MiR-200a enhances the migrations of A549 and SK-MES-1 cells by regulating the expression of TSPAN1

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MicroRNA-200a (miR-200a) has been reported to regulate tumour progression in several tumours; however, little is known about its role in non-small cell lung cancer cells (NSCLCs). Here, we found that miR-200a was up-regulated in A549 and SK-MES-1 cells compared with normal lung cells HELF. By a series of gain-of-function and loss-of-function studies, over-expression of miR-200a was indicated to enhance cells migration, and its knock-down inhibited migration of cells in NSCLC cell lines. Furthermore, miR-200a was identified to induce TSPAN1 expression which was related to migration. TSPAN1 was proved to induce migration, and so up-regulation of TSPAN1 by miR-200a may explain why over-expressing miR-200a promotes NSCLC cells migration.

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1. Introduction

Lung cancer has a higher death rate among all cancers, and is thus the leading cause of cancer-associated death. This trend is expected to continue until 2030. Lung tumours are usually classified into non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC) depending on their pathological and histological characteristics (Calbó *et al*. 2005). Regardless of subtype, 5-year survival rate for lung cancer is among the lowest of all cancers and metastasis is the primary cause of death from lung cancer (Granville and Dennis 2005). Almost 60% of brain metastases without a known primary tumour are from lung cancer (Lee *et al*. 2008). Tumour metastasis results from a multi-step cascading process that includes cell detachment, invasion, migration, intravasation and circulation, implantation, angiogenesis and proliferation (Jiang and Liu 2011). The lack of treatment options for patients with metastatic disease emphasizes the need for a better understanding of the biologic processes that drive metastasis.

TSPAN1 is a new member of transmembrane 4 superfamily (TM4SF), and several studies have revealed an increased expression of TSPAN1 in several types of cancer including liver (Chen *et al.* 2007), lung (Borczuk *et al.* 2003), ovarian cancer (Scholz *et al*. 2009), colorectal carcinoma (Chen *et al*. 2009), gastric carcinoma (Chen *et al*. 2008), etc., which pointed to a potential role of TSPAN1 in tumour progression. Likewise, suppression of TSPAN1 by RNA interference inhibits proliferation and invasion of colon cancer cells *in vitro* (Chen *et al.* 2010a, b). Knock-down of TSPAN1 by RNA silencing in human skin squamous carcinoma cells could inhibit proliferation and infiltration of cells (Chen *et al.* 2010a, b).

MicroRNAs (miRNAs) are endogenous short noncoding RNAs 22 nucleotides long, typically inactivate, developmentally important mRNAs by inhibiting their translation or directing sequence-specific degradation of complementary mRNA (Pontes and Pikaard 2008). MiRNAs play essential roles in a wide array of biological processes, including differentiation, proliferation, apoptosis and tumour development (Ventura and Jacks 2009), and frequently are misregulated in human cancers (Yu *et al*. 2008; Schaefer *et al*. 2010; Szczyrba *et al*. 2010). Of particular interest in the field of metastasis, the miR-200 family, which contains five members: miR-200a, -200b, -200c, -141 and 429, is a promising

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target. It has been shown that the miR-200 regulates epithelial-mesenchymal transition (EMT) and cell migration, invasion and metastasis in a variety of cancer cell lines, by targeting and inhibiting the expression of important mRNAs that are involved in EMT (ZEB1 and ZEB2) (Gregory *et al*. 2008; Park *et al*. 2008; Bendoraite *et al*. 2010), angiogenesis (Flt1, Ets-1) (Roybal *et al.* 2011; Chan *et al*. 2011), β-catenin/Wnt signalling (β-catenin) (Saydam *et al*. 2009; Xia *et al*. 2009), and cancer stem cell formation (Suz12) (Iliopoulos *et al*. 2010).

Recently, one study has shown that miR-200c can inhibit cells metastasis in non-small cell lung cancer (Ceppi *et al.* 2010). However, Korpal *et al.* reported that miR-200s promoted metastatic colonization by regulating the breast cancer cell secretome (Korpal *et al*. 2011). The miR-200 family has shown two faces in regulating metastasis. To date, the role of miR-200a in NSCLC has not been reported. In this study, we aim to investigate the effects of miR-200a in NSCLC cells and to elucidate its mechanism. This work reveals that elevation of miR-200a level enhanced cells migration, and down regulation of miR-200a inhibited cells migration in NSCLC cell lines. Moreover, we found that miR-200a induced the expression of TSPAN1 at both transcriptional level and translational level, and a specific site could exist in the gene's promoter. These results provide a better understanding on the function of miR-200a towards the migration of NSCLC cells.

2. Materials and methods

2.1 *Cell culture and reagents*

Lung cancer cell lines H446, SK-MES-1, A549 and normal lung cell HELF cells were obtained from Biomics Biotechnologies Co. Ltd (Nantong, China). H446 cells and SK-MES-1 cells were cultured in RPMI-1640 medium (GIBCO, USA) and minimum essential medium (MEM, GIBCO, USA), respectively. A549 and HELF cells were maintained in Dulbecco's modified Eagle's medium (DMEM, GIBCO,USA). All mediums were supplemented with 10% fetal bovine serum (FBS), penicillin (100U/ml) and streptomycin (100μg/ml). Hsa-miR-200a mimics (miR-200a mimics), hsa-miR-200a 5MM (miR-200a-5MM), hsa-miR-200a 3MM (miR-200a-3MM), hsa-miR-200a inhibitor (miR-200a inhibitor) and siTSPAN1 were chemically synthesized by Biomics Biotechnologies Co. Ltd (Nantong, China).

2.2 *Quantitative real-time PCR (qRT-PCR)*

Total RNA was extracted from cells with RISO™ RNA Isolation Reagent (Biomics, China). The isolated total RNA was reverse transcribed and quantified in the same reaction system using the EzOmicsTM One-Step qPCR Kit (Biomics, China) according to the manufacturer's guidelines. SYBR Green real-time RT-PCR was done using primers specific for TSPAN1 (forward 5′-GTGGCTTCACC AACTATACG-3′, reverse 5′- GACTGCATTAGTTCGGA TGT-3′), ZEB1 (forward 5′- GCCAATAAGC AAACGATT CTG -3′, reverse 5′- TTTGGCTGGATCACTTT CAAG-3′), EphA2 (forward 5′- TCAGCAGCAGCGACTTCGAGGC A-3′, reverse 5′-CAGTGGCCA GGGAAGGTGC A-3′), cortactin (forward 5′-AGCCGTCGCCCTGTACGA CT-3′, reverse 5′-G TACCGGCCCTTGCACACCC-3′), GAPDH (forward 5′-GAAGGTGA AGGTCGGAGTC-3′, reverse 5′- GAAGATGGTGATGGGATTTC-3′), and U6 (forward 5′- CTCGCTTCGGCAGCACA-3′, reverse5′-AACGCTTCACGAATTTG CGT-3′). The stem-loop primer for mature miR-200a was designed by Biomics (China). The PCR reactions were peformed at 42°C for 30min and 95°C for 7min, and subjected to 45 cycles at 95°C for 20s, 60°C for 20s and 72°C for 30s. After the cycles, the Tm of PCR products were examined at a rate of 0.5°C/10 s descending from 95°C to 55°C. The relative expression level of genes were determined using the 2-delta delta Ct analysis method. Samples were normalized to U6 or glyceraldehyde 3-phosphate dehydrogenase (GAPDH), as indicated.

2.3 *Wound healing assay*

A549 (1×10^5) and SK-MES-1(1.5×10^5) cells were seeded onto 24-well plates and allowed to adhere overnight, respectively. The cells were then transfected with miR-200a mimics (A549) or miR-200a inhibitor (SK-MES-1) at 80 nM final concentration using Lipofectamine 2000 transfection reagent (Invitrogen, USA) according to the manufacturer's specification. When cells reached about 95% confluence at 48 h post transfection, an artificial homogenous wound was made through the cells using a p20 tip, and the medium was replaced with low serum medium (2% FBS). At scheduled time points, cells that migrated into the wounded area were photographed under an upright microscope (40×objective). The mobility of cells was measured using the distance ratio ((0h distance-24h distance) /0h distance) analysis method. Fifteen randomly areas per group were selected, and the data were expressed as mean±SD. This experiment was repeated in three independent transfection experiments.

2.4 *Transwell migration assay*

A549 and SK-MES-1 cells were transfected with 80nM miR-200a mimics and miR-200a inhibitor, respectively, as mentioned above. At 48 h post transfection, cells were detached by treatment with 0.25% trypsin (Sangon, China),

centrifuged and resuspended into serum-free medium. 8×10^4 transfected cells in 100μl serum-free medium were added to the upper chamber, the lower chamber was filled with 600μl medium containing 10% FBS. The bottom membrane of upper chamber was a 8 μm pore polycarbonate membrane (Costar, Corning, NY). After 16 h incubation at 37°C, non-migration cells on the upper surface of the membrane were scrubbed gently with a cotton-tipped swab. The migration cells on the lower surface of the membranes were fixed with 10% methanol and stained with 0.2% crystal violet (Sangon, China). The inserts were rinsed twice with PBS and allowed to air dry. The stained cells were photographed under an upright microscope (100×objective) and quantified by manual counting in nine randomly selected areas. This experiment was performed in three independent transfection experiments.

2.5 *Western blotting*

A549 and SK-MES-1 (3×10^5) cells were seeded onto 6-well plates in 2 mL growth medium. The next day, cells were transfected with 80 nM miR-200a mimics or mimics control. After 48 hours, the transfected cells were harvested for immunoblot analysis. Whole-cell protein extracts were denatured, and 50μg were separated on 12% SDS-PAGE gels and transferred onto polyvinylidene difluoride membranes. After blocking in TBS-T which was supplied with 5% milk, membranes were hybridized with primary antibody overnight at 4°C. Primary antibody against TSPAN1 was received as a kind gift from Professor Chen Li of Nantong University (China), β-actin (Santa, USA) was used as internal control. All primary antibodies were used at a 1:200 dilution. After incubation with appropriate secondary antibody, results were detected using ECL chemiluminescence reagent (Beyotime, China).

2.6 *Statistical analysis*

Data were expressed as mean±SD. The statistical difference among different groups was analysed by two-tailed Student's t-test using the SPSS11.5. A *P*-value of less than 0.05 was considered to be statistically significant, and $P\leq0.01$ was significantly different.

3. Results

3.1 *Up-regulation of miR-200a in NSCLC cells*

We analysed expression level of mature miR-200a in lung cancer cells and normal lung cells, including H446 (small cell lung cancer cells), A549 (non-small cell lung cancer cells), SK-MES-1 (non-small cell lung cancer cells) and HELF (normal lung cells). Quantitative real-time PCR revealed that miR-200a was down-regulated in H446 cells compared with HELF cells, but up-regulated in A549 and SK-MES-1 cells (figure 1). Among these different cell lines we chose A549 and SK-MES-1 cells for further studies for the reason that both of them are NSCLC cells with up-regulation of miR-200a expression. As the miR-200a level in SK-MES-1 cells was 3-fold higher than that in A549 cells, we further chose SK-MES-1 cells for loss-of-function study, and A549 cells for the following gain-of-function study.

3.2 *Lung cancer cell migration regulated by miR-200a*

Up-regulation and down-regulation of miR-200a expression were achieved by transfection with synthetic miR-200a mimics and miR-200a inhibitor, respectively. Also, 48 h after transfection, miR-200a levels were examined by quantitative RT-PCR method as described in the Materials and methods section. The results showed that the transfection of miR-200a mimics dramatically increased the level of miR-200a by 151 folds in lung cancer cells, as compared to the control groups (figure 2A). Considering that the gene-silencing efficiency will be significant on the higher expression background, we utilized cells with over-expression of miR-200a to verify the effectiveness of miR-200a inhibitor. We proved that miR-200a inhibitor decreased miR-200a level, using co-transfection of miR-200a mimics and miR-200a inhibitor at different rates (figure 2B and C). In further experiments, wound healing and transwell migration assays were carried out to gain insight into the role of miR-200a towards migration of NSCLC cells. The results from the wound healing assay showed that over-expression of miR-200a enhanced A549 cells migration by 35.6% while down-regulation of miR-200a decreased the migration of SK-MES-1 cells by 22.1% (figure 3A). A similar and yet more obvious trend was seen in transwell migration assays: the migration of A549 cells was

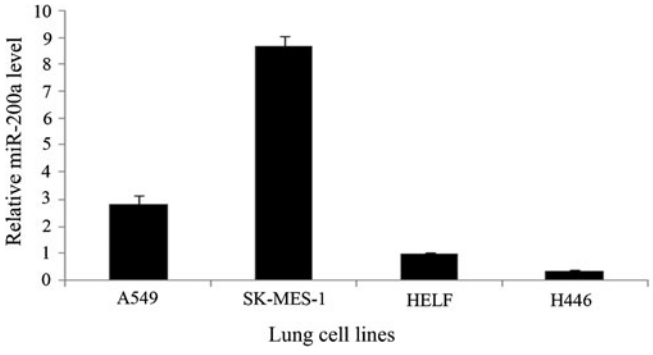

Figure 1. The relative levels of mature miR-200a in different lung cancer cell lines and normal lung cells. A549, SK-MES-1: non-small cell lung cancer (NSCLC) cells, HELF: normal lung cells. The value of miRNA-200a in HELF cells was designated as 1.

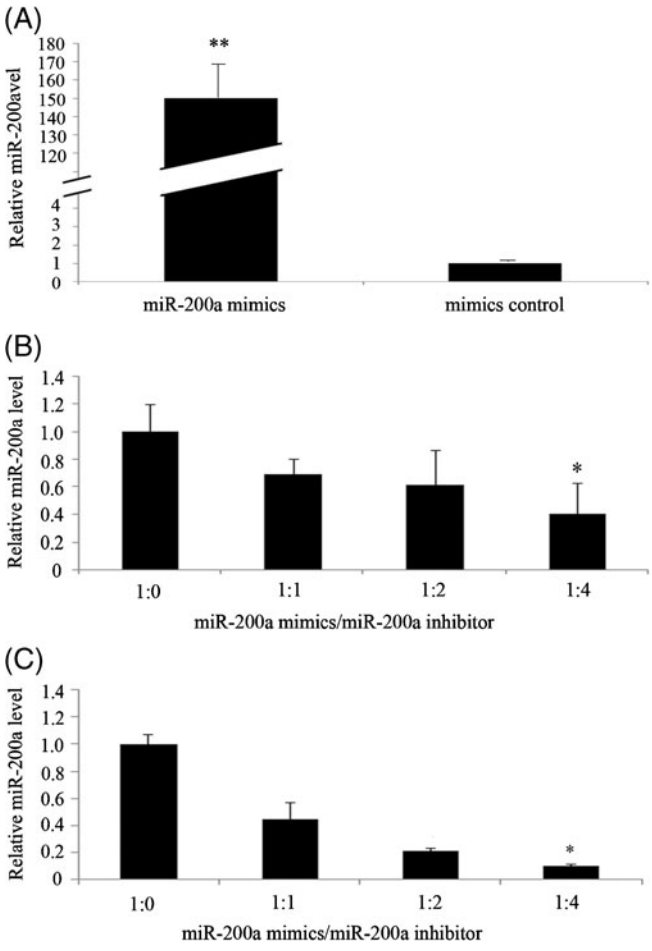

Figure 2. MiR-200a mimics and miR-200a inhibitor really play function. (**A**) Expression levels of mature miR-200a in A549 cells at 48 h after miR-200a mimics transfection. ** indicates $P<0.01$. (**B**) Mature miR-200a level at 48 h after co-transfecting with miR-200a mimics and miR-200a inhibitor with different rates in A549 cells. (**C**) Mature miR-200a level at 48 h after co-transfecting with miR-200a mimics and miR-200a inhibitor with different rates in SK-MES-1 cells. * represents $P<0.05$, as compared miR-200a mimics/inhibitor 1:4 group with 1:0 group.

increased by 53.8% when miR-200a was overexpression, while SK-MES-1 cells migration was reduced by 31.2% with the down-regulation of miR-200a (figure 3B). These results suggested that miR-200a played a promoting role in the migration of A549 and SK-MES-1 cells.

3.3 *MiR-200a-induced expression of TSPAN1*

We have revealed that miR-200a stimulated migration of NSCLC cells; however, the downstream targets remained unknown. Therefore, we attempted to identify the downstream targets of miR-200a by analyzing mRNA levels of TSPAN1, Eph receptor A2 (EphA2), ZEB1 and Cortactin, which were implicated in cell migration and invasion upon the miR-200a transfection. As shown in figure 4A, there was strong induction (4.3-fold) of TSPAN1 in miR-200a mimicking transfected cells compared to control miRNA transfected cells. The expression of Cortactin was somewhat up-regulated as well, although the up-regulation was only marginal. Induction of TSPAN1 gene was further confirmed by immunoblot analysis (figure 4B), the elevated protein level was strongly correlated with increased mRNA level. Such a correlation was further confirmed in the experiments in which mRNA and protein expression of TSPAN1 were down-regulated when miR-200a inhibitor was transfected (figure 4C and D).

3.4 *3.4 Inhibition of A549 and SK-MES-1 cells migration by suppression of TSPAN1*

To confirm the finding that TSPAN1 was the functional effector of miR-200a in promoting lung cancer cells migration, silencing of TSPAN1 by siRNA and its impact on A549 and SK-MES-1 cells migration was examined. At the first, we examined the effect of chemically synthesized siRNA: results from qRT-PCR and Western blotting showed that the expression level of TSPAN1 was exactly decreased by siTSPAN1, as compared to siControl-transfected groups (figure 5A). Wound healing assay (figure 5B) and transwell migration assay (figure 5C) demonstrated that suppression of TSPAN1 significantly inhibited A549 and SK-MES-1cells migration. Based on these results, it was concluded that TSPAN1 gene can promote the migration of NSCLC cells. MiR-200a induced expression of TSPAN1, and so TSPAN1 may be the cause of promoting role of miR-200a in NSCLC cells migration.

3.5 *MiR-200a may target the promoter of TSPAN1 to induce its expression by a sequence-specific mode*

In order to find out how miR-200a induced TSPAN1 expression, we scanned 1 kb promoter of TSPAN1 for sequences complementary to miR-200a using the University of California Santa Cruz genome browser (*http://genome.ucsc.edu/*). A potential binding sequence located at position −377 upstream to the transcription start site in the TSPAN1 promoter was identified (figure 6A). According to the potential site, two mutants of miR-200a to create mismatches with the target sites were designed and synthesized. Mutant derivatives with mutations of 5 bases (at −380 to −384 bases) and 9 bases (at −380 to −384 and −394 to −397 bases) in the miR-200a sequences were designed and named miR-200a-5MM (mutated at 5′ end) and miR-200a-3MM (mutated at both 5′ and 3′ end), respectively (figure 6B).

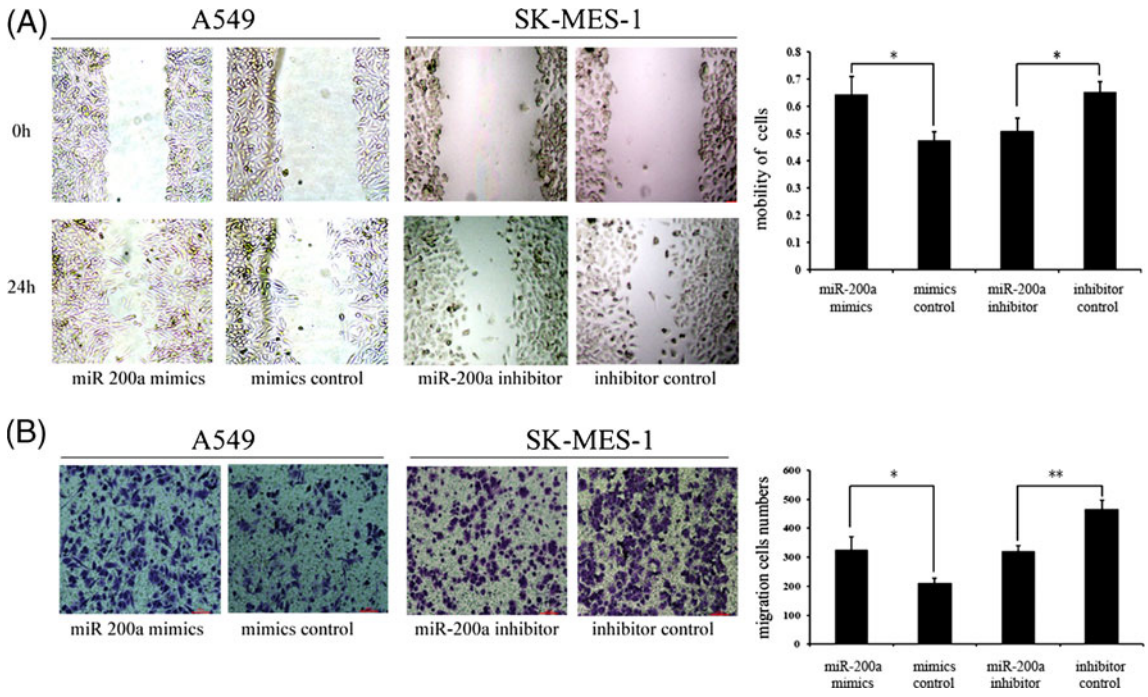

Figure 3. Effect of miR-200a expression on NSCLC cells migration. (**A**) Wound healing assay. Representative images photographed at 0 h (upper) and 24 h (lower) post-wounding were shown at magnification of 40×. (**B**) Transwell migration assay. The migration lung cancer cells were stained and counted under microscope (magnification of 100×) at 16 h after reseeding. Each experiment was performed at least three times and representative examples are shown here. **P*<0.05; ***P*<0.01, as compared to corresponding control.

In both NSCLC cells, neither miR-200a-5MM nor miR-200a-3MM were found to be capable of inducing TSPAN1 expression (figure 6C), indicating that miR-200a may target the promoter to play a role. All these results suggested that induction of TSPAN1 was specific to the sequence of miR-200a.

4. Discussion

Metastasis, the hallmark of malignant tumour, is the main cause for clinical death of most cancer patients. MiR-200a is one of the miR-200 family members which has been shown to

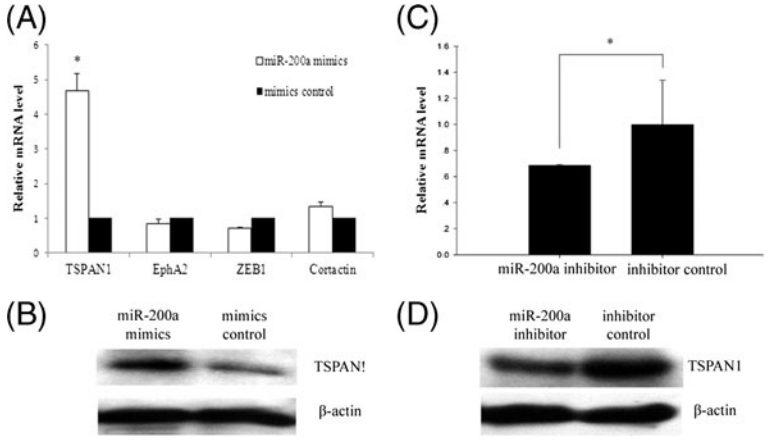

Figure 4. MicroRNA-200a regulates the expression of TSPAN1. (**A**) Effect of miR-200a on the target gene expression. The effect of miR-200a transfection on mRNA levels of predicted target genes in cells was quantified by qRT-PCR, and GAPDH served as a control. (**B**) The induction of TSPAN1 proteins by miR-200a transfection was detected by Western blotting analysis. (**C**) Down-regulation effect of miR-200a inhibitor on TSPAN1 expression was quantified by qRT-PCR. (**D**) Down-regulation effect of miR-200a inhibitor on TSPAN1 expression was assessed by Western blotting assays. **P* <0.05, as compared to corresponding control group.

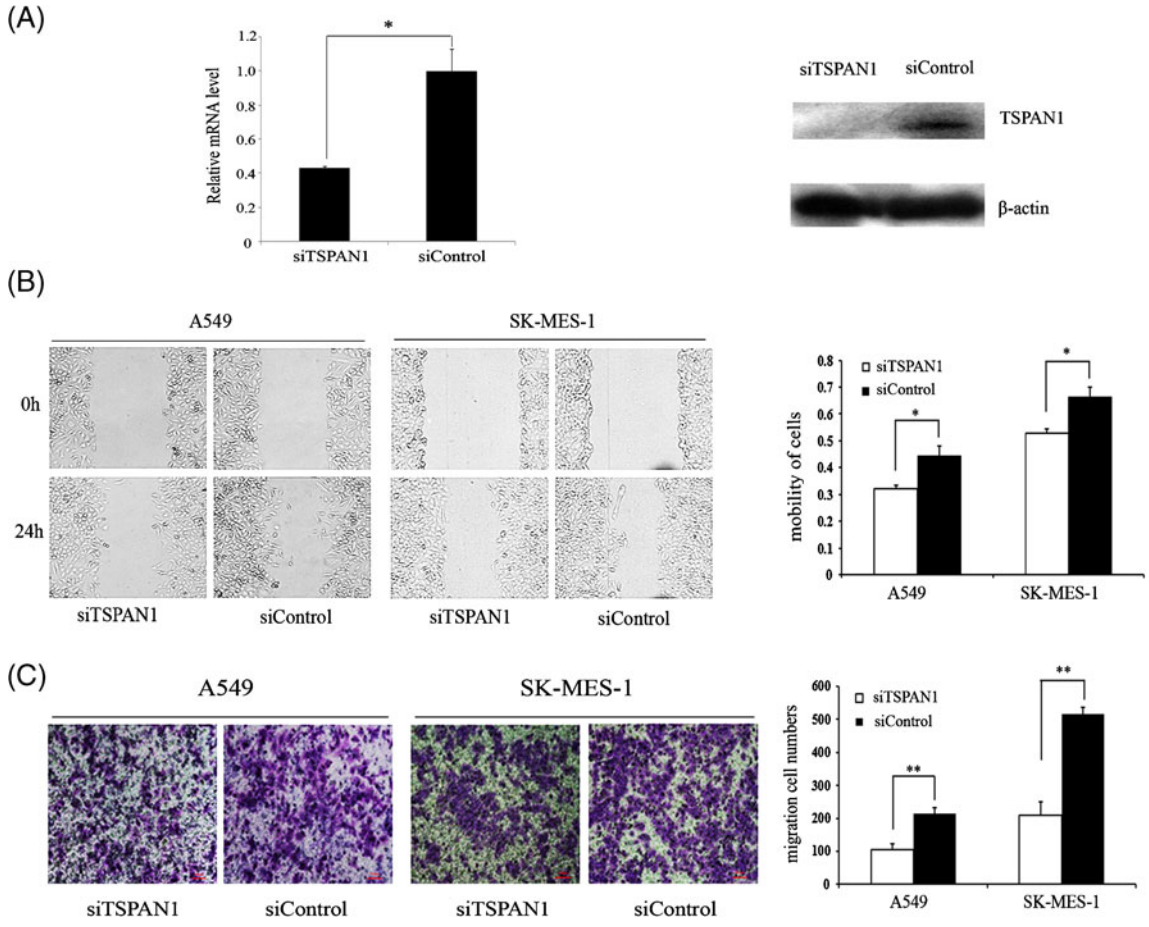

Figure 5. siTSPAN1 targeting TSPAN1 gene inhibited non-small cell lung cancer cells migration. (**A**) qRT-PCR and Western blotting analysis of TSPAN1 mRNA and protein expression in siTSPAN1 and siControl-transfected cells at 48h after transfection. GAPDH served as an internal control in qRT-PCR assay, and β-actin was shown as a control in Western blotting assay. (**B**) Wound healing assay. Representative images photographed at 0 h (upper) and 24 h (lower) post-wounding were shown at magnification of 40×. (**C**) Transwell migration assay. Migratory A549 and SK-MES-1 cells were stained and counted under microscope (magnification of 100×) at 16 h after reseeding. *P<0.05; **P<0.01 as compared with siControl-transfected group.

correlate strongly with metastasis of cancers (Perry and Anil 2010; Bracken *et al*. 2009). It has been reported that miR-200a has influences on EMT of breast cancer, targets to β-catenin inhibiting the cells growth in meningiomas, and regulates the migration of hepatocellular carcinoma (Saydam *et al*. 2009; Xia *et al*. 2010; Hung *et al*. 2012). To date, the function of miR-200a in NSCLC has not been reported. In this study, we aimed to investigate the role of miR-200a in cancer cell migration and to identify its targets in NSCLC cells.

According to the miR-200a expression level, A549 and SK-MES-1 were selected as models of NSCLC to study the role of miR-200a. Results from *in vitro* migration assays explained that over-expression of miR-200a increased the migration ability of A549 cells by 35.6% (wound healing assay) and 53.8% (transwell migration assay), while down-regulation of miR-200a decreased the migration of SK-MES-1 cells by 22.1% (wound healing assay) and 31.2% (transwell migration assay). The data presented here supported a promoting role for miR-200a in migration of NSCLC cells. Similarly, some other studies have also found that over-expression of miR-200a enhanced cells invasion in melanoma cell lines (Elson-Schwab *et al*. 2010). In addition, anti-miR-200a significantly inhibited the growth of endometrial endometrioid carcinoma cells where the expression level of miR-200a was up-regulated (Lee *et al*. 2011). Based on these results, a conclusion could be made that miR-200a serves such functions as an oncogene. However, a majority of functional studies concentrated on roles of down-regulation and suggested that miR-200a prevented tumour progression, migration and invasion by serving as a tumour suppressor (Saydam *et al*. 2009; Xia *et al*. 2009; Woo *et al*. 2004). The results in this work, in which miR-200a was

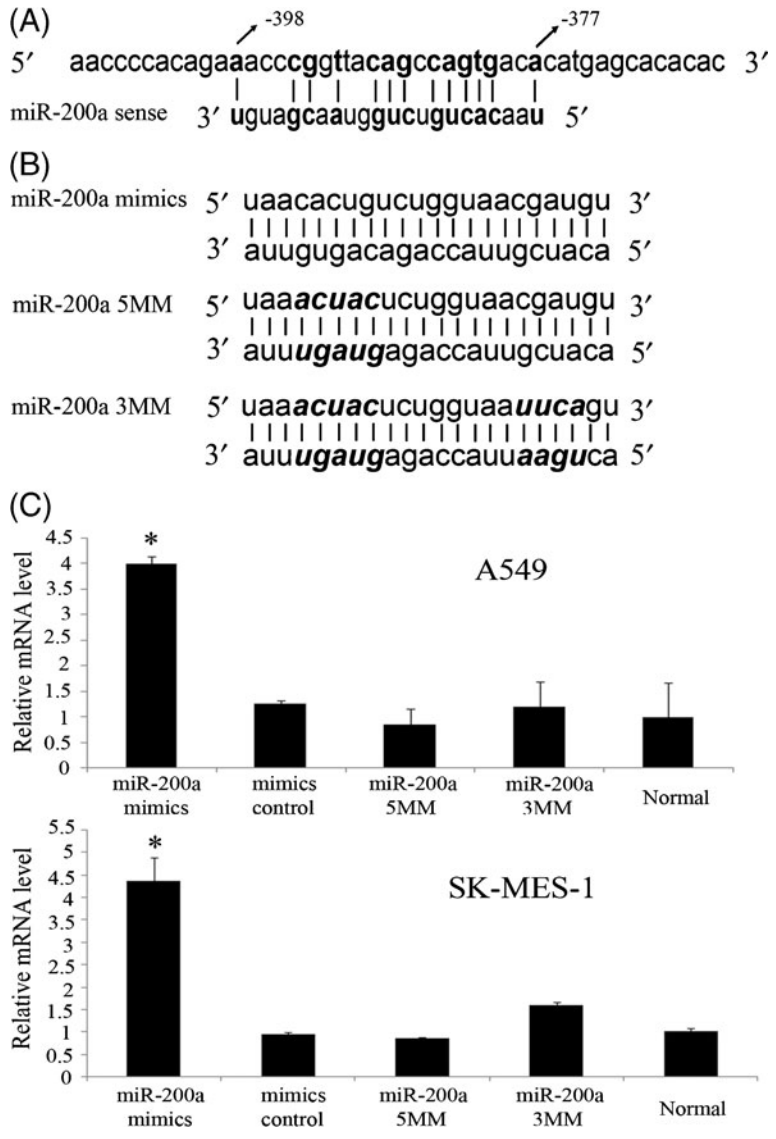

Figure 6. Sequence specificity for microRNA-200a (miR-200a) is illustrated. (**A**) Sequence of the miR-200a target site located at nucleotide −377 upstream to the transcription start sites. Bases in bold face corresponded to those bases in miR-200a that are complementary to the target sites. (**B**) Mutations of 5 base pairs at 5′ end or 9 base pairs both at 5′ and 3′ end of miR-200a resulted in the mutant derivatives miR-200a-5MM and miR-200a-3MM, respectively. The mutated bases are shown in bold and italic. (**C**) Relative TSPAN1 messenger RNA (mRNA) expression levels is illustrated in both A549 and SK-MES-1 cells and quantified by qRT-PCR. **P*<0.05, statistically significant.

demonstrated to enhance cells migration *in vitro*, lend weight to the point that the effects of miR-200a on tumour cell metastasis may be highly context-dependent. MiR-200a may play different roles, promoting or inhibiting tumour metastasis, in different cancers.

MiR-200a belongs to the miR-200 family that is classified into two subfamilies, miR-200a/141, AACACU, differing by only one nucleotide from the seed sequence of miR-200bc/429, AAUACU (Korpal and Kang 2008). The two subfamilies possess different seed sequences, and so in some aspects they may exhibit distinct functions. Recently, functional differences between miR-200 family members have been identified. In breast cancer cells, the two clusters of miR-200 family showed opposite roles on p27/Kip1, with increased p27/Kip1 from expression of the miR-200a/141 cluster but reduced p27/Kip1 from miR-200bc/429 cluster (Uhlmann *et al*. 2010). Moreover, miR-200a and miR-200c had opposite effects on morphology in melanoma cells, miR-200a resulting in a protrusion-associated elongated mode of invasion while miR-200c leading to a rounded, amoeboid-

like mode (Woo *et al*. 2004). These demonstrated that miRNAs of different seed sequences, even by only one nucleotide discrepancy, may cause different functions in the progression of cancers. Our result further supported these observations, by which we showed that miR-200a enhanced NSCLC cells migration whereas its subfamily miR-200c was reported to be of inhibition function in NSCLC cells in another study (Ceppi *et al*. 2010).

As demonstrated above, all these data suggested that miR-200a may play a role in NSCLC initiation and/or progression. Thus, it became necessary to identify the target genes of miR-200a involved in the processes. The four genes of TSPAN1, EphA2, ZEB1 and Cortactin are all related to cell migration and invasion. At mRNA levels, TSPAN1 showed 4- to 5-fold elevation, which was consistent with increased protein expression level. Therefore, we hypothesized that the promotion effect of miR-200a in NSCLC cells migration may be related to TSPAN1.

TSPAN1 is proved to play a role in the progression of several tumour types. Knock-down of TSPAN1 by RNA silencing in human skin squamous carcinoma cells could inhibit proliferation and infiltration of cells (Chen *et al.* 2010a, b). Suppression of TSPAN1 by RNA interference inhibits proliferation and invasion of colon cancer cells in vitro (Chen *et al.* 2010a, b). In order to assess the function of TSPAN1 on NSCLC cells, siRNA silencing the expression of TSPAN1 was studied. The obtained data showed that knock-down of TSPAN1 through siRNA silencing inhibited A549 and SK-MES-1 cells migration, suggesting TSPAN1 was required for the migration of the two NSCLC cells. These results support the standpoint that miR-200a plays a promoting role in NSCLC cells migration possibly through up-regulating TSPAN1 expression.

In the current study, we showed that expression of miR-200a in NSCLC cells promoted cells migration and induced TSPAN1 gene expression. MicroRNA is well known to silence expression of target genes with complementary sites in the UTR region. While miRNAs may also function to induce gene expression with complementary promoter sequence targets. Some reports have shown that miR-373 induced expression of E-Cadherin and CSDC2 genes (Place *et al*. 2008) and miR-205 induced the interleukin (IL) tumour suppressor genes IL24 and IL32 expression, with both of the miRNAs targeting at specific sites in the genes' promoters (Majid *et al*. 2010) . Over-expression of miR-200a thus induced TSPAN1 gene expression. It may indicate that miR-200a also targeted at complementary sites on promoter of TSPAN1. A target site existing in TSPAN1 gene promoter was identified in our study by comparing miR-200a sequence with promoter sequence of TSPAN1. Based on the target site, two mutant derivatives of miR-200a were designed and synthesized. Mutation of the nucleotides involved in the target recognition completely prevented gene induction by miR-200a, which suggested that complementarity with target sequences was necessary for the induction activity. MiR-200a mimics increased the expression of TSPAN1 comparing to mimics control molecules which were nonspecific. Moreover, introduction of miR-200a inhibitor led to down regulation of TSPAN1 gene expression. These lines of evidence indicated that miR-200a targeted the promoter of TSPAN1 to induce its expression by a sequence specific route. Here, we hypothesized that miR-200a induced the expression of TSPAN1 by targeting complementary promoter sequences, and our view has been supported by some of the preliminary experiments. However, the direct interaction between miR-200a and TSPAN1 promoter requires very extensive verification and several kinds of experiments.

Based on the above results, we concluded that miR-200a promoted NSCLC cells migration may be through inducing the expression of TSPAN1. We further hypothesized that there might exit a complementary site in the promoter sequence, which was verified by the preliminary data in the study. Although it was revealed that miR-200a enhanced migration of the two NSCLC cell lines *in vitro*, it would be worthwhile to examine the effect of miR-200a *in vivo* in future work, because metastatic phenotype may ascribe to multiple combinations of transforming mutations and alterations in miRNA expression (Jiang *et al*. 2010; Wang *et al*. 2010; Zhang *et al*. 2010).

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