Tumor suppressive role of miR-569 in lung cancer

YI PING ZHENG[1], LINXIA WU[1], JIE GAO[2] and YANFU WANG[1]

Departments of [1]Geriatrics and [2]General Surgery, The First Affiliated Hospital of Dalian Medical College, Dalian, Liaoning 116000, P.R. China

Received May 7, 2016; Accepted June 2, 2017

DOI: 10.3892/ol.2018.7869

Abstract. microRNAs (miRs) are targets for genomic aberrations and emerging treatments against cancer. It has been demonstrated that targeting miR-569 may potentially benefit patients with ovarian or breast cancer. However, the exact roles of miR-569 remain unclear in human lung cancer cells. Using the reverse transcription-quantitative polymerase chain reaction (RT-qPCR), it was demonstrated that miR-569 expression was consistently decreased in lung cancer cells. As well as cell proliferation and migration inhibition, apoptosis and cell arrest at the G1 phase were induced following reversion of miR-569 expression in lung cancer cells. The present study demonstrated that miR-569 was able to downregulate FOS and high mobility group A2 mRNA and protein expression using RT-qPCR and western blot analysis. The observed role of miRNA-569 in lung cancer cells in the present study suggested that it may be a novel and promising therapeutic target, and a novel biomarker for detecting lung cancer.

Introduction

Lung cancer has remained the most common type of cancer since 1985, with 1.8 million novel cases reported in 2012 (1). Despite decades of research and prolific cancer preventive strategies, the 5-year survival rate for patients with lung cancer remains poor, and lung cancer remains the primary cause of cancer-associated mortality (2,3). Globally, 1.6 million individuals die from lung cancer annually, accounting for 20% of all cancer mortalities (4). The poor prognosis of lung cancer is primarily due to late clinical diagnosis, metastases, multiple drug resistance and comorbidity (5). Lung cancer consists of two common forms: Small cell lung cancer (SCLC; 20%) and non-small cell lung cancer (NSCLC; 80%). NSCLC can be further divided into adenocarcinoma (40-45%), squamous cell carcinoma (20-25%) and large cell carcinoma (10-15%) subtypes (6). The primary molecular cause of NSCLC is not fully understood. Therefore, there is an urgent requirement to clarify the pathogenesis of these tumors, and identify a reliable and effective treatment strategy.

Cancer research in the area of endogenous microRNAs (miRNAs/miRs) has markedly increased. miRNAs are endogenous small (~22 nucleotides) non-coding RNAs that are able to suppress gene expression or protein translation by interacting with the 3'-untranslated region (3'-UTR) of the target mRNAs. In addition, miRNAs are also able to promote the expression of target genes (7). Single miRNAs do not target a single mRNA and the majority of mRNAs may be modulated by numerous miRNAs; this results in complex regulation of gene expression (8). Therefore, miRNAs are able to participate in various fundamental biological mechanisms (9). It has been demonstrated that miRNAs serve a pivotal role in lung cancer, with specific roles of miRNAs as tumor suppressors (let-7, miR-126, -145, -200 and -34) and oncogenes (miR-17, -92, -21, -31, -221 and -222) identified (10). Although the number of verified human miRNAs has increased in recent years, few have been functionally described (11). Among numerous miRNAs, miR-569 has been revealed to be a novel cancer-associated miRNA which is increased partially owing to amplification of 3q26.2. A previous *in vivo* and *vitro* study demonstrated that miR-569 contributes to ovarian and breast cancer cell survival and proliferation (12). Thus, miR-569 may be a feasible biomarker and target for these types of cancer.

Genetic aberrations, including mutations and copy number aberrations (CNAs), are signs of oncogenesis. CNAs have been identified to be associated with the survival time of patients with lung cancer (13,14). Non-coding miRNAs may be affected by CNAs and serve as drivers in oncogenesis (15). Amplification of 3q26.2 is prevalent in lung cancer (16). Accordingly, investigation of the expression of miR-569 at 3q26.2 in lung cancer cell (LCC) and its functional roles as well as the underlying molecular mechanisms may possess clinical value.

The results of the present study demonstrated that miR-569 was significantly decreased in LCC. Additionally, functional experiments indicated that miR-569 may be able to regulate cell proliferation, apoptosis and migration in LCC. Furthermore, the present study identified that FOS and high mobility group A2 (HMGA2) were potential targets of miR-569. The data

Correspondence to: Professor Yanfu Wang, Department of Geriatrics, The First Affiliated Hospital of Dalian Medical College, 193 Lianhe Road, Dalian, Liaoning 116000, P.R. China
E-mail: 2926243984@qq.com

Key words: miRNA-569, lung cancer, tumor suppressor, c-FOS, high mobility group A2

from the present study expands the current understanding of the specific roles and the underlying molecular mechanisms of miR-569 in LCC.

Materials and methods

Cell culture. The human lung cancer cell lines A549, H1299, HCC827 and 95D, and the normal human bronchial epithelial cell line HBE, were acquired from the Regenerative Medicine Center of The First Affiliated Hospital of Dalian Medical University (Dalian, China). All cell lines used were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with penicillin (100 U/ml), streptomycin (100 mg/ml) (Beijing Solarbio Science and Technology Co., Ltd., Beijing, China) and 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.). All cells were incubated at 37˚C in a humidified atmosphere containing 5% CO_2.

Target identification. Potential targets for miR-569 were identified using miRanda (www.microrna.org) and TargetScan (www.targetscan.org/vert_71).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. Total RNA was extracted from the cells using RNAiso Plus reagent (Takara Bio, Inc., Otsu, Japan), according to the manufacturer's protocol. The purity and concentration of RNA was measured using a NanoDrop 2000 instrument (Thermo Fisher Scientific, Inc.). The RNA was transcribed into cDNA using a microRNA Stem-Loop Reverse Transcription kit (GenePharma, Shanghai, China), according to the manufacturer's protocol. To evaluate c-FOS and HMGA2 expression, corresponding RNA was reverse-transcribed into cDNA using the PrimeScript™ RT Reagent kit with genomic DNA Eraser (Takara Bio, Inc.) 48 h after transfection in A549 cells. qPCR was performed with a TransStart Top Green qPCR SuperMix kit (TransGene Biotech Co., Ltd, Beijing, China) using the StepOne Plus system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The thermocycling settings were as follows: 30 sec at 94˚C, then 40 cycles of 5 sec at 94˚C and 30 sec at 60˚C. Small nuclear RNA(U6) was used as an internal marker of miRNA. For the analysis of c-FOS and HMGA2 expression, β-actin was used for normalization. All reactions were performed in triplicate. The $2^{-\Delta\Delta Cq}$ method was used for relative quantification of gene expression (17). The primers used are listed in Table I.

Transfection. The miR-569 mimic (5'-AGUUAAUGAAUC CUGGAAAGU-3', 5'-UUUCCAGGAUUCAUUAACUUU-3') and corresponding negative control (miR-NC; 5'-CAGUAC UUUUGUGUAGUACAA-3', 5'-ACGUGACACGUUCGG AGAATT-3') were synthesized by GenePharma. A549 cells were transfected with a final oligonucleotide concentration of 50 nM using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Transfection efficiency was observed using an inverted fluorescence microscope (Olympus Corporation, Tokyo, Japan) and flow cytometry (BD Biosciences, Franklin Lakes, NJ, USA) 6 h after transfection with a fluorescein miR negative control (miR-FAM-NC). To further measure transfection efficiency, RT-qPCR was performed on each experimental sample 48 h after transfection.

Cell proliferation. A total of 8,000 cells were cultured per well in 96-well plates prior to the addition of the miR-569 mimic or miR-NC into the well 1 day after seeding. Then a Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc., Kumamoto, Japan) was used to measure cell proliferation at 24, 48 and 72 h after transfection, and the absorbance of each well at 450 nm was determined using a microplate reader (BioTek Instruments, Inc., Winooski, VT, USA). A total of 5 replicates were used for each group in one experiment.

Cell cycle distribution and analysis of cellular apoptosis. Cell cycle distribution and apoptosis were examined using flow cytometry 48 h post-transfection. For the cell cycle assay, $1x10^6$ transfected cells were fixed in 75% pre-cooled ethanol overnight, rinsed twice with PBS and stained with propyl iodide dye solution (Beyotime Institute of Biotechnology, Haimen, China) at room temperature for 30 min in the dark prior to analysis by flow cytometry (BD Biosciences).

To analyze the effects of miR-569 on cell apoptosis, the transfected cells were trypsinized, centrifuged at 179 x g for 5 min at 4˚C and washed with ice-cold PBS, prior to being suspended in 500 μl matched binding buffer containing 5 μl annexin V-fluorescein isothiocyanate (FITC) and 5 μl propidium iodide (PI) from an Annexin V-FITC Apoptosis Detection kit (Vazyme, Piscataway, NJ, USA). Cells were incubated at room temperature for 15 min in the dark prior to analysis by flow cytometry using the Apoptosis Detection kit according to the manufacturer's protocol.

Wound-healing assay. Straight uniform lines were marked on the back of the 6-well plates with a ruler. When the transfected cells reached ~80% confluence, the unilaminar cells were scratched across each well using 10-μl pipette tips in order to evaluate cell migration by observing the ability of the cell to migrate into the wounded area. The wounds were recorded with images captured at0 and 24 h; thereafter, the scratch region was examined using ImageJ software version 1.38 (National Institutes of Health, Bethesda, MD, USA).

Western blot analysis. Transfected cells were lysed in radioimmunoprecipitation assay buffer (Beijing Solarbio Science and Technology Co., Ltd.). The whole cell protein concentration was determined using a bicinchoninic acid protein assay kit (Beijing Solarbio Science and Technology Co., Ltd.), according to the manufacturer's protocol. A total of 80 μg protein was loaded per lane for 12% SDS-PAGE. Separated proteins were electrotransferred onto polyvinylidene difluoride membranes and membranes were blocked with 5% skimmed milk powder in Tris Buffered Saline-Tween 20 for 1 h at room temperature. The membranes were sealed in micro-plastic bags containing polyclonal rabbit anti-human c-FOS (cat. no. BM4864; dilution, 1:200; Wuhan Boster Biological Technology, Ltd., Wuhan, China), HMGA2 (cat. no. 20795-1-AP; dilution, 1:1,000; Wuhan Sanying Biotechnology, Wuhan, China) or β-actin (cat. no. bs-0061R; dilution, 1:1,500; BIOSS, Beijing, China)

Table I. Reverse transcription-polymerase chain reaction primer sequences.

Name	Primer direction	Sequence (5'-3')
hsa-miR-569	F	AGACTGCTGAGTTAATGAATCCTG
	R	TATGGTTGTTCACGACTCCTTC
U6	F	CTCGCTTCGGCAGCACATATACT
	R	ACGCTTCACGAATTTGCGTGTC
High mobility group A2	F	CCAGGAAGCAGCAGCAAGA
	R	CCAGGCAAGGCAACATTGAC
c-FOS	F	TACTACCACTCACCCGCAGAC
	R	GAATGAAGTTGGCACTGGAGA
β-actin	F	ATCATGTTTGAGACCTTCAACA
	R	CATCTCTTGCTCGAAGTCCA

F, forward; R, reverse; hsa, human (*Homo sapiens*); miR, microRNA.

primary antibody. Following incubation with the primary antibody overnight at 4˚C, the blots were then incubated with the corresponding horseradish peroxidase-labeled goat anti-rabbit secondary antibody (cat. no. bs-0295G-HRP; dilution, 1:5,000; Wuhan Boster Biological Technology) at room temperature for 1 h. Protein bands were visualized using enhanced chemiluminescence (Beijing Solarbio Science and Technology Co., Ltd.) with FluorChemFC3 gel imaging software (version 3.4; Protein Simple; Bio-Teche, Minneapolis, MN, USA).

Statistical analysis. At least three repeats were performed for all experiments. The data are presented as the mean ± standard deviation. Statistical difference was determined by analysis of variance followed by Fisher's least significant difference or Student-Newman-Keuls post hoc test, or two-tailed Student's t-test using SPSS (version 21.0; IBM Corp., Armonk, NY, USA). P<0.05 was considered to indicate a statistically significant difference.

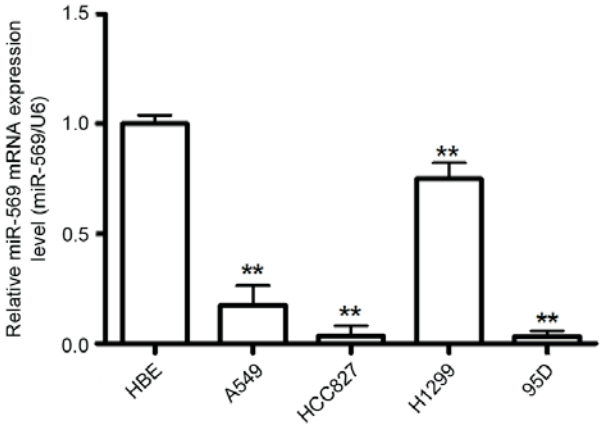

Figure 1. miR-569 expression is downregulated in four lung cancer cell lines compared with the normal human bronchial epithelial cell line HBE analyzed by reverse transcription-quantitative polymerase chain reaction. **P<0.05 vs. HBE cells. miR-569, microRNA-569.

Results

miR-569 is significantly decreased in human LCC. To investigate the potential role of miR-569 in LCC carcinogenesis, the present study examined miR-569 expression in a group of human LCC using RT-qPCR. The results demonstrated that miR-569 expression level was downregulated in the LCC compared with the normal cell line HBE (P<0.05; Fig. 1).

Ectopic overexpression of miR-569 is associated with adverse effects on LCC. To explore the cellular functions of miR-569 in LCC, first A549 cells were transfected with the fluorescently labelled negative control (miR-FAM-NC), which exhibited high transfection efficiency revealed using an inverted fluorescence microscope and flow cytometry. Additionally, RT-qPCR reflected the successful endogenic overexpression of miR-569: It was markedly increased following transfection with the miR-569 mimic compared with the miR-NC (P<0.05; Fig. 2A). CCK-8 and flow cytometry demonstrated that overexpression of miR-569 markedly prevented LCC proliferation (P<0.05; Fig. 2B) and induced cell apoptosis (P<0.05; Fig. 2C). In addition, as suggested by the flow cytometry, overexpression of miR-569 markedly induced G1 phase cell cycle arrest in lung adenocarcinoma A549 cells (P<0.05; Fig. 2D). A wound-healing assay was performed which revealed that overexpression of miR-569 significantly suppressed migration (P<0.05; Fig. 2E) in A549 cells.

miR-569 inhibits c-FOS and HMGA2 expression. miRanda and TargetScan identified that miR-569 may be associated with c-FOS and HMGA2 mRNA 3'-UTR binding sites. In contrast with the upregulated expression of c-FOS and HMGA2 identified in previous studies (18-21), it was observed in the present study that miR-569 expression was decreased in LCC. This suggested that c-FOS and HMGA2 were potential targets of miR-569. This was investigated in the present study by detecting endogenous c-FOS and HMGA2 expression of the miR-569 mimic-transfected cells in comparison with miR-NC-transfected cells. It was revealed that the miR-569 mimic markedly decreased the expression levels of HMGA2 and c-FOS mRNA (P<0.05; Fig. 3A and B) and protein

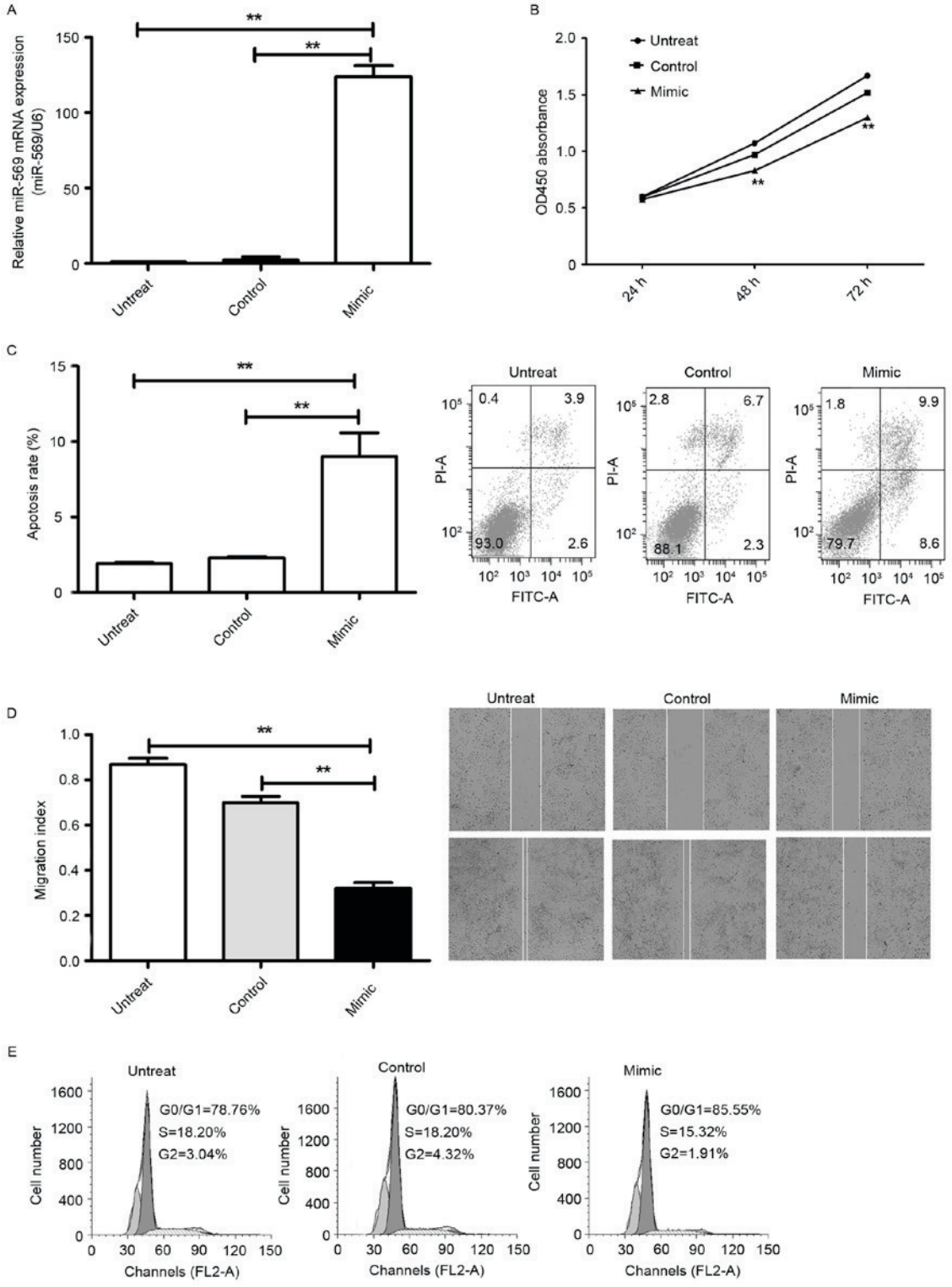

Figure 2. Ectopic overexpression of miR-569 inhibits cellular proliferation and invasion, induces cell cycle arrest at G0/G1 phase and apoptosis in A549 cells transfected with miR-569 mimic compared with miR-NC or untreated cells. (A) The reverse transcription-quantitative polymerase chain reaction revealed that miR-569 expression was significantly increased following transfection with the miR-569 mimic. Results were normalized to U6 snRNA. (B) The Cell Counting kit-8 assay analysis revealed that cell proliferation was suppressed in A549 cells transfected with the miR-569 mimic. (C) Flow cytometry revealed cell apoptosis was induced in A549 cells transfected with miR-569 mimic. (D) The wound-healing assay revealed that cell migration was suppressed in A549 cells transfected with miR-569 mimic. (E) Flow cytometry identified that the cell cycle was arrested at G0/G1. **P<0.05 vs. miR-NC or untreated. miR-NC, miR-569 negative control; miR, microRNA; NC, negative control; FITC, fluorescein isothiocyanate; PI, propidium iodide; untreat, untreated; OD, optical density.

(P<0.05; Fig. 3C). Taken together, the results of the present study suggest that c-FOS and HMGA2 are potential miR-569 targets in A549 cells.

Discussion

Previous studies have demonstrated that miRNAs may serve

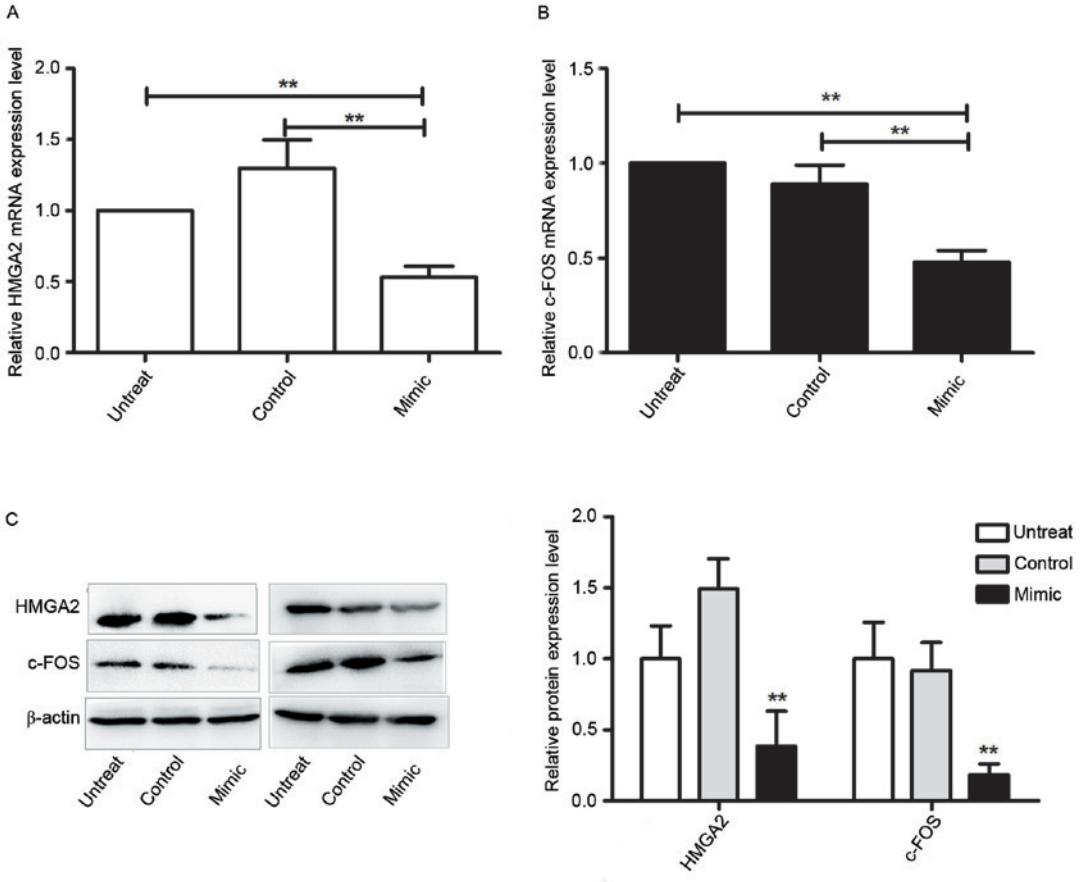

Figure 3. miR-569 inhibits HMGA2 and c-FOS expression in A549 cells. (A) HMGA2 mRNA expression was downregulated following transfection with the miR-569 mimic. β-actin served as the internal control. (B) c-FOS mRNA expression was downregulated following transfection with the miR-569 mimic, revealed using the reverse transcription-quantitative polymerase chain reaction. β-actin served as the internal control. (C) HMGA2 and c-FOS protein expression was suppressed following transfection with miR-569 mimic, revealed using western blot analysis. **P<0.05 vs. miR-NC or untreated. HMGA2, high mobility group A2; untreat, untreated; miR, microRNA.

key roles in cancer either as onco-miRNAs or tumor suppressive miRNAs in various types of cancer (22-24), including lung cancer (10). Despite this, the underlying molecular mechanisms between miRNAs in cancer remain somewhat unknown. A novel family of small single-stranded non-coding RNAs, miRNAs be affected by CNAs (25). Additionally, changes in DNA copy number, particularly at the 3q26.2 amplicon, have been identified as predictors of lung cancer (26). Thus, characterization of miR-569 associated with CNAs may expand current knowledge of the etiology underlying lung cancer, and provide new insights into molecular markers and targets for lung cancer therapy.

HMGA2 is a crucial regulator of tumorigenesis and embryogenesis (27). As a transcription factor, HMGA2 is usually upregulated and serves important roles in various biological processes of numerous malignant tumors including LCC (28-30). The 3'-UTR of HMGA2 is >3 kb, allowing it to bind to numerous miRNAs. It has previously been demonstrated that the let-7 miRNA family may prevent early lung cancer progression by specifically inhibit HMGA2 expression (31). In addition, other miRNAs, including miR-26 a and miR-98, are able to regulate HMGA2 expression to modulate cisplatin resistance of human NSCLC (32,33). c-FOS is the human homolog of the retroviral oncogene v-FOS (34). In combination with their Jun partners, FOS forms the activator protein 1 transcription factor complex protein, which has a leucine-zipper region to bind DNA, giving rise to changes of gene expression (35). As a proto-oncogene, c-FOS regulates diverse cellular functions in a variety of types of cancer including LCC (36,21).

To the best of our knowledge, the results of the present study demonstrate for the first time that miR-569 expression levels are markedly downregulated in LCC cells. Of note, miR-569 overexpression prevented cell proliferation and migration, and inversely induced cell apoptosis. In addition, upregulation of miR-569 inhibited HMGA2 and c-FOS mRNA and protein expression. Accordingly, the results of the present study demonstrated that miR-569 functions as a tumor suppressor in LCC, at least in part due to targeting HMGA2 and c-FOS, and contributed to the progression and metastasis of LCC.

To the best of our knowledge, the present study revealed for the first time that miR-569 functioned as a potential tumor suppressor gene by targeting HMGA2 and c-FOS in lung cancer. Which may provide a new breakthrough to explore the pathogenesis of lung cancer. However, the present study lacked tissue sample and *in vivo* murine experiments to provide further evidence of the role miR-569 serves in LCC. Therefore, further in-depth studies are required to support and expand the results from the present study.

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F: Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 136: E359-E386, 2015.
2. Wink KC, Roelofs E, Solberg T, Lin L, Simone CB II, Jakobi A, Richter C, Lambin P and Troost EG: Particle therapy for non-small cell lung tumors: Where do we stand? A systematic review of the literature. Front Oncol 4: 292, 2014.
3. Bray F, Jemal A, Grey N, Ferlay J and Forman D: Global cancer transitions according to the Human Development Index (2008-2030): A population-based study. Lancet Oncol 13: 790-801, 2012.
4. Siegel R, Naishadham D and Jemal A: Cancer statistics, 2013. CA Cancer J Clin 63: 11-30, 2013.
5. Grose D, Morrison DS, Devereux G, Jones R, Sharma D, Selby C, Docherty K, McIntosh D, Nicolson M, McMillan DC, *et al*: The impact of comorbidity upon determinants of outcome in patients with lung cancer. Lung Cancer 87: 186-192, 2015.
6. Mehta A, Dobersch S, Romero-Olmedo AJ and Barreto G: Epigenetics in lung cancer diagnosis and therapy. Cancer Metastasis Rev 34: 229-241, 2015.
7. Vasudevan S, Tong Y and Steitz JA: Switching from repression to activation: microRNAs can up-regulate translation. Science 318: 1931-1934, 2007.
8. van de Vrie M, Heymans S and Schroen B: MicroRNA involvement in immune activation during heart failure. Cardiovasc Drugs Ther 25: 161-170, 2011.
9. Garzon R, Marcucci G and Croce CM: Targeting microRNAs in cancer: Rationale, strategies and challenges. Nat Rev Drug Discov 9: 775-789, 2010.
10. Joshi P, Middleton J, Jeon YJ and Garofalo M: MicroRNAs in lung cancer. World J Methodol 4: 59-72, 2014.
11. Li F, Liu J and Li S: MicroRNA 106b ~25 cluster and gastric cancer. Surg Oncol 22: e7-10, 2013.
12. Chaluvally-Raghavan P, Zhang F, Pradeep S, Hamilton MP, Zhao X, Rupaimoole R, Moss T, Lu Y, Yu S, Pecot CV, *et al*: Copy number gain of hsa-miR-569 at 3q26.2 leads to loss of TP53INP1 and aggressiveness of epithelial cancers. Cancer Cell 26: 863-879, 2014.
13. Kawano O, Sasaki H, Okuda K, Yukiue H, Yokoyama T, Yano M and Fujii Y: PIK3CA gene amplification in Japanese non-small cell lung cancer. Lung Cancer 58: 159-160, 2007.
14. Go H, Jeon YK, Park HJ, Sung SW, Seo JW and Chung DH: High MET gene copy number leads to shorter survival in patients with non-small cell lung cancer. J Thorac Oncol 5: 305-313, 2010.
15. Esquela-Kerscher A and Slack FJ: Oncomirs-microRNAs with a role in cancer. Nat Rev Cancer 6: 259-269, 2006.
16. Qian J and Massion PP: Role of Chromosome 3q amplification in lung cancer. J Thorac Oncol 3: 212-215, 2008.
17. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
18. Di Cello F, Hillion J, Hristov A, Wood LJ, Mukherjee M, Schuldenfrei A, Kowalski J, Bhattacharya R, Ashfaq R and Resar LM: HMGA2 participates in transformation in human lung cancer. Mol Cancer Res 6: 743-750, 2008.
19. Wu Y, Song Y and Liu H: Expression and its clinical significance of HMGA2 in the patients with non-small cell lung cancer. Zhongguo Fei Ai Za Zhi 11: 377-381, 2008 (In Chinese).
20. Meyer B, Loeschke S, Schultze A, Weigel T, Sandkamp M, Goldmann T, Vollmer E and Bullerdiek J: HMGA2 overexpression in non-small cell lung cancer. Mol Carcinog 46: 503-511, 2007.
21. Volm M, Drings P and Wodrich W: Prognostic significance of the expression of c-fos, c-jun and c-erbB-1 oncogene products in human squamous cell lung carcinomas. J Cancer Res Clin Oncol 119: 507-510, 1993.
22. Gartel AL and Kandel ES: miRNAs: Little known mediators of oncogenesis. Semin Cancer Biol 18: 103-110, 2008.
23. Farazi TA, Spitzer JI, Morozov P and Tuschl T: miRNAs in human cancer. J Pathol 223: 102-115, 2011.
24. Garzon R and Marcucci G: Potential of microRNAs for cancer diagnostics, prognostication and therapy. Curr Opin Oncol 24: 655-659, 2012.
25. Holland AJ and Cleveland DW: Boveri revisited: Chromosomal instability, aneuploidy and tumorigenesis. Nat Rev Mol Cell Biol 10: 478-487, 2009.
26. Yang Z, Zhuan B, Yan Y, Jiang S and Wang T: Integrated analyses of copy number variations and gene differential expression in lung squamous-cell carcinoma. Biol Res 48: 47, 2015.
27. Wu J and Wei JJ: HMGA2 and high-grade serous ovarian carcinoma. J Mol Med (Berl) 91: 1155-1165, 2013.
28. Di Cello F, Hillion J, Hristov A, Wood LJ, Mukherjee M, Schuldenfrei A, Kowalski J, Bhattacharya R, Ashfaq R and Resar LM: HMGA2 participates in transformation in human lung cancer. Mol Cancer Res 6: 743-750, 2008.
29. Wu Y, Song Y and Liu H: Expression and its clinical significance of HMGA2 in the patients with non-small cell lung cancer. Zhongguo Fei Ai Za Zhi 11: 377-381, 2008 (In Chinese).
30. Meyer B, Loeschke S, Schultze A, Weigel T, Sandkamp M, Goldmann T, Vollmer E and Bullerdiek J: HMGA2 overexpression in non-small cell lung cancer. Mol Carcinog 46: 503-511, 2007.
31. Park SM, Shell S, Radjabi AR, Schickel R, Feig C, Boyerinas B, Dinulescu DM, Lengyel E and Peter ME: Let-7 prevents early cancer progression by suppressing expression of the embryonic gene HMGA2. Cell Cycle 6: 2585-2590, 2007.
32. Yang Y, Zhang P, Zhao Y, Yang J, Jiang G and Fan J: Decreased MicroRNA-26a expression causes cisplatin resistance in human non-small cell lung cancer. Cancer Biol Ther 17: 515-525, 2016.
33. Xiang Q, Tang H, Yu J, Yin J, Yang X and Lei X: MicroRNA-98 sensitizes cisplatin-resistant human lung adenocarcinoma cells by up-regulation of HMGA2. Pharmazie 68: 274-281, 2013.
34. Milde-Langosch K: The fos family of transcription factors and their role in tumourigenesis. Eur J Cancer 41: 2449-2461, 2005.
35. Chiu R, Boyle WJ, Meek J, Smeal T, Hunter T and Karin M: The c-Fos protein interacts with c-Jun/AP-1 to stimulate transcription of AP-1 responsive genes. Cell 54: 541-552, 1988.
36. Taniguchi S, Kawano T, Mitsudomi T, Kimura G and Baba T: Fos oncogene transfer to a transformed rat fibroblast cell line enhances spontaneous lung metastasis in rat. Jpn J Cancer Res 77: 1193-1197, 1986.