Ultrasound-targeted microbubble destruction enhances the anti-tumor action of miR-4284 inhibitor in non-small cell lung cancer cells

PENG TIAN[1], YANZHEN WANG[1] and WENYAN DU[2]

Departments of [1]Ultrasonics and [2]Science and Education, Zibo Central Hospital, Zibo, Shandong 255036, P.R. China

Received October 13, 2020; Accepted February 22, 2021

DOI: 10.3892/etm.2021.9983

Abstract. MicroRNAs (miRNAs/miRs) are known to be involved in various human cancer types. Ultrasound-targeted microbubble destruction (UTMD) may improve the transfection efficiency of exogenous genes into target tissues and organs, thereby improving cancer treatment. In the present study, the role of miR-4284 in non-small cell lung cancer (NSCLC) was investigated and the effect of UTMD-mediated inhibition of miR-4284 on tumor progression was further analyzed. The expression of miR-4284 in NSCLC cells and tissues was detected by reverse transcription-quantitative PCR. UTMD-mediated inhibition of miR-4284 was achieved by co-transfection of microvesicles and miR-4284 inhibitors into NSCLC cells. A Cell Counting Kit-8 assay was used to determine NSCLC cell proliferation, and the migration and invasion of NSCLC cells were examined by Transwell assays. Compared with that in the control group, the expression of miR-4284 was increased in NSCLC tissues and cells. Knockdown of miR-4284 in NSCLC cells inhibited cell proliferation, migration and invasion. UTMD improved the transfection efficiency of miR-4284 inhibitors in NSCLC cells, resulting in more significant inhibition of tumor cell proliferation, migration and invasion. In conclusion, the results indicated that the expression of miR-4284 was increased in clinical samples and cell lines of NSCLC and that knockdown of miR-4284 inhibited the proliferation, migration and invasion of tumor cells. UTMD-mediated miR-4284 inhibition further promoted this effect, indicating that this technique may represent a novel strategy for the treatment of NSCLC.

Correspondence to: Dr Wenyan Du, Department of Science and Education, Zibo Central Hospital, 54 Gongqingtuan West Road, Zhangdian, Zibo, Shandong 255036, P.R. China
E-mail: wenyan_du0928@163.com

Key words: ultrasound-targeted microbubble destruction, microRNA-4284, non-small cell lung cancer, proliferation, migration, invasion

Introduction

Despite the recent decline in the incidence and death rate, lung cancer still remains the leading cause of death from cancer in the US, accounting for ~27% of all cancer-related deaths (1). Only 17.7% of all patients with lung cancer are alive ≥5 years after diagnosis (2). Non-small cell lung cancer (NSCLC) is the most common subtype of lung cancer, with a higher incidence in developed countries (3). NSCLC accounts for >80% of all lung cancer cases (4). Thus, there is an urgent requirement for more accurate detection methods and more effective treatment options to address the high mortality rate of NSCLC. Ultrasound-targeted microbubble destruction (UTMD) was recently developed for the local release of drugs and genes (5). During UTMD, the gene is integrated into a microvesicle to induce its release upon reaching the target region, and the microbubble can promote the appearance of irreversible pores on the target cell membrane contributing to gene transfer to the nucleus, thereby enhancing the expression and transfection of the target gene (5,6). After the microvesicle enters the target tissues, ultrasound breaks the microbubbles, and then the drugs or genes that are carried by the microbubbles are directionally released for therapeutic purposes, leading to a therapeutic effect in the target tissues (7). Therefore, UTMD has been considered a promising mediator for target therapy in human malignancies.

MicroRNAs (miRNAs/miRs) are non-coding RNAs with a length of 22-26 nucleotides (8). Accumulating evidence has indicated that altered expression of miRNAs is crucial for carcinogenesis and miRNAs may either have tumor suppressor or oncogenic functions (9). In recent decades, the functional roles of miRNAs in tumor progression have been determined in different types of human cancer, which has increased the attention of researchers to the aberrant expression of miRNAs in tumor samples (10). miR-4284 is differentially expressed in several diseases, including cancer (11,12). Gene chip analysis has indicated that miR-4284 was associated with disease recurrence in lung adenocarcinoma (13). However, the biological roles of miRNA-4284 in NSCLC have rarely been investigated.

The present study aimed to evaluate the expression of miR-4284 in NSCLC tissues and cell lines and further explored the biological functions of miR-4284 in NSCLC progression. Several studies have reported on the enhancing effects of

UTMD on the functional role of miRNAs in disease progression, UTMD-mediated miR-767 inhibition has been indicated to result in a notable suppressive effect of tumor cell proliferation, migration and invasion, and UTMD has also been revealed to assist the exosome delivery of miR-21, serving a protective role in the heart (14,15). Therefore, the present study further compared the differences in biological functional changes between UTMD-mediated and conventional transfection of miR-4284 in NSCLC cells. The results may provide a theoretical basis for UTMD-mediated targeted therapy of miRNAs in NSCLC.

Materials and methods

NSCLC tissue collection. Between May 2017 and May 2019, a total of 65 patients with NSCLC underwent surgical tumor resection at Zibo Central Hospital (Shandong, China) and were diagnosed by histopathological examination. Cancer tissues and matched non-cancerous tissues were collected from each patient and all tissues were frozen in liquid nitrogen and stored at -80˚C for further use. Patients who received preoperative treatment were excluded from the study. The protocols for tissue collection and analysis were approved by the Ethics Committee of Zibo Central Hospital (Shandong, China; approval no. 170239) and written informed consent was provided by the patients prior to sampling.

Cell culture and conventional cell transfection. NSCLC cell lines, including SK-MES-1, A549, NCI-H460 (RRID: CVCL_0459) and H522, and the normal lung cell line NHBE were obtained from the Cell Bank of the Chinese Academy of Sciences. All cells were maintained in Dulbecco's modified Eagle's medium (Invitrogen; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (FBS; Invitrogen; Thermo Fisher Scientific, Inc.) in a humidified atmosphere with 5% CO_2 at 37˚C.

In this study, cell transfection was used to achieve the regulation of miR-4284 *in vitro*. The cell lines A549 and H460 were selected to perform the transfection experiments due to significantly higher expression of miR-4284 in these two cell lines compared with that in the normal cells. Inhibitor negative control (NC) and miR-4284 inhibitor were synthesized from Gene Pharmaceuticals and transfected into A549 and H460 cells using Lipofectamine 3000 (Invitrogen; Thermo Fisher Scientific, Inc.). Cells treated with transfection reagents alone were set as a mock group. After 48 h of transfection, the cells were used for further analysis.

Preparation of microbubbles and cell transfection. The method of obtaining microbubbles was according to the protocol of a previous study (16). It was performed by sonication of 0.4 mg/ml 1,2-distearoyl-3-trimethylammoniumpropane (Avanti Polar Lipids, Inc.) with 1 mg/ml polyethyleneglycol-2000 stearate (Avanti Polar Lipids, Inc.), 2 mg/ml distearoylphosphatidylcholine (Avanti Polar Lipids, Inc.) and perfluoropropane gas. miR-4284 inhibitor (5'-AUGGGGUAUGUGAGCCC-3') or non-targeting inhibitor-NC (5'-CAGUACUUUUGUGUAGUACAA-3') was incubated with the microbubbles for 30 min at 37˚C. According to the manufacturer's protocol, the mixtures were added to A549 and H460 cells and transfected with Lipofectamine 3000 (Invitrogen; Thermo Fisher Scientific, Inc.).

Table I. Demographics and clinical characteristics of patients with NSCLC.

Characteristics	Patients with NSCLC (n=65)
Age, years	59.8±13.9
Tumor size, cm	3.9±1.7
Sex	
Male	41
Female	24
Smoking status	
Never	22
Current/ever	43
Differentiation	
Well/moderate	39
Poor	26
TNM stage	
I-II	37
III-IV	28

Data are presented as the mean ± SD or count. TNM, tumor-node-metastasis; NSCLC, non-small cell lung cancer.

RNA extraction and reverse transcription-quantitative PCR. Total RNA was isolated from fresh tissue samples and cells using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). RNA was reversed transcribed into single-stranded complementary DNA with the PrimeScript reverse transcriptase kit (Takara Bio, Inc.), according to the manufacturer's protocol. The expression levels of miR-4284 were determined by quantitative PCR with the SYBR-Green I Master Mix kit (Invitrogen; Thermo Fisher Scientific, Inc.) on a 7500 real-time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The following thermocycling conditions were used for the qPCR: Initial denaturation at 95˚C for 10 min; followed by 40 cycles at 95˚C for 20 sec, 60˚C for 15 sec and 72˚C for 20 sec. U6 was used as an endogenous control for miR-4284. The final expression value was calculated using the $2^{-\Delta\Delta Cq}$ method (17). The sequences of primers used were as follows: miR-4284 forward, 5'-GCCGAGGGGCTCACATCACCCCAT-3' and reverse, 5'-CTCAACTGGTGTCGTGGA-3'; U6 forward, 5'-CTCGCTTCGGCAGCACA-3' and reverse, 5'-AACGCTTCACGAATTTGCGT-3'.

Cell Counting Kit 8 (CCK-8) assay. After cell transfection, the effect of miR-4284 on the proliferation of A549 and H460 cells was detected using a CCK-8 assay. Cells were seeded into a 96-well plate ($3x10^3$ cells/well) and incubated for 0, 24, 48 or 72 h. Subsequently, 10 μl of CCK-8 reagent (Beyotime Institute of Biotechnology) was added to each well and the plates were further incubated for 2 h. Cell proliferation was quantified by determining the optical density at 450 nm using a microplate reader.

Transwell assay. Transwell chambers (Corning, Inc.) were applied in the present study for the measurement of cell

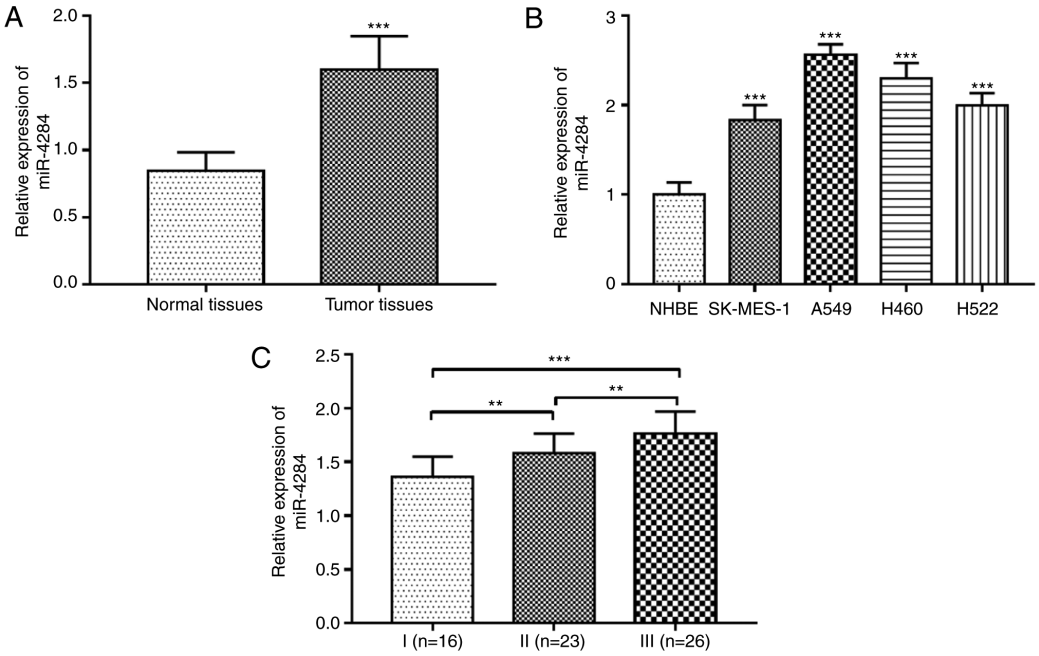

Figure 1. Expression of miR-4284 in NSCLC tissues and cell lines. (A) The expression of miR-4284 was increased in NSCLC vs. normal tissues. ***P<0.001 vs. normal tissues. (B) The expression level of miR-4284 was increased in four NSCLC cell lines compared with that in a normal lung cell line. ***P<0.001 vs. NHBE. (C) The expression level of miR-4284 exhibited differences among patients with different TNM stages: The level of miR-4284 in patients with stage II was higher than that in patients with stage I and the level in patients with stage III was higher than that in patients with stage II. **P<0.01, ***P<0.001. NSCLC, non-small cell lung cancer; miR, microRNA.

migration and invasion of NSCLC cells. Transwell chambers precoated with Matrigel (Corning, Inc.) were used for the invasion assay, while chambers without Matrigel coating were used for migration assay. The transfected cells were seeded into the upper chambers with serum-free medium at a density of $3x10^5$ cells/chamber, while the lower chambers were filled with culture medium supplemented with 10% FBS as a chemoattractant. The cells that had transgressed through the filter/membrane to the lower chambers were stained after 48 h of incubation and were counted under an inverted microscope (Olympus Corp.).

Statistical analysis. Values are expressed as the mean ± standard deviation and were analyzed in SPSS 21.0 (IBM Corp.) and GraphPad Prism 7.0 (GraphPad Software, Inc.). A paired Student's t-test was used to compare the differences between two groups and one-way ANOVA followed by Tukey's test was used for differences between multiple groups. P<0.05 was considered to indicate a statistically significant difference.

Results

Expression of miR-4284 in NSCLC tissues and cell lines. The expression level of miR-4284 was evaluated in 65 patients with NSCLC, including 41 males and 24 females with an average age of 59.8±13.9 years (Table I). The results indicated that the expression levels of miR-4284 in NSCLC tissues were significantly upregulated compared with those in matched normal tissues (P<0.001; Fig. 1A). Furthermore, compared with that in the normal NHBE cell line, miR-4284 expression levels were also observed to be increased in four NSCLC cell lines (SK-MES-1, A549, H460 and H522) (all P<0.001; Fig. 1B). In addition, the expression levels of miRNA-4284 were different among patients with different stages of TNM: The level in patients with stage II was higher than that in patients with stage I, it was higher in patients with stage III than that in patients with stage II and thus, it was the highest in patients with stage III (all P<0.01; Fig. 1C).

miR-4284 inhibition suppresses cell proliferation, migration and invasion of NSCLC cells. Next, cell experiments were performed to determine the role of miR-4284 in NSCLC. The cell lines A549 and H460 were subjected to cell transfection, as they had significantly higher miR-4284 expression compared with that in the normal cell line NHBE, and among the four cells lines (SK-MES-1, A549, H460 and H522) the expression level of miR-4284 was higher in A549 and H460 cell lines. After transfection with inhibitor, the expression levels of miR-4284 in the A549 and H460 cell lines decreased compared with those in the corresponding negative controls (all P<0.01; Fig. 2A). A cell proliferation assay indicated that decreased expression of miR4284 inhibited cell proliferation (all P<0.05; Fig. 2B). In addition to cell proliferation, the regulatory effects of miR-4284 on the migration and invasion of the A549 and H460 cell lines were further analyzed, revealing that knockdown of miR-4284 inhibited cell migration and invasion (all P<0.05; Fig. 2C and D)

UTMD enhances the cell transfection efficiency of miR-4284. UTMD is able to improve the transfection efficiency of foreign genes into target tissues and organs (6). The effect of UTMD on

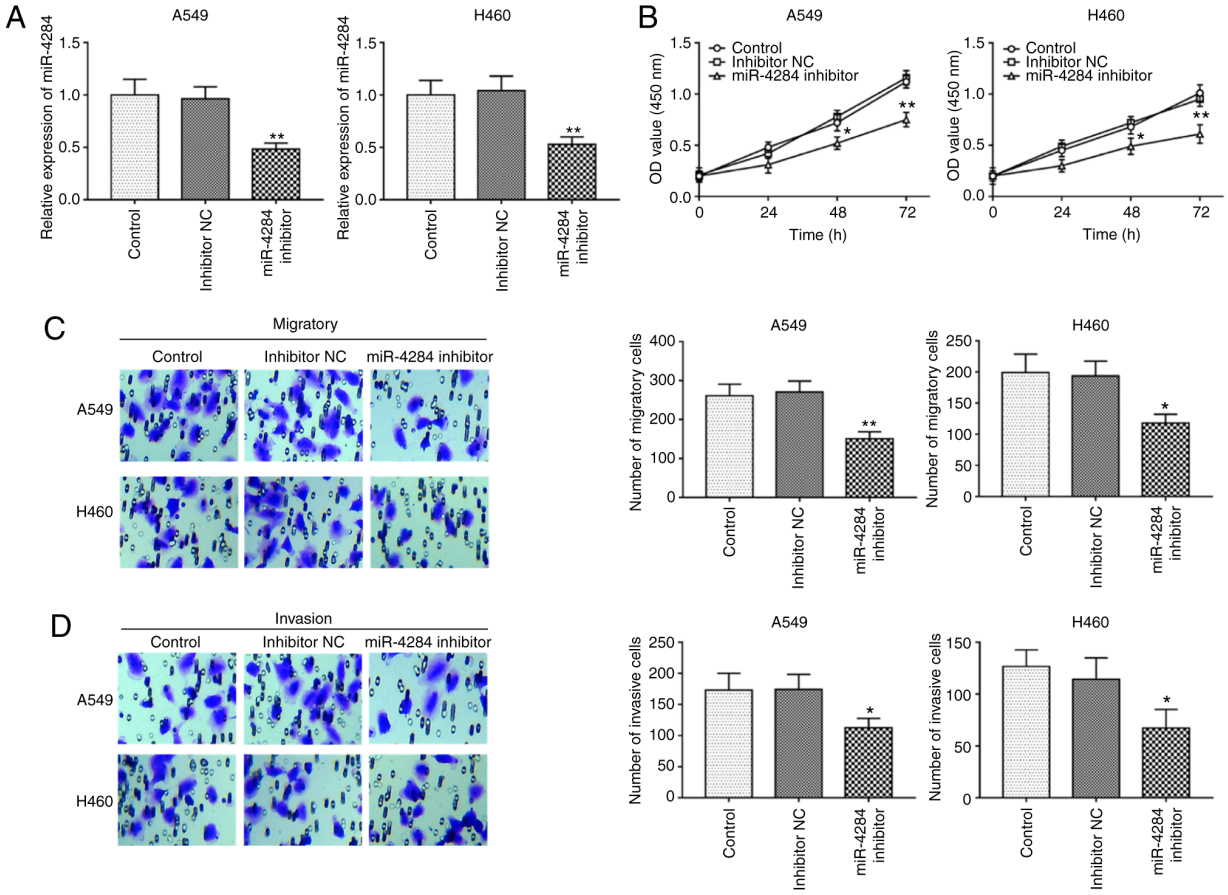

Figure 2. miR-4284 inhibition suppresses cell proliferation, migration and invasion of non-small cell lung cancer cells. (A) The expression of miR-4284 in cells was significantly downregulated after transfection with inhibitor of miR-4284. (B) Inhibition of miR-4284 in cells resulted in a decrease in cell proliferation. (C and D) Inhibition of miR-4284 in cells resulted in a decrease of (C) cell migration and (D) invasion. In addition to quantitative bar graphs, representative images from Transwell assays are provided (magnification, x200). *P<0.05, **P<0.01 vs. control. miR, microRNA; OD, optical density; NC, negative control.

the transfection efficiency of miR-4284 was thus investigated. In A549 and H460 cells, UTMD significantly increased the inhibitory effects of miR-4284 inhibitor on miR-4284 in both A549 and H460 cells, which manifested as markedly decreased miR-4284 expression levels induced by UTMD-mediated miR-4284 inhibitor compared with the inhibition achieved by miR-4284 inhibitor alone (all P<0.05; Fig. 3).

Effects of UTMD-mediated miR-4284 transfection on NSCLC cell proliferation, migration and invasion. As presented in Fig. 4A, UTMD-mediated knockdown of miR-4284 significantly inhibited the proliferation of A549 and H460 cells compared with that in the mock group. As expected, further inhibition of cell proliferation was observed in cells co-transfected with UTMD and miR-4284 inhibitor compared with that in cells transfected with miR-4284 inhibitor alone (all P<0.05). Similarly, further experiments suggested that cell migration and invasion were significantly reduced after *in vitro* transfection with UTMD-mediated miR-4284 inhibitor compared with those in the mock group and the miR-4284 inhibitor group (all P<0.05; Fig. 4B and C).

Discussion

Lung cancer is the most common cause of cancer-related death worldwide (18). The number of patients with NSCLC accounts for 85% of all patients with lung cancer and the 5-year overall survival rate is only 15% (19). NSCLC is the leading cause of malignancy-associated mortality worldwide (20). Lung cancer is a molecularly heterogeneous disease with differences in the growth rate, invasive ability, sensitivity to drugs and prognosis of the tumor during its progression (21). Thus, the heterogeneity provides ample opportunity for multiple treatment approaches and target pathways. MiRNAs are small non-coding RNAs that regulate gene expression at the post-transcriptional level, and increasing evidence suggests that miRNAs are involved in carcinogenesis and the development of human cancers (22,23). For instance, in colorectal cancer (CRC), studies have indicated the tumor suppressor function of miR-421; therefore, the miR-421/mating-type locus (MAT1) axis is expected to be one of the targets for CRC targeted therapy (24). miR-4319 is able to inhibit the proliferation of CRC by targeting ankyrin repeat and BTB/POZ domain containing protein 1 and miR-4319 may become a meaningful treatment for CRC (25). Yang *et al* (26) confirmed that miR-497 may be used as a biomarker for cancer diagnosis and prognosis and is a promising therapeutic target for future clinical applications. Ji *et al* (27) studied the effect of UTMD of miR-133a on breast cancer treatment and determined that it inhibited tumor growth and improved the survival rate in a breast cancer model of mice, which may indicate the safety and effectiveness of the UTMD method for

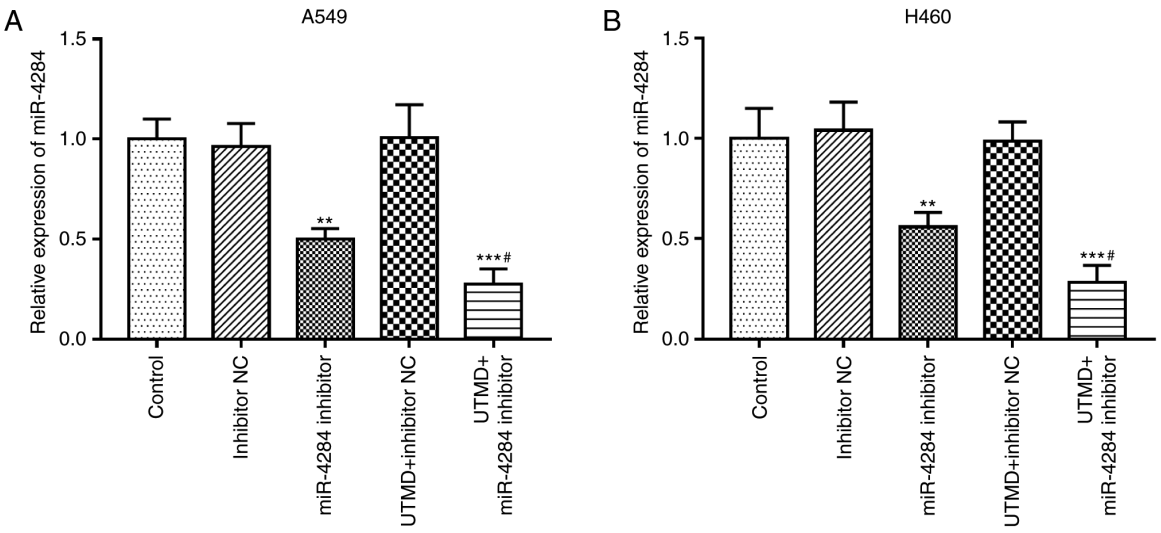

Figure 3. UTMD enhances the cell transfection efficiency of miR-4284. (A and B) UTMD significantly increased the efficacy of miR-4284 inhibitor to decrease the expression level of miR-4284 in the (A) A549 and (B) H460 cell lines, as it increased the efficiency of transfection. **P<0.01, ***P<0.001 vs. control; $^{\#}$P<0.05 vs. miR-4284 inhibitor. miR, microRNA; NC, negative control; UTMD, ultrasound-targeted microbubble destruction.

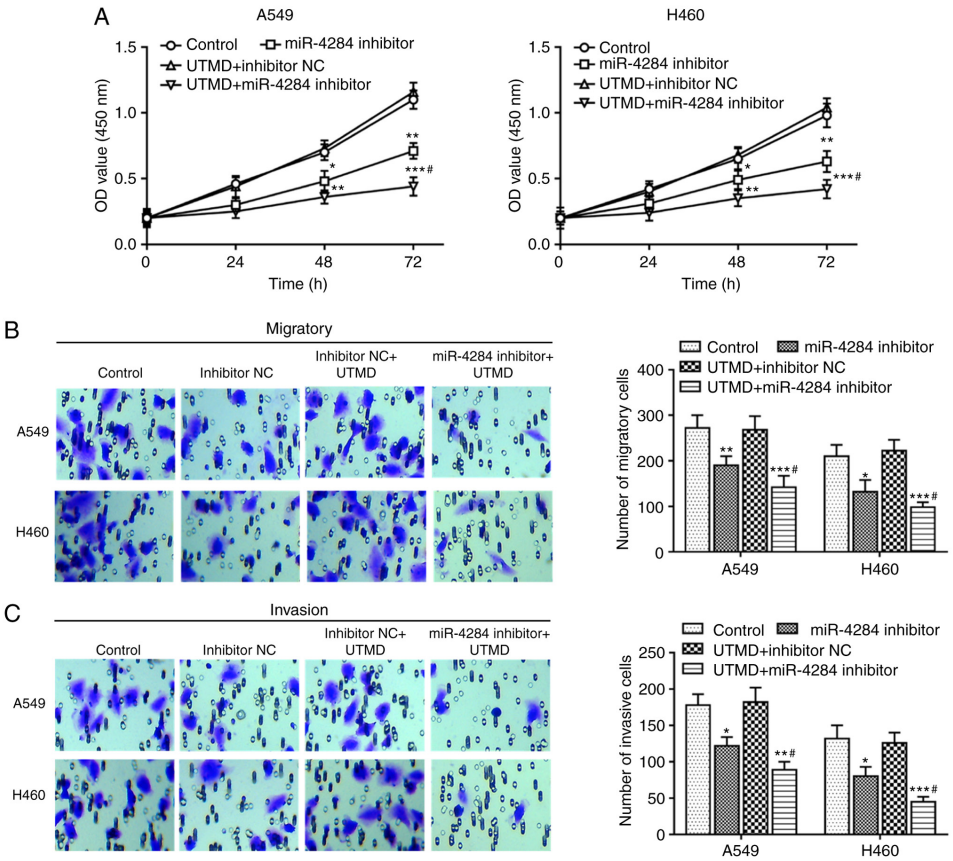

Figure 4. Effects of UTMD-mediated miR-4284 transfection on non-small cell lung cancer cell proliferation, migration and invasion. (A) UTMD-mediated inhibition of miR-4284 further inhibited cell proliferation compared with transfection of miR-4284 inhibitor alone. (B and C) UTMD-mediated inhibition of miR-4284 further inhibited (B) cell migration and (C) invasion compared with inhibitor of miR-4284 alone. In addition to quantitative bar graphs, representative images from Transwell assays are provided (magnification, x200). *P<0.05, **P<0.01, ***P<0.001 vs. control; $^{\#}$P<0.05 vs. miR-4284 inhibitor. miR, microRNA; NC, negative control; UTMD, ultrasound-targeted microbubble destruction; OD, optical density.

miRNA delivery in the regulation of tumorigenesis. However, the role of miR-4284 in NSCLC has remained elusive.

In the present study, it was determined that miR-4284 was aberrantly expressed in NSCLC. Previous studies have indicated that the expression of miR-4284 was altered in various diseases, suggesting that miR-4284 may be involved in their pathogenesis (12,28). For instance, Liu *et al* (29) reported that bone marrow-derived mesenchymal stem cells

(BMSCs) had a stronger ability to inhibit osteoclastogenesis through the miR-4284/C-X-C motif chemokine ligand 5 axis, which provided a novel perspective on pathological osteogenesis mechanisms of BMSCs. miR-4284 may serve as a tissue and prognostic biomarker for diffuse large B-cell lymphoma (30). In gastric cancer, the expression level of miR-4284 was significantly upregulated, which indicated that it may represent a novel diagnostic and prognostic biomarker for this tumor type (11). In the present study, the expression levels of miR-4284 was revealed to be upregulated in NSCLC tumors and in the A549 and H460 cell lines as compared with those in the corresponding normal control group. In addition, the expression level of miR-4284 exhibited a gradual enhancement with the increase of the TNM stage and was the highest in tumors from stage III patients. Furthermore, cell proliferation assays suggested that the proliferation ability of NSCLC cells decreased after miR-4284 silencing. Cell migration and invasion experiments were further performed, further demonstrating inhibitory effects of miR-4284: When the expression level of miR-4284 decreased, the cell migration and invasion ability declined. Therefore, miR-4284 may have an oncogenic role in NSCLC.

UTMD is a promising targeted gene delivery method that has been successfully applied in the treatment of numerous diseases over the past decade (31). As a potential drug/gene delivery system, UTMD may be used to improve the permeability of biological barriers and enhance the therapeutic effect of tumors (32). For instance, UTMD technology is able to significantly facilitate the co-transmission of gemcitabine and miR-21i and thus provides a promising strategy for the effective treatment of pancreatic cancer (33). In the study of Yang *et al* (34), miR-let-7b was transfected into ovarian cancer stem cells by flow cytometry, and the results indicated that miR-let-7b transfection efficiency using UTMD was significantly higher. In NSCLC, the expression level of miR-767 could be successfully downregulated by miR-767 inhibitor and UTMD further enhanced the transfection efficacy of miR-767 inhibitor, while downregulation of miR-767 was indicated to inhibit the proliferation, migration and invasion of tumor cells (15). In the present study, UTMD improved the transfection efficiency of a miR-4284 inhibitor in NSCLC cells. UTMD-mediated transfection further promoted the decrease in the proliferation, migration and invasion ability of NSCLC cells induced by miR-4284 inhibitor. This was due to UTMD enhancing the transfection efficiency of the inhibitor of miR-4284 *in vitro*, which thereby enhanced the efficacy of miR-4284 inhibition to impair the progression of NSCLC.

In conclusion, the present study indicated that the expression of miR-4284 was increased in NSCLC tissues and cell lines compared with that in the corresponding normal controls. Decreased expression of miR-4284 was able to inhibit tumor cell proliferation, migration and invasion. In addition, UTMD enhanced the transfection efficiency of miR-4284 inhibitors, leading to more significant inhibition of the biological function of NSCLC cells. Therefore, miR-4284 may be a potential therapeutic target for NSCLC and UTMD-mediated delivery of inhibitors of miR-4284 may be a promising therapeutic strategy for NSCLC. There are still certain limitations to the present study, such as the lack of elucidation of the detailed mechanism of the role of miR-4284 in NSCLC pathogenesis. In addition, the present study provided *in vitro* results and it is esteemed that the effects of UTMD introduction methods *in vivo* and their detailed molecular mechanisms will be further addressed in depth in future studies.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Project of Zibo Key Research and Development Plan (grant no. 2018kj010104).

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

PT and WD designed and conceived the study, conducted the clinical studies, analyzed the clinical data and wrote the manuscript. YW conducted the cell experiments and analyzed the cell experimental data. All authors read and approved the manuscript.

Ethics approval and consent to participate

Written informed consent was obtained from each patient and the experimental procedures were approved by the Ethics Committee of Zibo Central Hospital (Shandong, China; approval no. 170239).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Smith RA, Andrews KS, Brooks D, Fedewa SA, Manassaram-Baptiste D, Saslow D, Brawley OW and Wender RC: Cancer screening in the United States, 2018: A review of current American Cancer Society guidelines and current issues in cancer screening. CA Cancer J Clin 68: 297-316, 2018.
2. Miller KD, Siegel RL, Lin CC, Mariotto AB, Kramer JL, Rowland JH, Stein KD, Alteri R and Jemal A: Cancer treatment and survivorship statistics, 2016. CA Cancer J Clin 66: 271-289, 2016.
3. Travis WD, Brambilla E, Nicholson AG, Yatabe Y, Austin JHM, Beasley MB, Chirieac LR, Dacic S, Duhig E, *et al*: The 2015 World Health Organization Classification of Lung Tumors: Impact of Genetic, Clinical and Radiologic Advances Since the 2004 Classification. J Thorac Oncol 10: 1243-1260, 2015.
4. Cai J, Fang L, Huang Y, Li R, Xu X, Hu Z, Zhang L, Yang Y, Zhu X, Zhang H, *et al*: Simultaneous overactivation of Wnt/β-catenin and TGFβ signalling by miR-128-3p confers chemoresistance-associated metastasis in NSCLC. Nat Commun 8: 15870, 2017.
5. Chen H and Hwang JH: Ultrasound-targeted microbubble destruction for chemotherapeutic drug delivery to solid tumors. J Ther Ultrasound 1: 10, 2013.

6. Tinkov S, Bekeredjian R, Winter G and Coester C: Microbubbles as ultrasound triggered drug carriers. J Pharm Sci 98: 1935-1961, 2009.
7. Yang H, Sun Y, Wei J, Xu L, Tang Y, Yang L, Zhang X and Lu Y: The effects of ultrasound targeted microbubble destruction (UTMD) carrying IL 8 monoclonal antibody on the inflammatory responses and stability of atherosclerotic plaques. Biomed Pharmacother Oct 118: 109161, 2019.
8. Lee Y, Jeon K, Lee JT, Kim S and Kim VN: MicroRNA maturation: Stepwise processing and subcellular localization. EMBO J 21: 4663-4670, 2002.
9. Barbato S, Solaini G and Fabbri M: MicroRNAs in Oncogenesis and Tumor Suppression. Int Rev Cell Mol Biol 333: 229-268, 2017.
10. Dan B, Luo J, Li K and Chen S: Prognostic value of miR-375 for survival outcomes in various cancers: A systematic review and meta-analysis. Oncol Res Treat 41: 47-50, 2018.
11. Li Y, Shen Z, Jiang H, Lai Z, Wang Z, Jiang K, Ye Y and Wang S: MicroRNA 4284 promotes gastric cancer tumorigenicity by targeting ten-eleven translocation 1. Mol Med Rep 17: 6569-6575, 2018.
12. Yang F, Nam S, Brown CE, Zhao R, Starr R, Ma Y, Xie J, Horne DA, Malkas LH, Jove R, *et al*: A novel berbamine derivative inhibits cell viability and induces apoptosis in cancer stem-like cells of human glioblastoma, via up-regulation of miRNA-4284 and JNK/AP-1 signaling. PLoS One 9: e94443, 2014.
13. Sim J, Kim Y, Kim H, Shin SJ, Kim DH, Paik SS and Jang K: Identification of recurrence-associated microRNAs in stage I lung adenocarcinoma. Medicine (Baltimore) 97: e10996, 2018.
14. Sun W, Zhao P, Zhou Y, Xing C, Zhao L, Li Z and Yuan L: Ultrasound targeted microbubble destruction assisted exosomal delivery of miR-21 protects the heart from chemotherapy associated cardiotoxicity. Biochem Biophys Res Commun 532: 60-67, 2020.
15. Li X, Xu M, Lv W and Yang X: Ultrasound-targeted microbubble destruction-mediated miR-767 inhibition suppresses tumor progression of non-small cell lung cancer. Exp Ther Med 19: 3391-3397, 2020.
16. Leong-Poi H, Kuliszewski MA, Lekas M, Sibbald M, Teichert-Kuliszewska K, Klibanov AL, Stewart DJ and Lindner JR: Therapeutic arteriogenesis by ultrasound-mediated VEGF165 plasmid gene delivery to chronically ischemic skeletal muscle. Circ Res 101: 295-303, 2007.
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. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A: Global cancer statistics, 2012. CA Cancer J Clin 65: 87-108, 2015.
19. Akhurst T: Staging of non-small-cell lung cancer. PET Clin 13: 1-10, 2018.
20. Soria JC, Massard C and Le Chevalier T: Should progression-free survival be the primary measure of efficacy for advanced NSCLC therapy? Ann Oncol 21: 2324-2332, 2010.
21. Schwartz AG and Cote ML: Epidemiology of lung cancer. Adv Exp Med Biol 893: 21-41, 2016.
22. Connell LC, Harding JJ and Abou-Alfa GK: Advanced Hepatocellular Cancer: The Current State of Future Research. Curr Treat Options Oncol 17: 43, 2016.
23. Wang Z, Sha HH and Li HJ: Functions and mechanisms of miR 186 in human cancer. Biomed Pharmacother 119: 109428, 2019.
24. Xue L and Yang D: miR 421 inhibited proliferation and metastasis of colorectal cancer by targeting MTA1. J BUON 23: 1633-1639, 2018.
25. Huang L, Zhang Y, Li Z, Zhao X, Xi Z, Chen H, Shi H, Xin T, Shen R and Wang T: miR-4319 suppresses colorectal cancer progression by targeting ABTB1. United European Gastroenterol J 7: 517-528, 2019.
26. Yang G, Xiong G, Cao Z, Zheng S, You L, Zhang T and Zhao Y: miR-497 expression, function and clinical application in cancer. Oncotarget 7: 55900-55911, 2016.
27. Ji Y, Han Z, Shao L and Zhao Y: Evaluation of in vivo antitumor effects of low-frequency ultrasound-mediated miRNA-133a microbubble delivery in breast cancer. Cancer Med 5: 2534-2543, 2016.
28. Munari E, Marchionni L, Chitre A, Hayashi M, Martignoni G, Brunelli M, Gobbo S, Argani P, Allaf M, Hoque MO, *et al*: Clear cell papillary renal cell carcinoma: micro-RNA expression profiling and comparison with clear cell renal cell carcinoma and papillary renal cell carcinoma. Hum Pathol 45: 1130-1138, 2014.
29. Liu W, Wang P, Xie Z, Wang S, Ma M, Li J, Li M, Cen S, Tang S, Zheng G, *et al*: Abnormal inhibition of osteoclastogenesis by mesenchymal stem cells through the miR-4284/CXCL5 axis in ankylosing spondylitis. Cell Death Dis 10: 188, 2019.
30. Tamaddon G, Geramizadeh B, Karimi MH, Mowla SJ and Abroun S: miR-4284 and miR-4484 as putative biomarkers for diffuse large B-cell lymphoma. Iran J Med Sci 41: 334-339, 2016.
31. Wu J and Li RK: Ultrasound-targeted microbubble destruction in gene therapy: A new tool to cure human diseases. Genes Dis 4: 64-74, 2016.
32. Shi D, Guo L, Sun X, Shang M, Meng D, Zhou X, Liu X, Zhao Y and Li J: UTMD inhibit EMT of breast cancer through the ROS/miR-200c/ZEB1 axis. Sci Rep 10: 6657, 2020.
33. Lin L, Fan Y, Gao F, Jin L, Li D, Sun W, Li F, Qin P, Shi Q, Shi X, *et al*: UTMD-Promoted Co-Delivery of gemcitabine and miR-21 inhibitor by dendrimer-entrapped gold nanoparticles for pancreatic cancer therapy. Theranostics 8: 1923-1939, 2018.
34. Yang C, Li B, Yu J, Yang F, Cai K and Chen Z: Ultrasound microbubbles mediated miR-let-7b delivery into $CD133^+$ ovarian cancer stem cells. Biosci Rep 38: 38, 2018.