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ABBS

Original Article

Down-regulation of MTA1 protein leads to the inhibition of migration, invasion, and angiogenesis of non-small-cell lung cancer cell line

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Metastasis-associated protein 1 (MTA1) high expression has been detected in a wide variety of human aggressive tumors and plays important roles in the malignant biological behaviors such as invasion, metastasis, and angiogenesis. However, the specific roles and mechanisms of MTA1 protein in regulating the malignant behaviors of non-small-cell lung cancer (NSCLC) cells still remain unclear. To elucidate the detailed functions of MTA1 protein, we down-regulated the MTA1 protein expression in NSCLC cell line by RNA interference (RNAi) *in vitro*, and found that down-regulation of MTA1 protein significantly inhibited the migration and invasion potentials of 95D cells. Further research revealed that down-regulation of MTA1 protein significantly decreased the activity of matrix metalloproteinase-9, which could be the mechanism responsible for the inhibition of 95D cells migration and invasion. In addition, the tube formation assay demonstrated that the number of complete tubes induced by the conditioned medium of MTA1-siRNA 95D cells was significantly smaller than that of 95D cells. These findings demonstrate that MTA1 protein plays important roles in regulating the migration, invasion, and angiogenesis potentials of 95D cells, suggesting that MTA1 protein down-regulation by RNAi might be a novel therapeutic approach to inhibit the progression of NSCLC.

Keywords metastasis-associated protein 1; RNA interference; angiogenesis; non-small-cell lung cancer

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Introduction

Non-small-cell lung cancer (NSCLC) accounts for ~75%–80% of all primary lung cancer and has been a major public health problem worldwide [1,2]. Despite major advances made in diagnosis and therapy, the prognoses of NSCLC patients are still dismal due to invasion and metastasis [3,4]. Therefore, identification of novel biomarkers involved in NSCLC invasion and metastasis that could be utilized as possible therapeutic targets is important to improve clinical outcomes.

Among a number of malignant tumor-related genes that have been discovered in the last few years, metastasis-associated gene (MTA) is a family of malignant tumor progression-related genes including three distinct genes (*MTA1*, *MTA2*, and *MTA3*), of which, *MTA1* is the first gene detected in this family and its protein product has been detected to be highly expressed in a wide range of human malignant tumors [5]. In addition, metastasis-associated protein 1 (MTA1) also plays important roles in enhancing the malignant properties of various human cancer cell lines *in vitro*, such as proliferation, migration, invasion, metastasis, and angiogenesis [6–10]. However, no data are available regarding the roles of MTA1 protein in NSCLC cell line.

Thus, to identify the roles of MTA1 protein in the malignant properties of NSCLC cell line, we performed this study to investigate the biological functions of MTA1 protein in regulating the migration, invasion, and angiogenesis potentials of human NSCLC 95D cell line by RNA interference (RNAi) *in vitro*.

Materials and Methods

Cell lines and cell culture

The human NSCLC cell line 95D was purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai, China) and cultured in Rosewell's Park Memorial Institute (RPMI) 1640 medium (Hyclone, Logan, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Gaithersburg, USA). Human umbilical vein endothelial cells (HUVECs) were purchased from the American Type Culture Collection (ATCC, Manassas, USA) and cultured in endothelial cell growth medium M199 (Invitrogen, Carlsbad, USA) supplemented with 15% FBS, 1 mg/ml low serum growth supplements (Invitrogen), and 2 mM glutamine. All cells were incubated in a humidified atmosphere of 5% CO_2 at 37°C.

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Small interfering RNA transfection

The small interfering RNA (siRNA) targeting *MTA1* gene and negative control siRNA were chemically synthesized by GenePharma Co., Ltd (Shanghai, China). The sequences of different siRNA used in this study are listed in **Table 1**. All the siRNAs were dissolved in sterilized and RNase-free water and the final concentration was 20 μM. The siRNA transfection was performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Briefly, the 95D cells were seeded in a six-well plate at a density of 5×10^5 cells/well and incubated until ~80% confluence, the cells were washed twice with phosphate-buffered solution (PBS) and then Opti-MEM I reduced serum medium (Invitrogen) was added to each well. Then siRNA and Lipofectamine 2000 were mixed gently and incubated together in Opti-MEM I reduced serum medium at room temperature for 20 min to form siRNA-Lipofectamine complex. The cells were incubated in the siRNA-Lipofectamine complex-containing medium for 6 h, then the medium was replaced with RPMI 1640 medium containing 10% FBS. The cells continued to be incubated for 48 h and then harvested for analysis of MTA1 mRNA and protein expression. A sequence non-specific to any known gene was used as the negative control-siRNA, and a carboxyfluorescein-conjugated control-siRNA was used to monitor the transfection rate.

Preparation of conditioned medium

The MTA1-siRNA 95D cells, control-siRNA 95D cells, and 95D cells were cultured under serum-free conditions in RPMI 1640 medium for 24 h, respectively. Then the supernatant was collected, centrifuged, filtered through a 0.22 μm filter (Millipore, Billerica, USA), and stored at −20°C until used in the enzyme-linked immunosorbent assay (ELISA), gelatin zymography assay, and tube formation assay.

Real-time quantitative PCR

The real-time quantitative polymerase chain reaction (qRT-PCR) was performed as described previously [11]. Briefly, total RNA of cells was extracted using Trizol reagent (TaKaRa, Tokyo, Japan). First strand cDNA was synthesized from mRNA using a Primescript™ RT reagent kit (TaKaRa). qRT-PCR was carried out using SYBR Green PCR kit (TaKaRa) by a Light Cycler system (Roche Molecular Biochemicals, Mannheim, Germany). The following primers were used for amplification of *MTA1*: sense primer 5′-CAGCTACGAGCAGCACAACG-3′ and antisense primer 5′-TGTCCGTGGTTTGCCAGA-3′. glyceraldehyde phosphate dehydrogenase (*GAPDH*) was amplified as an internal control using sense primer 5′-GGTGGTCTCCTCT GACTTCAACA-3′ and antisense primer 5′-GTTGCTG TAGCCAAATTCGTTGT-3′. The cycling conditions were as follows: initial denaturation at 95°C for 30 s, followed by 40 cycles at 95°C for 5 s, 60°C for 30 s, and 72°C for 15 s. Comparative threshold cycle method ($2^{-\triangle\triangle CT}$) was used to analyze the relative changes in gene expression [12]. The experiment was repeated twice with triplicate measurements in each experiment.

Western blot analysis

Western blot was performed as described in the previous report [13]. The protein of cells was extracted using the Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime, Shanghai, China) with protease inhibitors, and then the protein concentration was detected using the Enhanced BCA Protein Assay Kit (Beyotime). The protein lysates were subject to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then electrotransferred to polyvinylidene fluoride (PVDF) membranes (Millipore). After blocking with 5% fat-free milk in Tris-buffered saline Tween 20 (pH 7.6), the PVDF membranes were incubated with anti-MTA1 polyclonal antibody (1 : 200, Santa Cruz, Santa Cruz, USA) and anti-β-actin monoclonal antibody (1 : 1000, Santa Cruz) overnight at 4°C, followed by respective horseradish peroxidase-conjugated secondary antibody (1 : 1000 for MTA1 and 1 : 5000 for β-actin, Beyotime) for 1.5 h at room temperature. Signals were detected using the Chemilucent ECL Detection System (Millipore) according to the instructions and exposed to X-ray films. The intensity of each band was quantified using the Image J software version 1.43 (National Institutes of Health, Bethesda, USA). The experiment was repeated twice with triplicate measurements in each experiment.

Wound healing assay

The wound healing assay was performed as described previously [10,14]. Briefly, the MTA1-siRNA 95D cells, control-siRNA 95D cells, and 95D cells were seeded in a six-well plate with the same numbers in complete medium, respectively, and incubated until the cells grown to ~80% confluence. A sterile pipette tip was used to scratch wounds on each monolayer with the same width, then the plates were washed with PBS to remove the detached cells and the remaining cells were cultured in serum-free 1640 medium. Photos were subsequently taken at 0, 12, 24, and 36 h. The closure of the wounds was enumerated by the distance of cells moved into the wounded area. The experiment was repeated twice with triplicate measurements in each experiment.

Transwell invasion assay

The invasion assay was performed using transwell chambers (Corning, New York, USA) with 50 μl Matrigel-precoated (BD, San Diego, USA) polycarbonate membrane (8.0 μm pore size) as described in the previous

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Table 1 The sequences of siRNA used in this study

siRNA	Sequences (5′–3′)	
MTA1 siRNA-1	Sense	CCAUGGAUACUCUCCACAATT
	Antisense	UUGUGGAGAGUAUCCAUGGCG
MTA1 siRNA-2	Sense	CCAGCAUCAUUGAGUACUATT
	Antisense	UAGUACUCAAUGAUGCUGGTC
MTA1 siRNA-3	Sense	GGACAUAUUGGAAGAAAUATT
	Antisense	UAUUUCUUCCAAUAUGUCCAA
Negative control siRNA	Sense	UUCUCCGAACGUGUCACGUTT
	Antisense	ACGUGACACGUUCGGAGAATT
carboxyfluorescein-conjugated control siRNA	Sense	UUCUCCGAACGUGUCACGUTT
	Antisense	ACGUGACACGUUCGGAGAATT

report [13]. Briefly, the MTA1-siRNA 95D cells, control-siRNA 95D cells, and 95D cells were collected and resuspended in the serum-free RPMI 1640 medium at a concentration of 1×10^5 cells/ml, respectively. Then the cell suspensions were added into the top chambers (200 μl/well) and the bottom chambers were filled with RPMI 1640 medium containing 10% FBS (600 μl/well), followed by a 24 h incubation at 37°C. The cells that did not penetrate the polycarbonate membrane were swabbed using cotton bud, then the cells transmembraned through and adhered to the bottom of polycarbonate membrane were stained with 4′,6-diamidino-2-phenylindole dye (1 μg/ml) for 10 min, and were photographed under an Olympus fluorescence microscope and counted manually. The average of three randomly selected ×400 fields' cell counts was recorded as the value of each chamber. The experiment was repeated twice with triplicate measurements in each experiment.

ELISA assay

The matrix metalloproteinase-9 (MMP-9) level in the conditioned medium was determined by an ELISA kit (R&D Systems, Abingdon, UK) as described previously [15]. Samples of the supernatant were prepared in PBS and incubated at 40°C in a 96-well plate until dry. The plate was then washed three times with PBS and blocked with 2% bovine serum albumin for 1 h at room temperature, washed three times with PBS, and incubated with primary antibody of MMP-9 (1 : 1000, Santa Cruz) diluted with PBS containing 0.05% Tween 20 for 1 h. The wells were then washed three times with PBS and incubated with horseradish peroxidase-conjugated secondary antibody for 4 h. After washing three times with PBS, the color was developed using 3,3′,5,5′-tetramethylbenzidine peroxidase solution and the absorbance was read at 450 nm. The experiment was repeated twice with triplicate measurements in each experiment.

Gelatin zymography assay

The activity of MMP-9 in conditioned medium was measured by gelatin zymography assays as described previously [15]. Briefly, the conditioned medium was mixed with SDS loading buffer and then subjected to 0.1% gelatin-10% SDS-PAGE at 80 V for 2 h. The gel was washed twice with 2.5% Triton X-100 for 1.5 h to remove SDS and subsequently incubated in assay solution (50 mM Tris-HCl, 200 mM NaCl, 5 mM $CaCl_2$, 0.02% Brij-35) at 37°C for overnight. The gel was stained with 0.5% Coomassie brilliant blue R-250 (Bio-Rad, Hercules, USA) for 2 h and subsequently de-stained in methanol and acetic acid solution for 0.5 h, and then rinsed twice in the de-staining solution to visualize the clear bands against the aqua-blue-stained gelatin background. The gel was photographed and the intensity of each band was quantified using the Image J software version 1.43 (National Institutes of Health). The experiment was repeated twice with triplicate measurements in each experiment.

Endothelial cell tube formation assay

The tube formation assay was performed as described previously [16]. Briefly, the Matrigel (BD) was thawed at 4°C for overnight and mixed homogeneously using cooled pipette tips. Each well of pre-chilled 96-well plate was bottom-coated with a thin layer of Matrigel (50 μl) and incubated at 37°C for 1 h to polymerize. HUVECs (1×10^4 cells) were seeded in each well with different conditioned medium from MTA1-siRNA 95D cells, control-siRNA 95D cells, and 95D cells, respectively. The cells were incubated in a humidified atmosphere of 5% CO_2 at 37°C for 24 h. Tube-forming ability was quantified by counting the total number of complete tubes, and the average of three randomly selected ×200 fields' complete tube counts was recorded as the value of per well. The experiment was repeated twice with triplicate measurements in each experiment.

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Statistical analysis

All statistical analyses were performed with SPSS 13.0 statistical software (SPSS Inc., Chicago, USA). The data are presented as mean ± SD for each group and comparisons were performed using Student's *t*-test. $P < 0.05$ is considered statistically significant.

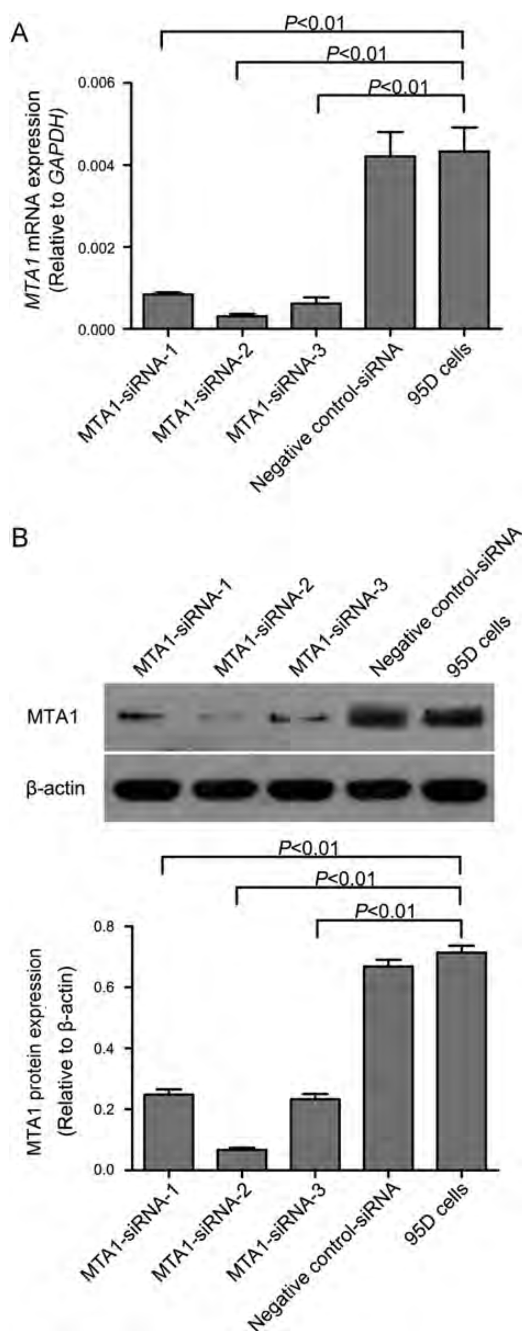

Figure 1 Effects of MTA1 siRNA on the expression of MTA1 mRNA and protein (A) The results of qRT-PCR showed that all the three MTA1-targeted siRNAs specifically silenced the *MTA1* mRNA expression in 95D cells, of which, MTA1-siRNA-2 showed the most powerful RNAi effects. (B) Western blot showed that all the three MTA1-targeted siRNAs specifically silenced the MTA1 protein expression of 95D cells, of which, MTA1-siRNA-2 showed the most powerful RNAi effects.

Results

Effects of MTA1 siRNA on the expression of MTA1 mRNA and protein

Three pieces of siRNA targeting *MTA1* gene were employed for function analysis in 95D cells, and the results of qRT-PCR demonstrated that all the three pieces of siRNA could down-regulate the expression of *MTA1* mRNA significantly [**Fig. 1(A)**, $P < 0.01$, respectively], of which, the *MTA1*-siRNA-2 showed the most powerful RNAi effects, while the negative control-siRNA did not significantly affect the expression of *MTA1* mRNA [**Fig. 1(A)**, $P > 0.05$]. Western blot results demonstrated that all the three pieces of siRNA also could down-regulate the expression of MTA1 protein significantly [**Fig. 1(B)**, $P < 0.01$, respectively], of which, the MTA1-siRNA-2 showed the most powerful RNAi effects and then was selected to further study the functions of MTA1 protein *in vitro*, while the negative control-siRNA did not significantly affect the expression of MTA1 protein [**Fig. 1(B)**, $P > 0.05$].

Down-regulation of MTA1 protein decreased the migration of 95D cells

The association between MTA1 protein and migration potential of 95D cells was explored by wound healing assay, and the results demonstrated that the healing ability of MTA1-siRNA 95D cells was significantly lower than that of 95D cells at 24 and 36 h after wound scratched ($P < 0.01$, respectively), but not significantly at 12 h after wound scratched (**Fig. 2**, $P > 0.05$), while no statistically significant differences of healing ability were detected between control-siRNA 95D cells and 95D cells at 12, 24, and 36 h after wound scratched (**Fig. 2**, $P > 0.05$, respectively).

Down-regulation of MTA1 protein decreased invasion of 95D cells

The association between MTA1 protein and invasion potential of 95D cells was explored by transwell invasion assay, and the results demonstrated that the number of MTA1-siRNA 95D cells (19.33 ± 2.08) penetrated to the lower side of the polycarbonate membrane was significantly smaller than that of 95D cells (51.67 ± 2.52, **Fig. 3**, $P < 0.01$), while no statistically significant difference of the number of penetrated cells was detected between control-siRNA 95D cells (48.00 ± 4.58) and 95D cells (51.67 ± 2.52, **Fig. 3**, $P > 0.05$).

Down-regulation of MTA1 protein decreased MMP-9 activity of 95D cells

The association between MTA1 protein and MMP-9 protein levels in the conditioned medium was explored by ELISA assay, and the results showed that down-regulation

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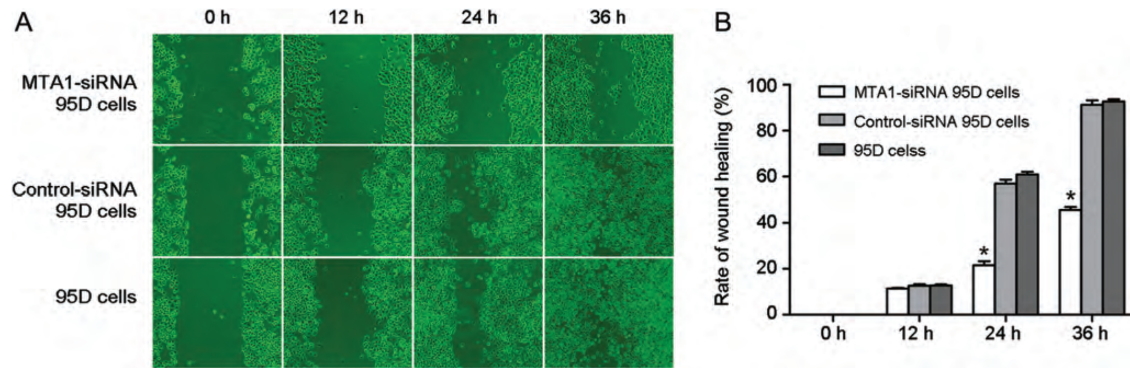

Figure 2 Down-regulation of MTA1 protein decreased the migration of 95D cells (A) The representative pictures of wound healing assay (×200). (B) The quantitative analysis of the migration potential of 95D cells (*$P < 0.01$).

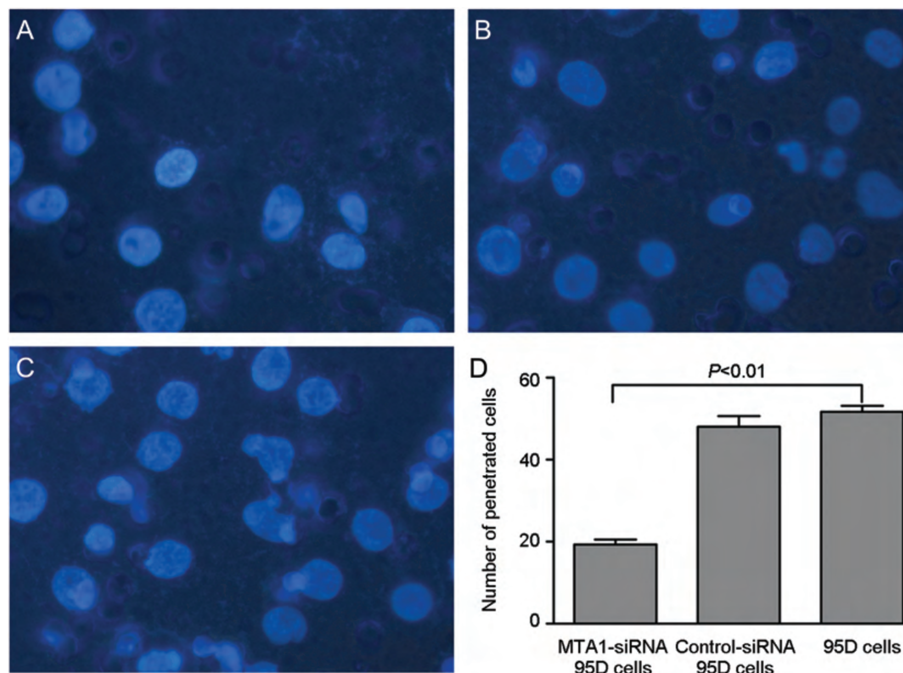

Figure 3 Down-regulation of MTA1 protein decreased the invasion of 95D cells Transwell invasion assay: (A) MTA1-siRNA 95D cells; (B) control-siRNA 95D cells; (C) 95D cells (×400). (D) The quantitative analysis of the invasion potential of 95D cells (*$P < 0.01$).

of MTA1 protein expression did not significantly decrease MMP-9 protein levels of 95D cells (**Fig. 4**, $P = 0.081$). Then we further investigated the association between MTA1 protein and MMP-9 activity by gelatin zymography assay, and the results demonstrated that the MMP-9 activity in the conditioned medium of MTA1-siRNA 95D cells was significantly lower than that of 95D cells (**Fig. 5**, $P < 0.01$), while no statistically significant difference of MMP-9 activity was detected between control-siRNA 95D cells and 95D cells (**Fig. 5**, $P > 0.05$).

Down-regulation of MTA1 protein decreased tube formation of HUVECs induced by conditioned medium

The association between MTA1 protein and tube formation of HUVECs was explored by tube formation assay induced by conditioned medium, and the results demonstrated that the number of complete tubes induced by the conditioned medium of MTA1-siRNA 95D cells (6.33 ± 2.52) was significantly smaller than that of 95D cells (43.00 ± 5.29, **Fig. 6**, $P < 0.01$), while no statistically significant difference of number of complete tubes was detected between control-siRNA 95D cells (40.33 ± 3.06) and 95D cells (43.00 ± 5.29, **Fig. 6**, $P > 0.05$).

Discussion

Invasion and metastasis is an extremely complex process and the major cause of therapeutic failure in patients with NSCLC, the detailed molecular mechanisms are still not completely clear and still under intensive investigations [4].

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Recent advances in the molecular biology have resulted in the discovery of a wide range of molecules closely related to human malignant tumor progression [17].

MTA1 protein, as one of transcriptional co-repressors, functions with histone deacetylase involved in the nucleosome remodeling and histone deacetylation complex and promotes malignant tumor metastasis by regulating many metastasis-related genes [18–21]. In addition, a transcriptional co-activator function of MTA1 protein has also been demonstrated [22,23]. It has been reported that MTA1 protein is involved in the aggressive potential of a wide range of human solid malignant tumors and various cancer cell lines [5–10]. However, the roles of MTA1 protein in regulating the migration and invasion of NSCLC cell line have not been investigated to date.

Recently, MTA1 protein has attracted widespread attention as one of the key molecules that plays indispensable roles in the angiogenesis of human malignant tumors [24–28]. Definitive studies further showed that under hypoxic conditions, MTA1 protein expression was strongly induced and deacetylated the hypoxia-inducible factor-1α (HIF-1α), leading to the enhanced transcriptional activity and stability of HIF-1α protein, and then increased the expression of vascular endothelial growth factor and promoted tumor angiogenesis [29,30]. On the basis of the available data, it is very likely that MTA1 protein plays important and critical roles in the angiogenesis of a wide variety of human malignant tumors. The angiogenic activities of MTA1 protein in NSCLC has been reported in our previous study [31]. However, the roles of MTA1 protein in angiogenesis of NSCLC cell line have not yet been addressed.

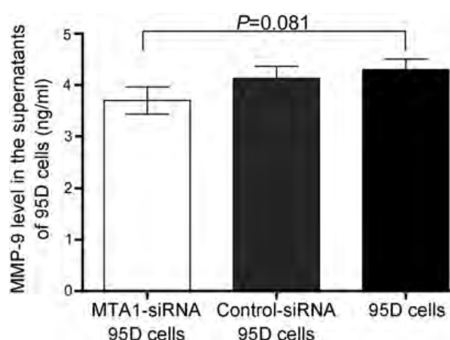

Figure 4 Down-regulation of MTA1 protein did not significantly decrease MMP-9 protein levels of 95D cells ELISA assay showed that down-regulation of MTA1 protein expression by RNAi decreased MMP-9 protein levels of 95D cells in the supernatants insignificantly.

To further specifically confirm the regulation of migration, invasion, and angiogenesis of 95D cells by MTA1 protein, siRNA-mediated down-regulation of MTA1 protein was employed. 95D cells were transfected with three siRNAs specific for MTA1 gene, and the MTA1-targeted siRNAs specifically and significantly down-regulated the MTA1 protein expression, while the level of MTA1 protein was unaffected with negative control-siRNA, demonstrating the highly selectivity and efficacy of these chemically synthesized siRNAs. After MTA1 protein was down-regulated by MTA1-siRNA, the migration and invasion potentials of 95D cells significantly decreased, which is consistent with the previous report [32], indicating that MTA1 protein mediated regulation of the aggressive phenotypes of 95D cells *in vitro*, while the regulating pathways is not clear. It is well known that MMP-9 is critically involved in the processes of cancer cell migration and invasion [33,34]. The regulatory role of MTA1 protein on MMP-9 has been investigated in different malignant tumors; however, the results were controversial [35–37]. This may be due to the complex process involving MTA1 protein regulates MMP-9 or MTA1 protein plays different regulatory roles on MMP-9 in different malignant tumors. Up to now, there were no data available regarding the regulatory roles of MTA1 protein on MMP-9 in NSCLC cell lines. Our results showed that down-regulation of MTA1 protein expression by RNAi did not significantly decrease MMP-9 protein levels of 95D cells, then we further performed gelatin zymography assay to detect the MMP-9 activity after MTA1 RNAi and found that MTA1-siRNA transfection decreased MMP-9 activity in the conditioned

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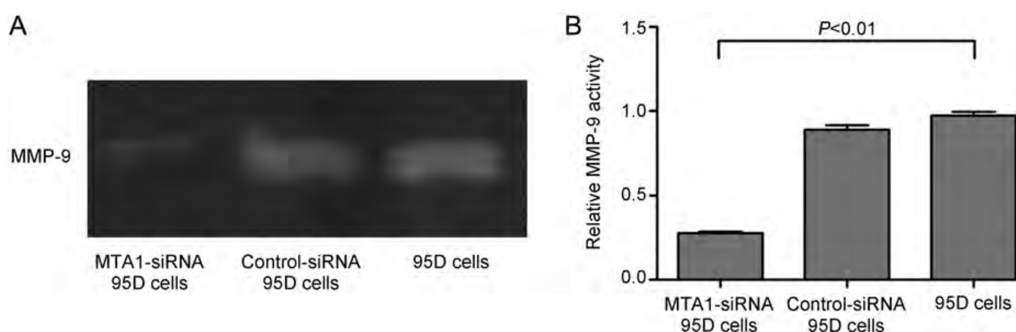

Figure 5 Down-regulation of MTA1 protein decreased MMP-9 activity of 95D cells (A) Gelatin zymography assay. (B) The quantitative analysis of the MMP-9 activity of 95D cells.

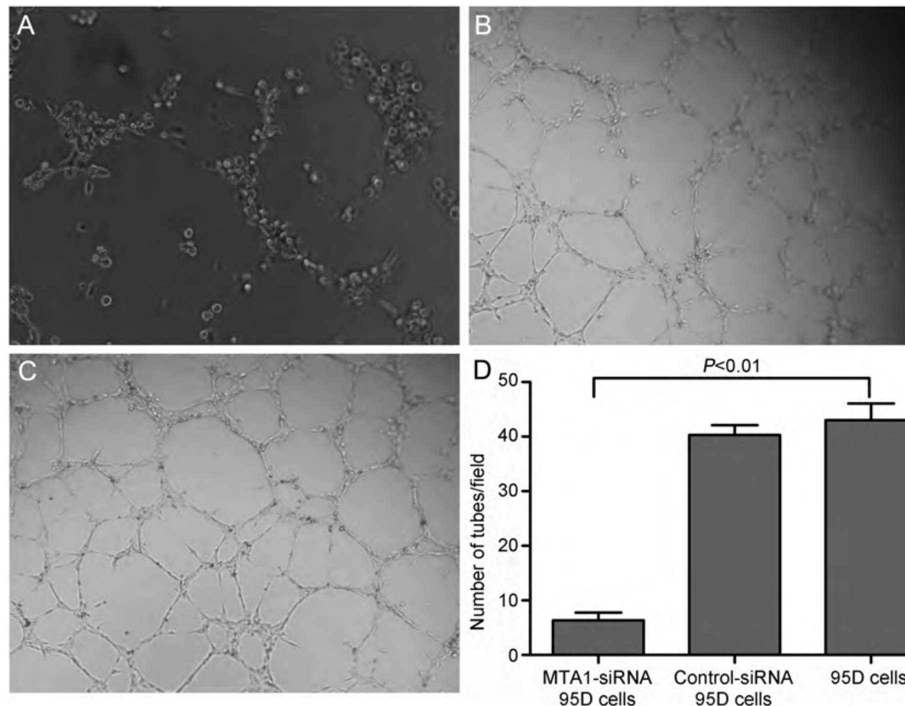

Figure 6 Down-regulation of MTA1 protein decreased tube formation of HUVECs induced by conditioned medium Tube formation assay: (A) MTA1-siRNA 95D cells; (B) control-siRNA 95D cells; (C) 95D cells (×200). (D) The quantitative analysis of the tube formation of HUVECs induced by conditioned medium.

medium significantly, indicating that MTA1 protein exerts its effects on migration and invasion of 95D cells partly by regulating the MMP-9 activity, rather than MMP-9 protein levels, and then providing a novel mechanism underlying the relation between MTA1 protein and aggressive phenotype of 95D cells. However, the exact mechanisms involved in MTA1 protein mediating MMP-9 activity still needs to be elucidated at molecule level.

NSCLC is an angiogenesis-dependent tumor and angiogenesis plays central roles in the progression and blood-borne metastases [38,39], then we analyzed the possible angiogenic potential of MTA1 protein by tube formation assay *in vitro*. Interestingly, our results showed that down-regulation of MTA1 protein by RNAi in 95D cells could significantly decrease the tube formation of HUVECs induced by conditioned medium, demonstrating a novel role for MTA1 protein in tumor angiogenesis and the angiogenic potential of MTA1 protein represents a novel therapeutic target for anti-angiogenesis in patients with NSCLC. However, the exact mechanisms necessary for MTA1 protein to exert its angiogenic functions in regulating tumor angiogenesis still remain to be elucidated by further in-depth studies.

Taken together, our studies provided direct experimental evidence for the first time that MTA1 protein mediated regulation of the migration, invasion, and angiogenesis potentials of 95D cells *in vitro*. These findings provided new insights and potentially clinical utility for MTA1 protein as an effective therapeutic target for NSCLC by inhibiting migration, invasion, and angiogenesis of cancer cells.

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References

1 Brognard J, Clark AS, Ni Y and Dennis PA. Akt/protein kinase B is constitutively active in non-small cell lung cancer cells and promotes cellular survival and resistance to chemotherapy and radiation. Cancer Res 2001, 61: 3986–3997.
2 Ren H, Tang X, Lee JJ, Feng L, Everett AD, Hong WK and Khuri FR, *et al*. Expression of hepatoma-derived growth factor is a strong prognostic predictor for patients with early-stage non-small-cell lung cancer. J Clin Oncol 2004, 22: 3230–3237.
3 Hoffman PC, Mauer AM and Vokes EE. Lung cancer. Lancet 2000, 355: 479–485.
4 Steeg PS. Tumor metastasis: mechanistic insights and clinical challenges. Nat Med 2006, 12: 895–904.
5 Toh Y and Nicolson GL. The role of the MTA family and their encoded proteins in human cancers: molecular functions and clinical implications. Clin Exp Metastasis 2009, 26: 215–227.

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6 Nawa A, Nishimori K, Lin P, Maki Y, Moue K, Sawada H and Toh Y, *et al*. Tumor metastasis-associated human MTA1 gene: its deduced protein sequence, localization, and association with breast cancer cell proliferation using antisense phosphorothioate oligonucleotides. J Cell Biochem 2000, 79: 202–212.

7 Mazumdar A, Wang RA, Mishra SK, Adam L, Bagheri-Yarmand R, Mandal M and Vadlamudi RK, *et al*. Transcriptional repression of oestrogen receptor by metastasis-associated protein 1 corepressor. Nat Cell Biol 2001, 3: 30–37.

8 Nicolson GL, Nawa A, Toh Y, Taniguchi S, Nishimori K and Moustafa A. Tumor metastasis-associated human MTA1 gene and its MTA1 protein product: role in epithelial cancer cell invasion, proliferation and nuclear regulation. Clin Exp Metastasis 2003, 20: 19–24.

9 Hofer MD, Menke A, Genze F, Gierschik P and Giehl K. Expression of MTA1 promotes motility and invasiveness of PANC-1 pancreatic carcinoma cells. Br J Cancer 2004, 90: 455–462.

10 Qian H, Lu N, Xue L, Liang X, Zhang X, Fu M and Xie Y, *et al*. Reduced MTA1 expression by RNAi inhibits in vitro invasion and migration of esophageal squamous cell carcinoma cell line. Clin Exp Metastasis 2005, 22: 653–662.

11 Zhu X, Guo Y, Li X, Ding Y and Chen L. Metastasis-associated protein 1 nuclear expression is associated with tumor progression and clinical outcome in patients with non-small cell lung cancer. J Thorac Oncol 2010, 5: 1159–1166.

12 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 2001, 25: 402–408.

13 Li L, Tian H, Yue W, Zhu F, Li S and Li W. Human mesenchymal stem cells play a dual role on tumor cell growth in vitro and in vivo. J Cell Physiol 2011, 226: 1860–1867.

14 Liang CC, Park AY and Guan JL. In vitro scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro. Nat Protoc 2007, 2: 329–333.

15 Bae CH, Chen SM, Lee HM, Song SY and Kim YD. The effect of doxycycline on PMA-induced MUC5B expression via MMP-9 and p38 in NCI-H292 cells. Clin Exp Otorhinolaryngol 2011, 4: 177–183.

16 Wang Z, Banerjee S, Kong D, Li Y and Sarkar FH. Down-regulation of Forkhead Box M1 transcription factor leads to the inhibition of invasion and angiogenesis of pancreatic cancer cells. Cancer Res 2007, 67: 8293–8300.

17 Sidransky D. Emerging molecular markers of cancer. Nat Rev Cancer 2002, 2: 210–219.

18 Kumar R, Wang RA and Bagheri-Yarmand R. Emerging roles of MTA family members in human cancers. Semin Oncol 2003, 30: 30–37.

19 Fearon ER. Connecting estrogen receptor function, transcriptional repression, and E-cadherin expression in breast cancer. Cancer Cell 2003, 3: 307–310.

20 Manavathi B and Kumar R. Metastasis tumor antigens, an emerging family of multifaceted master coregulators. J Biol Chem 2007, 282: 1529–1533.

21 Singh RR and Kumar R. MTA family of transcriptional metaregulators in mammary gland morphogenesis and breast cancer. J Mammary Gland Biol Neoplasia 2007, 12: 115–125.

22 Gururaj AE, Singh RR, Rayala SK, Holm C, den Hollander P, Zhang H and Balasenthil S, *et al*. MTA1, a transcriptional activator of breast cancer amplified sequence 3. Proc Natl Acad Sci USA 2006, 103: 6670–6675.

23 Gururaj AE, Holm C, Landberg G and Kumar R. Breast cancer-amplified sequence 3, a target of metastasis-associated protein 1, contributes to tamoxifen resistance in premenopausal patients with breast cancer. Cell Cycle 2006, 5: 1407–1410.

24 Jang KS, Paik SS, Chung H, Oh YH and Kong G. MTA1 overexpression correlates significantly with tumor grade and angiogenesis in human breast cancers. Cancer Sci 2006, 97: 374–379.

25 Ryu SH, Chung YH, Lee H, Kim JA, Shin HD, Min HJ and Seo DD, *et al*. Metastatic tumor antigen 1 is closely associated with frequent postoperative recurrence and poor survival in patients with hepatocellular carcinoma. Hepatology 2008, 47: 929–936.

26 Miyake K, Yoshizumi T, Imura S, Sugimoto K, Batmunkh E, Kanemura H and Morine Y, *et al*. Expression of hypoxia-inducible factor-1alpha, histone deacetylase 1, and metastasis-associated protein 1 in pancreatic carcinoma: correlation with poor prognosis with possible regulation. Pancreas 2008, 36: 1–9.

27 Yoo YG, Na TY, Seo HW, Seong JK, Park CK, Shin YK and Lee MO. Hepatitis B virus X protein induces the expression of MTA1 and HDAC1, which enhances hypoxia signaling in hepatocellular carcinoma cells. Oncogene 2008, 27: 3405–3413.

28 Kai L, Wang J, Ivanovic M, Chung YT, Laskin WB, Schulze-Hoepfner F and Mirochnik Y, *et al*. Targeting prostate cancer angiogenesis through metastasis-associated protein 1 (MTA1). Prostate 2011, 71: 268–280.

29 Yoo YG, Kong G and Lee MO. Metastasis-associated protein 1 enhances stability of hypoxia-inducible factor-1alpha protein by recruiting histone deacetylase 1. EMBO J 2006, 25: 1231–1241.

30 Moon HE, Cheon H, Chun KH, Lee SK, Kim YS, Jung BK and Park JA, *et al*. Metastasis-associated protein 1 enhances angiogenesis by stabilization of HIF-1alpha. Oncol Rep 2006, 16: 929–935.

31 Li SH, Tian H, Yue WM, Li L, Li WJ, Chen ZT and Hu WS, *et al*. Overexpression of metastasis-associated protein 1 is significantly correlated with tumor angiogenesis and poor survival in patients with early stage non-small cell lung cancer. Ann Surg Oncol 2011, 18: 2048–2056.

32 Zhu X, Zhang X, Wang H, Song Q, Zhang G, Yang L and Geng J, *et al*. MTA1 gene silencing inhibits invasion and alters the microRNA expression profile of human lung cancer cells. Oncol Rep 2012, 28: 218–224.

33 Littlepage LE, Sternlicht MD, Rougier N, Phillips J, Gallo E, Yu Y and Williams K, *et al*. Matrix metalloproteinases contribute distinct roles in neuroendocrine prostate carcinogenesis, metastasis, and angiogenesis progression. Cancer Res 2010, 70: 2224–2234.

34 Choi JY, Jang YS, Min SY and Song JY. Overexpression of MMP-9 and HIF-1α in breast cancer cells under hypoxic conditions. J Breast Cancer 2011, 14: 88–95.

35 Yan C, Wang H, Toh Y and Boyd DD. Repression of 92-kDa type IV collagenase expression by MTA1 is mediated through direct interactions with the promoter via a mechanism, which is both dependent on and independent of histone deacetylation. J Biol Chem 2003, 278: 2309–2316.

36 Li JR, Li MQ, Bao JT and Li JZ. Correlation between expression of metastasis-associated gene 1 and matrix metalloproteinase 9 and invasion and metastasis of breast cancer. Zhonghua Yi Xue Za Zhi 2008, 88: 2278–2280.

37 Jiang Q, Zhang H and Zhang P. ShRNA-mediated gene silencing of MTA1 influenced on protein expression of ER alpha, MMP-9, cyclinD1 and invasiveness, proliferation in breast cancer cell lines MDA-MB-231 and MCF-7 in vitro. J Exp Clin Cancer Res 2011, 30: 60–70.

38 Shijubo N, Kojima H, Nagata M, Ohchi T, Suzuki A, Abe S and Sato N. Tumor angiogenesis of non-small cell lung cancer. Microsc Res Tech 2003, 60: 186–198.

39 D'Amico TA. Angiogenesis in non-small cell lung cancer. Semin Thorac Cardiovasc Surg 2004, 16: 13–18.

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