Suppression of MGAT3 expression and the epithelial–mesenchymal transition of lung cancer cells by miR-188-5p

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ABSTRACT

Background: To investigate the effect of miR-188-5p overexpression on the invasion and migration of cultured lung cancer cells, and on related cellular mechanisms that underlie epithelial mesenchymal transition (EMT).

Methods: Human lung cancer cell line 95D was transfected with miR-188-5p mimic. Quantitative real-time polymerase chain reaction (qRT-PCR) and Western blot were performed to quantify the expression levels of genes including E-cadherin, Snail, α-SMA, and MGAT3. Changes in cell motility, invasion and proliferation were studied using scratch migration assay, transwell invasion assay, and colony formation assay, respectively. The expression levels of EMT-related proteins and MGAT3 protein were also determined via immunofluorescent staining. The ability of miR-188-5p to regulate its target gene, MGAT3, was assessed using dual luciferase activity assay.

Results: Lung cancer cell line 95D showed the lowest miR-188-5p expression level thus was used in this study. Transfection with miR-188-5p mimic significantly suppressed migration, invasion and clonal formation potency of 95D cells. Dual luciferase activity assay implicated that miR-188-5p exerts its negative regulatory effect on MGAT3 expression through recognizing the 3’ untranslated region (3’UTR) of the MGAT3 gene. Over-expression of miR-188-5p in 95D cells also remarkably increased E-cadherin protein expression and decreased the expression levels of Snail and α-SMA, which suppressed the EMT process.

Conclusion: MiR-188-5p reduces the expression of MGAT3 and inhibits the metastatic properties of a highly invasive lung cancer cell line, probably via targeted regulation of EMT process. Further research to explore the potential therapeutic value of miR-188-5p, both as a biomarker and as a drug candidate for the management of metastatic lung cancer may be warranted.

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At a glance of commentary

Scientific background on the subject

Lung cancer is among the most common causes of cancer deaths in the world. The high incidence of metastasis is a major contributor to the poor prognosis of patients. miR-188-5p was identified as a lung cancer suppressor, but its role in lung cancer metastasis is not fully understood.

What this study adds to the field

Over-expression of miR-188-5p suppressed the invasion and migration of cultured lung cancer cell line 95D. We observed that the expression of MGAT3, a key component of EMT, was down-regulated by miR-188-5p expression. Our study suggests value in further exploring the potential therapeutic value of miR-188-5p for metastatic lung cancer management.

Background

Lung cancer is among the most common causes of cancer deaths in the United States and around the world [1]. Although major progresses have been made in the development of targeted therapies, the overall survival rate is still unfavorable (5-year overall survival < 20%) [2]. The high incidence of metastasis is a major contributor to the poor prognosis for patients with lung cancer; in one study, > 90% patients with metastatic lung cancer had tumor lesions in the brain, bone or liver [3]. Studies have shown that epithelial–mesenchymal transition (EMT) is a critical cellular mechanism involved in tumor metastasis [4]. Inhibition of EMT process offers a potential therapeutic strategy for the management of tumor progression [5]. Indeed, identification of targets for the purpose of inhibiting EMT is a key active area of current cancer therapeutics research [6–8].

The gene MGAT3 encodes the glycosyltransferase N-acetylglucosaminyltransferase-III (GnT-III), which has been suggested to be a metastasis suppressor that affects cell adhesion and migration. Transfection of the MGAT3 gene into B16 mouse melanoma cells with high metastatic capacity has been shown to suppress lung metastasis in vivo [9], and increased glycosylation of E-cadherin in vitro. E-cadherin is a crucial cell adhesion molecule which is often altered in EMT. Glycosylated E-cadherin exhibited delayed turnover and increased cell–cell aggregation, which thereby might contribute to the suppression of metastasis [10]. A recent study found that MGAT3 expression was dramatically decreased during EMT, and later recovered when cells returned to an epithelial-like phenotype. This change in MGAT3 expression leads to a variation in the expression levels of the enzymatic product of GnT-III, and specific modifications of E-cadherin expression levels [11].

MicroRNAs (miRs) are a group of small non-coding RNA molecules associated with the post-transcriptional down regulation of specific genes by turning off corresponding protein expression and promoting mRNA degradation [12]. Since their initial discovery, the roles of miR molecules in tumor biology have been extensively investigated, especially those with a prospect to be used as a pre-diagnosis biomarkers and/or those as a potential therapeutic targets or disease management tools [13]. In particular, the involvement of microRNA (such as miR-145) in lung cancer metastasis has been well characterized [14]. During tumor EMT, miRs were found to perform critical functions, some of which serve as triggers for lung cancer metastasis [15], whilst others act to inhibit metastasis via down-regulating the expression of tumor-related gene [16]. Among the various miR molecules identified to be tumor-associated, miR-188 has been previously characterized as a tumor suppressor for non-small cell lung cancer (NSCLC) both in vitro in lung cancer cells and in vivo in a murine lung cancer model. MiR-188 over-expression was found to suppress the proliferation and migration of NSCLC cells, as well as promote cell apoptosis [17]. However, whether or not miR-188-5p is involved in the EMT of lung cancer remains unclear.

In this study, we examine the role of miR-188-5p in mediating lung cancer cell migration and invasion using a cell culture model. Our results show that over-expression of miR-188-5p significantly suppressed the invasion and migration of cultured lung cancer cell line 95D. Mechanistic studies show that miR-188-5p strongly suppressed MGAT3 gene expression, which is associated with an impaired EMT process in tumor cells. To the best of our knowledge, our results provide the first direct evidence for the mechanism of lung cancer inhibition by miR-188-5p. In addition, our findings support the potential use of miR-188-5p as a biomarker for lung cancer metastasis and underline its role as a potential therapeutic target against metastatic lung cancer.

Materials and methods

Cell culture and transfection

Different lung cancer cell lines such as A549, 95D, H446, H460 were obtained from the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). Cells were kept in RPMI-1640 medium (Gibco, NY, US) containing 10% fetal bovine serum (FBS) and were incubated in a 37 °C chamber with 5% CO2 perfusion.

Cell transfection was performed using the cationic liposome method and the Lipofectamine™ 2000 reagent (Invitrogen, Carlsbad, US) following the manufacturer’s instructions. In brief, cells at log-phase were rinsed twice with phosphate buffered saline (PBS), and were treated with 1 mL trypsin. Cell suspensions were prepared, enumerated and adjusted to 5 × 10^6 cells/mL. Two-milliliter cell suspension was added to a 6-well plate and incubated. Oligonucleotide sequences for miR-188-5p mimic (forward, S’ -CAUCC CUUGC AUGGU GGAGG G-3’; reverse, S’ -CUCCA CCAUG CAAAG GAUGU U-3’) and controlled negative control (NC) sequence (forward, S’ -UUCUC CGAAC GUGUC ACGUT T-3’; reverse, S’ -ACUGU ACAGG UUCGG AGAATT-3’) were synthesized by GenePharma (Shanghai, China). These were prepared into 20 μM working solution in diethyl pyrocarbonate (DEPC)-
treated water. Twenty-four hours before transfection, 4–5 × 10^5 cells per well were seeded onto 6-well plate. These were cultured using 2 mL antibiotic-free complete medium till reaching 70–90% confluence within 24 h. Cells were left to rest for 4–6 h after transfection (with 50 pmol mimic per 10,000 cells) before being switched to fresh serum-containing medium and used for further experiments.

**Scratch assay**

Cells were seeded onto 6-well plate at a density of 5 × 10^5 cells per well. When cells reached 90% confluence, serum-free culture medium containing 1 μg/mL mitomycin C was added and incubated for 1 h. Sterilized pipette tip was used to plot three parallel lines to equally divide each well. Floating cells were removed by rinsing with PBS and serum-free culture medium was added for continuous incubation in a 37 °C incubator with 5% CO₂. The migration of cell was observed and images obtained at 0 h and 24 h. The width of scratch and the migration distance were used as markers to evaluate migration. These were measured under AE31 phase-contrast microscope (Motic, Xiamen, China). Each group (miR-188-5p mimic transfected group and Negative Control group) had three wells and the experiment was performed in triplicate.

**Transwell assay for cell invasion**

Transwell chamber was used to examine the migration efficiency of miR-188-5p transfected cells. Approximately 2 × 10^4 cells in serum-free medium were seeded onto the upper chamber (pore size 8.0 μm) which was pre-coated with Matrigel (BD Biosciences, US). The lower chamber contained RPMI-1640 medium with 30% FBS. The chamber was incubated in a 24-well plate for 24 h, and excess cells were rinsed using PBS. Cells were fixed and stained with 0.5% crystal violet for 5 min. Cells undergoing invasion were imaged and counted using PBS. Cells were fixed and stained with Wright-Giemsa dye for 5 min. Images were obtained under a microscope, and cell clone was defined as a clone with > 50 cells. Clonal formation rate = (clone number/number of inoculated cells) × 100%.

**Dual luciferase activity assay**

9SD cells were cultured till 90% confluence. These were rinsed twice with PBS, and digested with 0.25% trypsin. Cells were collected and centrifuged for 3 min. One-milliliter complete medium was added for enumeration. The cells were seeded onto 12-well plate with three wells for each group and incubated. After 24 h, reporter gene plasmid (pmirGLO, Item nr E133A, Promega, Wisconsin, USA), miR-188-5p mimic (forward, 5'-CAUCC CUUGC AUUGG GAAGG G-3'; reverse, 5'-CUCCA CCAUG GAAGG GAUGU U-3), or NC sequence (forward, 5'-UUCUC CGAAC GUGUC ACGUT T-3'; reverse, 5'-ACGUG ACAGG UUCCG AGAATT-3') was used for transfecting cultured cells. Twenty-four hours after transfection, cells were collected by centrifugation, mixed with 0.5 mL lysis buffer and incubated for 15 min at RT. 50 μL IARII buffer was added into each tube. After adding 10 μL test sample, firefly luciferase activity was measured (E1910, Promega, US). By adding 50 μL Stop Glo reagent, Renilla luciferase activity was measured. The ratio of firefly and renilla luciferase activity was then calculated as the internally calibrated activity of the reporter.

### Quantitative real-time polymerase chain reaction (qRT-PCR)

Cultured cells were lysed and RNA was extracted using extraction kit (TIANGEN, Beijing, China). cDNA was synthesized using a reverse transcription kit following the instructions in the manual (BioTeke, Beijing, China). Real-time PCR analysis was performed using the specific primers listed in Table 1. PCR conditions were: denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 10 s

| Name       | Sequence (5’-3’)                   | Tm (°C) |
|------------|------------------------------------|---------|
| miR-188-5p | CGATATTTCACCCCTTGTGATGTT          | 61.4    |
| miR-188-5p | GTCAGGGCTCCAGGTATTC               | 59.2    |
| U6- F      | CTGCCCTGCCACACCA                  | 60.4    |
| U6- R      | AAGCCTTCAGAGATTTTCCGT            | 59.7    |
| MGAT3 F    | CCACGAGTAGAAGTGAGAC              | 57.6    |
| MGAT3 R    | AATTTGTAGTAGTGAGTGA              | 53.3    |
| E-cadherin F | CAGGCTCTCCCTGTTGCGTCTG        | 57      |
| E-cadherin | CAGGCTCTCCCTGTTGCGTCTG         | 57      |
| α-SMA F    | CCTGAAGACATCCCAACCT               | 61.2    |
| α-SMA R    | ACCATCTCCAGAGTGAGCGAGCG         | 63.7    |
| snail F    | CCATTCTGAGGGAGGG                 | 61.4    |
| snail R    | CCAGTGGAGGGTGCTGAGCCTTGT        | 60.9    |
| β-actin F  | CTTAAGTTGATGGAAGAGCTT           | 62      |
| β-actin R  | CTGACGACCTCAGGTTCCAGTT          | 64.4    |
each, annealing at 60 °C for 20 s, followed by elongation at 72 °C for 30 s. Relative expression level of target gene was calculated against beta-actin gene, or U6 (endogenous control for miR) using 2-∆∆Ct approach. The amplified products were quantitated with SYBR Green fluorescence (Solarbio, Beijing, China).

Western blotting

Total proteins were extracted using RIPA lysis buffer. Total proteins were quantified using BCA kit (Wanleibio, Shenyang, China). Proteins were separated by SDS-PAGE, and were transferred onto PVDF membrane (Millipore, Billerica, MA, US). The membrane was blocked in defatted milk powder, and was incubated at 4 °C overnight in primary antibody (mouse anti-E-cadherin at 1:500; rabbit anti-α-SMA at 1:500; goat anti-Snail 1:400 and goat anti-MGAT3 1:200; Wanleibio, Shenyang, China). On the following day, the membrane was rinsed in PBS, and was incubated in secondary antibody (goat anti-mouse IgG-HRP at 1:500; goat anti-rabbit IgG-HRP at 1:500; donkey anti-goat IgG-HRP at 1:500; Wanleibio, Shenyang, China) for 1 h at room temperature. ECL chromogenic substrate (BD Bioscience, US) was added for development, and was imaged using a gel imaging system. Relative protein expression was quantified against the beta-actin band.

Immunofluorescent staining

Cells were seeded on coverslips and grown until reaching 50–70% confluence. The culture medium was then removed and the cells were rinsed with PBS three times before fixation with 4% paraformaldehyde for 15 min. After rinsing with PBS (three times, 5 min each), the cells were incubated with 0.1% Triton X-100 at RT for 30 min, followed by three washes of PBS (5 min each). Cells on slides were blocked with 1% BSA for 15 min at RT. Primary antibody (mouse anti-E-cadherin at 1:200; goat anti-Snail at 1:200; rabbit anti-α-SMA at 1:200; and goat anti-MGAT3 at 1:50) was added for overnight incubation at 4 °C. On the following day, excess antibody was removed by rinsing with PBS, and fluorescent labelled secondary antibody (1:300) was added and incubated at RT for 1 h and subsequently stained with DAPI. Coverslips were mounted and images were obtained under a laser scanning confocal microscope BX3 (Olympus, Tokyo, Japan).

Statistical analysis

All data are presented as mean ± standard deviation. Parametric data were compared by Student's t-test while one-way ANOVA with Dunnett's post hoc analysis was used for multiple comparisons. p < 0.05 was considered indicative of statistically significant between-group difference. All statistical analyses were performed using SPSS software (ver.18) (IBM, US).
Results

miR-188-5p negatively regulates migration and invasion of lung cancer cells

We first evaluated the expression levels of miR-188-5p in the commonly used lung cancer cell lines such as A549, 95D, H446, H460. qRT-PCR results showed that 95D cell line had the lowest level of miR-188-5p expression [Fig. 1A]. Hence, we selected 95D cell line as the in vitro model for further assays. After transfection with miR-188-5p mimic, 95D cells showed significantly lower migration rate [Fig. 1B]. Transwell assay revealed that the number of invading cells in the miR-188-5p mimic transfected group was significantly lower than that in the miR-188-5p NC group [Fig. 1C]. Taken together, miR-188-5p can inhibit both migration and invasion of lung cancer cells in vitro. Furthermore, results of colony formation assay showed that miR-188-5p effectively reduced the proliferation of cancer cells [Fig. 1D]. To summarize, these observations strongly support the role of miR-188-5p as a tumor suppressor, possibly acting on more than one aspect of tumor development.

Transfection with miR-188-5p mimic regulates the expression of MGAT3

Potential binding sites for miR-188-5p on MGAT3 were predicted. The online prediction tools (Targetscan and miRDB) in combination with target sequence database suggested a strong binding site of miR-188-5p on MGAT3 [Fig. 2A], which was later substantiated by dual luciferase activity assay. Using a reporter luciferase gene (in the background of pmirGLO) fused to the 3'UTR of MGAT3, we found remarkably decreased
luciferase activity ratio after transfection with miR-188-5p mimic. However, no significant changes in luciferase activity was observed with the use of mimic NC sequence or a mutant form of 3’UTR sequence [Fig. 2B]. These data suggest that miR-188-5p may directly regulate the expression of MGAT3.

Transfection with miR-188-5p mimic regulates the expression of EMT-related proteins via transcriptional mechanisms

We investigated the effect of miR-188-5p on the expression of major proteins involved in the EMT process. Western blotting revealed elevated expression levels of E-cadherin and depressed levels of Snail, α-SMA, and MGAT3 proteins in 95D cells transfected with miR-188-5p mimic [Fig. 2C]. Further, qRT-PCR results also confirmed that miR-188-5p mimic transfection remarkably, at mRNA level, increased the expression levels of E-cadherin, and decreased the expressions of Snail, α-SMA, and MGAT3 gene transcripts [Fig. 2D]. These results collectively showed that miR-188-5p inhibited EMT process in lung cancer cells.

We subjected 95D cells to immunofluorescent staining and examined the expressions of EMT-related proteins after transfection with miR-188-5p mimic. Consistent with the results of qRT-PCR, 95D cells with miR-188-5p over-expression showed prominent up-regulation of E-cadherin. Further, we observed decreased expressions of Snail, α-SMA and MGAT3 proteins [Fig. 3].

Discussion

In sharp contrast to mature somatic cells, tumor cells exhibit unlimited potential for proliferation, ectopic seeding, and
unregulated growth capacity, all of which required for tumor metastasis. A complex regulatory network governs the process of tumor metastasis, among which EMT is one of the critical processes that endows cells at the primary cancer site the ability to migrate to distant sites for the formation of secondary lesions [18]. Dysregulation of effective surveillance for tumor metastasis plays a crucial role in oncogenesis. This study found that overexpression of miR-188-5p suppressed the migration of cultured lung cancer cells via inhibition of EMT related gene expression. These results are consistent with those of previous studies that revealed the involvement of multiple miR molecules in tumor cell EMT and metastasis [19]. We believe that the findings presented in this study provide the theoretical foundation for developing novel therapeutic options for the management of metastatic lung cancer.

In lung cancer patients, miR-188 has been identified as a circulating marker for disease progression, as miR-188 level showed a direct correlation with patient survival [20]. In this study, we further investigated the molecular mechanisms by which miR-188-5p mediates tumor cell biology. Our results supported that miR-188-5p inhibited EMT process. After miR-188-5p over-expression, we found consistent results such as upregulation of E-cadherin and down-regulation of Snail and α-SMA genes. The interplay between E-cadherin and miR molecules during cancer progression has been previously reported in the literature [21]. Similar miR-directed modulation was also demonstrated for Snail signaling [22] and for α-SMA modulation [23]. In addition, the luciferase assay augmented the notion that MGAT3 gene expression is inhibited by miR-188-5p. As a glycosyltransferase gene, MGAT3 modulates various cellular events in addition to the regulation of carbohydrate chain function on cell surface. MGAT3 protein was shown to exhibit a strong correlation with tumor metastasis [24]. To the best of our knowledge, no studies have demonstrated that any miR is involved in the post-transcriptional regulation of MGAT3 gene. A previous study has shown that MGAT3 can affect the function of E-cadherin proteins during EMT process [11]. The regulatory relationship between E-cadherin and MGAT3 appears to be bidirectional, as E-cadherin has also shown to regulate MGAT3 gene transcription [25]. However, while the previous results have shown a decrease in MGAT3 expression during EMT [11], the results from the present study suggested that low MGAT3 levels were associated with high E-cadherin levels, which leads to suppressed EMT. Our results serve to elaborate the regulatory network for the MGAT3 gene and provide preliminary insights into the mechanism underlying miR-188-5p-induced tumor suppression.

Additional sequence analysis and luciferase reporter-based assays are required to thoroughly investigate the mechanism underlying the regulation of EMT-related genes by miR-188-5p and the role of MGAT3 in this process. Overexpression of miR-188-5p did not completely inhibit the migration and invasion of the 95D lung cancer cells, indicating the complex nature of the cognate regulatory mechanisms underlying the process. Many miRNAs have been shown to be involved in the EMT of lung cancer cells, such as the miRNA-200 family [26]. Next generation sequencing analysis has shown dysregulation of 39 miRNA in NSCLC, where 28 were upregulated and 11 were downregulated [20]. We therefore cannot rule out the possibility that miR-188-5p may also directly modulate the expression of other genes associated with EMT process. These questions may be addressed by additional bioinformatics analysis and experimental confirmation such as the luciferase reporter system used in the present study. In addition, comparison of the effects of these different miRNAs as well as studying the possible synergistic effects of their combinatorial applications could be of interest for future research and possibly clinical studies.

Another limitation of the present study is that the effect of miR-188-5p overexpression was only studied in 95D cells, which are known to be highly invasive and has been shown in the present study to express a low level of miR-188-5p. Further studies that examine the effect of overexpression of miR-188-5p on the EMT process in other lung cancer cell lines, as well as inhibition of miR-188-5p expression in lung cancer cells with high levels of miR-188-5p could offer a more comprehensive understanding of the roles of miR-188-5p in lung cancer metastasis.

In summary, our results collectively present a working model wherein miR-188-5p suppresses the EMT activity and contributes to reduce tumor cell migration and invasion. This was an in vitro study that was inspired by a human study that showed a correlation between miR-188-5p and lung cancer metastasis [17,20]; future experiments using animal models are required to further validate the role of miR-188-5p in mediating tumor progression and metastasis. These should provide more information regarding metastatic regulation by miR, and help identify therapeutic targets for the management of metastatic lung cancer.

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**Conflicts of interest**

Authors declare no conflicts of interest.

**REFERENCES**

[1] Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics. CA A Cancer J Clin 2014;64:9–29.
[2] Coleman MP, Forman D, Bryant H, Butler J, Rachet B, Maringe C, et al. Cancer survival in Australia, Canada, Denmark, Norway, Sweden, and the UK, 1995-2007 (the International Cancer Benchmarking Partnership): an analysis of population-based cancer registry data. Lancet 2011;377:127–38.
[3] Riihimäki M, Hemminki A, Fallah M, Thomsen H, Sundquist K, Sundquist J, et al. Metastatic sites and survival in lung cancer. Lung Cancer 2014;86:78–84.
[4] Mittal V. Epithelial mesenchymal transition in aggressive lung cancers. Adv Exp Med Biol 2016;890:37–56.
[5] Niu H, Wu B, Jiang H, Li H, Zhang Y, Peng Y, et al. Mechanisms of RhoGDI2 mediated lung cancer epithelial-mesenchymal transition suppression. Cell Physiol Biochem 2014;34:2007–16.

[6] Chanvorachote P, Chammn S, Ninsontia C, Phiboonchaiyanan PP. Potential anti-metastasis natural compounds for lung cancer. Anticancer Res 2016;36:5707–17.

[7] Niu H, Zhang Y, Wu B, Zhang Y, Jiang H, He P. Matrine induces the apoptosis of lung cancer cells through downregulation of inhibitor of apoptosis proteins and the Akt signaling pathway. Oncol Rep 2014;32:1087–93.

[8] Niu H, Wang J, Li H, He P. Rapamycin potentiates cytotoxicity by docetaxel possibly through downregulation of Survivin in lung cancer cells. J Exp Clin Cancer Res 2011;30:28.

[9] Yoshimura M, Nishikawa A, Ihara Y, Taniguchi S, Taniguchi N. Suppression of lung metastasis of B16 mouse melanoma by N-acetylglucosaminyltransferase III gene transfection. Proc Natl Acad Sci U S A 1995;92:8754–8.

[10] Yoshimura M, Ihara Y, Matsuzawa Y, Taniguchi N. Aberrant N-linked glycosylation of E-cadherin enhances cell-cell binding to suppress metastasis. J Biol Chem 1996;271:13811–5.

[11] Finho SS, Oliveira P, Cabral J, Carvalho S, Huntsman D, Gartner F, et al. Loss and recovery of Mgat3 and Gnt-III Mediated E-cadherin N-glycosylation is a mechanism involved in epithelial-mesenchymal-epithelial transitions. PloS One 2012;7:e33191.

[12] Mohr AM, Mott JL. Overview of microRNA biology. Semin Liver Dis 2015;35:3–11.

[13] Hayes J, Peruzzi PP, Lawler S. MicroRNAs in cancer: biomarkers, functions and therapy. Trends Mol Med 2014;20:460–9.

[14] Ling DJ, Chen ZS, Zhang YD, Liao QD, Feng JX, Zhang XY, et al. MicroRNA-145 inhibits lung cancer cell metastasis. Mol Med Rep 2015;11:3108–14.

[15] Li J, Yang S, Yan W, Yang J, Qin YJ, Lin XL, et al. MicroRNA-19 triggers epithelial-mesenchymal transition of lung cancer cells accompanied by growth inhibition. Lab Invest A J Tech Meth Pathol 2015;95:1056–70.

[16] Yu T, Li J, Yan M, Liu L, Lin H, Zhao F, et al. MicroRNA-193a-3p and -5p suppress the metastasis of human non-small-cell lung cancer by downregulating the ERBB4/PIK3R3/mTOR/S6K2 signaling pathway. Oncogene 2015;34:413–23.

[17] Zhao L, Ni X, Zhao L, Zhang Y, Jin D, Yin W, et al. MicroRNA-188 acts as tumor suppressor in non-small-cell lung cancer by targeting MAP3K3. Mol Pharm 2018;15:1682–9.

[18] Liu Y, Chen LP. The regulation of cell polarity in the progression of lung cancer. J Canc Res Therapeut 2013;9:580–5.

[19] Legras A, Pecuchet N, Imbeaud S, Pallier K, Didelot A, Rousset H, et al. Epithelial-to-Mesenchymal transition and MicroRNAs in lung cancer. Cancers 2017;9:101.

[20] Gallach S, Jantus-Lewintre E, Calabuig-Farinas S, Montaner D, Alonso S, Sirera R, et al. MicroRNA profiling associated with non-small cell lung cancer: next generation sequencing detection, experimental validation, and prognostic value. Oncotarget 2017;8:56143–57.

[21] Wang TS, Gao W, Chan JY. Interactions between E-cadherin and microRNA deregulation in head and neck cancers: the potential interplay. BioMed Res Int 2014;2014:126038.

[22] Abba ML, Patil N, Leupold JH, Allgayer H. MicroRNA regulation of epithelial to mesenchymal transition. J Clin Med 2016;5:8.

[23] Rajasekaran S, Rajaguru P, Sudhakar Gandhi PS. MicroRNAs as potential targets for progressive pulmonary fibrosis. Front Pharmacol 2015;6:254.

[24] Taniguchi N, Kizuka Y. Glycans and cancer: role of N-glycans in cancer biomarker, progression and metastasis, and therapeutics. Adv Cancer Res 2015;126:11–51.

[25] Pinho SS, Reis CA, Paredes J, Magalhães AM, Ferreira AC, Figueiredo J, et al. The role of N-acetylglucosaminyltransferase III and V in the post-transcriptional modifications of E-cadherin. Hum Mol Genet 2009;18:2599–608.

[26] Chen L, Gibbons DL, Goswami S, Cortez MA, Ahn YH, Byers LA, et al. Metastasis is regulated via microRNA-200/ZEB1 axis control of tumour cell PD-L1 expression and intratumoral immunosuppression. Nat Commun 2014;5:5241.