Regular paper

S100A4 promotes invasion and angiogenesis in breast cancer MDA-MB-231 cells by upregulating matrix metalloproteinase-13

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S100A4 is a member of the S100 family of calcium-binding proteins that is directly involved in tumor metastasis. In the present study, we examined the potential role of S100A4 in metastasis in breast cancer and its relation with matrix metalloproteinase-13 (MMP-13). Analysis of 100 breast cancer specimens including 50 with and 50 without lymph node metastasis showed a significant upregulation of S100A4 and MMP-13 expression in metastatic breast cancer tissues. Positive immunoreactivity for S100A4 was associated with MMP-13 expression. Overexpression of S100A4 in the MDA-MB-231 breast cancer cell line upregulated MMP13 expression leading to increased cell migration and angiogenesis. SiRNA-mediated silencing of S100A4 downregulated MMP13 expression and suppressed cell migration and angiogenesis. Moreover, neutralization of MMP-13 activity with a specific antibody blocked cell migration and angiogenesis in MDA-MB-231/S100A4 cells. *In vivo* siRNA silencing of S100A4 significantly inhibited lung metastasis in transgenic mice. The present results suggest that the S100A4 gene may control the invasive potential of human breast cancer cells by modulating MMP-13 levels, thus regulating metastasis and angiogenesis in breast tumors. S100A4 could therefore be of value as a biomarker of breast cancer progression and a novel therapeutic target for human breast cancer treatment.

INTRODUCTION

S100A4, also known as mts1, p9Ka, FSP1, CAPL, calvasculin, pEL98, metastasin, 18A2, and 42A, was cloned in the 1980s and early 1990s from various cell systems (Garrett *et al.*, 2006). The human S100A4 gene is located in a frequently rearranged gene cluster on chromosome 1q21 and is composed of four exons, of which the first two are noncoding (Garrett *et al.*, 2006). The 101-amino acid protein has a molecular mass of approximately 11.5 kDa and is characterized by the presence of two Ca^{2+}-binding EF-hands. Upon Ca^{2+}-binding, S100A4 undergoes a conformational change, forming a hydrophobic pocket essential for the recognition of target proteins (Malashkevich *et al.*, 2008).

A number of studies have demonstrated the involvement of the S100A4 gene product in the promotion of metastasis. Introduction of the S100A4 gene into non-metastatic tumor cell lines and suppression of its activity in cells with known metastatic potential proved its involvement in metastasis formation (Ford *et al.*, 1995; Maelandsmo *et al.*, 1996; Klingelhöfer *et al.*, 1997; Lloyd *et al.*, 1998; Wang *et al.*, 2006). Furthermore, transgenic mouse models overexpressing the S100A4 gene showed enhanced metastatic activity (Ambartsumian *et al.*, 1996; Levett *et al.*, 2002; Liu *et al.*, 2010). Recent studies showed that S100A4 expression is associated with metastatic breast cancers (Rudland *et al.*, 2000; Ismail *et al.*, 2008), and is upregulated in cancer tissues compared with normal breast tissue, suggesting that S100A4 plays a role in the metastatic phenotype. However, the mechanisms by which S100A4 promotes tumor progression or metastasis have not been elucidated.

Matrix metalloproteinases (MMPs) play an essential role in tumor metastasis and invasion, and several experimental strategies have established an intimate connection between S100A4 and certain members of the MMP family. In a recent study, treatment of mouse endothelial SVEC 4-10 cells with the S100A4 protein caused the transcriptional activation of collagenase 3 (MMP-13) mRNA followed by the subsequent release of the protein from the cells. The resulting enhancement of the proteolytic activity associated with MMP-13 was demonstrated by beta-casein zymography. These findings suggest that S100A4 may induce the synthesis of extracellular matrix (ECM) degrading enzymes resulting in the remodeling of the ECM (Schmidt-Hansen *et al.*, 2004).

In vitro and *in vivo* models of metastatic breast cancer have provided evidence supporting the direct involvement of S100A4 in tumor progression and metastasis (Simpson *et al.*, 2003; Jenkinson *et al.*, 2004). Clinical evidence suggests that matrix metalloproteinase-13 (MMP-13) could be a useful prognostic marker for predicting the development and progression of breast cancer (Zhang *et al.*, 2008).

In the present study, the expressions of S100A4 and MMP-13 and their relationship with breast cancer progression and metastasis were analyzed *in vivo* and *in vitro* to gain insight into the mechanisms of S100A4-induced metastatic progression.

MATERIALS AND METHODS

Tissue sample collection. One-hundred paraffin-embedded archival specimens of primary breast cancer from patients with and without lymph node metastasis

(n = 50 each) were included in the study. None of the patients had received pretreatment.

Cell culture. MDA-MB-231(MDA-231) human breast cancer cells were purchased from the American Type Culture Collection and cultured in DMEM supplemented with 10% fetal bovine serum in a humidified CO_2 incubator at 37°C. High expression levels of S100A4 (Matsuura*et al.,* 2010) and MMP-13 (McKay *et al.,* 2002) have been reported in the MDA-231 cell line.

Immunohistochemistry. Specimens were cut into 4 μm sections and mounted on positively charged slides, incubated for 30 min at 60°C, deparaffinized by conventional methods, and washed in Tris-buffered saline buffer. Antigen retrieval was performed for 20 min in 10 mmol/L of sodium citrate buffer (pH 6.0) heated at 95°C in a steamer, followed by cooling for 20 min. After blocking endogenous peroxidase activity with a 3% aqueous H_2O_2 solution for 5 min, sections were incubated with polyclonal anti-S100A4 (1:100, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) and anti-MMP-13 (1:150, Santa Cruz) antibodies. The percentage of carcinoma cells with cytoplasmic staining was calculated from two sections of each specimen, 10 fields/section, at 200× magnification. Specimens were classified into two groups according to the expression of S100A4: specimens with <1% S100A4 staining were designated as negative (–) and those with ≥1% were designated as positive (+). For MMP-13, specimens with <5% stained cells were designated as negative (–) and those with ≥5% were designated as positive.

siRNA transfection. Pre-designed siRNAs were purchased from Invitrogen. The siRNA targeted sequences were 5'-AACGAGGTGGACTTCCAAGAG-3' for S100A4, and 5'-AATTCTCCGAACGTGTCTCGT-3' for the non-silencing control siRNA. The siRNAs were transfected into MDA-231 cells using Lipofectamine™ RNAi Max (Invitrogen) for 48 h according to the manufacturer's instructions.

Plasmid construction and transfection. The pGEM-S100A4 plasmid was procured from Biovector Science Lab, Inc., and S100A4-cDNA was isolated from pGEM-S100A4 using the BamH I restriction endonuclease. The digested fragment was then ligated into the pcDNA3.1 vector (Invitrogen) previously digested with BamH I and treated with calf intestinal alkaline phosphatase. The resulting construct was verified by direct sequencing. For transfection studies, MDA-231 cells were plated at a density of 1×10^6 cells per well in six-well plates and incubated for 24 h in complete medium. The cells were then transfected with 2–4 μg of the S100A4 construct using Lipofectamine™. The same amount of empty vector was transfected as a positive control. Dulbecco's Modified Eagle's Medium (DMEM) containing G418 (600 μg/mL) was used for selection of stable transfectants, and DMEM with 300 μg/mL G418 was used for maintenance of the stable transfectants.

Western blotting. Cells were harvested by trypsinization after transfection with the pcDNA3.1-S100A4 plasmid (24 h) or S100A4 siRNA plasmid (48 h), washed with PBS, and lysed overnight at –20°C in lysis buffer (20 mmol/L Tris/HCl, pH 7.6, 100 mmol/L NaCl, 20 mmol/L KCl, 1.5 mmol/L $MgCl_2$ and 0.5% Nonidet P-40) containing phosphatase inhibitors (10 mmol/L NaF, 1 mmol/L Na_3VO_4 and 50 mmol/L β-glycerophosphate) and protease inhibitors (20 μg/mL leupeptin, 10 μg/mL pepstatin A and 4 μg/mL aprotinin). Debris was sedimented by centrifugation for 10 min at $14\,000 \times g$, and the protein concentration of the supernatant was determined using a Bio-rad protein de-

tection assay kit. Proteins in the total cell lysate (40 μg of protein) were separated by 10% *sodium dodecyl sulfate* polyacrylamide gel electrophoresis (SDS/PAGE) and electrotransferred to a polyvinylidene difluoride membrane (Immobilon-P membrane; Millipore, Bedford, MA). After blocking the membranes in a solution of 5% skim milk and 0.1% Tween-20 in PBS, membrane-bound proteins were probed with primary antibodies against S100A4 (1:100) and MMP-13 (1:200). The membrane was washed and then incubated with horseradish peroxidase-conjugated secondary antibodies for 30 min. Antibody-bound protein bands were detected using enhanced chemiluminescence reagents (Amersham Pharmacia Biotech, Piscataway, NJ) and visualized by autoradiography with Kodak X-Omat Blue film (Perkin Elmer Life Sciences, Boston, MA).

RT-PCR. Total RNA was isolated from the transfected cells using the Qiagen RNeasy kit (Qiagen, Inc., Valencia, CA) according to the manufacturer's protocol, and the OneStep RT-PCR kit (Qiagen) was used for detecting mRNA expression of S100A4 and MMP-13. First-strand cDNA was prepared using Omniscript and Sensiscript reverse transcriptases at 50°C for 30 min. PCR amplification was performed under the following conditions: 95°C for 15 min, followed by 35 cycles at 94°C for 1 min, 56°C for 1 min, and 72°C for 1 min. The final extension was completed at 72°C for 10 min. The primers used are shown below: S100A4 forward, 5'-CAGATCCTGACTGCTGCCATGGCG-3'; S100A4 reverse, 5'-ACGTGTCTGAAGGAGCCATGGTGG-3'; MMP-13 forward, 5'-CTATCCTGGCCACCTTCTTC-3'; MMP-13-reverse, 5'-GGGACCATTTGAGTGTTCTAGG-3'; GAPDH-forward, 5'-CCACCCATGGCAAATTCCATCGCA-3'; GAPDH reverse, 5'-TCTA GACGGCAGGTGAGGTCCACC-3'.

Gelatin zymography. An equal number of cells (1×10^6) was transfected with the S100A4 siRNA and pcDNA3.1-S100A4 plasmids, and after 24–48 h, the conditioned media were harvested, concentrated, and electrophoresed (10 μg of protein) under nonreducing conditions. The gelatinolytic activity of MMP-13 was determined with a zymography kit (Invitrogen) according to the manufacturer's protocol.

Cell motility and invasion assay. To assess the motility and invasion of MDA-231 cells, the upper chambers (8 μm pore size) of transwells (Costar) were coated with collagen at 4°C; Matrigel (0.5 μg; Collaborative Research) diluted with cold water was then dried onto each filter overnight at room temperature. Transwells were washed with PBS and cells transfected with the pcDNA3.1-S100A4 plasmid for 36 h or the S100A4 siRNA plasmid for 48 h were added to the upper chamber using serum-free DMEM/bovine serum albumin. LPA was added to the lower chamber as a chemoattractant. The chambers were incubated for 2 h at 37°C in the presence of 10% CO_2. The cells that attached to the bottom side of the membrane were stained and counted using crystal violet. Assays were performed in triplicate and repeated several times.

***In vitro* angiogenesis assay (Chicken embryo chorioallantoic membrane assay)**. Fertilized white Leghorn hen eggs were incubated in a humidified chamber at 37.8°C and 67% humidity. At day 3 of development, a window was cut into the egg shell and sealed again with adhesive plaster. The eggs were further incubated for 6 days. At 9 days, the window was opened and the chorioallantoic membranes (CAMs) of the chicken embryos were transduced with supernatant of MDA-231 cells [after transfection with pcDNA3.1-S100A4 plasmid

Table 1. Relationship between S100A4 and MMP-13 expression and node status

Node Status	n	S100A4 Negative	S100A4 Positive	X^2	P	MMP-13 Negative	MMP-13 Positive	X^2	P
Node+	50	11 (22%)	39(78%)	5.17	0.003	9 (18%)	41 (82%)	4.83	0.008
Node-	50	29 (58%)	21(42%)			33 (66%)	17 (34%)		

(24 h) or S100A4 siRNA plasmid (48 h)]. Eggs were returned to the incubator for four additional days. At 13 days, the eggs were opened and the CAM with the Thermanox™ ring was analyzed and photographed under a stereo microscope fitted with a digital camera and flexible cold light (Olympus SZ51, Olympus E410). Blood vessels were counted inside the ring area (20 mm²). The allantoic vascular plexus was subclassified into large vessels (diameter > 50 μm) and microvessels (diameter < 50 μm).

***In vivo* experimental metastasis assays.** MDA-231 cells (1×10^6) transfected with S100A4 siRNA for 48 h or stably transfected with the pcDNA3.1-S100A4 plasmid were injected into the tail vein of 6- to 8-week-old mice (n = 6). After 21 days, the mice were sacrificed, and the lungs were fixed in Bouin's solution. Lung tumors were analyzed first by obtaining a surface tumor count, and then by assessing tumor area in histologic sections from multiple depths throughout the lungs.

Statistical analysis. All statistical analyses were performed using SPSS13.0 software. The results were expressed as means ±S.D. Differences between the groups were assessed by the Student's *t*-test or Pearson's chi-square test (χ^2) and $P < 0.05$ was considered to indicate statistical significance.

RESULTS

Relationship between S100A4 and MMP-13 expression and node status

S100A4 and MMP-13 protein expression was detected in the cytoplasm of 60% and 58% of breast cancer specimens, respectively (Fig. 1). Increased S100A4 and MMP-13 expression levels were correlated with lymph node metastases ($P < 0.01$, all) (Table 1), and there was a significant correlation between the expression levels of S100A4 and MMP-13 in breast cancer cells ($P = 0.001$) (Table 2). These results indicate that metastasis from breast cancer is associated with significantly increased expression of S100A4 and MMP13.

siRNA mediated knock-down of S100A4 expression in MDA-MB-231 cell

S100A4 siRNA transfection of cells decreased the levels of S100A4 protein (Fig. 2A) and mRNA (Fig. 2B) in a dose-dependent manner as shown by western blotting and RT-PCR. Non-silencing siRNAs had no effect on the S100A4 protein level (data not shown). These data confirmed the siRNA mediated knock-down of S100A4 expression. Based on the lowest levels of S100A4 expression, a 48 h siRNA transfection was used in all subsequent experiments.

S100A4 overexpression in MDA-MB-231 cells

Overexpression of S100A4 was confirmed by western blotting 36 h after transfection. Based on the highest levels of S100A4 expression, a 36 h transfection

was used for further studies. MDA-231 cells transfected with the pcDNA3.1-S100A4 plasmid showed significantly increased S100A4 levels compared with vector control-transfected cells (Fig. 2C).

Effect of S100A4 on motility, invasion and angiogenesis of MDA-MB-231 cells

The S100A4 gene has been reported to confer invasive characteristics on various cancer cells. We therefore examined the effect of S100A4 gene suppression or overexpression on the motility, invasion and angiogenesis of the highly invasive and metastatic MDA-231 cell line using in vitro chemoinvasion and **chicken embryo CAM** assays. As shown in Figure 2D, suppression of S100A4 expression using siRNA effectively blocked MDA-231 cell motility and invasion, whereas S100A4 overexpression promoted MDA-231 cell motility and invasion, suggesting that S100A4 plays an essential role in metastasis. The effect of S100A4 on angiogenesis was assessed using an in vitro CAM assay. The supernatant of S100A4-siRNA or S100A4 cDNA-transfected MDA-231 cells was added to the CAMs of chicken embryos and the total tube area was quantified. S100A4-siRNA-transfected MDA-231 cell supernatants effectively decreased the total tube area, whereas S100A4 cDNA-transfected MDA-231 cell supernatants increased the total tube area (Fig. 2E).

S100A4 regulates MMP-13 expression and its enzymatic activity

The effect of S100A4 silencing or overexpression on the expression and activity of MMP-13 was assessed. S100A4 siRNA transfection significantly decreased MMP-13 mRNA (Fig. 3A) and protein (Fig. 3B) levels, whereas S100A4 overexpression significantly increased MMP-13 mRNA (Fig. 3A) and protein (Fig. 3B) levels in MDA-231 cells. Transfection with mock vector had no effect. The effect of S100A4 knockdown or overexpression on the activity of MMP-13 was examined by gelatin zymography. S100A4 gene silencing significantly
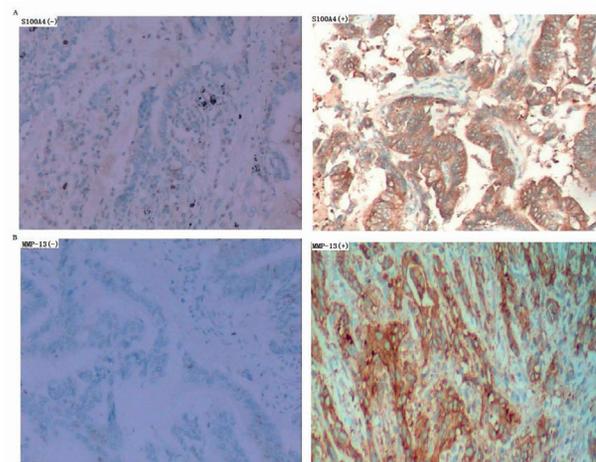
Figure.1. Representative immunohistochemistry of an infiltrating ductal carcinoma.
(A) S100A4; **(B)** MMP-13. The brown stain represents the indicated antigen and the nuclei are counterstained in blue. ×100.

Table 2. Relationship between S100A4 and MMP-13 expression levels

S100A4 expression	No.	MMP-13 expression		X²	P
		Positive	Negative		
Positive	60 (60%)	46 (76.7%)	14 (23.3%)		
Negative	40 (40%)	12 (30%)	28 (70%)	6.75	0.001

decreased MMP-13 enzymatic activity, and S100A4 over-expression significantly increased MMP-13 enzymatic activity in MDA-231 cells (Fig. 3C). These results indicated that the S100A4-mediated decrease or increase of MMP-13 mRNA and protein levels led to the inhibition or enhancement of MMP-13 enzymatic activity ($P<0.05$).

S100A4 overexpression promotes metastasis through MMP-13 upregulation

To determine whether the effect of S100A4 on promoting invasion and angiogenesis in MDA-231 cells is mediated by MMP-13, cells were pretreated with 0.1 units of a neutralizing MMP-13 antibody for 12 h before transient transfection with S100A4 cDNA (4 μM) for 36 h. The results showed that the enhanced invasion and angiogenesis caused by S100A4 overexpression and MMP-13 protein and mRNA upregulation (Fig. 3A and B) were suppressed by MMP-13 neutralization, which inhibited the upregulation of MMP-13 by S100A4 cDNA transfection (data not shown) and the invasive phenotype of MDA-231 cells (Fig. 4A and B).

S100A4 regulates the metastatic potential of MDA-MB-231 cells

The effect of S100A4 on metastasis was examined in vivo using a mouse tumor model. Mice transfected with S100A4 siRNA showed a 60% reduction of the number of surface tumors compared to control mice (22.7 vs. 58.6; Fig. 5A). In contrast, overexpression of S100A4

had a significant effect on the development of lung metastases, causing a 50% increase in the number of surface tumors compared to the controls (S100A4 cDNA transfected mice median tumor number=109.4; Fig. 5B). To further examine the metastatic lesions, the lungs were sectioned at different depths and the specimens were assessed morphometrically to determine the percent of lung parenchyma occupied by tumor lesions. Similar to the surface count results, a significant reduction in tumor burden was observed in the lungs of S100A4 siRNA transfected mice compared to littermate controls. The 73% reduction in total tumor area in the lungs correlated well with the 60% reduction in the number of surface tumor lesions (Fig. 5C). In contrast, S100A4 cDNA transfected mice showed a significant increase in tumor burden in the lungs compared to littermate controls. The 50% increase in total tumor area in the lungs correlated well with the 50% increase in the number of surface tumor lesions (Fig. 5D).

DISCUSSION

Metastasis is generally defined as the spread of malignant cells from the primary tumor to distant sites through the circulation. Studies in rodents have provided evidence supporting direct involvement of S100A4 in tumor progression and metastasis. The role of S100A4 in cancer has been examined extensively in breast cancer models, in which overexpression of S100A4 in nonmetastatic mammary tumor cells confers a metastatic phenotype (Davies *et al.*, 1993; Grigorian *et al.*, 1996). Transgenic mice overexpressing S100A4 in the mammary epithelium are phenotypically indistinguishable from wild-type mice (Ambartsumian *et al.*, 1996), demonstrating that S100A4 itself is not tumorigenic. However, transgenic mouse models of breast cancer have shown that S100A4 expression correlates with metastasis. The link
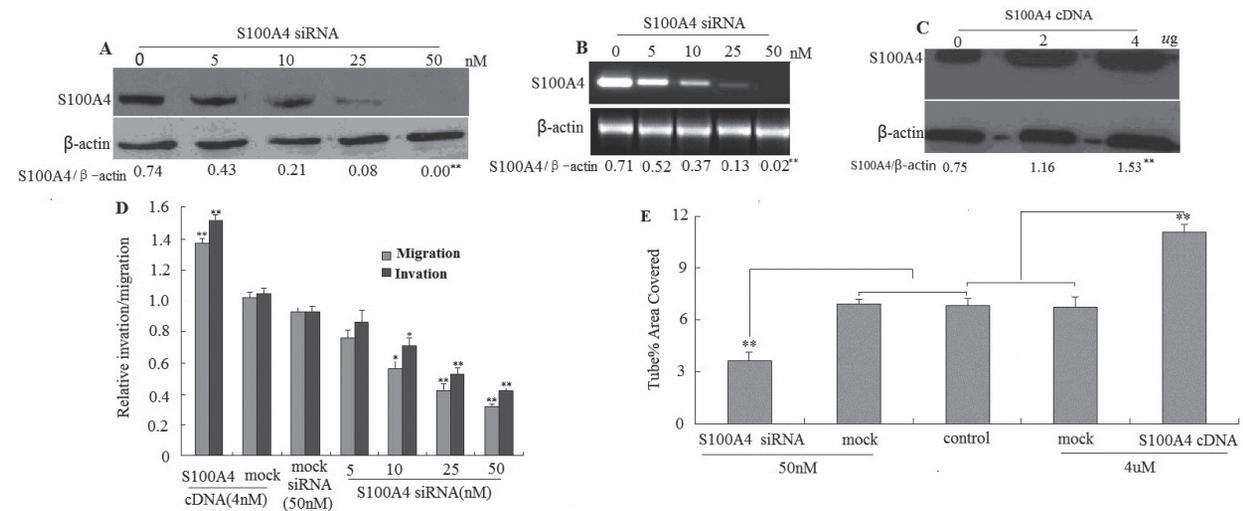
Figure 2. siRNA-mediated knockdown of S100A4 expression in MDA-MB-231 cells
(A and **B)** Representative images showing S100A4 protein and mRNA expression levels in S100A4-siRNA transfected cells as determined by western blotting (A) and RT-PCR (B). **(C)** Representative images showing S100A4 protein levels in S100A4 cDNA-transfected cells as determined by western blotting. **(D)** The motility and invasion ability of MDA-231 cells was assessed using a transwell cell motility assay as described in the Materials and Methods section. Each bar represents the mean ±S.E. Statistical analyses were performed using the Student's t-test. **$P<0.01$ or *$P<0.05$ compared with the controls. All experiments were repeated three times with similar results. **(E)** The supernatant of MDA-231 cells transfected with S100A4 siRNA inhibited angiogenesis, and the supernatant of MDA-231 cells transfected with S100A4 cDNA enhanced angiogenesis in the CAMs of hen embryos with a statistically significant decrease or increase in the percentage of tube area covered compared with untreated groups (*$P<0.01$).
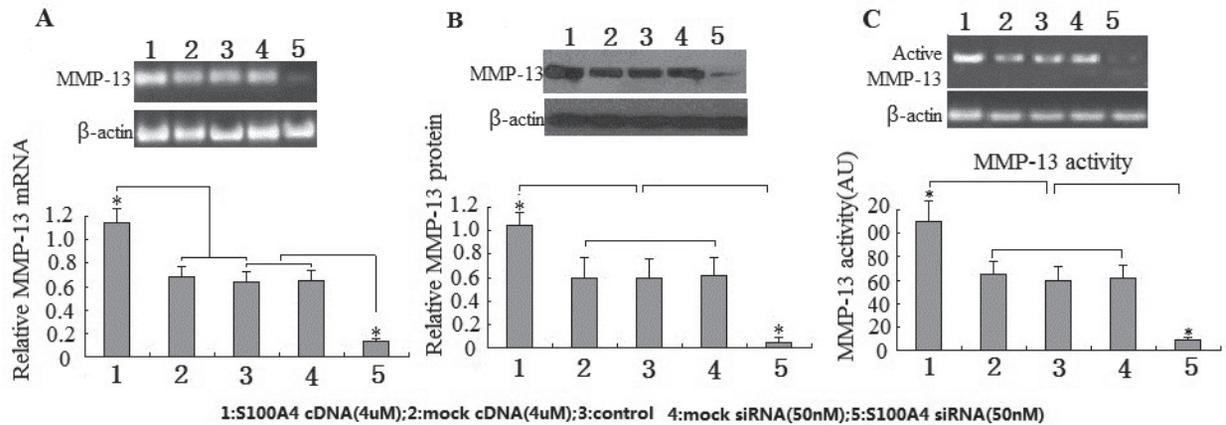
Figure 3. Effect of S100A4 gene knockdown or overexpression on the expression and activity of MMP-13
(A) Effect of S100A4 overexpression or silencing on MMP-13 mRNA levels in MDA-231 cells assessed by RT-PCR. **(B)**, Effect of S100A4 overexpression or silencing on MMP-13 protein levels in MDA-231 cells assessed by western blotting. **(C)** Effect of S100A4 overexpression or silencing on MMP-13 enzymatic activity in MDA-231 cells assessed by gelatin zymography. All experiments were repeated three times with similar results.

between S100A4 and metastasis is further supported by knockdown experiments, as inhibition of S100A4 expression by antisense or anti-ribozyme techniques suppresses the metastatic capacity of S100A4-expressing tumor cells in animal models of lung carcinoma (Maelandsmo *et al.,* 1996). However, the exact mechanisms underlying the metastasis-promoting effects of S100A4 are less well defined.

As a member of the S100 family of Ca^{2+} binding proteins, S100A4 functions intra- and extracellularly. When released into the extracellular space, it promotes the differentiation of neurons and osteoclasts (Novitskaya *et al.,* 2000; Duarte *et al.,* 2003). Furthermore, S100A4 acts as an angiogenic factor by stimulating the motility of endothelial cells (Ambartsumian *et al.,* 2001). It has been shown that S100A4 is released by tumor and normal cells (Ambartsumian *et al.,* 2001; Duarte *et al.,* 2003). Taken together, these findings indicate that extracellular S100A4 may generate a microenvironment that promotes invasion and motility of both endothelial and tumor cells, and therefore facilitates the dissemination of tumor cells in the organism.

Our results showed that S100A4 overexpression was associated with lymph node metastasis from breast cancer. Furthermore, S100A4 expression was significantly correlated with the levels and activity of MMP-13. These results indicate an association between breast cancer metastasis and increased levels of S100A4 and MMP13, and suggest that S100A4 may promote metastasis through MMP13 upregulation. We also showed that S100A4 overexpression increased the invasiveness of MDA-231 cells and stimulated angiogenesis, whereas S100A4 knockdown reversed this effect *in vitro*. Furthermore, downregulation of S100A4 expression suppressed lung metastasis in a transgenic mouse model, whereas overexpression of the protein had the opposite effect.

The ability to migrate is a prerequisite for a cancer cell to escape the primary tumor and enter the circulation. However, to travel through the surrounding stroma, these locally invading cells must be able to proteolytically degrade extracellular matrix components. Matrix metalloproteinases (MMPs) play an essential role in this process.

MMP-13 (collagenase-3) was originally cloned from a human breast tumor cDNA library (Freije *et al.,* 1994), and has since been shown to be elevated in a variety of cancers (Acuff *et al.,* 2006). MMP-13 was reported to affect the levels of a functionally diverse range of proteins, including those known to be involved in the promotion of tumor growth, angiogenesis and metastasis (Neutzner *et al.,* 2007). An association between S100A4 expression,
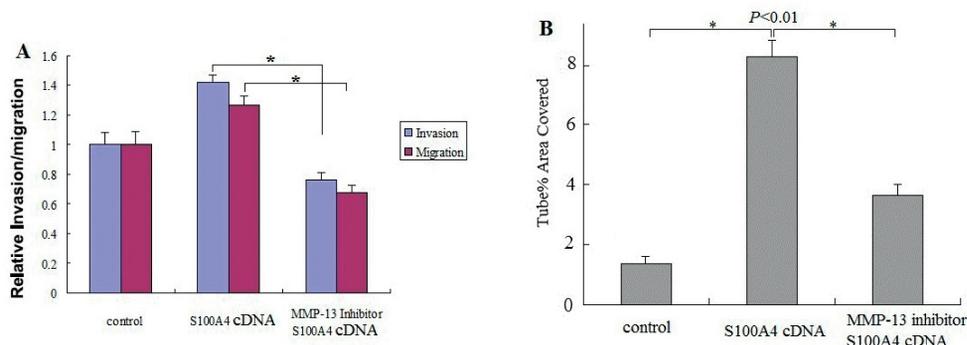
Figure 4. MMP-13 inhibition suppresses S100A4-induced invasion and angiogenesis
(A) Representative histogram showing the invasive capability of MDA-231 cells transfected with S100A4 cDNA and treated with an MMP-13 inhibitor assessed in a chemoinvasion chamber. **(B)** Representative histogram showing the tube percent area covered in MDA-231 cells transfected with S100A4 cDNA and treated with an MMP-13 inhibitor. Each bar represents the mean ±S.E.; *$P < 0.01$. All experiments were repeated three times with similar results.
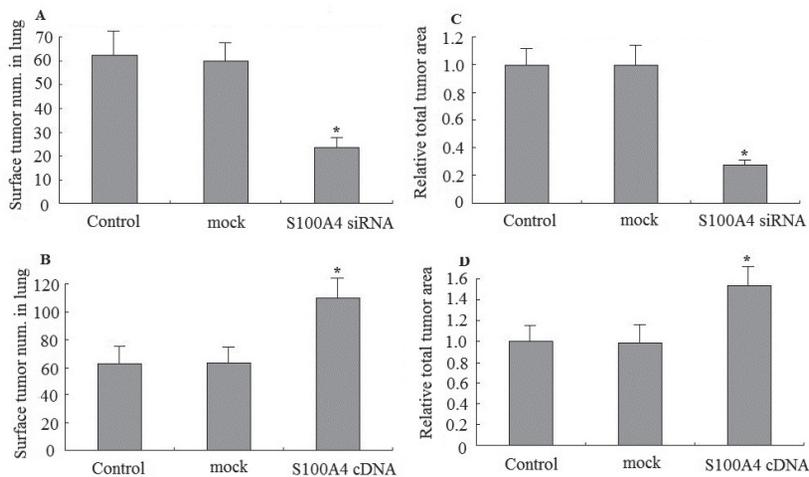
Figure.5. Effect of S100A4 on lung metastasis
(**A** and **B**) Representative histograms showing the surface tumor number in the S100A4 siRNA and S100A4 cDNA groups. (**C** and **D**) Representative histograms showing the total tumor area in the lungs of the S100A4 siRNA and S100A4 cDNA groups. Each bar represents mean ±S.E.; *$P < 0.01$. All experiments were repeated three times with similar results.

MMP-13 activity and metastatic potential of human tumor cells has been reported (Schmidt-Hansen *et al.,* 2004).

In the present study, S100A4 was shown to stimulate invasion and metastasis, promote angiogenesis, and upregulate MMP-13. Conversely, S100A4 silencing suppressed invasion and metastasis, inhibited angiogenesis and downregulated MMP-13. The S100A4-induced stimulation of invasiveness and metastasis was attenuated by treatment with anti-MMP-13, a specific inhibitor of MMP-13 activity. Taken together, our findings suggest that the metastasis and angiogenesis promoting effect of S100A4 may be mediated by the regulation of MMP-13.

Considering the role of S100A4 in tumor progression, our findings that its presence in the extracellular space induces the expression of MMP-13 offers a new mechanistic explanation for its role as a metastasis stimulating protein.

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