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ORIGINAL ARTICLE

Sevoflurane inhibits invasion and migration of lung cancer cells by inactivating the p38 MAPK signaling pathway

Hua Liang · Miaoning Gu · Chengxiang Yang · Hanbing Wang · Xianjie Wen · Qiaoling Zhou

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Abstract
Purpose Sevoflurane is used widely during lung cancer surgery. However, the effect of sevoflurane on the invasion and migration of lung carcinoma cells remains unclear. The aims of this study were to explore the role of matrix metalloproteinase (MMP)-2 and MMP-9 in the effect of sevofluane on the invasion and the role of fascin and ezrin on the effect of sevofluane on the migration of human lung adenocarcinoma A549 cells. We also investigated whether sevoflurane regulates the expression of these molecules through the p38 mitogen-activated protein kinase (MAPK) signaling pathway.
Methods The invasion of cells was evaluated using the Transwell invasion assay, and the migration of cells was determined using the wound healing assay. The expression of MMP-2, MMP-9, ezrin, fascin, and phospho-p38 MAPK in cells was determined by western blotting.
Results A significant inhibition of cell invasion and migration was found in A549 cells which had been treated with sevoflurane. The data also revealed that sevoflurane could decrease the phosphorylation level of p38 MAPK, which is involved in the downregulation of MMP-2, MMP-9, fascin, and ezrin expression, accompanied by a concomitant inhibition of the invasion and migration of A549 cells. SB203580, a p38 MAPK inhibitor, augmented the downregulation of the expression of these proteins.
Conclusion The anti-invasion effect of sevoflurane on A549 cells was associated with a downregulation of both MMP-2 and MMP-9 expression, while the anti-migration effect was associated with a downregulation of both fascin and ezrin expression. These effects could occur partly as a result of inactivation of the p38 MAPK signaling pathway.

Keywords Sevoflurane · Lung cancer cell · Invasion · Migration · p38 MAPK

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Introduction

Lung cancer is the most common cancer worldwide, and non-small cell lung carcinoma accounts for approximately 80% of all lung cancers [1]. Surgical resection is a commonly used therapy for patients with non-small cell lung carcinoma. However, tumor metastasis after lung cancer surgery often occurs, which is the leading cause of deaths in lung cancer patients [2, 3].

The invasion and migration of tumor cells are essential features of the metastatic process [4]. Anesthetics and anesthesia techniques have an impact on the invasion and migration ability of tumor cells that can possibly affect the long-term outcome of patients who have undergone cancer surgery [5]. Therefore, it is important that the choice of anesthetics and anesthesia technique to be used during cancer surgery takes into account inhibitory properties against the invasion and migration ability of cancer cells in order to decrease the risk of metastasis [6].

Sevoflurane, a volatile anesthetic agent, is used widely during lung cancer surgery. The anti-proliferative effect of sevoflurane on colon cancer SW620 cells and larynx cancer Caco-2 cells has been demonstrated in two studies [7, 8],

but the effect of sevofluane on the invasion and migration of lung cancer cells remains unclear.

Matrix metalloproteinase (MMP)-2 and MMP-9 produced by cancer cells can degrade extracellular matrix components and are of critical importance in tumor invasion [9]. Fascin and ezrin can enhance the motility of cells that are associated with the migration process of cancer cells [10, 11]. However, the effect of sevoflurane on the expression of MMP-2, MMP-9, fascin, and ezrin is still undefined.

In this in vitro study, we explored the role of MMP-2 and MMP-9 on the effect of sevoflurane on invasion and that of fascin and ezrin on the effect of sevoflurane on the migration of human lung adenocarcinoma A549 cells. Because p38 mitogen-activated protein kinase (p38 MAPK) plays an important role in the invasion and migration of cancer cells [12, 13], we also investigated whether sevoflurane regulates the expression of MMP-2, MMP-9, fascin, and ezrin through the p38 MAPK signaling pathway.

Materials and methods

Major reagents

RPMI-1640 and fetal bovine serum (FBS) was purchased from the HyClone Corporation (New York, NY). Sevoflurane was purchased from Maruishi Pharmaceutical Co. (Sevofrane; Osaka, Japan). TRIZOL reagent was purchased from GIBCO (Invitrogen, Carlsbad, CA). Rabbit anti-human MMP-2 monoclonal antibody and rabbit anti-human MMP-9 polyclonal antibody were purchased from Abcam Inc.(Cambridge, UK), mouse anti-human fascin monoclonal antibody was purchased from the Labvision and Neomarker Corp. (Fremont, CA), rabbit anti-human ezrin monoclonal antibody, rabbit anti-human p38 MAPK antibody, rabbit anti-human p-p38 MAPK antibody, and SB203580 (p38 MAPK inhibitor) were purchased from Cell Signaling Technology (CST; Danvers, MA), and horseradish peroxidase (HRP)-conjugated secondary antibodies, goat anti-rabbit immunoglobulin G (IgG), and goat anti-mouse IgG were purchased from Southern Biotechnology Associates (Birmingham, AL). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was obtained from Abcam Inc. All other reagents were procured locally.

Cell line and cell culture

The human pulmonary adenocarcinoma cell line A549 was obtained from Shanghai Cell Biology Medical Research Institute, Chinese Academy of Sciences, and maintained in RPMI-1640 supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin. The cells were incubated in 5% CO_2 humidified at 37°C for growth.

Protocol of A549 cell exposure to sevoflurane

A549 cells in the exponential growth phase were inoculated onto plates and cultured in a CO_2 incubator (ThermoFisher Scientific, Waltham, MA) for 24 h. In accordance with the experimental protocol previously described [14, 15], cell culture plates were placed in an airtight glass chamber with inlet and outlet connectors. The inlet port of the chamber was connected to the anesthesia machine (Cicero–EM 8060; Dräger, Germany), and sevoflurane was supplied into the chamber by a sevoflurane vaporizer (Sevorane; Abbott, Abbot Park, IL) attached to the anesthesia machine. The concentrations of sevoflurane in the chamber were monitored at the chamber outlet port by using a gas monitor (PM 8060; Dräger). A549 cells were allocated into four groups: control group, 1.7% sevoflurane group, 3.4% sevoflurane group, and 5.1% sevoflurane group. The control group was treated with 95% air/5% CO_2 at 6 L/min for 2, 4, and 6 h; the sevoflurane group was treated with 1.7, 3.4, or 5.1% sevoflurane mixed with 95% air/5% CO_2 at 6 L/min for 2, 4, and 6 h, respectively. Concentrations of sevoflurane dissolved in the cell culture medium were detected by a gas chromatography (GC model 6890 N; Agilent Technologies, Palo Alto, CA, USA) connected to a mass spectrometer detector (MSD model 5975B; Agilent Technologies). The conditions of the GC system were as follows: temperature of sampling port, 240°C; temperature of detector, 250°C; DB-5 column, 60 m × 0.32 mm × 0.25 μm; sampling volume, 0.5 mL. The conditions of the MSD were: electron bombardment ion source, ionizing potential, 70 eV; temperature of ion source, 250°C; mass scan range, 50–550 I*m*/*z*. A linear standard curve was drawn. Sevoflurane concentration was selected as the *x*-axis and peak area as the *y*-axis. A linear correlation was obtained in the range of 0.012–0.28 mg/L (coefficient correlation 0.9962). Based on above-mentioned method, concentrations of sevoflurane in the culture medium were measured after different hours of incubation with different concentrations of sevoflurane (monitored at the chamber outlet port): 207 ± 8 (1.7%; incubation for 6 h), 421 ± 5 (3.4%; incubation for 6 h), and 639 ± 10 μmol/L (5.1%; incubation for 6 h). There were no significant differences in sevoflurane concentration at the different incubation time points (2, 4, 6 h) at each sevoflurane concentration.

Transwell invasion assay

A549 cells were seeded in 24-well plates (2×10^5 cells per well). After being exposed to different concentrations of sevoflurane for 2, 4, and 6 h, respectively, the cells were placed in a CO_2 incubator for an additional 24 h of culture. Thereafter, the Transwell chambers, which incorporated a polycarbonate filter membrane (diameter 6.5 mm, pore size

8 μm; Corning Costar, Corning, NY), were used to evaluate cell invasiveness. The polycarbonate filter membrane at the bottom of the Transwell chamber was coated with 50 μL Matrigel (BD BioSciences, San Jose, CA) and air dried in a laminar hood overnight. The upper compartment of the Transwell chamber was filled with serum-free RPMI-1640 medium (100 μL per well), and the lower compartment was filled with the same medium (600 μL per well). The cells were harvested and washed twice with phosphate buffered saline (PBS), then re-suspended with 0.2% bovine serum albumin (BSA) and incubated at 37°C for 2 h. The cells were then inoculated into the upper compartment of the Transwell chambers (2×10^5 cells per well). RPMI-1640 medium with FBS was added into the lower compartment (600 μL per well). The cells were cultured at 37°C in 5% CO_2 atmosphere for 24 h. Cells that did not penetrate the polycarbonate membrane at the bottom of the chamber were wiped off gently using a cotton swab. The membrane was then removed, fixed with methanol for 15 min, and stained with 0.5% crystal violet. Five vision fields were selected randomly under a microscope (MSHOT/MC30 imaging system; Micro-shot Technology, Guangzhou, China), and the number of cells that penetrated the membrane was counted.

Wound healing assay

A549 cells were seeded in 6-well plates (1×10^6 cells per well). After being treated with different concentrations of sevoflurane for 4 h, the cells were placed in a CO_2 incubator for an additional 24 h of culture. Cell migration was then measured by using a wound healing assay. When the cells reached 100% confluence, cell layers were wounded with a plastic micropipette tip to generate a clean wound area across the center of the well. The cell debris was aspirated away and replaced by 2.5 mL of fresh serum-free medium, which allowed cells to migrate. A549 cells were then placed in the CO_2 incubator. The "wound closure" was assessed at the 0, 12, and 24 h time points by microscopic examination. Five randomly selected points along each wound were marked, and the horizontal distance of migrating cells from the initial wound was measured.

Reverse transcription-PCR

A549 cells were seeded in 6-well plates (1×10^6 cells per well). After being treated with different concentrations of sevoflurane for 4 h, the cells were placed in a CO_2 incubator for an additional 24 h of culture. Thereafter, the expressions of MMP-2, MMP-9, fascin, and ezrin were analyzed by reverse transcription (RT)-PCR. Total RNA was isolated from proliferating cells using the TRIZOL reagent according to the manufacturer's instructions (Life Technologies, Carlsbad, CA) and quantified by spectrophotometry at a wavelength of 260 nm. RT reactions and PCR were performed using reverse transcriptase, Oligo (DT) primers, and Taq DNA polymerase. The primers used were those for MMP-2 (forward: 5′-CTGGAGATACAATGAGGTGAAG-3′; reverse: 5′-TCAGCAGCCTAGCCAGTC-3′), MMP-9 (forward: 5′-TACCACCTCGAACTTTGACA-3′; reverse: 5′-AGGGCGAGGACCATAGAG-3′), fascin (forward: 5′-AGCTGCTACTTTGACATCGA-3′; reverse: 5′-TCATGAGGAAGAGCTCTGAGT-3′), and ezrin (forward: 5′-AGCTGTGAAGAGACTCTGTTTG-3′; reverse: 5′-CTTAGCTGTGAAGGAGAAAGC -3′). GAPDH was used as an internal control. The primers for GAPDH were 5′-GGGAAACTGTGGCGTGAT-3′ (forward) and 5′-GAGTGGGTGTCGCTGTTGA-3′ (reverse). The reaction was initiated with denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 60°C for 40 s (annealing), and 72°C for 30 s (extension), with a terminal extension step at 72°C for 7 min and a final holding stage at 4°C. The PCR products were electrophoresed on a 2% agarose gel, stained with ethidium bromide, and visualized with an ultra-violet transilluminator. The electrophoresis products were analyzed using GDS-8000 System (UVP, Upland, CA). Relative gene expression was defined as the ratio of target gene expression to GAPDH expression.

Western blot analysis

A549 cells were cultured in 6-well plates (1×10^6 cells per well). After being treated with different concentrations of sevoflurane for 2–6 h, the cells were placed in a CO_2 incubator for an additional 24 h of culture. Thereafter, the expressions of phospho-p38 MAPK, p38 MAPK, MMP-2, MMP-9, fascin, and ezrin were analyzed by western blot analysis. The cells were washed with PBS and lysed with lysis buffer. The lysates were incubated on ice for 30 min and centrifuged at 14,000*g* for 15 min. The supernatants were collected and analyzed for protein concentration using a bicinchoninine acid assay. Protein samples were separated on a 12.5% sodium dodecyl sulfate-polyacrylamide gel and then electrophoretically transferred onto a polyvinylidene difluoride membrane (Millipore, Bedford, MA). The membrane was blocked overnight at 4°C in TBS–Tween 20 (TBST) buffer containing 5% non-fat milk, washed with TBST (3 washes, 5 min each), and incubated for 2 h at 37°C with 1/1000 dilution of MMP-2 antibodies, 1/1000 dilution of MMP-9 antibodies, 1/1000 dilution of fascin antibodies, 1/5000 dilution of ezrin antibodies, 1/1000 dilution of p38 MAPK antibodies, 1/1000 dilution of p-p38 MAPK antibodies, and 1/10000 dilution of GAPDH antibodies, respectively. After being washed with TBST (3 washes, 5 min each), the membranes were incubated with HRP-conjugated goat anti-rabbit IgG (1/5000

dilution) or rabbit anti-mouse IgG secondary antibody (1/4000 dilution) at 37°C for 1 h. Protein signals were detected using enhanced chemiluminescence reagent (Millipore) and quantified by densitometry using Bio-Rad Quantity One software (Bio-Rad, Hercules, CA).

p38 MAPK inhibitor treatment

To investigate the effect of SB203580 (p38 MAPK inhibitor) on sevoflurane-mediated anti-invasion and anti-migration effects, s confluent cell culture was pre-incubated for 8 h with 20 μM SB203580 prior to exposure to 3.4% sevoflurane for 4 h. The invasion and migration of cells were evaluated at 24 h post-sevoflurane treatment. The expressions of phospho-p38 MAPK, p38 MAPK, MMP-2, MMP-9, fascin, and ezrin were detected at 24 h post-sevoflurane treatment.

Statistical analysis

Data analysis was performed using SPSS for Windows software (v.11.0; SPSS, Chicago, IL). Data were expressed as mean ± standard deviation (SD). Differences in group were analyzed by using repeated measure analysis of variance (ANOVA). Differences among groups were assessed using one-way ANOVA, followed by Duncan's test for post hoc comparisons. $P < 0.05$ was considered to indicate statistical significance.

Results

Sevoflurane inhibited A549 cell invasion

To observe the effect of sevoflurane on the invasion of A549 cells, we determined the number of invasive cells using the cell invasion assay. As shown in Fig. 1, sevoflurane was able to significantly decrease the number of A549 cells that penetrated the membrane relative to the group control (all $P < 0.05$, ANOVA). The inhibition effect was enhanced with increased sevoflurane action time (all $P < 0.05$, repeated measure ANOVA).

Sevoflurane downregulated the expression of invasion-related molecules

To investigate the molecular mechanism underlying the effect of sevoflurane on A549 cell invasion, we observed the effect of sevoflurane on the expression of MMP-2 and MMP-9 at the mRNA and protein levels. The RT-PCR and western blot analyses revealed that sevoflurane downregulated mRNA and protein levels of MMP-2 and MMP-9 (all $P < 0.05$, ANOVA) (Fig. 2).

Sevoflurane inhibited the migration of A549 cells

The effect of sevoflurane on A549 cell migration was assessed by determining the migration distance using the wound healing assay. Compared with the group control, treatment with 1.7, 3.4, and 5.1% sevoflurane was able to significantly inhibit the migration distance of cells at 12 h post-treatment (all $P < 0.05$, ANOVA). The migration distance of those A549 cells treated with 1.7% sevoflurane did not change significantly at 24 h post-treatment as compared to the control group ($P = 0.17$, ANOVA), while the migration distance of A549 cells treatment with 3.4 and 5.1% sevoflurane, respectively, did decrease significantly at 24 h post-treatment compared to the control group ($P < 0.01$, ANOVA) (Fig. 3).

Sevoflurane downregulated expression of the migration-related molecules

The related molecular mechanism underlying the effect of sevoflurane on the migration of A549 cells was investigated by determining the effect of sevoflurane on the expression of fascin and ezrin at the mRNA and protein levels. The RT-PCR and western blot analyses revealed that sevoflurane downregulated fascin and ezrin at both the mRNA and protein level (all $P < 0.05$, ANOVA) (Fig. 4).

Sevoflurane decreased the phosphorylation levels of p38 MAPK

The role of p38 MAPK on the effects of sevoflurane on the invasion and migration of A549 cells was investigated by examining the levels of phospho-p38 MAPK. The cells were exposed to 3.4% sevoflurane for 2–6 h, and the phosphorylation levels of p38 MAPK were detected by western blot analysis at 24 h post-sevoflurane treatment. The effect of sevoflurane on reducing the levels of p38 MAPK phosphorylation was enhanced with increasing duration of sevoflurane treatment (all $P < 0.05$, ANOVA). The total levels of p38 MAPK remained unaltered (all $P > 0.05$, ANOVA) (Fig. 5).

Effect of SB203580 on sevoflurane-induced p38 MAPK signaling pathway

To further explore whether the effects of sevofluane downregulation of MMP-2, MMP-9, fascin, and ezrin expression occurred via inhibition of the p38 MAPK signaling pathway, we pretreated A549 cells with a p38 MAPK inhibitor (SB203580; 20 μM) for 8 h prior to exposing the cells to 3.4% sevoflurane for 4 h. The SB203580 pretreatment inhibited the invasion and migration of A549 cells relative to the group control (all $P < 0.05$, ANOVA).

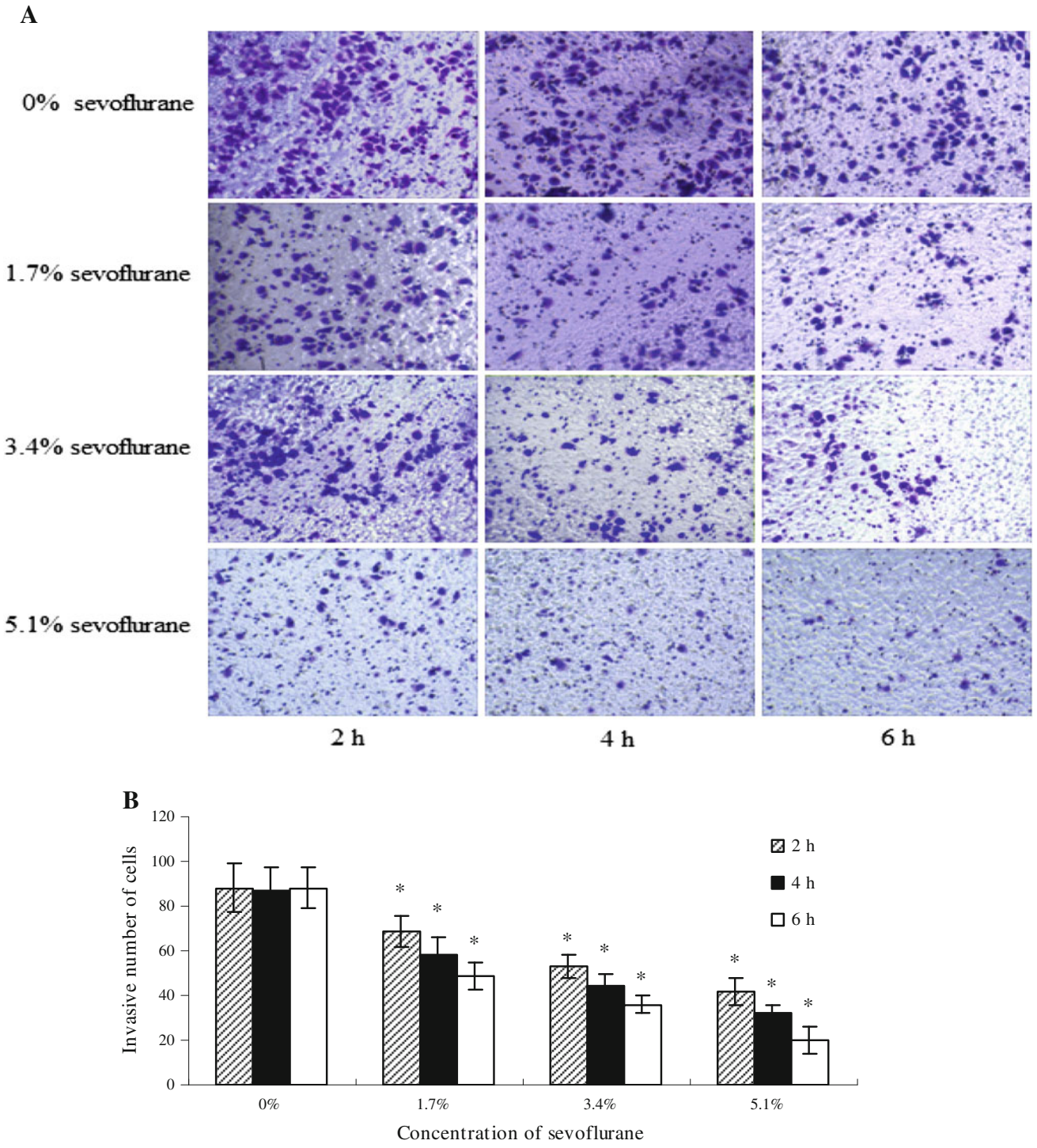

Fig. 1 Effect of sevoflurane on A549 cell invasion. **a** Cells were exposed to different concentrations (0, 1.7, 3.4, and 5.1%) of sevoflurane for 2, 4, and 6 h, and the invasion of cells was evaluated by the Transwell invasion assay at the 24 h post-sevoflurane treatment (×200 magnification). **b** Representation of the invasive cells at different time points and at different concentrations of sevoflurane. Data are shown as the mean ± standard deviation (SD) of five independent experiments. *Asterisks* indicate a statistically significant difference ($P < 0.05$) between a given group and its corresponding control group

The combination treatment of SB203580 with 3.4% sevoflurane resulted in a stronger inhibition of invasion and migration than that of sevoflurane treatment or SB203580 treatment alone (all $P < 0.05$, ANOVA) (Figs. 6, 7).

The results of the western blot analysis revealed that the SB203580 treatment both decreased the levels of phospho-p38 MAPK and reduced MMP-2, MMP-9, fascin, and ezrin expression (all $P < 0.05$, ANOVA). The combination

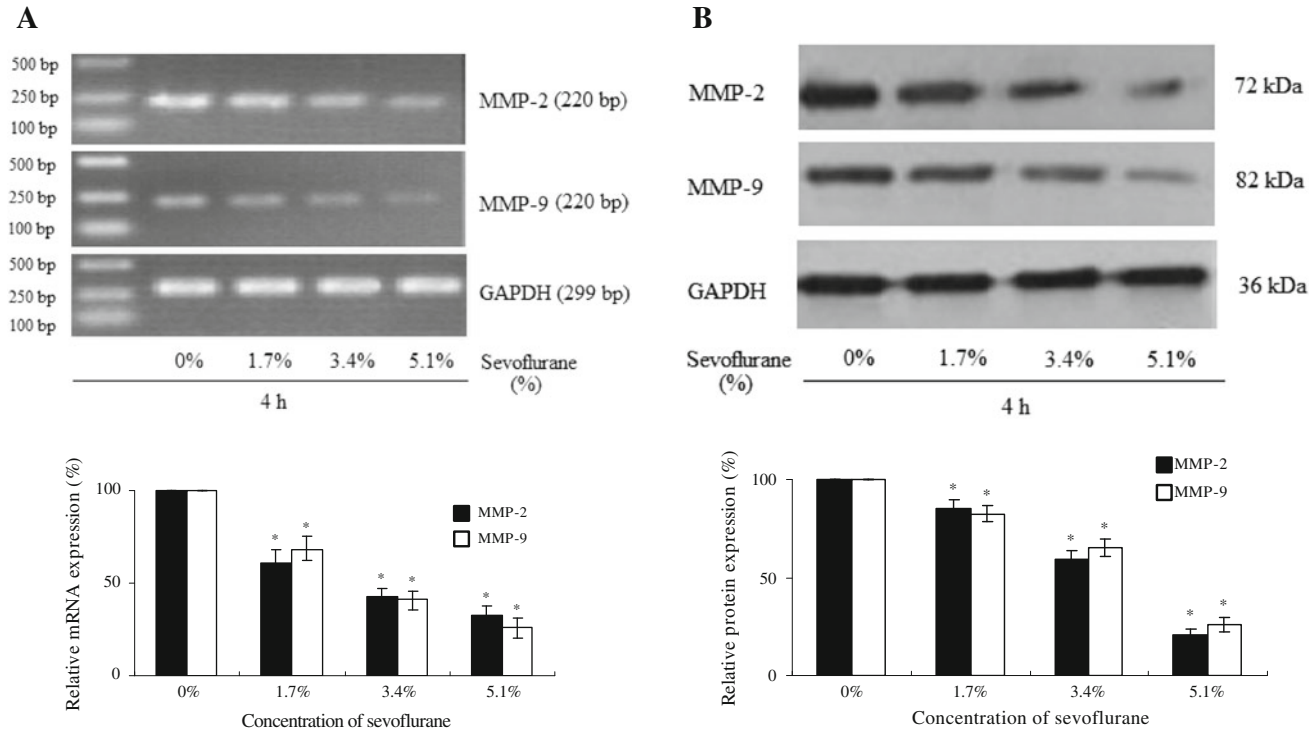

Fig. 2 Effect of sevoflurane on the expression of molecules related to A549 cell invasion. **a** Cells were exposed to different concentrations (0, 1.7, 3.4, and 5.1%) of sevoflurane for 4 h, and the expression of matrix metalloproteinase (*MMP*)-2 and MMP-9 mRNA was evaluated by reverse transcription (RT)-PCR at 24 h post-sevoflurane treatment. The histograms represent MMP-2 and MMP-9 mRNA expression at different concentrations of sevoflurane. **b** Cells were exposed to different concentrations (0, 1.7, 3.4, and 5.1%) of sevoflurane for 4 h, and the expression of MMP-2 and MMP-9 protein was detected by western blot analysis at 24 post-sevoflurane treatment. The histograms represent the protein expression of MMP-2 and MMP-9 at different concentrations of sevoflurane. Data are shown as the mean ± SD of three independent experiments. *Asterisks* indicate a statistically significant difference ($P < 0.05$) between a given group and its corresponding control group. *GAPDH* Glyceraldehyde 3-phosphate dehydrogenase

treatment of SB203580 with 3.4% sevoflurane was able to downregulate the level of phospho-p38 MAPK and inhibit the expression of these molecules more significantly than that of the sevoflurane treatment or SB203580 treatment alone (all $P < 0.05$, ANOVA) (Fig. 8).

Discussion

Bronchi and pulmonary alveoli are directly exposed to sevoflurane during sevoflurane anesthesia. The aim of our study was to elucidate the effect of sevoflurane on the invasion and migration of lung cancer cells, the mechanisms of which are still unclear, using human lung carcinoma A549 cells. To simulate the clinical anesthesia setting, we treated A549 cells for 2–6 h with three commonly used concentrations of sevoflurane. Our results indicate that sevoflurane was able to inhibit the invasion and migration of A549 cells and to downregulate the expression of MMP-2, MMP-9, fascin, and ezrin. They also suggest that the effects of sevoflurane in downregulating the expression of MMP-2, MMP-9, fascin, and ezrin occur in part through inactivation of the p38 signaling pathway.

Metastasis of cancer cells consists of a series of complex, continuous, and multi-step process that includes the detachment of the tumor cells from the primary site, the degradation of the extracellular matrix, and penetration of the cells through the blood vessel walls. All of these processes are associated with the invasive and migration characteristics of cancer cells. It has been demonstrated that surgical procedures may augment the invasion and migration potential of cancer cells [16] and thus promote their ability to disseminate during the perioperative period [17]. Inhibition of the invasion and migration potential of cancer cells would thus exert a positive influence on lung cancer mortality rates [4]. Consequently, attenuation of the metastasis potential of cancer cells during the perioperative period is a new and challenging topic for anesthesiologists. In our study, the results of cell invasion and wound healing assays demonstrated that sevoflurane was able to significantly suppress the invasion and migration of A549 cells, respectively. These findings may have some practical implications for lung cancer surgery.

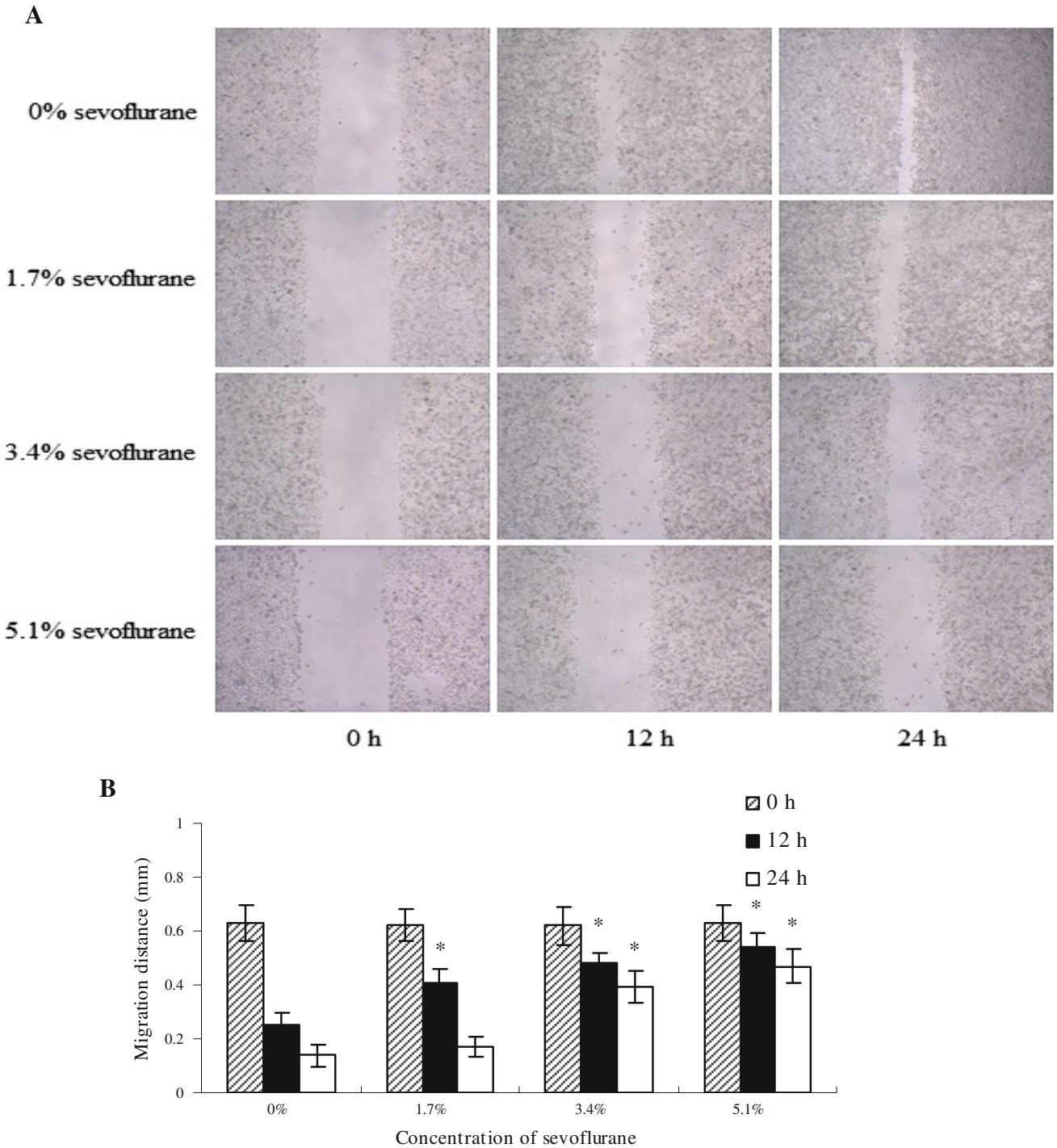

Fig. 3 Effect of sevoflurane on the migration of A549 cells. **a** Cells were exposed to different concentrations (0, 1.7, 3.4, and 5.1%) of sevoflurane for 4 h. After an additional 24 h of culture, the migration distance of cells was evaluated by the wound healing assay at the 0, 12, and 24 h time points (×200 magnification). **b** The histograms represent migration distance at the different time points and at different concentrations of sevoflurane. Data are shown as the mean ± SD of five independent experiments. *Asterisks* indicate a statistically significant difference ($P < 0.05$) between a given group and its corresponding control group

Matrix metalloproteinases belong to a family of zinc-dependent proteinases. MMP-2 and MMP-9 are two members of this family. Their primary function is the degradation of proteins in the extracellular matrix, a process which plays an important role in the invasion process [18, 19]. High levels of MMP-2 and MMP-9 have been found to correlate with a poor prognosis of lung cancer patients [20]. In line with the results of the cell

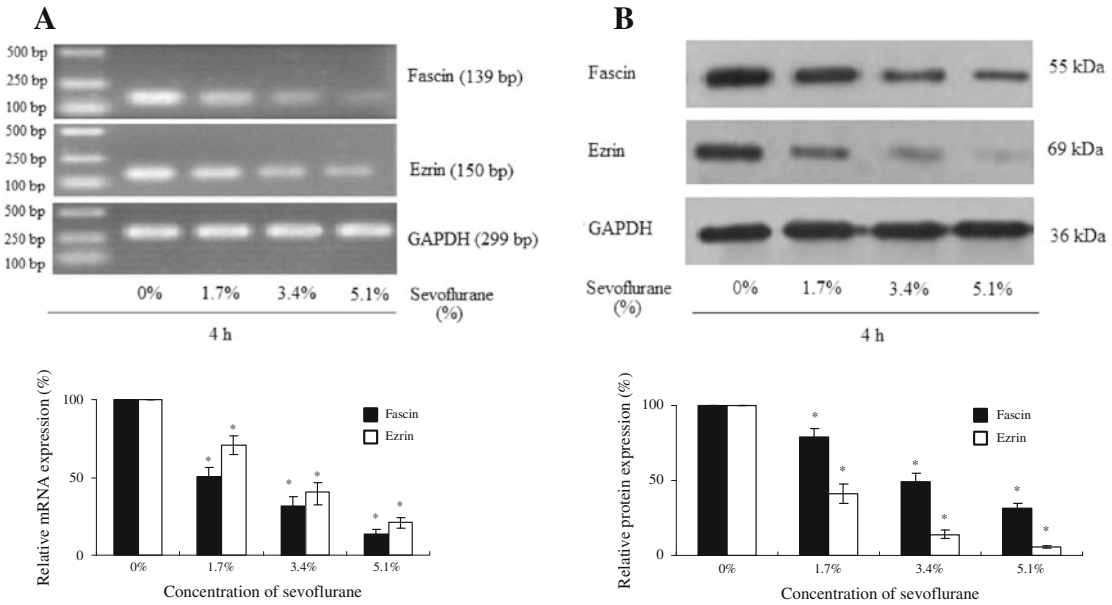

Fig. 4 Effect of sevoflurane on the expression of molecules related to the migration of A549 cells. **a** Cells were exposed to different concentrations (0, 1.7, 3.4, and 5.1%) of sevoflurane for 4 h, and the expression of fascin and ezrin mRNA was evaluated by RT-PCR at 24 h post-sevoflurane treatment. The histograms represent the expression of fascin and ezrin mRNA at different concentrations of sevoflurane. **b** Cells were exposed to different concentrations (0, 1.7, 3.4, and 5.1%) of sevoflurane for 4 h, and the expression of fascin and ezrin protein was detected by western blot analysis at 24 h post-sevoflurane treatment. The histograms represent expression of fascin and ezrin protein at different concentrations of sevoflurane. Data are shown as the mean ± SD of three independent experiments. *Asterisks* indicate a statistically significant difference ($P < 0.05$) between a given group and its corresponding control group

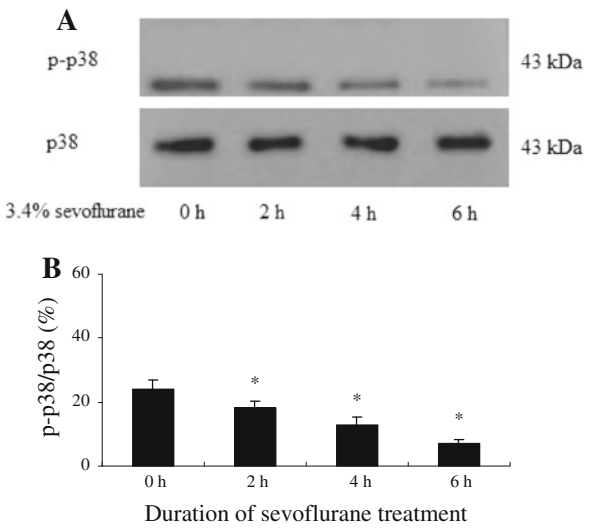

Fig. 5 Effect of sevoflurane on the phosphorylation levels of the p38 mitogen-activated protein kinase (*MAPK*) of A549 cells. **a** Cells were exposed to 3.4% sevoflurane for 2, 4, and 6 h, and the phosphorylation levels of p38 MAPK were detected by western blot analysis at 24 post-sevoflurane treatment. **b** The histograms represent the phosphorylation levels of p38 MAPK at different time points post-sevoflurane treatment. Data are shown as the mean ± SD of three independent experiments. *Asterisks* indicate a statistically significant difference ($P < 0.05$) between a given group and its corresponding control group. *p-p38* Phospho-p38

invasion assay, we found that sevoflurane downregulated MMP-2 and MMP-9 expression at both the mRNA and protein levels, suggesting that sevoflurane-mediated anti-invasion activity is related to the inhibition of MMP-2 and MMP-9 expression.

Fascin is a key actin-bundling protein, and cancer cells expressing high levels of fascin exhibit increased migration ability [10, 21]. Various studies have revealed that the upregulation of fascin is correlated with a clinically aggressive phenotype and poor prognosis [22, 23]. Ezrin, a cytoskeleton organizer, is involved in cell migration through its action as a molecular linker between the actin cytoskeleton and plasma membrane. High levels of ezrin expression can promote the migration of cancer cells [24, 25] and correlate with a poor prognosis of cancer patients [26]. Consistent with the results of the wound healing assay, the results of the RT-PCR and western blot analyses showed that sevoflurane was able to inhibit the expression of fascin and ezrin at the mRNA and protein levels, suggesting that sevoflurane-mediated anti-migration activity is associated with the downregulation of fascin and ezrin expression.

p38 MAPK is a member of the MAPK super-family. It can be activated in response to different extracellular stimuli and has distinct downstream targets [27, 28]. p38 MAPK has been implicated in cancer development, and its

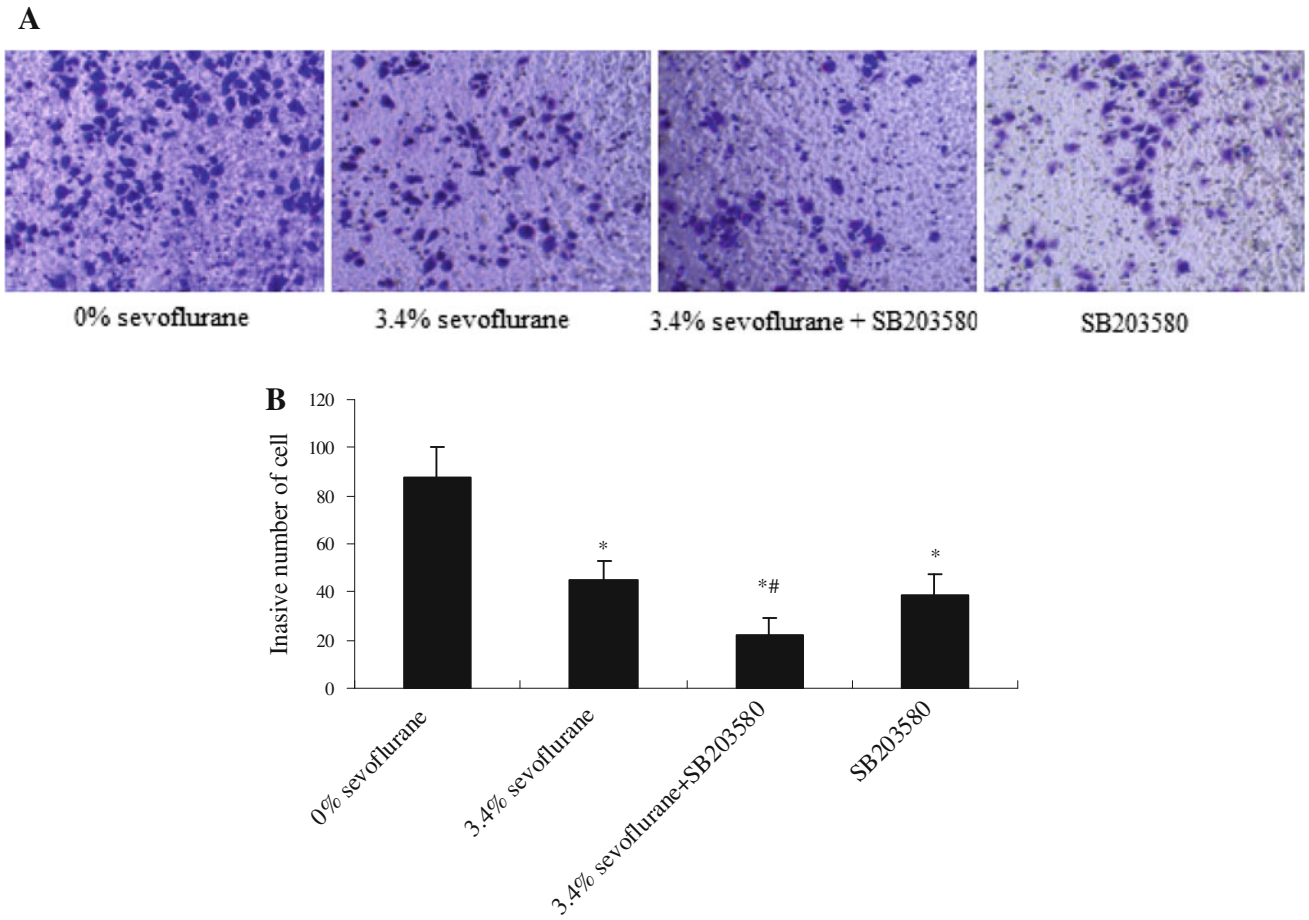

Fig. 6 Effect of SB203580 on sevoflurane-mediated anti-invasion activity of A549 cells. **a** Cells were pretreated with p38 MAPK inhibitor (SB203580; 20 μM) for 8 h and then treated either with 3.4% sevoflurane, or not treated, for 4 h. The invasion of cells in each group was evaluated using the Transwell invasion assay at 24 h post-sevoflurane treatment (×200 magnification). **b** The histograms represent the number of invasive cells of each group. Data are shown as the mean ± SD of five independent experiments. *Asterisks* indicate a statistically significant difference ($P < 0.05$) between a given group and its control group. *Hash* indicates a statistically significant difference ($P < 0.05$) between a given group and each of the other three groups

activation is associated with an increased invasion and migration ability of tumor cells [29]. In this study, we found that sevoflurane was able to inhibit the phosphorylation levels of p38 MAPK, suggesting that sevoflurane-mediated anti-invasion and anti-migration activity was associated with inactivation of the p38 MAPK signaling pathway. Results of the cell invasion and wound healing assay showed that the combination treatment of SB203580 with sevoflurane was able to inhibit the invasion and migration of A549 cells more significantly than the sevoflurane or SB203580 treatment alone, while the results of the western blot analysis showed that the combination treatment of SB203580 with sevoflurane could downregulate phospho-p38 MAPK expression and inhibit MMP-2, MMP-9, fascin, and ezrin expression more potently than the sevoflurane or SB203580 treatment alone. These findings suggest that the inhibition of MMP-2, MMP-9, fascin, and ezrin expression by sevoflurane may occur in part through inactivation of the p38 signaling pathway. Sevoflurane combined with SB203580 had an additive effect of inhibiting the invasion and migration of A549 cells.

Loop and Roesslein [14, 15] found that sevoflurane activates the p38 MAPK signaling pathway and increases phosphorylation of p38 MAPK. Our results are not consistent with their results, possibly due to our use of a different category of cell line (Loop and Roesslein [14, 15] used human T lymphocytes and Jurkat T-cells). However, the cell line investigated in this study is a human lung adenocarcinoma A549 cell line.

Our results show that sevoflurane was able to inhibit the invasion and migration of A549 cells. However, metastasis of cancer is not regulated only by the direct effects of general anesthetic on the tumor cells but is also affected by the effects of general anesthetic on immune cells [6]. Therefore, it is important to obtain a balance between these two states.

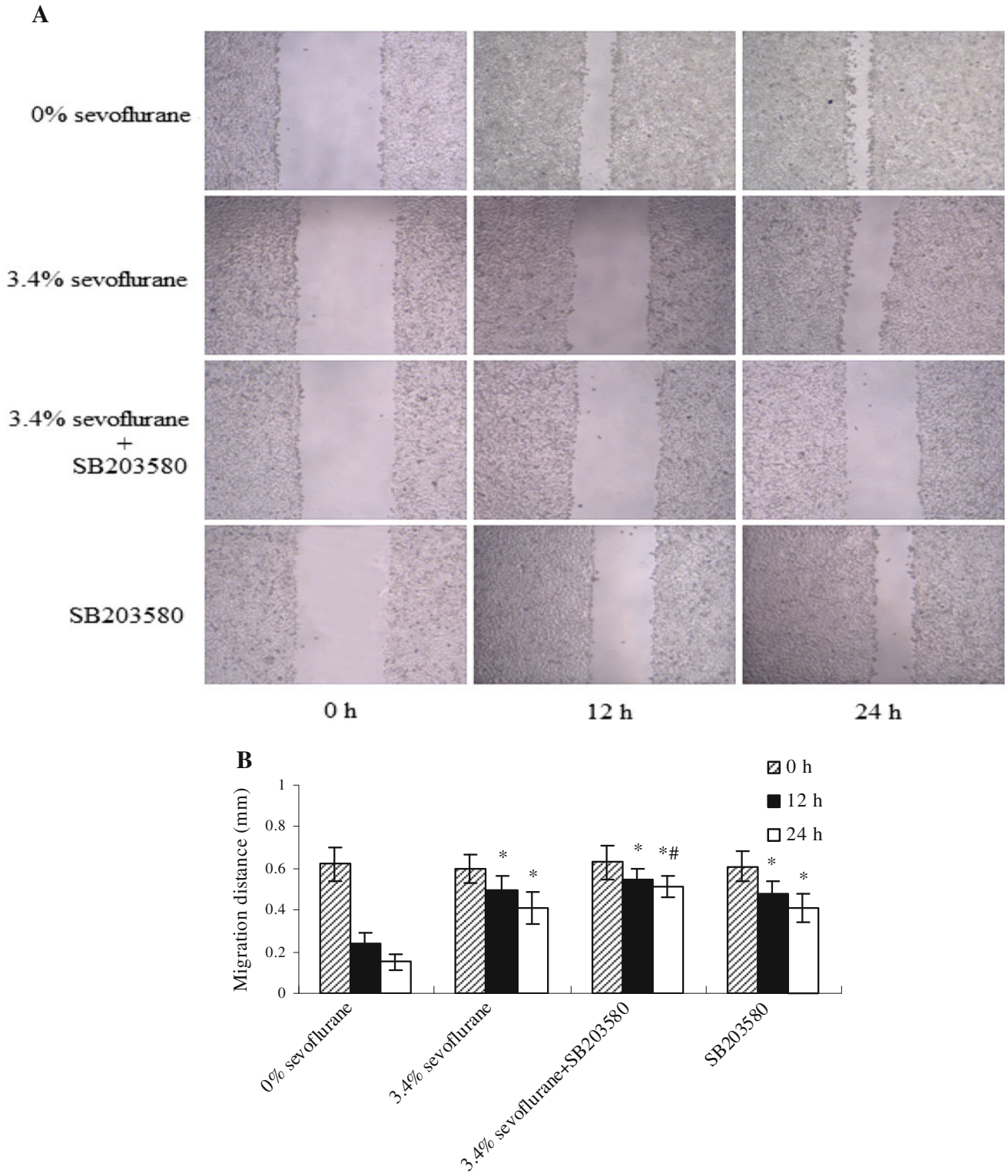

Fig. 7 Effect of SB203580 on sevoflurane-mediated anti-migration activity of A549 cells. **a** Cells were pretreated with p38 MAPK inhibitor (SB203580; 20 μM) for 8 h and then treated either with 3.4% sevoflurane, or not treated, for 4 h. The migration of cells in each group was evaluated using the wound healing assay at 24 h post-sevoflurane treatment (×200 magnification). **b** The histograms represent the migration distance of cells in each group. Data are shown as the mean ± SD of five independent experiments. *Asterisks* indicate a statistically significant difference ($P < 0.05$) between a given group and its control group. *Hash* indicates a statistically significant difference ($P < 0.05$) between a given group and each of the other three corresponding group

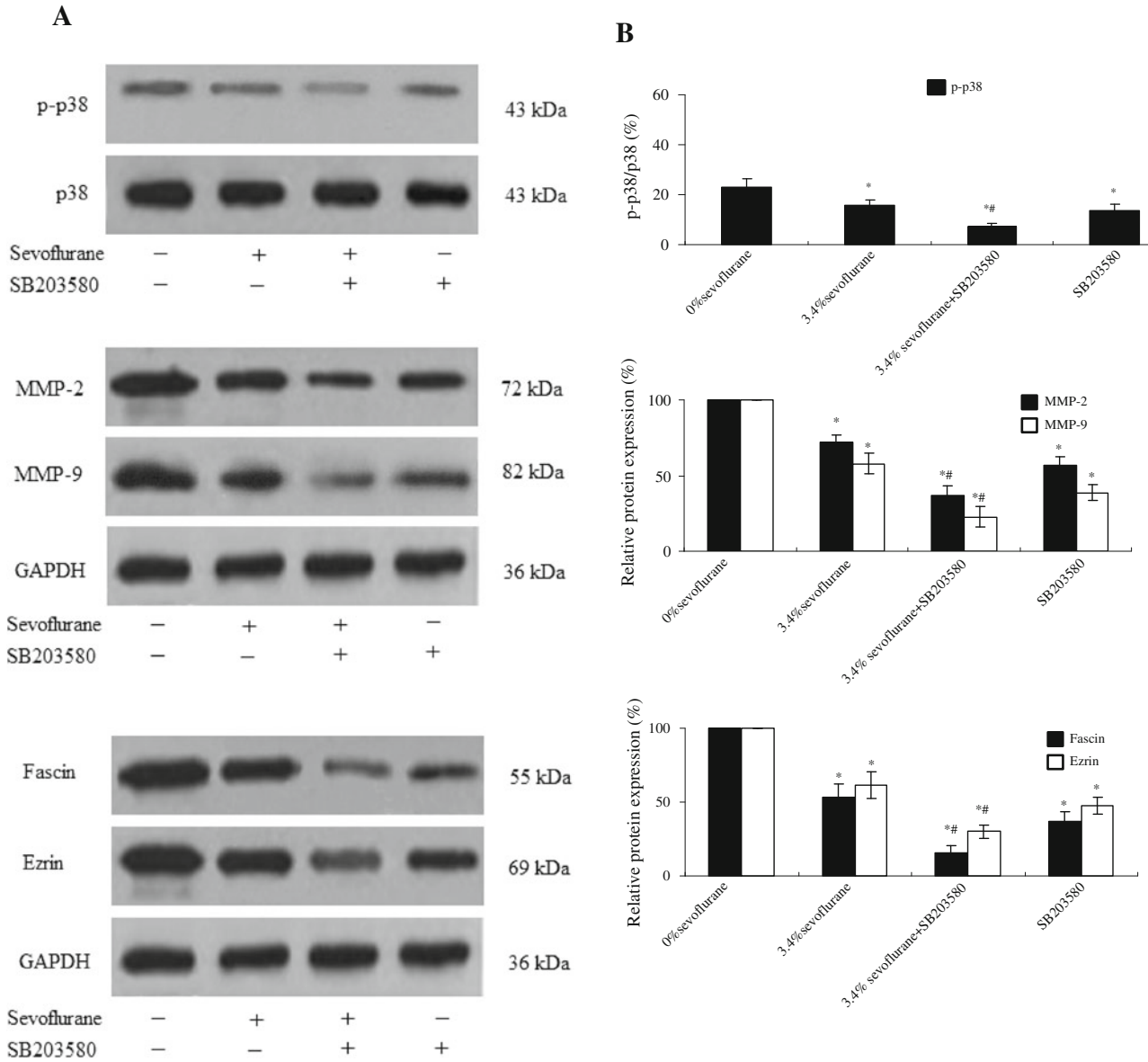

Fig. 8 Effect of SB203580 on sevoflurane-induced p38 MAPK signaling pathway of A549 cells. **a** Cells were pretreated with p38 MAPK inhibitor (SB203580; 20 μM) for 8 h and then treated with 3.4% sevoflurane, or not treated, for 4 h. The expression of p-p38 MAPK, p38 MAPK, MMP-2, MMP-9, fascin, and ezrin of the A549 cells in each group were detected by western blot analysis at 24 h post-sevoflurane treatment. **b** The histograms represent p-p38 MAPK, MMP-2, MMP-9, fascin, and ezrin expression of the cells of each. Data are shown as the mean ± SD of three independent experiments. *Asterisks* indicate a statistically significant difference ($P < 0.05$) between a given group and its corresponding control group. *Hashes* indicate a statistically significant difference ($P < 0.05$) between a given group and each of the other three corresponding groups

In conclusion, the results from our study demonstrate that (1) sevoflurane was able to inhibit the invasion and migration of human lung adenocarcinoma A549 cells; (2) sevoflurane-mediated anti-invasion activity was associated with a downregulation of MMP-2 and MMP-9 expression and that anti-migration activity was associated with a downregulation of fascin and ezrin expression; (3) the effects of sevoflurane downregulation of MMP-2, MMP-9, fascin, and ezrin expression may occur, at least in part, through inactivation of the p38 MAPK signaling pathway.

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