-25 nucleotides. They are extensively distributed in mammals, plants, and viruses, and participate in a series of life activities[4,5]. Scholars[6] have discovered the crucial role of miRNAs in the occurrence and progression of tumors. Based on the specific functions, the tumor-related miRNAs are served as oncogenes or tumor-suppressor genes[7]. A plenty of abnormally expressed miRNAs have been discovered in NSCLC, which are closely involved in the malignant progression of NSCLC[8]. Liu et

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al[9] demonstrated that the overexpression of miR-454 suppresses NSCLC cells to proliferate, migrate, and invade by binding STAT3. Want et al[10] highlighted the role of miR-124 in inhibiting the proliferative rate of NSCLC by downregulating STAT3. Hence, the clarification of the molecular mechanism of miRNAs underlying the occurrence and progression of NSCLC contributes to develop miRNA-based drugs targeting NSCLC.

It is reported that the miR-200 family could be utilized as intervention targets for tumor diseases, thus improving the prognosis of affected patients. There are 5 members in the miR-200 family. Based on their locations in the gene cluster, the 5 members were classified into two groups (miR-200a/b/429 and miR-200c/141), serving a carcinogenic or tumor-suppressor effect[11,12]. MiRNA-200a-3p is the most dysregulated member in the miR-200 family. It is closely related to multiple types of cancer[13-15]. Nevertheless, the specific role of miRNA-200a-3p in NSCLC remains unclear.

This study focused on the regulatory effect of miRNA-200a-3p on epithelial-mesenchymal transition (EMT) and cellular behaviors of NSCLC.

Patients and Methods

Sample Collection

A total of 44 NSCLC patients were enrolled in this study. NSCLC tissues and adjacent normal tissues were surgically harvested and preserved. The clinical data and follow-up information of the enrolled NSCLC patients were collected. All subjects volunteered to participate in the study and signed the written informed consent. This study was approved by the Ethics Committee of The First Affiliated Hospital of Nanjing Medical University.

Cell Culture and Transfection

Normal lung epithelial cell line (BEAS-2B) and lung carcinoma cell lines (A549, PC9, H292, and SPC-A1) were provided by Cell Bank (Shanghai, China). The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA). The medium was replaced every two days. The cell passage was conducted every 6 days at a ratio of 1:3.

The cells were cultured until 80-90% confluence and subjected to transfection using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Six hours later, the complete medium was replaced. The transfected cells for 24-48 h were harvested for the following experiments.

Cell Counting Kit-8 (CCK-8)

The cells were seeded in a 96-well plate with 2×10^3 cells per well. At the appointed time points, the absorbance value at 450 nm of each sample was recorded using the CCK-8 kit (Dojindo Laboratories, Kumamoto, Japan) for depicting the viability curves.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

The extraction of the total cellular RNA was performed using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and RNA was subjected to reverse transcription. The extracted complementary deoxyribose nucleic acid (cDNA) was used as a template for PCR using the SYBR Green method (TaKaRa, Otsu, Shiga, Japan). QRT-PCR reaction conditions were as follows: 94°C for 30 s, 55°C for 30 s, and 72°C for 90 s, for a total of 40 cycles. The relative level of the target gene was calculated by the $2^{-\Delta\Delta Ct}$ method. The primer sequences were listed as follows: miRNA-200a-3p: F: 5'-ACATCGTTACCAGACAGTGTTA-3', R: 5'-ACATCGTTACCAGACAGTGTTA-3'; IRS2: F: 5'-TTGACTTCTTGTCCCACCACTTG-3', R: 5'-GCTGAGCGTCTTCTTTTAATGATACT-3'. GAPDH was served as the internal control for IRS2. U6 was the internal control of miRNA-200a-3p. U6, forward, 5'-CTCGCTTCGGCAGCACA-3'; reverse, 5'-AACGCTTCACGAATTT GCGT-3'. GAPDH, forward, 5'-AGGTCGGTGTGAACGGATTTG-3'; reverse, 5'-TGTAGACCATGTAGTTGAGGTCA-3'

Transwell Assay

The cell density was adjusted to 3×10^4 cells/ml. 100 μl of suspension was applied in the upper side of the transwell chamber (Corning, Corning, NY, USA) and pre-coated with diluted Matrigel. In the bottom side, 600 μL of medium containing 20% FBS was applied. After 24 h of incubation, the cells migrated to the bottom side were fixed in methanol for 15 min, stained with crystal violet for 20 min, and counted using a microscope. The number of migratory cells was counted in 5 randomly selected fields per sample (magnification 200×).

Western Blot

The total protein was extracted from cells using radioimmunoprecipitation assay (RIPA) and quantified by bicinchoninic acid (BCA) method (Beyotime, Shanghai, China). The protein sample was loaded for electrophoresis and transferred on polyvinylidene difluoride (PVDF) membranes

(Millipore, Billerica, MA, USA). After blockage of non-specific antigens in PBS containing 5% skim milk for 2 hours, the membranes were subjected to incubation with primary and secondary antibodies. The bands were exposed by enhanced chemiluminescence (ECL) and analyzed by Image Software (NIH, Bethesda, MD, USA).

Dual-Luciferase Reporter Gene Assay

The cells were co-transfected with miR-NC/miRNA-200a-3p mimic and IRS2-WT/IRST-MT using Lipofectamine 2000. After transfection for 48 hours, the co-transfected cells were lysed for determining the Luciferase activity using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA).

Statistical Analysis

The Statistical Product and Service Solutions (SPSS) 19.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 7.0 (La Jolla, CA, USA) were used for data analyses. The data were expressed as mean ± standard deviation. The differences between the two groups were analyzed by the *t*-test. The survival analysis was conducted by introducing the Kaplan-Meier method, followed by the log-rank test for comparing the difference between the curves. The Spearman correlation test was carried out to identify the relationship between the expression levels of miRNA-200a-3p and IRS2. $p<0.05$ was considered as statistically significant.

Results

MiRNA-200a-3p Was Downregulated in NSCLC

The miRNA-200a-3p level was detected in 44 paired cases of NSCLC tissues and adjacent normal tissues by qRT-PCR. It is shown that

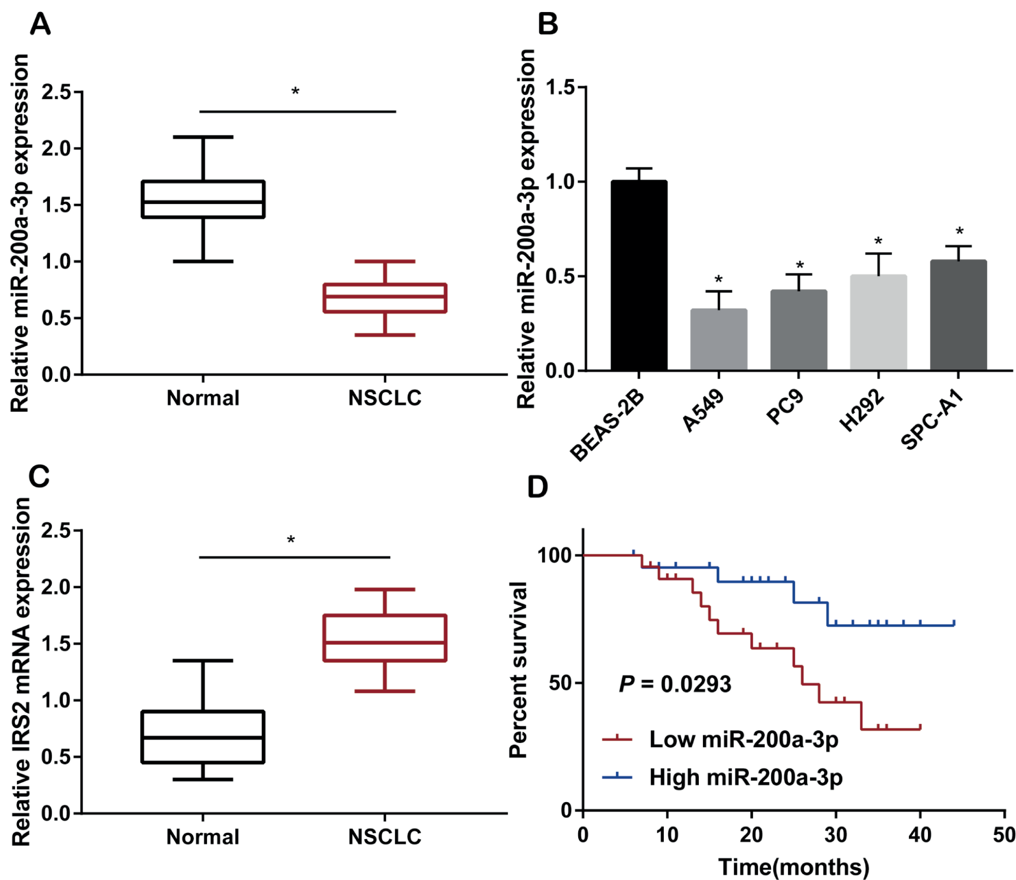

Figure 1. MiR-200a-3p was downregulated in NSCLC. **A,** Relative level of miR-200a-3p in NSCLC tissues and adjacent normal tissues. **B,** Relative level of miR-200a-3p in normal lung epithelial cell line (BEAS-2B) and lung carcinoma cell lines (A549, PC9, H292, and SPC-A1). **C,** Relative level of IRS2 in NSCLC tissues and adjacent normal tissues. **D,** The Kaplan-Meier curves showed survival in NSCLC patients with high-level or low-level miR-200a-3p.

Table I. Correlation between miR-200a-3p level and pathological characteristics of NSCLC patients (n=44).

Clinicopathologic features	Number of cases	MiR-200a-3p expression		*p*-value
		Low (n=22)	High (n=22)	
Age (years)				0.507
≤50	20	8	12	
>50	24	12	12	
Gender				0.763
Male	22	12	10	
Female	22	11	11	
Tumor size				0.046*
≤3 cm	19	8	11	
>3 cm	25	18	7	
TNM stage				0.028*
I-II	18	8	10	
III-IV	26	20	6	
Distant metastasis				0.036*
Yes	24	16	8	
No	20	7	13	

miRNA-200a-3p was downregulated in NSCLC tissues relative to normal ones (Figure 1A). In lung carcinoma cell lines, miRNA-200a-3p was identically downregulated (Figure 1B). IRS2 level in NSCLC tissues was detected as well, which was upregulated in NSCLC tissues than that of normal ones (Figure 1C). In addition, the relationship between miRNA-200a-3p level and pathological characteristics of NSCLC was identified. As shown in Table I, the miRNA-200a-3p level was closely correlated to tumor size, TNM staging, and lymphatic metastasis of NSCLC patients. The Kaplan-Meier curves revealed a worse survival in NSCLC patients expressing a low level of miRNA-200a-3p (Figure 1D).

Overexpression of MiRNA-200a-3p Attenuated the Proliferative and Metastatic Abilities of NSCLC

The transfection efficacies of miRNA-200a-3p mimic and miRNA-200a-3p inhibitor were examined in A549 cells (Figure 2A). The overexpression of miRNA-200a-3p markedly decreased the viability in A549 cells, whereas the knockdown of miRNA-200a-3p achieved the opposite trend (Figure 2B). The transfection of miRNA-200a-3p mimic reduced the migratory and invasive cell numbers in A549 cells. Conversely, the transfection of miRNA-200a-3p inhibitor enhanced the numbers of the migratory and invasive cells (Figure 2C). The protein levels of EMT-related genes were examined by Western blot. As the results showed, the transfection of miRNA-200a-3p mimic upregulated the E-cadherin and downregulated the N-cadherin and Vimentin. The knockdown of miRNA-200a-3p in A549 cells resulted in the opposite trends in the protein levels of E-cadherin, N-cadherin, and Vimentin (Figure 2D).

IRS2 Was the Direct Target for MiRNA-200a-3p

Through prediction in TargetScan, IRS2 was found to be the direct target of miRNA-200a-3p (Figure 3A). The Luciferase activity was markedly reduced after the co-transfection of miRNA-200a-3p mimic and IRS2-WT, verifying the binding between miRNA-200a-3p and IRS2 (Figure 3B). The Spearman correlation test illustrated a negative correlation between the expression levels of miRNA-200a-3p and IRS2 in NSCLC tissues (Figure 3C). Furthermore, the transfection of miRNA-200a-3p mimic downregulated IRS2 level in A549 cells. On the contrary, the transfection of miRNA-200a-3p inhibitor upregulated IRS2 level (Figure 3D). Hence, IRS2 was proved to be the direct target for miRNA-200a-3p, and its level was negatively regulated by miRNA-200a-3p in NSCLC.

IRS2 Reversed the Regulatory Effect of MiRNA-200a-3p on NSCLC

To further uncover the interaction between miRNA-200a-3p and IRS2, a series of rescue experiments were conducted. The downregulated level of IRS2 in A549 cells overexpressing miRNA-200a-3p was reversed by IRS2 overexpression (Figure 4A). The transfection of miRNA-200a-3p

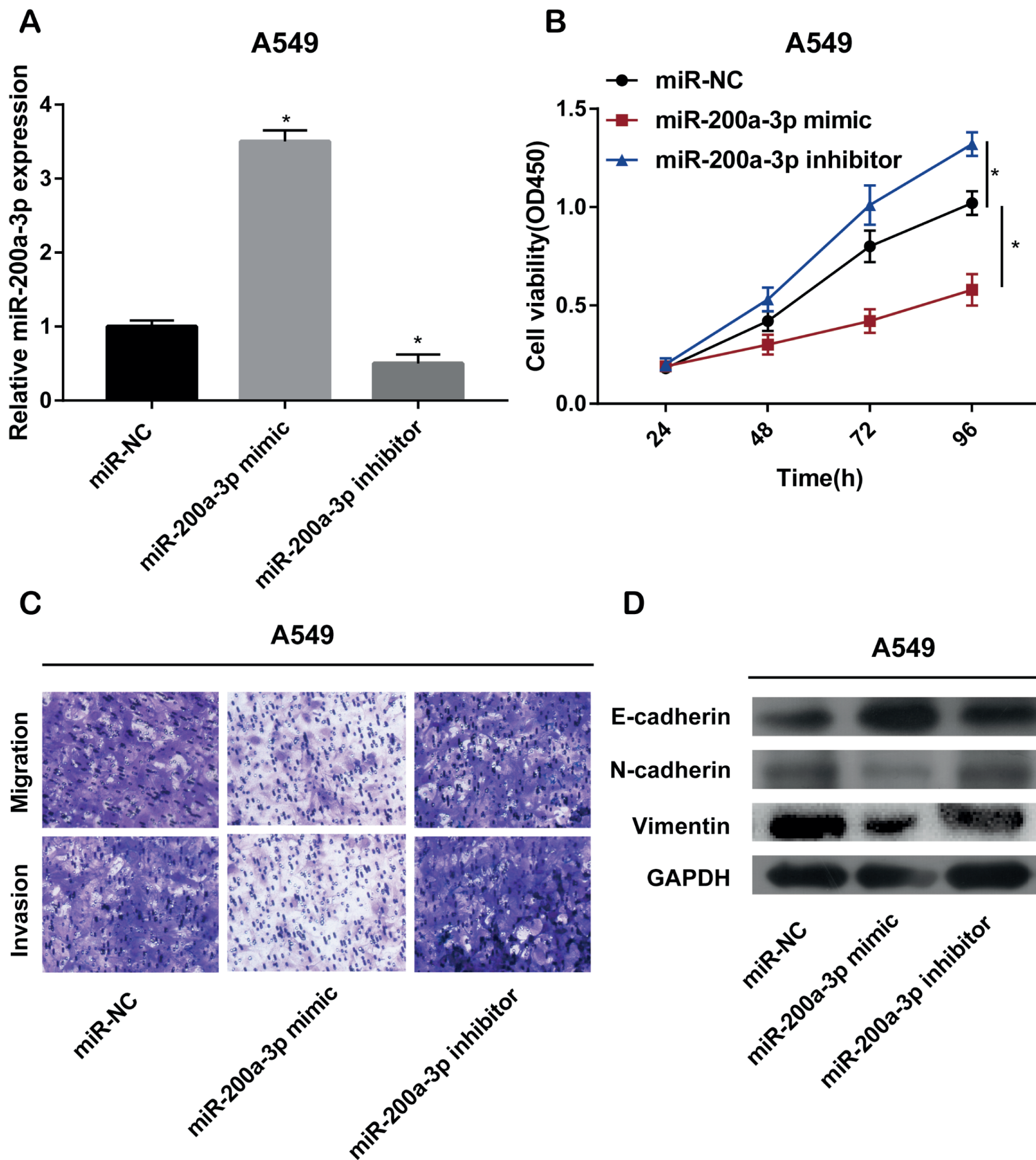

Figure 2. Overexpression of miR-200a-3p suppressed the proliferative, migratory, and invasive abilities of NSCLC. **A,** Transfection efficacies of miR-200a-3p mimic and miR-200a-3p inhibitor in A549 cells. **B,** Viability in A549 cells transfected with miR-NC, miR-200a-3p mimic, or miR-200a-3p inhibitor. **C,** Migration and invasion in A549 cells transfected with miR-NC, miR-200a-3p mimic, or miR-200a-3p inhibitor (magnification 200×). **D,** Protein levels of E-cadherin, N-cadherin, and Vimentin in A549 cells transfected with miR-NC, miR-200a-3p mimic, or miR-200a-3p inhibitor.

mimic reduced the viability in A549 cells, which was partially reversed by the co-transfection of pcDNA-IRS2 (Figure 4B). Identically, the reduced migratory and invasive cell numbers in A549 cells overexpressing miRNA-200a-3p were elevated after the overexpression of IRS2 (Figures 4C, 4D). It is believed that the overexpression of IRS2 reversed the regulatory effects of miRNA-200a-3p on proliferative, migratory, and invasive abilities of NSCLC.

Discussion

In recent years, the high mortality and morbidity of NSCLC pose a huge threat to the global public health[16-18]. NSCLC is a kind of heterogeneous tumor, which is pathologically classified into squamous cell carcinoma (accounting for 30%), adenocarcinoma (40%), and large cell carcinoma (15%)[19,20]. With the advance in early-stage diagnosis and therapeutic strategies, the incidence of

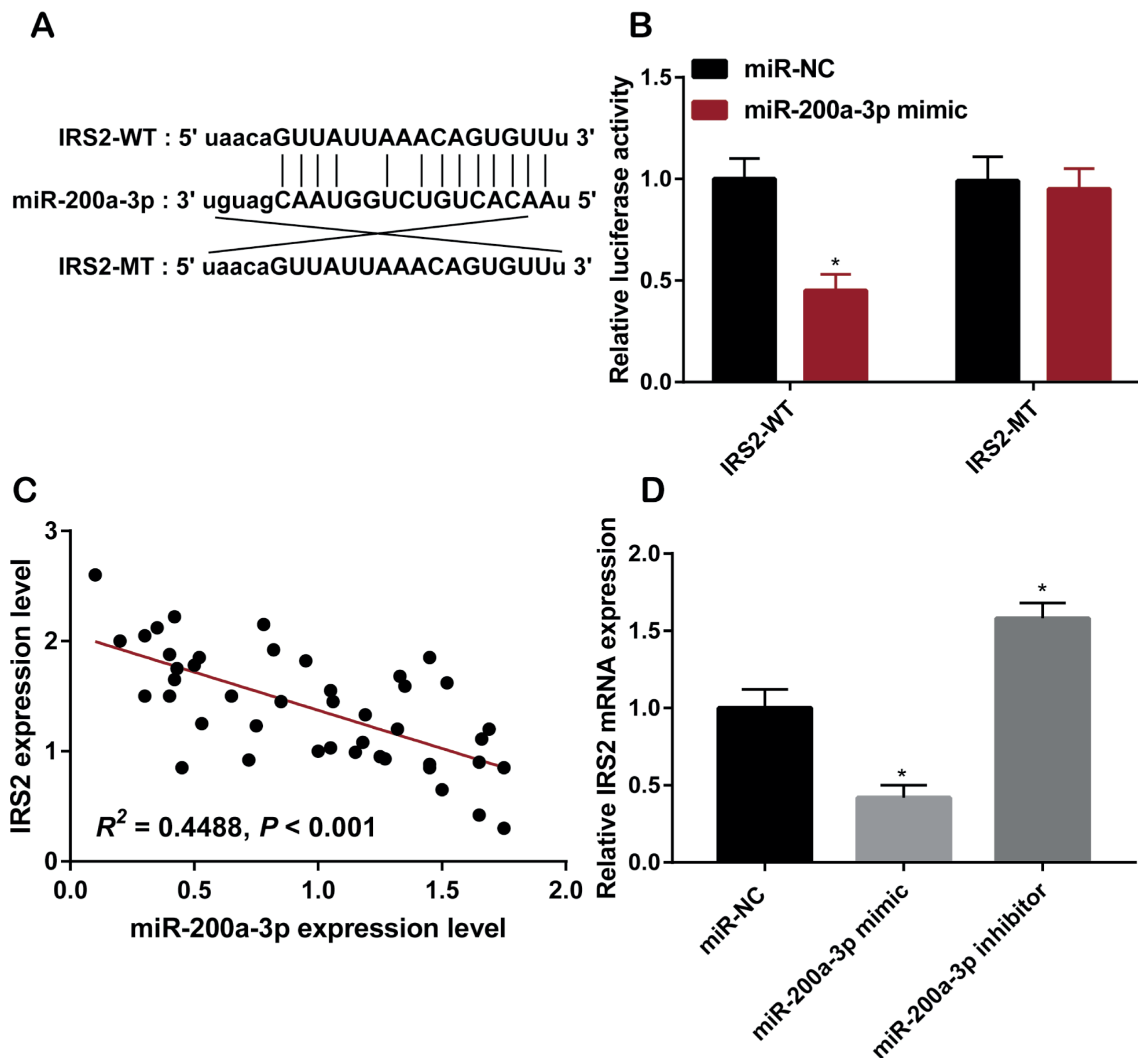

Figure 3. IRS2 was the direct target for miR-200a-3p. **A,** Binding sites between IRS2 and miR-200a-3p. **B,** Luciferase activity in A549 cells co-transfected with miR-NC/miR-200a-3p mimic and IRS2-WT/IRST-MT. **C,** A negative correlation between the expression levels of miR-200a-3p and IRS2. **D,** Relative level of IRS2 in A549 cells transfected with miR-NC, miR-200a-3p mimic, or miR-200a-3p inhibitor.

NSCLC is slowly declined. However, the 5-year survival of NSCLC remains low[21]. Invasiveness and metastasis are the major characteristics of malignant tumors, which are the leading causes of treatment failure.

MiRNAs are able to regulate the gene expressions at post-transcriptional level by binding to 3'UTR of target genes[22]. They are involved in the diagnosis, treatment, and drug-resistance of NSCLC. NSCLC-associated miRNAs could be utilized as hallmarks for elevating the sensitivity and specificity in NSCLC diagnosis[23,24]. Gregory et al[25] reported that miRNA-200a-3p exerts the tumor-suppressor effect by mediating EMT of tumor cells. In this study, miRNA-200a-3p was found to be downregulated in NSCLC tissues and cell lines. The overexpression of miRNA-200a-3p remarkably attenuated A549 cells to proliferate and metastasize. A series of investigations[26-30] have demonstrated the involvement of EMT-related miRNAs in the progression of NSCLC, including miR-33a, miR-135a, miR-134, miR-149, and miR-23a. Our study showed that the knockdown of miRNA-200a-3p downregulated E-cadherin and upregulated N-cadherin and Vimentin in A549 cells. It is suggested that miRNA-200a-3p was able to induce EMT in NSCLC and served as a tumor suppressor.

IRS2 locates on the 13q34 region, which is a member of the insulin receptor substrate (IRS) family. It is considered as an adaptor protein for additional surface receptors, including the closely related insulin-like growth factor 1 receptor (IGF-1R)[31,32]. Upregulated IRS2 could be observed in many types of cancer, including NSCLC[33]. Zhang et al[34] demonstrated that the overexpression of miR-338-3p inhibits proliferative, migratory, and invasive abilities, but promotes apoptosis of NSCLC cells by targeting IRS2. Wang et al[35] found that miR-431 inhibits the adipogenic differentiation of bone marrow mesenchymal stem cells by targeting IRS2. In our work, through bioinformatics prediction and experimental verification, miRNA-200a-3p was proved to bind to IRS2 and

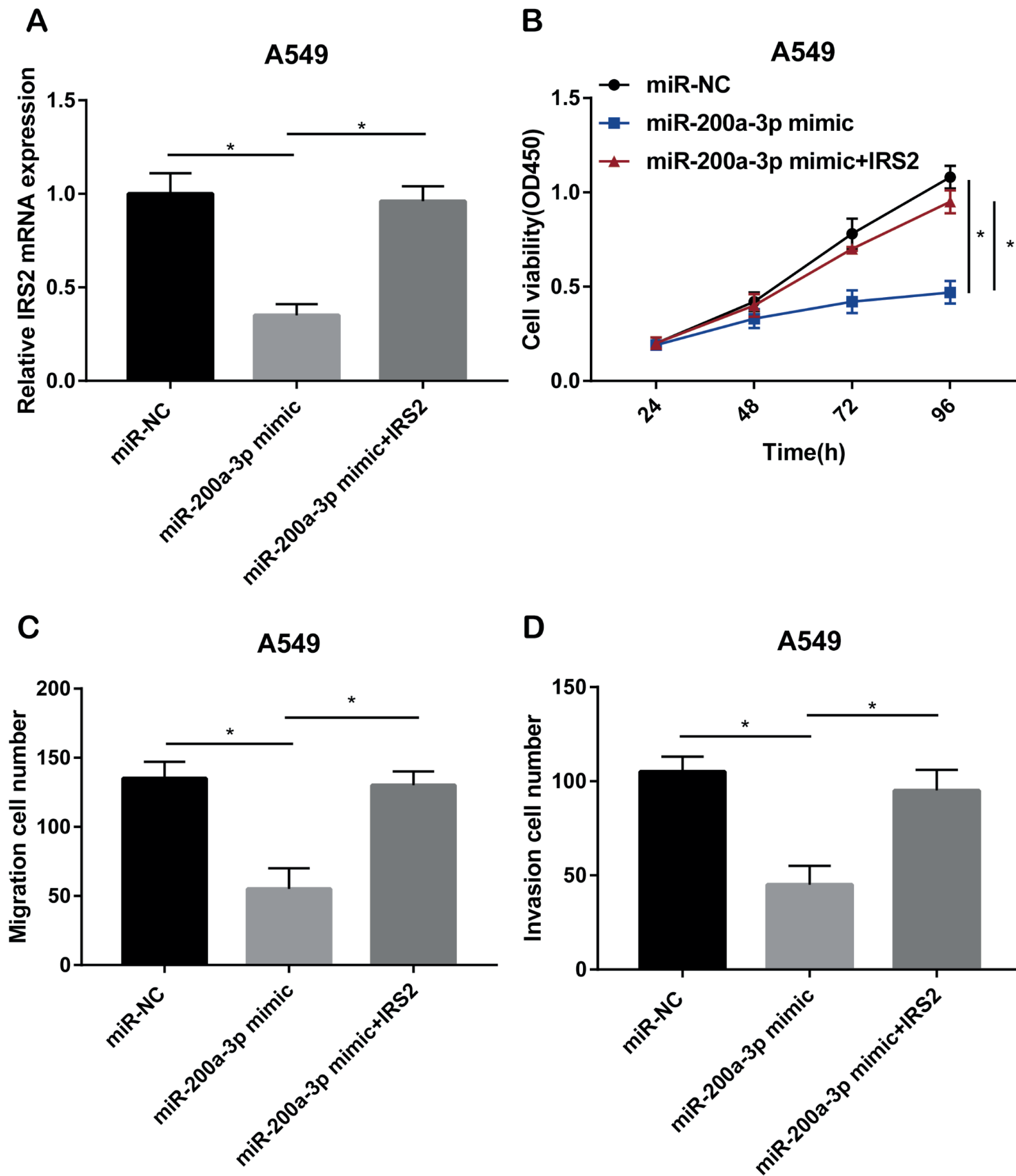

Figure 4. IRS2 reversed the regulatory effect of miR-200a-3p on NSCLC. A549 cells were transfected with miR-NC, miR-200a-3p mimic, or miR-200a-3p mimic + pcDNA-IRS2. **A,** Relative level of IRS2 in A549 cells. **B,** Viability in A549 cells at 24, 48, 72, and 96 h. **C,** Migratory cell number. **D,** Invasive cell number.

negatively regulated its level in A549 cells. Moreover, the overexpression of IRS2 could partially reverse the regulatory effect of miRNA-200a-3p on cellular behaviors of A549 cells. Collectively, our study illustrated that miRNA-200a-3p suppressed the malignant progression of NSCLC by inhibiting IRS2 level.

Conclusions

It has been demonstrated in this report that miRNA-200a-3p suppresses the proliferative and metastatic abilities of NSCLC by targeting IRS2, thus alleviating the progression of NSCLC.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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References

1) Lin CK, Hsu YT, Christiani DC, Hung HY, Lin RT. Risks and burden of lung cancer incidence for residential petrochemical industrial complexes: a meta-analysis and application. Environ Int 2018; 121: 404-414.
2) Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136: E359-E386.
3) Fountzilas E, Levva S, Mountzios G, Polychronidou G, Maniadakis N, Kotoula V, Fountzilas G. Treating EGFR-mutated oncogene-addicted advanced non-small-cell lung cancer in the era of economic crisis in Greece: challenges and opportunities. J Glob Oncol 2018; 4: 1-12.
4) Balça-Silva J, Sousa Neves S, Gonçalves AC, Abrantes AM, Casalta-Lopes J, Botelho MF, Sarmento-Ribeiro AB, Silva HC. Effect of miR-34b overexpression on the radiosensitivity of non-small cell lung cancer cell lines. Anticancer Res 2012; 32: 1603-1609.
5) Montani F, Bianchi F. Circulating cancer biomarkers: the macro-revolution of the micro-RNA. EBioMedicine 2016; 5: 4-6.
6) Shao Y, Liang B, Long F, Jiang SJ. Diagnostic microRNA biomarker discovery for non-small-cell lung cancer adenocarcinoma by integrative bioinformatics analysis. Biomed Res Int 2017; 2017: 2563085.
7) Guan P, Yin Z, Li X, Wu W, Zhou B. Meta-analysis of human lung cancer microRNA expression profiling studies comparing cancer tissues with normal tissues. J Exp Clin Cancer Res 2012; 31: 54.
8) Yu H, Guan Z, Cuk K, Brenner H, Zhang Y. Circulating microRNA biomarkers for lung cancer detection in Western populations. Cancer Med 2018; 7: 4849-4862.
9) Liu S, Ge X, Su L, Zhang A, Mou X. MicroRNA-454 inhibits non-small cell lung cancer cells growth and metastasis via targeting signal transducer and activator of transcription-3. Mol Med Rep 2018; 17: 3979-3986.
10) Wang M, Meng B, Liu Y, Yu J, Chen Q, Liu Y. MiR-124 inhibits growth and enhances radiation-induced apoptosis in non-small cell lung cancer by inhibiting STAT3. Cell Physiol Biochem 2017; 44: 2017-2028.
11) Pan Y, Liang H, Chen W, Zhang H, Wang N, Wang F, Zhang S, Liu Y, Zhao C, Yan X, Zhang J, Zhang CY, Gu H, Zen K, Chen X. MicroRNA-200b and microRNA-200c promote colorectal cancer cell proliferation via targeting the reversion-inducing cysteine-rich protein with Kazal motifs. RNA Biol 2015; 12: 276-289.
12) Hu X, Macdonald DM, Huettner PC, Feng Z, El NI, Schwarz JK, Mutch DG, Grigsby PW, Powell SN, Wang X. A miR-200 microRNA cluster as prognostic marker in advanced ovarian cancer. Gynecol Oncol 2009; 114: 457-464.
13) Zang Y, Tai Y, Wan B, Jia X. MiR-200a-3p promotes the proliferation of human esophageal cancer cells by post-transcriptionally regulating cytoplasmic collapsin response mediator protein-1. Int J Mol Med 2016; 38: 1558-1564.
14) Ding M, Sun X, Zhong J, Zhang C, Tian Y, Ge J, Zhang CY, Zen K, Wang JJ, Zhang C, Wang C. Decreased miR-200a-3p is a key regulator of renal carcinoma growth and migration by directly targeting CBL. J Cell Biochem 2018; 119: 9974-9985.
15) Xia H, Ng SS, Jiang S, Cheung WK, Sze J, Bian XW, Kung HF, Lin MC. MiR-200a-mediated downregulation of ZEB2 and CTNNB1 differentially inhibits nasopharyngeal carcinoma cell growth, migration and invasion. Biochem Biophys Res Commun 2010; 391: 535-541.
16) Fitzmaurice C, Dicker D, Pain A, Hamavid H, Moradi-Lakeh M, MacIntyre MF, Allen C, Hansen G, Woodbrook R, Wolfe C, Hamadeh RR, Moore A, Werdecker A, Gessner BD, Te AB, McMahon B, Karimkhani C, Yu C, Cooke GS, Schwebel DC, Carpenter DO, Pereira DM, Nash D, Kazi DS, De Leo D, Plass D, Ukwaja KN, Thurston GD, Yun JK, Simard EP, Mills E, Park EK, Catala-Lopez F, DeVeber G, Gotay C, Khan G, Hosgood HR, Santos IS, Leasher JL, Singh J, Leigh J, Jonas JB, Sanabria J, Beardsley J, Jacobsen KH, Takahashi K, Franklin RC, Ronfani L, Montico M, Naldi L, Tonelli M, Geleijnse J, Petzold M, Shrime MG, Younis M, Yonemoto N, Breitborde N, Yip P, Pourmalek F, Lo-

tufo PA, Esteghamati A, Hankey GJ, Ali R, Lunevicius R, Malekzadeh R, Dellavalle R, Weintraub R, Lucas R, Hay R, Rojas-Rueda D, Westerman R, Sepanlou SG, Nolte S, Patten S, Weichenthal S, Abera SF, Fereshteh-nejad SM, Shiue I, Driscoll T, Vasankari T, Alsharif U, Rahimi-Movaghar V, Vlassov VV, Marcenes WS, Mekonnen W, Melaku YA, Yano Y, Artaman A, Campos I, MacLachlan J, Mueller U, Kim D, Trillini M, Eshrati B, Williams HC, Shibuya K, Dandona R, Murthy K, Cowie B, Amare AT, Antonio CA, Castaneda-Orjuela C, van Gool CH, Violante F, Oh IH, Deribe K, Soreide K, Knibbs L, Kereselidze M, Green M, Cardenas R, Roy N, Tillmann T, Li Y, Krueger H, Monasta L, Dey S, Sheikhbahaei S, Hafezi-Nejad N, Kumar GA, Sreeramareddy CT, Dandona L, Wang H, Vollset SE, Mokdad A, Salomon JA, Lozano R, Vos T, Forouzanfar M, Lopez A, Murray C, Naghavi M. The global burden of cancer 2013. JAMA Oncol 2015; 1: 505-527.

17) Liu Z, Jiang L, Zhang G, Li S, Jiang X. MiR-24 promotes migration and invasion of non-small cell lung cancer by targeting ZNF367. J BUON 2018; 23: 1413-1419.

18) Zheng HE, Wang G, Song J, Liu Y, Li YM, Du WP. MicroRNA-495 inhibits the progression of non-small-cell lung cancer by targeting TCF4 and inactivating Wnt/β-catenin pathway. Eur Rev Med Pharmacol Sci 2018; 22: 7750-7759.

19) Mendes F, Antunes C, Abrantes AM, Goncalves AC, Nobre-Gois I, Sarmento AB, Botelho MF, Rosa MS. Lung cancer: the immune system and radiation. Br J Biomed Sci 2015; 72: 78-84.

20) Sangodkar J, Katz S, Melville H, Narla G. Lung adenocarcinoma: lessons in translation from bench to bedside. Mt Sinai J Med 2010; 77: 597-605.

21) Kohler J. Second-line treatment of NSCLC-the pan-erbB inhibitor afatinib in times of shifting paradigms. Front Med (Lausanne) 2017; 4: 9.

22) Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.

23) Sozzi G, Boeri M, Rossi M, Verri C, Suatoni P, Bravi F, Roz L, Conte D, Grassi M, Sverzellati N, Marchiano A, Negri E, La Vecchia C, Pastorino U. Clinical utility of a plasma-based miRNA signature classifier within computed tomography lung cancer screening: a correlative MILD trial study. J Clin Oncol 2014; 32: 768-773.

24) Montani F, Marzi MJ, Dezi F, Dama E, Carletti RM, Bonizzi G, Bertolotti R, Bellomi M, Rampinelli C, Maisonneuve P, Spaggiari L, Veronesi G, Nicassio F, Di Fiore PP, Bianchi F. MiR-test: a blood test for lung cancer early detection. J Natl Cancer Inst 2015; 107: djv063.

25) Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G, Vadas MA, Khew-Goodall Y, Goodall GJ. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. Nat Cell Biol 2008; 10: 593-601.

26) Yang L, Yang J, Li J, Shen X, Le Y, Zhou C, Wang S, Zhang S, Xu D, Gong Z. MircoRNA-33a inhibits epithelial-to-mesenchymal transition and metastasis and could be a prognostic marker in non-small cell lung cancer. Sci Rep 2015; 5: 13677.

27) Shi H, Ji Y, Zhang D, Liu Y, Fang P. MiR-135a inhibits migration and invasion and regulates EMT-related marker genes by targeting KLF8 in lung cancer cells. Biochem Biophys Res Commun 2015; 465: 125-130.

28) Li J, Wang Y, Luo J, Fu Z, Ying J, Yu Y, Yu W. MiR-134 inhibits epithelial to mesenchymal transition by targeting FOXM1 in non-small cell lung cancer cells. FEBS Lett 2012; 586: 3761-3765.

29) Ke Y, Zhao W, Xiong J, Cao R. MiR-149 inhibits non-small-cell lung cancer cells EMT by targeting FOXM1. Biochem Res Int 2013; 2013: 506731.

30) Cao M, Li Y, Lu H, Meng Q, Wang L, Cai L, Dong X. MiR-23a-mediated migration/invasion is rescued by its target, IRS-1, in non-small cell lung cancer cells. J Cancer Res Clin Oncol 2014; 140: 1661-1670.

31) White MF. IRS proteins and the common path to diabetes. Am J Physiol Endocrinol Metab 2002; 283: E413-E422.

32) He W, Craparo A, Zhu Y, O'Neill TJ, Wang LM, Pierce JH, Gustafson TA. Interaction of insulin receptor substrate-2 (IRS-2) with the insulin and insulin-like growth factor I receptors. Evidence for two distinct phosphotyrosine-dependent interaction domains within IRS-2. J Biol Chem 1996; 271: 11641-11645.

33) Kusakabe M, Kutomi T, Watanabe K, Emoto N, Aki N, Kage H, Hamano E, Kitagawa H, Nagase T, Sano A, Yoshida Y, Fukami T, Murakawa T, Nakajima J, Takamoto S, Ota S, Fukayama M, Yatomi Y, Ohishi N, Takai D. Identification of G0S2 as a gene frequently methylated in squamous lung cancer by combination of in silico and experimental approaches. Int J Cancer 2010; 126: 1895-1902.

34) Zhang P, Shao G, Lin X, Liu Y, Yang Z. MiR-338-3p inhibits the growth and invasion of non-small cell lung cancer cells by targeting IRS2. Am J Cancer Res 2017; 7: 53-63.

35) Wang Y, Yang L, Liu X, Hong T, Wang T, Dong A, Li J, Xu X, Cao L. MiR-431 inhibits adipogenic differentiation of human bone marrow-derived mesenchymal stem cells via targeting insulin receptor substance 2. Stem Cell Res Ther 2018; 9: 231.