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MiRNA-199 inhibits malignant progression of lung cancer through mediating RGS17

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Abstract. – OBJECTIVE: MicroRNAs (miRNAs) are endogenous, non-coding small RNAs, which play an important part in regulating organismal and pathological processes. Previous studies have shown that miRNA-199 acts as a tumor suppressor gene. However, we aimed to explore the characteristics and function of miRNA-199 in lung cancer (LCa), so as to further study its relationship with clinicopathological parameters and prognosis.

PATIENTS AND METHODS: Quantitative Real-time polymerase chain reaction (qRT-PCR) was used to detect miRNA-199 expression in 75 pairs of LCa tissues and normal adjacent tissues. In addition, the relationship between miRNA-199 expression and pathological features along with the prognosis of LCa patients were investigated. Besides, the expression level of miRNA-199 in LCa cells was further validated by qRT-PCR. In addition, miRNA-199 overexpression expression model was constructed in LCa cell lines H1299 and SPCA1. Cell counting kit-8 (CCK-8), cell cloning experiments, transwell invasion and migration assays were performed to analyze the effect of miRNA-199 on the biological function of LCa cells. Finally, the potential mechanism was explored using Western blot.

RESULTS: qRT-PCR results displayed that the expression level of miRNA-199 in LCa tissues was significantly lower than that of the normal tissues. Compared with patients with high miRNA-199 expression, patients with lowly-expressed miRNA-199 had higher rates of lymph node metastases and distant metastases, and their overall survival rates were lower. In addition, the proliferation, invasion and metastasis of the miRNA-199 overexpression group were significantly increased than that in the negative control group. Western Blot results showed that the expression of key proteins in the EMT pathway, such as N-cadherin, Vimentin, β-catenin and MMP9 significantly increased in miRNA-199 overexpression group. Moreover, we also found that miRNA-199 and RGS17 have mutual regulation, which inhibited the malignant progression of LCa.

CONCLUSIONS: miRNA-199 expression was down-regulated in LCa and was significantly associated with LCa stage, distant metastasis, and poor prognosis. Besides, miRNA-199 may inhibit the malignant progression of LCa by interacting with RGS17.

Key Words:
miRNA-199, RGS17, Lung cancer, Malignant progression.

Introduction

As one of the most common cancers in the world, lung cancer (LCa) has been the leading cause of death in both male and female[1,2]. The incidence of lung cancer in China is increasing year by year, becoming one of the most important tumors seriously affecting the life and health of the people[3]. With the rapid development of molecular biology and genetic diagnosis technology, it is believed that the possible mechanism of LCa at present is the long-term interaction of genetic and environmental factors, which induces cell malignant transformation and irreversible genetic changes. The main characteristics are the activation of oncogenes and inactivation of tumor suppressor genes, whose changes eventually lead to the loss of control of signal transduction involving key physiological functions of cells including proliferation, apoptosis, and differentiation[4,5]. Although great achievements have been made in exploring the mechanisms of LCa, whether there are other genes or mechanisms of epigenetic regulation involved in the occurrence and development still need of further investigations. Diagnostic and therapeutic difficulties resulting from LCa pathogenesis have not yet been fully elucidated, which is one of the important reasons for its high morbidity and mortality[5,6]. In recent years, a large number of clinical trials have been adjusted for the treatment of lung cancer, such as the combination of chemotherapeutic drugs or increasing the dose intensity; however, these adjustments did not

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achieve a good therapeutic effect[7, 8]. The side effects of high-dose chemotherapy and drug resistance in tumor cells have limited the effectiveness of chemotherapy[8, 9]. The treatment of lung cancer enters a stagnation period and urgently requires new treatment options. Finding genes that are abnormally expressed in LCa and finding key genes or new immunotherapeutic targets in the development and metastasis of LCa may open new ideas for the treatment of lung cancer[10]. Therefore, elucidating the molecular mechanism of lung cancer metastasis, predicting, diagnosing, and judging prognosis of lung cancer metastasis is a vital part of LCa research. MicroRNA (miRNA) is a type of eukaryotic endogenous small-molecule single-stranded RNA, which is usually 18 to 25 nt in length. MicroRNA could bind to the 3'untranslated region (3'UTR) of messenger RNA, resulting in the degradation or translational inhibition of mRNA to regulate target gene post-transcriptional expressions[11, 12]. MiRNA and mRNA are not completely complementary paired, thereby exerting a targeted regulatory function[13, 14]. Therefore, misexpression of miRNAs leads to a large number of protein misexpressions[15]. A large number of experiments have shown that the abnormal expression of miRNA is closely related to malignant tumors, and that miRNA can also be used as some tumor markers[16, 17]. Kai et al[17] have found that miRNAs are unbalanced in a variety of tumors and show certain tissue specificities. It can promote the proliferation, invasion and metastasis of tumor cells through various mechanisms, and plays a vital regulatory part in the occurrence and development of tumors[18]. MiRNA has been found to be abnormally expressed in various tumor tissues including hepatocellular carcinoma, renal cell carcinoma, breast carcinoma, and colorectal carcinoma, and is closely related to the occurrence and development of these tumors[19-22]. However, the molecular regulation mechanisms of miRNAs in tumors are not yet fully understood. In general, miRNAs may involve in chromosome recombination, gene imprinting, epigenetic regulation, nucleocytoplasmic transport, mRNA splicing and translation, and participate in all aspects of gene regulation. Thus, miRNAs play an important part in biological processes such as cell proliferation, cell cycle, differentiation, apoptosis, and metastasis of tumor cells[23,24]. However, the function of miRNAs in LCa has rarely been reported. In addition, the involvement of miRNAs in the epithelial to mesenchymal transition (EMT) mutation process in LCa has not been explained yet[25].

In this study, we detected the expression of miRNA-199 in 75 pairs of LCa tissues and adjacent tissues, and explored the effects of miRNA-199 on the biological functions of LCa cells. Previous studies have pointed out that miRNA-199 can inhibit tumor cell division and metastasis and control the development of tumors. Thus, we aimed to explore the role of miRNA-199 in the pathological parameters and prognosis of LCa and the mechanisms involved in tumorigenesis.

Patients and Methods

LCa Samples Collection

Tumor and paracancerous tissue specimens of 75 pairs of LCa were obtained from patients receiving resection surgery. According to the 8th UICC/AJCC lung cancer tumor node metastasis (TNM) staging criteria, all patients included were diagnosed as LCa by postoperative pathological analysis and had not received radiotherapy or chemotherapy and other anti-cancer treatments before surgery. This study has been approved by the Ethics Supervision Committee of Shanxi Cancer Hospital. Patients and their family members had been fully informed that their specimens would be used for scientific research and signed relevant informed consent forms.

Cell Culture and Reagents

Five human LCa cell lines (A549, SPC-A1, H1299, PC-9, and H358) and a normal human bronchial epithelial cell BEAS-2B were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Rockville, MD, USA) with 10% fetal bovine serum (FBS) (Life Technologies, Gaithersburg, MD, USA) in a 37°C, 5% CO2 incubator.

Transfection

Negative controls (si-RNA) and siRNA containing the miRNA-199 overexpression sequence (si-miRNA-199) were constructed by Zima (Shanghai, Cina). Cells were plated in 6-well plates and siRNA transfections were performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. 48 hours after the transfection, cells were collected for quantitative Real-time polymerase chain reaction (qRT-PCR) analysis and cell function experiments.

Cell Proliferation Assays

Cells transfected for 48 h were collected and seeded in 96-well plates (2000 cells per well). Af-

ter that, cells were cultured for different hours at 6 h, 24 h, 48 h, and 72 h, respectively, and added with cell counting kit-8 (CCK-8) (Dojindo Laboratories, Kumamoto, Japan) reagent. After another incubation for 2 hours, the optical density (OD) value of each well at 490 nm absorbance wavelength was measured by a microplate reader.

Colony Formation Assay

The cells transfected for 48 h were collected, seeded in a 6-well plate in a density of 200 cells/well and cultured in complete medium for 2 weeks. The medium was changed after one week, and then replaced twice a week. After the formation of the clones, the medium should be aspirated and washed twice with phosphate-buffered saline (PBS). The cells were fixed in methanol for 20 min and then stained with 0.1% crystal violet for 20 min. After washed 3 times with PBS, cells were photographed for colony formation observation.

Transwell Assay

After 48 hours of transfection, the cells were collected and resuspended in serum-free medium. After cell counting, the diluted cell density was adjusted to 2.0×10^5 /mL. Transwell chambers with or without Matrigel were placed in 24-well plates. 200 μL of suspension were added to the upper chamber while 500 μL of culture medium containing 10% FBS were added to the lower chamber. Cells were placed in a 37°C incubator. After 48 hours, the chamber was taken out and fixed with 4% paraformaldehyde for 30 min. Next, chamber was stained with crystal violet for 15 min. The inner surface of the basement membrane of the chamber was carefully cleaned to remove inner cells. Microscopically stained transmembrane cells were visualized in the basement membrane of the chamber and 5 fields were randomly selected.

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

Total RNAs of LCa cell lines and tissues were isolated by TRIzol (Invitrogen, Carlsbad, CA, USA). Reverse transcription was performed to synthesize cDNA using Primescript RT Reagent (TaKaRa, Otsu, Shiga, Japan). qRT-PCR reactions were performed using SYBR reagent (TaKaRa, Otsu, Shiga, Japan) by Real-time PCR System (Applied Biosystems, Foster City, CA, USA). The following primers were used for qRT-PCR reactions: miRNA-199: F: 5'-GCGCCCAGTGTTCAG-3', R: 5'-GTGCGAGGTCCGAGT-3'; RGS17: F: 5'-CAGAGGCCCAACAACACCTG-3', R: 5'-TGTGGGTCTTCCCGCATTTT-3'; U6: F: 5'-TGCGGTGCTCGTCTGCGCAGC-3', R: 5'-CCAGTGCGAGGTCCGGAT-3'; β-actin: F: 5'-CCTGGCACCCAGCACAAT-3', R: 5'-TGCCGTAGGTGTCCCTTTG-3'.

Western Blot

Cells were digested with trypsin and total protein concentrations were calculated by bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, USA). The extracted proteins were separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). After blocking in nonfat milk, the immunoblots were incubated with the following primary antibodies: N-cadherin, Vimentin, β-catenin and MMP9 and GAPDH, and the secondary antibodies were anti-mouse and anti-rabbit, both purchased from Cell Signaling Technology (Danvers, MA, USA). Protein bands were detected using enhanced chemiluminescence (ECL) machine (Thermo Fisher Scientific, Waltham, MA, USA).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 software (IBM, Armonk, NY, USA) were used for statistical analysis. Data were shown as mean ± standard deviation ($\overline{x}\pm s$). Differences were calculated by Students' *t*-test, χ^2-test or the Fisher's exact probability method. Kaplan-Meier method was applied to evaluate the survival time of patients, while Log-rank test was performed to compare the differences between different curves. Statistics were considered significant at $p<0.05$.

Results

miRNA-199 was Lowly Expressed in LCa Tissues and LCa Cell Lines

We detected the expression level of miRNA-199 in 75 pairs of LCa tissues and their corresponding paratumor tissues and LCa cell lines by qRT-PCR. The results revealed that compared with tumor-free tissues, miRNA-199 expression level was significantly lower in LCa tissues (Figure 1A, 1B). Besides, the level of miRNA-199 was markedly lower in LCa cells than that in the normal bronchial epithelial cell line BEAS-2B (Figure 1C). MiRNA-199 expression was the lowest especially in the H1299 and SPCA1 cell lines, so we chose these two cells for subsequent experiments. These above results suggested that miRNA-199 was lowly expressed in LCa tissues and the relevant cell lines.

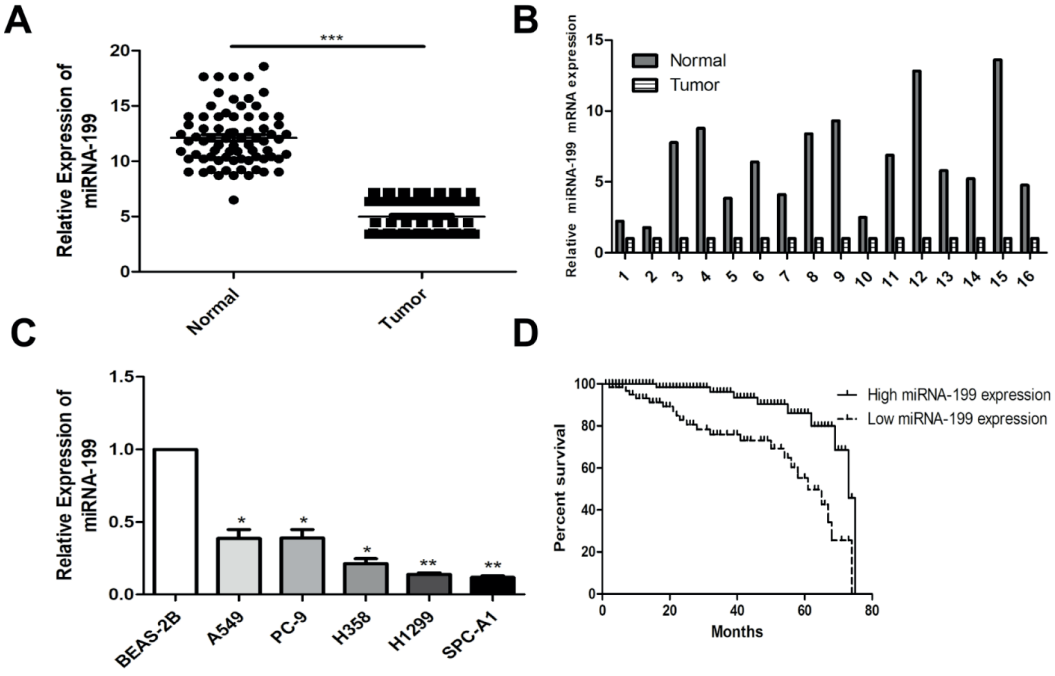

Figure 1. ***A-B,*** The expression of miRNA-199 in 75 pairs of LCa tissue was significantly increased. ***C,*** Expression levels of miRNA-199 in 6 LCa cell lines (HLCa, SPCA1, SLCaP-9607, H1299, 143B, SaLCa-2) and normal skeletogenous cell (BEAS-2B). ***D,*** Kaplan-Meier survival curves of patients with LCa based on miRNA-199 expression. Patients in the low expression group had a significantly more unfavorable prognosis than that in high expression group.

miRNA-199 Expression was in Correlation with Clinical Stage, Lymph Node and Distance Metastasis and Overall Survival in LCa Patients

Based on qRT-PCR results of miRNA-199 expression in 75 pairs of LCa tissues and adjacent tissues, patients were divided into the high expression group and the low expression group. The number of each group was counted. x^2-test was then applied to analyze the relationship between miRNA-199 expression and the age, gender, clinical stage, and distant metastasis of patients with LCa. As listed in Table I, miRNA-199 level was not in correlation with the age and gender of patients with LCa

Table I. Association of miR-199 expression with clinicopathologic characteristics of lung cancer.

		miR-199 expression		
Parameters	No. of cases	Low (n)	High (n)	*p*-value
Age (years)				0.435
< 60	32	20	12	
≥ 60	43	23	20	
Gender				0.294
Male	27	22	15	
Female	38	18	20	
T stage				0.021
T1-T2	42	13	29	
T3-T4	33	19	14	
Lymph node metastasis				0.045
No	45	15	30	
Yes	30	17	13	
Distance metastasis				0.036
No	60	22	38	
Yes	15	10	5	

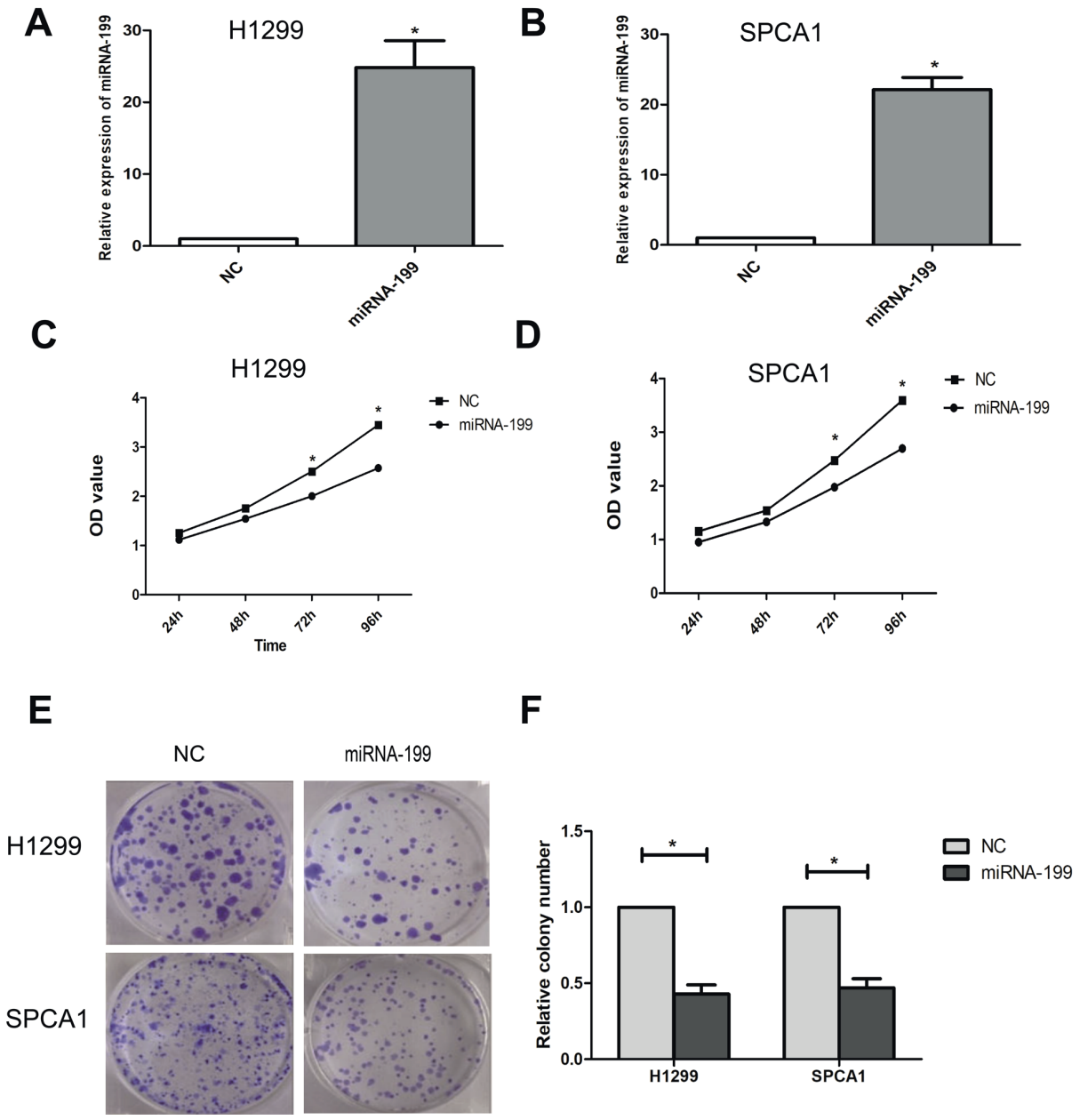

Figure 2. ***A-B,*** qRT-PCR were used to verify the efficiency of miRNA-199 overexpression in H1299 and SPCA1 cell lines. ***C-D,*** Growth curve analysis showing the cell growth of H1299 and SPCA1 cells with miRNA-199 knockdown. ***E-F,*** The efficiencies of cell colony formation in H1299 and SPCA1 cells with miRNA-199 knockdown.

but was related to pathological stage, lymph node metastasis, and distant metastasis. Meanwhile, in order to investigate the relationship between the miRNA-199 expression and the prognosis of patients with LCa, we collected relevant follow-up data. The Kaplan-Meier survival curve revealed that low expression of miRNA-199 was associated with poor prognosis of LCa, and the lower the expression level of miRNA-199, the worse the prognosis was (Figure 1D). These results showed that miRNA-199 might be a new biological indicator for predicting the prognosis of LCa.

Overexpression of miRNA-199 Promoted Cell Proliferation

To investigate the effect of miRNA-199 on the proliferation of LCa cells, we first constructed the miRNA-199 overexpression expression cell mod-

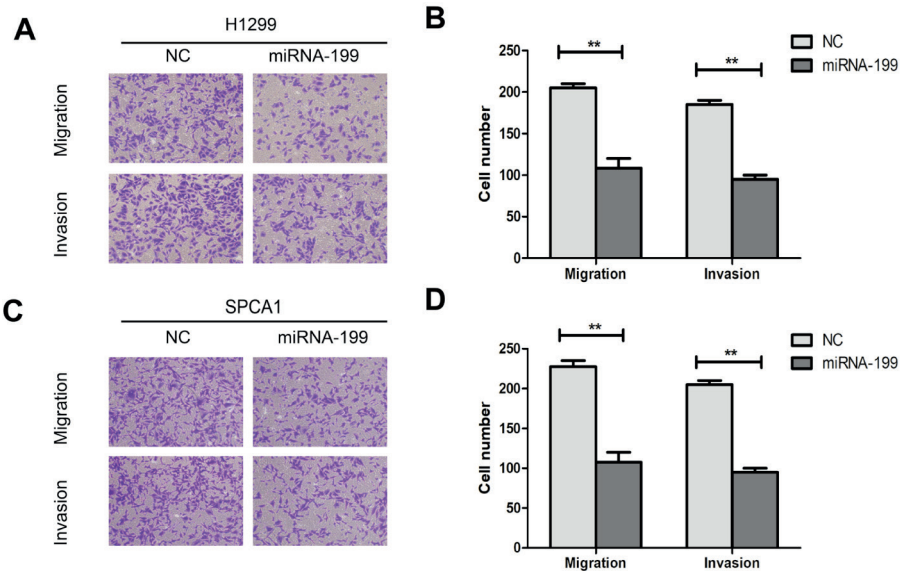

Figure 3. ***A-B,*** H1299 cell transfected with si-miRNA-199 displayed significantly lower migration and invasion capacity. ***C, D,*** SPCA1 cell transfected with si-miRNA-199 displayed significantly lower migration and invasion capacity.

el (Figure 2A and 2B) and used CCK8 to examine cell proliferation. As shown in Figure 2C and 2D, the miRNA-199 overexpression group had a significantly reduced cell proliferation rate compared to the NC group. Similarly, we observed the same trend from the cell cloning experiments (Figure 2E and 2F). These results revealed that overexpression of miRNA-199 promoted cell proliferation.

Overexpression of miRNA-199 Promoted Cell Migration and Invasion

Next, we performed transwell migration invasion experiments to explore the effect of miRNA-199 on the migration and invasion of LCa cell lines. In H1299, results showed that compared with the NC group, the number of LCa cells transmembrane in the transwell chamber was significantly reduced after miRNA-199 overexpression, suggesting that the migration and invasion ability was weakened (Figure 3A and 3B). In addition, in the SPCA1 cell line, the experimental results were consistent with the above results (Figure 3C and 3D). These results demonstrated that miRNA-199 overexpression promoted cell migration and invasion.

Overexpression of miRNA-199 Changed the Expression of EMT Signaling Pathway

In order to investigate the potential mechanism of miRNA-199 in promoting cell proliferation and migration, we detected the expression of N-cadherin, Vimentin, β-catenin, and MMP9 in the EMT pathway after overexpression of miRNA-199 by Western Blot. The results showed that these above-mentioned protein expression levels were significantly changed after overexpression of miRNA-199 (Figure 4A), suggesting that overexpression of miRNA-199 changed the expression of EMT signaling pathway.

RGS17 Modulated miRNA-199 Expression in Human Lung Cells

To further explore the ways in which miRNA-199 inhibited the malignant progression of lung cancer, we discovered that there may be some relations between RGS17 and miRNA-199 through related bioinformatics analysis. qRT-PCR and Western Blot were performed to detect the expression of RGS17 in 75 pairs of LCa and its corresponding tumor-free tissues and LCa cell lines. Results showed that the expression level of RGS17-related pathway protein in LCa tissue was significantly increased compared with that in the tumor-free tissue, and the difference was statistically significant (Figure 4B). Meanwhile, RGS17 expression was also markedly higher in LCa cells compared with that of BEAS-2B cells (Figure 4C).

In addition, in order to further explore the interaction between miRNA-199 and RGS17 in LCa cells, we found that miRNA-199 increased significantly after overexpressing RGS17 in LCa cells, which confirmed the mutual regulation of miRNA-199 and RGS17. Therefore, we selected 16 pairs

of samples from 75 pairs of LCa tissues and detected the mRNA expression level of miRNA-199 and RGS17 by qRT-PCR. Results displayed that the expression of miRNA-199 and RGS17 was in a negative correlation in H1299 and SPCA1 cell lines (Figure 4D). In addition, in order to explore the relationship between RGS17 and the prognosis of patients with LCa, we collected relevant follow-up data. The Kaplan-Meier survival curve showed that high expression of RGS17 was associated with poor prognosis of LCa. The lower the expression level of miRNA-199, the worse the prognosis (p<0.05; Figure 4E). Meanwhile, as shown in Figure 5, we found that RGS17 was significantly higher in LCa cells compared to BEAS-2B (Figure 5). At the cellular level, we constructed a small RGS17 interference and used Western blot to examine the transfection efficiency of RGS17. In the H1299 cell line with suppressed miRNA-199 expression, the invasion and metastasis of LCa cells was impaired and RGS17 expression was decreased when Si-RGS17 was added.

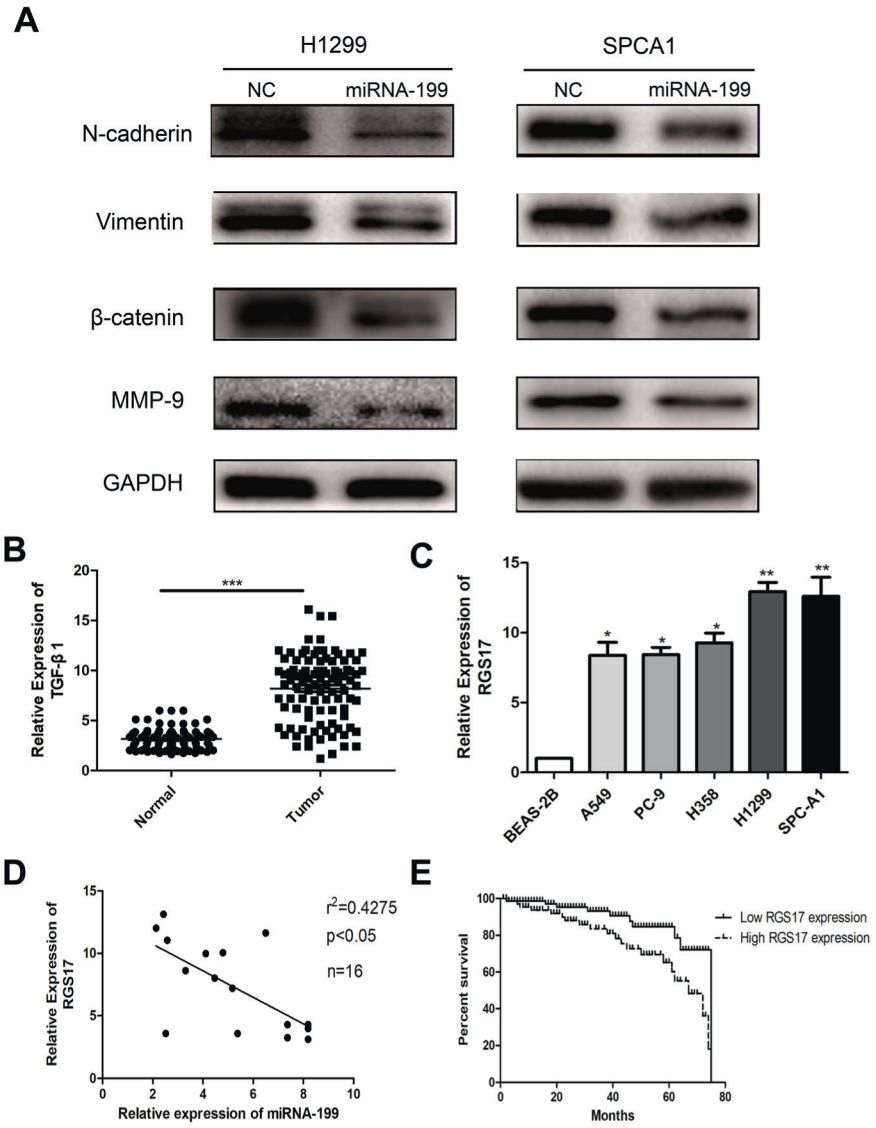

Figure 4. ***A,*** Overexpression of miRNA-199 expression significantly decreased the expression of EMT signal pathway, including N-cadherin, Vimentin, β-catenin and MMP9. ***B, C,*** The mRNA expression level of RGS17 relative to GAPDH in human LCa tissues and corresponding adjacent tissues, and cell lines were detected by using qRT-PCR. ***D,*** A negative correlation was found between miRNA-199 and RGS17 in tumor samples. ***E,*** Kaplan-Meier survival curves of patients with LCa based on RGS17 expression. Patients in the high expression group had a significantly more unfavorable prognosis than that in low expression group.

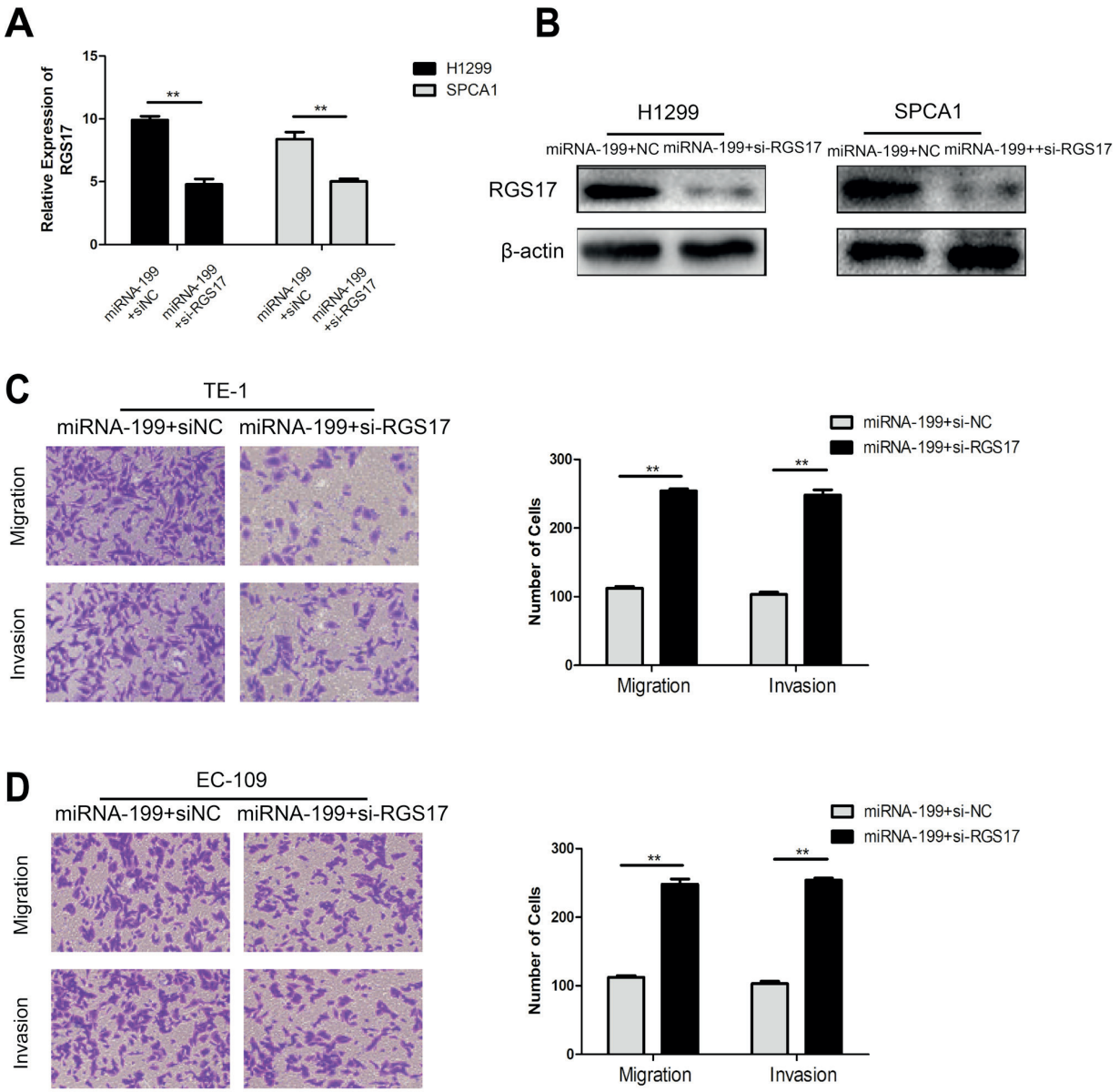

Figure 5. ***A,*** The expression of RGS17 was verified by qRT-PCR in co-transfected cell lines. ***B,*** Western blot was used to verify the expression of RGS17. ***C-D,*** The roles of miRNA-199 and RGS17 in the regulation of LCa cell migration and invasion were examined by transwell assay. A representative data set is displayed as mean ± SD values. *$p<0.05$, **$p<0.01$.

Discussion

As one of the most common cancers worldwide, LCa has been the leading cause of death in both male and female[1,2]. Molecular genetic changes in lung cancer cells, such as changes in gene copy number and disruption of coding sequences, have important effects on tumor phenotype[7]. At present, the study of multiple tumor signaling pathways has promoted the development of many new therapeutic targets[8]. In recent years, the incidence and mortality of LCa in China have gradually increased. The early diagnosis rate of LCa patients in China is extremely low, and most of them have advanced to the middle and late stages of treatment. Therefore, the majority of LCa is of advanced stage[3, 4]. Lung cancer has a high degree of malignancy; meanwhile, it is easy to relapse and metastasize and has a poor prognosis. Early diagnosis and accurate judgment of prognosis can contribute to the timely and standardized treatment of diseases and improve clin-

ical outcomes. Therefore, active exploration and finding suitable early diagnosis and prognostic judgement molecular markers have important clinical significance[5]. Genetics, diet, unhealthy lifestyles, and precancerous lesions are all closely related to their occurrence. Clinically, more than half of LCa patients have had micrometastases before receiving radical surgery, which is a direct cause of postoperative metastasis and recurrence of lung cancer[5, 6]. Researches of early diagnosis, metastasis, recurrence of LCa, and adjuvant therapy after advanced LCa have become the focus of current research[2]. Recent studies have revealed that miRNA plays vital parts in many diseases, including tumors. There are several abnormally expressed miRNAs in LCa, which may act as a crucial part in the diagnosis, treatment and prognosis of LCa[11, 12]. Therefore, finding miRNAs that are abnormally expressed in LCa and analyzing their association with clinical outcomes will improve the diagnosis and treatment of LCa and the clinical outcomes.

In recent years, many molecular targets regulated by miRNAs have been continuously revealed. Evidence[17] shows that miRNAs play a vital part in the biological behavior of lung cancer, and may provide a new direction for the future diagnosis and treatment of lung cancer. MiRNA is an endogenous non-coding RNA molecule consisting of 22 nucleotides in length, which binds to the 3′ UTR of the target gene mRNA to degrade its target mRNA or inhibit its translation, thereby regulating downstream gene expression[16]. MiRNAs can affect cell proliferation, apoptosis, sensitivity to chemotherapy and radiotherapy, tumor metastasis, and may even define the phenotype of cancer stem cells[17]. Revealing the roles and mechanisms of miRNAs is conducive to revealing the complex molecular mechanisms of LCa and providing targets for the development of new anti-lung cancer drugs[17,21,22]. We investigated the features of miRNA-199 in LCa, as well as the role of miRNA-199 in LCa expression and progression of malignant progression. We validated the level of miRNA-199 in 75 pairs of LCa tissues and paracancerous tissues and found that the expression of miRNA-199 was significantly downregulated and positively correlated with the pathological stage, lymph node metastasis, distant metastasis, and poor prognosis of LCa. Therefore, we consider that miRNA-199 may play a role in suppressing cancer in LCa. To further explore the effect of miRNA-199 on the biological function of LCa, we constructed a miRNA-199 overexpression model using small over-expressed RNA. CCK8, cell cloning experiments, invasion and migration experiments showed that miRNA-199 can inhibit the occurrence and development of LCa and play an important role in LCa; however, its specific molecular mechanism was not yet clear. The EMT signaling pathway is an important signaling pathway involved in tumorigenesis[26]. Since the concept that epithelial cell could transfer to mesenchymal cells was mentioned, investigations[27] have shown that it is closely related to the occurrence and development of epithelial cell malignancies. Currently, both *in vivo* and *in vitro* experimental evidence have suggested that EMT plays a pivotal role in the primary and secondary metastases of breast cancer, colon cancer, lung cancer, prostate cancer, pancreatic cancer, and liver cancer[28-30]. Therefore, the analysis of the occurrence and regulation mechanism of EMT is of great significance in finding a target for the treatment of malignant tumors, especially tumor cell metastases[31]. These findings show that EMT pathway is expected to play a benign part in the treatment of cancer. In this experiment, we found that the expression of key proteins such as N-cadherin, Vimentin, β-catenin, and MMP9 in the EMT pathway was significantly reduced after overexpression of miRNA-199 by Western Blot, indicating that miRNA-199 played important parts in promoting invasion and transfer through EMT signaling pathway. The G protein is located on the inner surface of the cell membrane and functions as a bridge between cell membrane surface receptors and intracellular effectors and regulates the activity of downstream effectors such as the adenylyl cyclase signaling pathway[32]. The G protein-signaling pathway acts as a key part in the growth and regeneration of normal hepatocytes, while its expression and function was abnormal in hepatocellular carcinoma patients and animal models[33]. It has been reported that the G protein-coupled receptor 87 (GPR87) can promote the proliferation and migration of liver cancer cells. Besides, GPR 5 is abnormally expressed in liver cancer cells and participates in regulating the phenotype and survival of liver cancer cells[34,35]. In addition to the above-mentioned studies on G protein and GPR in liver cancer, RGS has gradually attracted people's attention[35]. Among them, RGS17 gene amplification is closely related to increased cell differentiation, local and distant metastasis, migration, decreased apoptosis, accelerated angiogenesis, tumor invasion, local and distant metastasis[36]. In this study, we found that miRNA-199

and RGS17 interact with each other through cell recovery experiments. Further deepening the understanding of the biological function of RGS17 gene and its role in the development of tumors will be more helpful for the diagnosis, treatment and prognosis evaluation of tumors. This undoubtedly brings the gospel to many cancer patients and their families, bringing new hope and dawn to the conquest of cancer by humans. To prove whether miRNA-199 can promote the development of LCa by regulating EMT signaling pathway, we detected the expression of key proteins N-cadherin, Vimentin, β-catenin, and MMP9 in EMT signaling pathway after overexpression of miRNA-199 by Western Blot. Results suggested that miRNA-199 could promote the invasion and metastasis of LCa through the EMT signaling pathway. In addition, our findings also showed that the expression of RGS17 was significantly altered after overexpression of miRNA-199, suggesting that miRNA-199 may inhibit the malignant progression of LCa by regulating RGS17.

Conclusions

We found that the expression of miRNA-199 in LCa was significantly reduced, which was significantly associated with LCa stage, distant metastasis, and poor prognosis. Thus, miRNA-199 may be able to inhibit the malignant progression of LCa by regulating RGS17.

Conflict of Interests

The Authors declare that they have no conflict of interests.

References

1) Wang N, Mengersen K, Kimlin M, Zhou M, Tong S, Fang L, Wang B, Hu W. Lung cancer and particulate pollution: a critical review of spatial and temporal analysis evidence. Environ Res 2018; 164: 585-596.
2) Aktas ON, Ozturk AB, Erman B, Erus S, Tanju S, Dilege S. Role of natural killer cells in lung cancer. J Cancer Res Clin Oncol 2018; 144: 997-1003.
3) Ma J, Xu H, Wang S. Immunosuppressive role of myeloid-derived suppressor cells and therapeutic targeting in lung cancer. J Immunol Res 2018; 2018: 6319649.
4) Lu J, Zhan Y, Feng J, Luo J, Fan S. MicroRNAs associated with therapy of non-small cell lung cancer. Int J Biol Sci 2018; 14: 390-397.
5) Lin X, Liu L, Fu Y, Gao J, He Y, Wu Y, Lian X. Dietary cholesterol intake and risk of lung cancer: a meta-analysis. Nutrients 2018; 10: 185.
6) Wang LY, Cui JJ, Guo AX, Yin JY. Clinical efficacy and safety of afatinib in the treatment of non-small-cell lung cancer in Chinese patients. Onco Targets Ther 2018; 11: 529-538.
7) Liu X, Chen L, Liang T, Tian XD, Liu Y, Zhang T. Withaferin A induces mitochondrial-dependent apoptosis in non-small cell lung cancer cells via generation of reactive oxygen species. J BUON 2017; 22: 244-250.
8) Du L, Morgensztern D. Chemotherapy for advanced-stage non-small cell lung cancer. Cancer J 2015; 21: 366-370.
9) Su LD, Peng JM, Ge YB. Formyl peptide receptor 2 mediated chemotherapeutics drug resistance in colon cancer cells. Eur Rev Med Pharmacol Sci 2018; 22: 95-100.
10) Fathi Z, Syn NL, Zhou JG, Roudi R. Molecular epidemiology of lung cancer in Iran: implications for drug development and cancer prevention. J Hum Genet 2018; 63: 783-794.
11) Ultimo S, Martelli AM, Zauli G, Vitale M, Calin GA, Neri LM. Roles and clinical implications of microRNAs in acute lymphoblastic leukemia. J Cell Physiol 2018; 233: 5642-5654.
12) Ross CL, Kaushik S, Valdes-Rodriguez R, Anvekar R. MicroRNAs in cutaneous melanoma: role as diagnostic and prognostic biomarkers. J Cell Physiol 2018; 233: 5133-5141.
13) Mahdiannasser M, Karami Z. An innovative paradigm of methods in microRNAs detection: highlighting DNAzymes, the illuminators. Biosens Bioelectron 2018; 107: 123-144.
14) Sethi S, Sethi S, Bluth MH. Clinical implication of MicroRNAs in molecular pathology: an update for 2018. Clin Lab Med 2018; 38: 237-251.
15) Yuan H, Mischoulon D, Fava M, Otto MW. Circulating microRNAs as biomarkers for depression: many candidates, few finalists. J Affect Disord 2018; 233: 68-78.
16) Hayder H, O'Brien J, Nadeem U, Peng C. MicroRNAs: crucial regulators of placental development. Reproduction 2018; 155: R259-R271.
17) Kai K, Dittmar RL, Sen S. Secretory microRNAs as biomarkers of cancer. Semin Cell Dev Biol 2018; 78: 22-36.
18) Fujii T, Shimada K, Nakai T, Ohbayashi C. MicroRNAs in smoking-related carcinogenesis: biomarkers, functions, and therapy. J Clin Med 2018; 7: 98.
19) Yan J, Ying S, Cai X. MicroRNA-mediated regulation of HMGB1 in human hepatocellular carcinoma. Biomed Res Int 2018; 2018: 2754941.
20) He YH, Chen C, Shi Z. The biological roles and clinical implications of microRNAs in clear cell renal cell carcinoma. J Cell Physiol 2018; 233: 4458-4465.
21) Bahrami A, Aledavood A, Anvari K, Hassanian SM, Maftouh M, Yaghobzade A, Salarzaee O, ShahidSales S, Avan A. The prognostic and therapeutic appli-

cation of microRNAs in breast cancer: tissue and circulating microRNAs. J Cell Physiol 2018; 233: 774-786.

22) Rahmani F, Avan A, Hashemy SI, Hassanian SM. Role of Wnt/beta-catenin signaling regulatory microRNAs in the pathogenesis of colorectal cancer. J Cell Physiol 2018; 233: 811-817.

23) Aghabozorg AS, Ghaderian SM. The role of microRNAs in cardiovascular disease. Int J Mol Cell Med 2013; 2: 50-57.

24) Wang H, Peng R, Wang J, Qin Z, Xue L. Circulating microRNAs as potential cancer biomarkers: the advantage and disadvantage. Clin Epigenetics 2018; 10: 59.

25) Uddin A, Chakraborty S. Role of miRNAs in lung cancer. J Cell Physiol 2018; 9: 773-776.

26) Antony J, Huang RY. AXL-driven EMT state as a targetable conduit in cancer. Cancer Res 2017; 77: 3725-3732.

27) Zare M, Bastami M, Solali S, Alivand MR. Aberrant miRNA promoter methylation and EMT-involving miRNAs in breast cancer metastasis: diagnosis and therapeutic implications. J Cell Physiol 2018; 233: 3729-3744.

28) Matysiak M, Kapka-Skrzypczak L, Jodlowska-Jedrych B, Kruszewski M. EMT promoting transcription factors as prognostic markers in human breast cancer. Arch Gynecol Obstet 2017; 295: 817-825.

29) Chruscik A, Gopalan V, Lam AK. The clinical and biological roles of transforming growth factor beta in colon cancer stem cells: a systematic review. Eur J Cell Biol 2018; 97: 15-22.

30) Hamilton G, Rath B. Mesenchymal-epithelial transition and circulating tumor cells in small cell lung cancer. Adv Exp Med Biol 2017; 994: 229-245.

31) Cai Z, Cao Y, Luo Y, Hu H, Ling H. Signalling mechanism(s) of epithelial-mesenchymal transition and cancer stem cells in tumour therapeutic resistance. Clin Chim Acta 2018; 483: 156-163.

32) Laschet C, Dupuis N, Hanson J. The G protein-coupled receptors deorphanization landscape. Biochem Pharmacol 2018; 153: 62-74.

33) Kadowaki M, Venerando R, Miotto G, Mortimore GE. Mechanism of autophagy in permeabilized hepatocytes: evidence for regulation by GTP binding proteins. Adv Exp Med Biol 1996; 389: 113-119.

34) Ochiai S, Furuta D, Sugita K, Taniura H, Fujita N. GPR87 mediates lysophosphatidic acid-induced colony dispersal in A431 cells. Eur J Pharmacol 2013; 715: 15-20.

35) Pluznick JL. Extra sensory perception: the role of Gpr receptors in the kidney. Curr Opin Nephrol Hypertens 2014; 23: 507-512.

36) Nunn C, Mao H, Chidiac P, Albert PR. RGS17/RGSZ2 and the RZ/A family of regulators of G-protein signaling. Semin Cell Dev Biol 2006; 17: 390-399.