Matrine inhibits the metastatic properties of human cervical cancer cells via downregulating the p38 signaling pathway

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Abstract. Matrine is a traditional Chinese herbal medicine that shows antitumor efficacy for many types of cancer. The present study evaluated the antitumor efficacy of matrine on cervical cancer and to investigate the underlying mechanisms. We performed MTT assays, and *in vitro* invasion and migration assays, and P1 L6 found that matrine significantly inhibited cervical cancer cell growth by inducing apoptosis, and suppressed the invasion and migration ability of cervical cancer cells *in vitro* in a concentration-dependent manner. Mechanistically, we found that matrine decreased the expression and activity of the extracellular matrix factors, matrix metalloproteinases-2 (MMP-2) and MMP-9 via the suppression of p38 signaling pathway. In addition, when cervical cancer cells were grown as xenografts in nude mice, intraperitoneal (i.p.) injection of matrine induced a significant dose-dependent decrease in tumor growth. Taken together, these findings suggest that a potential mechanism by which matrine inhibits the growth and metastasis of cervical cancer through downregulating the p38 signaling pathway.

Introduction

Cervical cancer is the fourth most common cause of cancer-related mortality in women worldwide (1). In the clinical, surgery may be used alone or with radiation therapy and chemotherapy for cervical cancer treatment, depending on the stage of the cancer and the patients' preferences. However, metastasis and recurrence of invasive cervical cancer will lead to poor prognosis and treatment failure. Therefore, it is urgent to develop alternative treatment options which are more effective and less toxic. In recent years, considerable attention has been given to the application of traditional Chinese medicine (TCM) in anticancer therapy (2-4). Matrine, which was approved by Chinese FDA (CFDA) in 1995, is derived primarily from *Sophora* species plants and has a chemical formula of $C_{15}H_{24}N_2O$ (5). Matrine exhibits a wide range of pharmacological effects and has long been applied to treat viral hepatitis (6), neuropathic pain (7), isoproterenol-induced cardiotoxicity inflammatory and other diseases (8,9). In addition, increasing evidence has revealed that matrine displays anticancer effects in various cancers, such as gastric (10), rhabdomyosarcoma (11), acute myeloid leukemia (12), osteosarcoma (13), prostate (14), breast (15) and lung cancer (16). Moreover, the antitumor mechanisms of matrine have been demonstrated to involve the blockade of cell cycle progression, the induction of apoptosis, the regulation of oncogene expression, the inhibition of cytokine production and the modulation of signaling pathways (17-19).

Metastasis is a multi-step process that involves various cytophysiological changes, among which the degradation of the extracellular matrix (ECM) is very important (20). Matrix metalloproteinases-2 (MMP-2) and MMP-9 play important roles in degrading basement membranes and are intricately involved in cancer invasion and metastasis (21-23). The mitogen-activated protein kinases (MAPKs) are important components of intracellular signaling networks that regulate cell proliferation, differentiation, cellular stress responses, apoptosis and tumor progression (24,25). The activation of p38 signaling pathway, which belongs to MAPKs, is correlated to the development of cancer (26-28). Tumor cells need to modulate p38 activity to successfully metastasize (29). Recent studies showed that p38 signaling pathway participates in the invasion and metastasis of cervical cancer (30-32). Notably, Zhang *et al* (33) reported that matrine inhibited HeLa cell adhesion and migration through the inhibition of the activity of cAMP-dependent protein kinase (PKA) and vasodilator-stimulated phosphoprotein (VASP). On the other hand, Tan *et al* (34) recently reported that matrine activated the p38 pathway and promoted caspase-dependent apoptosis by inducing the generation of ROS in non-small cell lung cancer cells. Therefore, in the present study we aimed to investigate the effects of matrine against the invasion and metastasis of cervical cancer and explore whether the mechanism of its actions is associated with the p38 signaling pathway.

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Materials and methods

Reagents. Matrine, dimethyl sulfoxide dissolving (DMSO), SB203580 and anisomycin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin and streptomycin were purchased from Invitrogen (Carlsbad, CA, USA). Anti-p38, anti-phospho-p38 (p-p38), anti-MMP-2, anti-MMP-9, anti-Akt, anti-pAkt, anti-NF-κB p65 and anti-p-p65, antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). Anti-β-actin antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Cell culture. Human cervical cancer cell lines HeLa and C33A were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA) and were cultured in DMEM supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin. All cells were incubated at 37˚C with 5% CO_2.

Cell viability assay. Cell survival was assessed using standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay kit (Sigma-Aldrich) according to the manufacturer's instructions. Briefly, cells were seeded in 96-well culture plate (Corning Costar, Corning, NY, USA) at $2x10^4$ cells/well in quadruplicate. The cells were treated with different concentrations of matrine (0-400 μg/ml) for 24, 48 or 72 h, then cells were washed twice with phosphate-buffered saline (PBS) and incubated with 20 μl MTT (5 mg/ml) for 4 h at 37˚C. The solution was then discarded and 150 μl DMSO were added to each well. The optical density was measured in a microplate reader at 562 nm. Each experiment was performed in triplicate.

Flow cytometric analysis of apoptosis. Apoptosis was analyzed *in vitro* using a fluorescence-activated cell sorter (FACS) Annexin V assay kit (BD Biosciences, San Jose, CA, USA) according to the manufacturer's instructions. Briefly, the cells were exposed to oxymatrine at concentrations of 0, 25, 50 and 100 μg/ml for 48 h, then harvested and resuspended in binding buffer. Follow staining in duplicate with 5 μl of Annexin V-FITC and 5 μl of propidium iodide (PI) for 30 min in the dark, the samples were analyzed by flow cytometry using a FACSCalibur system (BD Biosciences). The percentage of apoptotic cells is referred as the apoptotic index. Each experiment was performed in triplicate.

In vitro invasion and migration assays. The *in vitro* invasion and migration activity was measured according to the methods previously described (35). HeLa and C33A cervical cells were pretreated with 0, 25, 50 and 100 μg/ml matrine or SB203580 (10 μM) or anisomycin (5 μM/l) for 24 h, surviving cells were harvested and seeded to Boyden chamber (Neuro Probe, Inc., Cabin John, MD, USA) at 10^5 cells/well in serum-free medium and then incubated for 24 h at 37˚C. At the end point, the cells on the upper side of inserts were completely removed by swabbing, while the cells on the bottom side of the filter were fixed, stained and counted. For invasion assay, 50 μl Matrigel (25 μg/ml; BD Biosciences, Bedford, MA, USA) was applied to 8-mm pore size polycarbonate membrane filters.

Quantitative real-time RT-PCR (qRT-PCR). Total RNAs were prepared using the RNeasy Mini kit (Invitrogen). cDNA was synthesized with SuperScript II Reverse Transcriptase (Invitrogen). qRT-PCR was performed using an iQ5 Real-Time PCR Detection System (Bio-Rad Laboratories, Richmond, CA, USA) with SYBR Premix Ex Taq™ II (Takara Bio, Tokyo, Japan) according to the manufacturer's instructions. Gene-specific primers were as follows: β-actin: forward, CCATCG TCCACCGCAAAT and reverse, CATGCCAATCTCATCT TGTTT; MMP-2: forward, CTCATCGCAGATGCCTGGAA and reverse, TTCAGGTAATAGGCACCCTTGAAGA; MMP-9: forward, GTCCACCCTTGTGCTCTTCC and reverse, GCCACCCGAGTGTAACCAT. The analysis of the relative gene copy number data for MMP-2 and MMP-9 was performed using the comparative ΔΔCT method and were normalized by the endogenous β-actin in each sample. All experiments had at least biological duplicates and assay triplicates.

Gelatin zymography. The cells were treated with different concentrations of matrine at 37˚C for 24 h and samples of conditioned media were collected. Briefly, the conditioned medium was adjusted to the same quantity of total protein, and then treated with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) non-reducing sample buffer without boiling. Samples were separated by 0.1% gelatin-8% SDS-PAGE electrophoresis. Afterwards, the gels were soaked twice in 2.5% Triton X-100 for 30 min at room temperature (RT), and incubated in reaction buffer (10 mM $CaCl_2$, 40 mM Tris-HCl and 0.01% NaN_3, pH 8.0) at 37˚C for 12 h. Gels were rinsed with distilled water, stained with Coomassie brilliant blue R-250 staining solution (Bio-Rad Laboratories). The gelatinolytic activities were densitometrically quantified and analyzed by an image analysis system (Bio-Rad Laboratories).

Western blot analysis. Cells were suspended in lysis buffer (40 mmol/l Tris-HCl, 1 mmol/l EDTA, 150 mmol/l KCl, 100 mmol/l $NaVO_3$, 1% Triton X-100, 1 mmol/l PMSF, pH 7.5) and the lysates were collected. Equal amounts of proteins were separated by 10% SDS-PAGE gel electrophoresis and transferred onto polyvinylidene fluoride (PVDF) membranes (Milipore, Billerica, MA, USA). The membranes were subsequently blocked in defatted milk (Heilongjiang Wondersun, Harbin, China) (5% in Tris-buffered saline with Tween-20 buffer) at RT for 1 h to block non-specific binding and then incubated overnight at 4˚C with antibodies against p38, p-P38, MMP-2, MMP-9, Akt, p-Akt, p65, p-p65 or β-actin. The membranes were then incubated with an HRP goat anti-mouse or anti-rabbit IgG antibody for 1 h at RT. The bands were detected with an enhanced chemiluminescence kit (Amersham ECL Plus; GE Healthcare, Freiburg, Germany) and exposed by autoradiography. The densitometric analysis was performed using ImageJ software (NIH Image, Bethesda, MD, USA) and the results were expressed as arbitrary units (a.u.).

Animal and tumor xenograft assays. To assess *in vivo* tumorigenicity, 4-to-6-weeks-old BALB/c athymic nude mice were purchased from the Experimental Animal Center of Xi'an Jiaotong University Medical College (Xi'an, China). Suspensions of HeLa tumor cells ($1x10^6$ viable cells/mouse)

were implanted into the right flank region of BALB/c nude mice. At 48 h after the injection (day 1), the mice were randomly divided into two groups (n=6). The animals were pair-matched so that the median tumor volume for each group was similar: the treatment group received matrine at 50 mg/kg per day via intragastric administration; the control group received an equal volume of normal saline. The tumor volumes were measured twice weekly with calipers and calculated according to the standard formula: (length x width^2)/2. After 3 weeks of drug administration, the mice were sacrificed and the tumors were dissected and weighed. The experimental protocols were approved by the Animal Care and Use Committee of the Medical School of Xi'an Jiaotong University.

Statistical analysis. Statistical analyses were performed using the GraphPad Prism 5.01 software (GraphPad Software, Inc., La Jolla, CA, USA). All data are expressed as mean ± standard deviation (SD). The Student's t-test was used for comparisons between two groups and one-way or two-way analysis of variance (ANOVA) test was used to analyze the statistical differences between the groups under different conditions. Correlation analysis was performed by the Z-test. P<0.05 was regarded as statistically significant. All statistical tests and corresponding P-values were two-sided.

Results

Matrine inhibits the proliferation of human cervical cancer cells by inducing apoptosis. The inhibitory effects of matrine at various concentrations (0-400 μg/ml) on the growth of HeLa and C33A cervical cancer cells were assessed by MTT assay. As shown in Fig. 1A and B, at the concentration of 50-100 μg/ml of matrine, HeLa and C33A cells showed a sharp decrease in cell viability (P<0.05), and the growth inhibition rates were increased with elevated matrine concentration (>100 μg/ml) (P<0.01), suggesting that matrine inhibits the growth of cervical cancer cells in a dose-dependent manner *in vitro*. In addition, during the prolonged treatments (48 and 72 h), matrine showed a significantly greater inhibitory effect than that at 24 h (P<0.01).

It has been reported that matrine exhibits antitumor effects by inducing apoptosis of various cancer cells (36,37). Thus, to examine the effects of matrine on cervical cancer cell apoptosis, matrine-treated cells were stained with Annexin V/PI and subjected to flow cytometric analysis. As shown in Fig. 1C, matrine induced a significant increase in the proportion of apoptotic HeLa and C33A cells, matrine at concentration of 25, 50 and 100 μg/ml caused 8.91±2.11, 19.53±7.17 and 38.41±8.13% of HeLa cells and 5.31±2.29, 15.37±3.01 and 28.91±5.63% of C33A cells to undergo apoptosis, respectively. Based on previous *in vitro* studies (33), we selected concentrations of 25, 50 and 100 μg/ml matrine for subsequent investigation to rule out the cytotoxicity. Taken together, these results suggest that matrine significantly inhibits the growth of cervical cancer cells at a dose- and time-dependent manner by inducing apoptosis.

Matrine inhibits the migration and invasion of cervical cancer cells. Abnormal growth and metastasis of cancer cells are regarded as the important biological characteristics

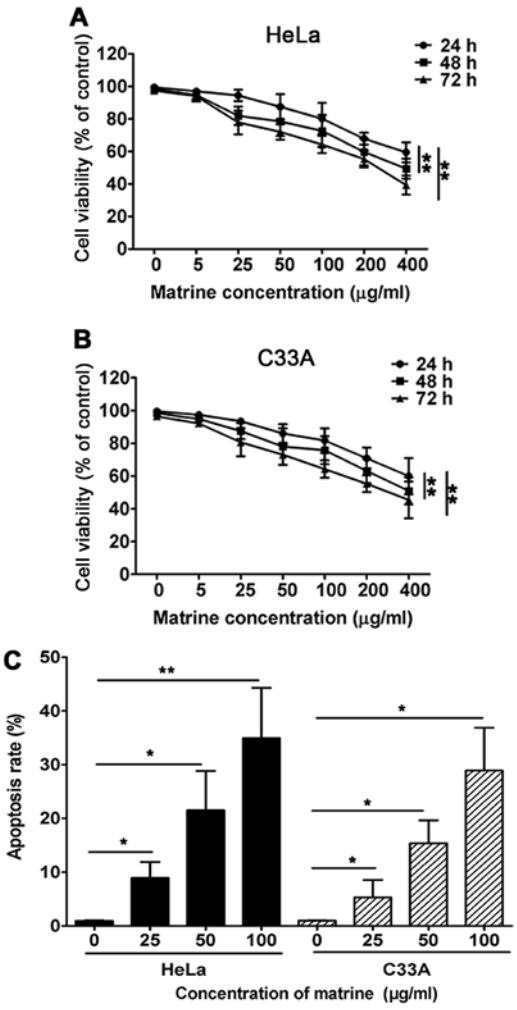

Figure 1. Matrine inhibits the proliferation of cervical cancer cells by inducing apoptosis. (A) HeLa and (B) C33A cell viability was measured using MTT assay after exposure to matrine (0-400 μg/ml) for 24, 48 and 72 h. The error bars represented the mean ± SD from three independent experiments. Two-way analysis of variance showed a significant interaction (P<0.01). Bonferroni's post-tests showed a significant difference between the 24- and the 48-h incubations (P<0.01) and between 24- and 72-h incubations (P<0.01) with various concentrations of matrine. *P<0.05 and **P<0.01 vs. control group. (C) After HeLa and C33A cells were treated with matrine at 0, 25, 50 and 100 μg/ml for 48 h, apoptosis was detected with Annexin V-FITC/PI staining using flow cytometry, and apoptosis rate was calculated and expressed as the mean ± SD (n=3). *P<0.05 and **P<0.01 vs. control group.

of cancers. Next, we examined the effects of matrine on the migration and invasion of cervical cancer cells. Matrine at the concentrations of 25, 50 and 100 μg/ml significantly reduced the rate of HeLa cells migration compared with the control group (Fig. 2A). Matrine at concentrations of 25, 50 and 100 μg/ml caused 40.53±2.05, 49.85±7.48 74.29±4.26% inhibition of cell migration, respectively (Fig. 2C). In addition, the invasion assay revealed similar results (Fig. 2B and D), the inhibition of HeLa cell invasion was 40.1±1.95, 78.84±3.05 and 92.74±3.75%, at concentrations of 25, 50 and 100 μg/ml, respectively. Similar inhibitory effects of matrine on cell migration and invasion were observed in C33A cells (Fig. 2). These findings suggest that matrine inhibits the migration and invasion of cervical cancer cells in a concentration-dependent manner.

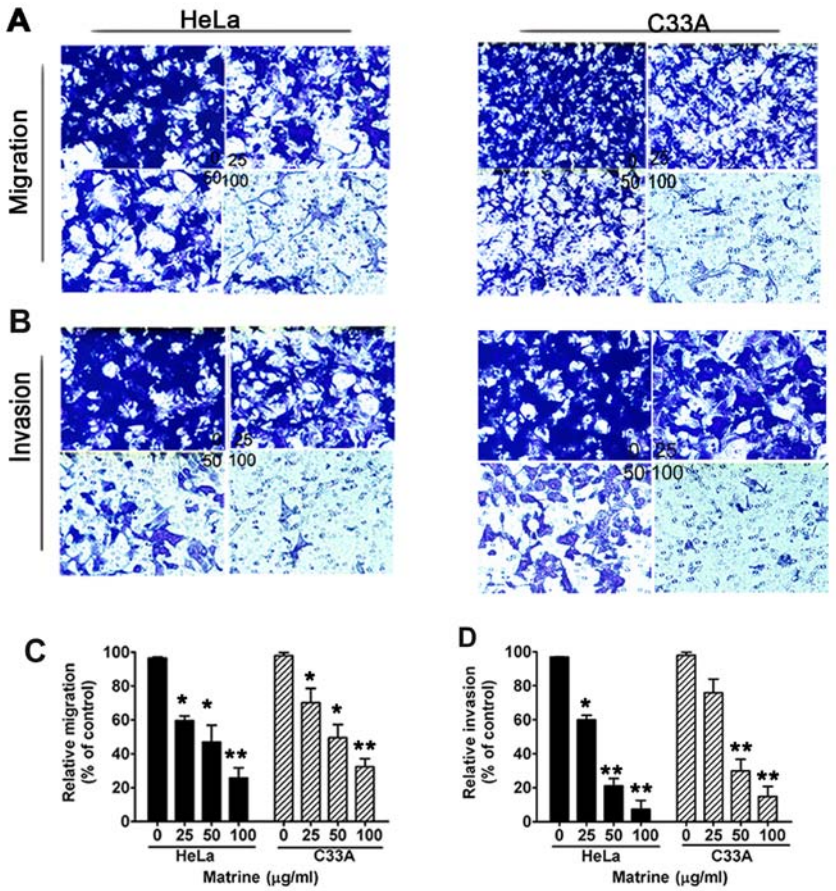

Figure 2. Matrine inhibits the invasion and migration of cervical cancer cells. HeLa and C33A cells were treated for 24 h with 0.1% DMSO as the control or various concentrations of matrine (0, 25, 50 and 100 μg/ml), and (A) cell migration and (B) invasion were analyzed as described in Materials and methods. Representative images of 3 independent experiments are shown. Magnification, x200. (C) Quantitative analysis of HeLa and C33A cell migration. (D) Quantitative analysis of HeLa and C33A cell invasion. Data were expressed as the mean ± SD (n=3). Statistical analysis was carried out using the one-way ANOVA followed by Bonferroni post-tests. *P<0.05 and **P<0.01 vs. control group.

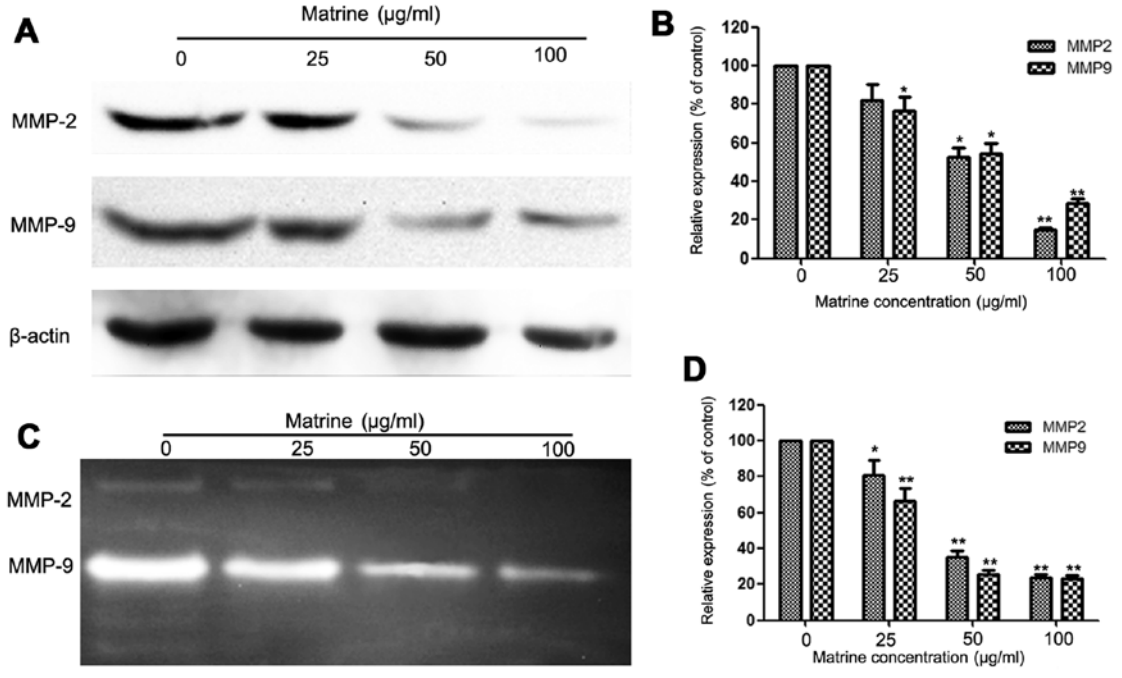

Figure 3. Matrine suppresses the expression and activity of MMP-2 and MMP-9 in HeLa cells. (A) HeLa cells were treated with matrine (0, 25, 50 and 100 μg/ml) for 24 h and protein levels of MMP-2 and MMP-9 were detected by western blot analysis. (B) Quantification of relative protein levels of MMP-2 and MMP-9 in HeLa cells. (C) HeLa cells were treated with matrine (0, 25, 50 and 100 μg/ml) for 24 h and then the activities of MMP-2 and MMP-9 were analyzed. (D) Quantification of the activity of MMP-2 and MMP-9 in HeLa cells. Values represented the means ± SD of three independent experiments. *P<0.05 and **P<0.01 vs. control group.

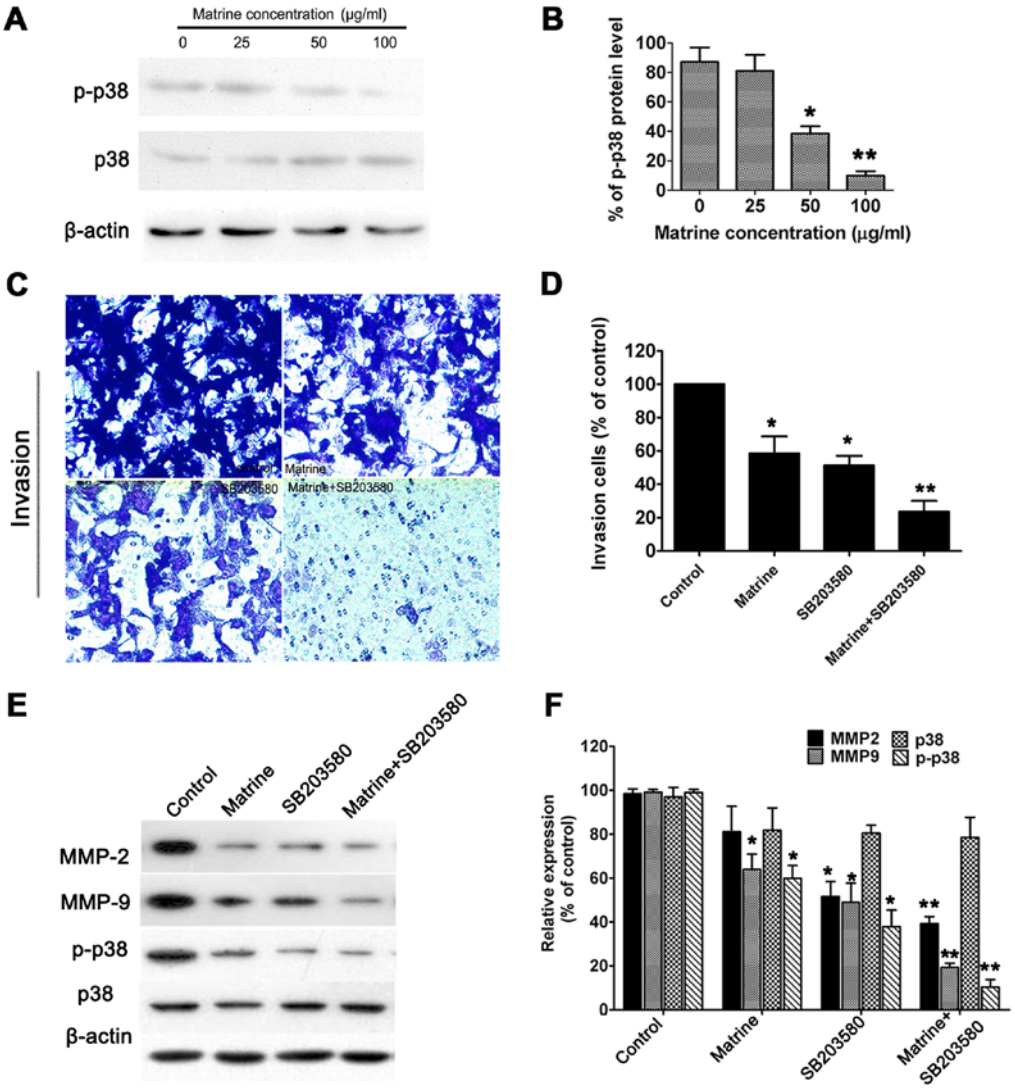

Figure 4. Matrine inhibits the migration and invasion of HeLa cells via downregulating p38 signaling pathway. (A) Western blot analysis of the levels of p38 and p-p38. (B) Densitometry analysis of p38 and p-p38 levels. Values represent the means ± SD of three independent experiments. *P<0.05 and **P<0.01 vs. control group. (C) Cells were pretreated with 10 μM SB203580 for 30 min and then incubated in the presence or absence of 50 μg/ml matrine for 24 h. Cell invasion was analyzed by Transwell chamber invasion assay. (D) Quantitative analysis of invasion rate of HeLa cells shown in C. (E) Western blot analysis of the levels of MMP-2, MMP-9, p38 and p-p38. (F) Quantitative analysis of protein levels shown in E. Values represent the means ± SD of three independent experiments. Statistical analysis was carried out using the two-way ANOVA followed by Bonferroni post-tests. *P<0.05 and **P<0.01 vs. control group.

Matrine suppresses the expression and activity of MMP-2 and MMP-9. The complex dynamics of tumor invasion and metastasis often involves the destruction of extracellular matrix (ECM) by enzymes such as serine proteases, threonine proteases and matrix metalloproteinases (MMPs) (38). To further explore the potential mechanism by which matrine inhibits cervical cancer invasion and metastasis, we examined the expression and activity of MMP-2 and MMP-9 in HeLa cells exposed to different concentrations of matrine. Western blot analysis showed that matrine significantly reduced the protein levels of MMP-2 and MMP-9 in a concentration-dependent manner (Fig. 3A and B). Gelatin zymography showed that the activities of MMP-2 and MMP-9 were significantly inhibited by matrine in a concentration-dependent manner (Fig. 3C). MMP-2 activity was reduced to 79.98±8.73, 37.5±4.67 and 22.08±1.67%, and MMP-9 activity was reduced to 70.24±7.87, 23.68±4.25 and 21.60±1.39%, in cells treated with 25, 50 and 100 μg/ml of matrine, respectively (Fig. 3D). Collectively, these data suggest that matrine significantly inhibits the expression and activity of MMP-2 and MMP-9.

Matrine inhibits the p38 signaling pathway in cervical cancer cells. Activation of p38 is required for the invasion of human cervical cancer cells (39). Thus, we wondered whether the anti-metastatic effect of matrine is related to the modulation of p38 signaling pathway in cervical cancer cells. Western blot analysis showed that the level of phosphorylated (activated) p38 in HeLa cells was downregulated upon matrine treatment in a concentration-dependent manner (Fig. 4B and C). To confirm that the inhibitory effects of matrine on cell invasion and MMP-2 and MMP-9 expression was associated with the downregulated p38 signaling pathway, HeLa cells were pretreated with a p38 inhibitor (SB203580, 10 μM) for 30 min and then incubated in the presence or absence of matrine (50 μg/ml) for 24 h. The results showed that treatment with SB203580 and matrine

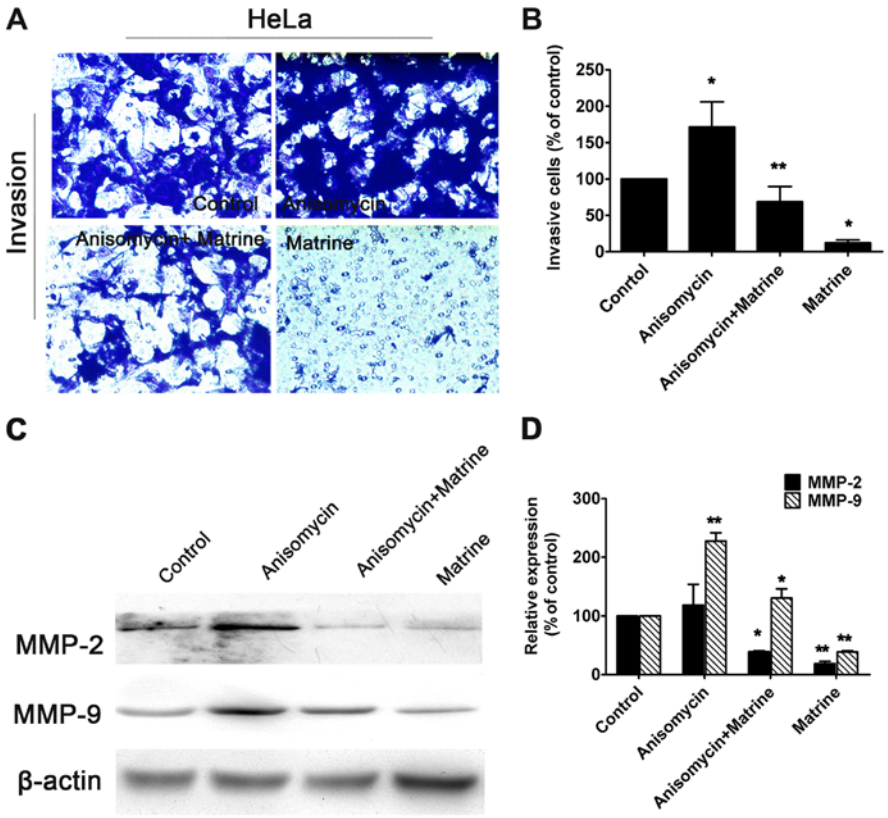

Figure 5. Effects of the p38 activator anisomycin and matrine on HeLa cell invasion. (A) Cells were pretreated with 5 μM/l anisomycin for 30 min and then incubated in the presence or absence of 100 μg/ml matrine for 24 h. Cell invasion was measured using the Boyden chamber invasion assay. (B) Quantitative analysis of invasion rate of HeLa cells shown in A. (C) HeLa cells were treated with matrine or anisomycin for 24 h and protein levels of MMP-2 and MMP-9 were detected by western blot analysis. (D) Quantitative analysis of MMP-2 and MMP-9 protein levels shown in C. Values represent the means ± SD of three independent experiments. Statistical analysis was carried out using the two-way ANOVA followed by Bonferroni post-tests. *P<0.05 and **P<0.01 vs. control group.

significantly reduced both cell invasion (Fig. 4D and F) and the levels of MMP-2, MMP-9 and p-P38 (Fig. 4E and G). Furthermore, anisomycin, a p38 activator, blocked the effects of matrine (Fig. 5). Taken together, these results reveal that the inhibition of cervical cancer cell invasion and MMP-2 and MMP-9 expression by matrine is mediated by the suppression of the p38 signaling pathway.

In vivo inhibition of cervical cancer tumor growth by matrine. To evaluate the effects of matrine on tumor growth *in vivo*, we performed nude mouse xenograft assay of HeLa cells as previously described (40). The time courses of HeLa xenograft growth with and without matrine treatment are shown in Fig. 6A. The average volume of xenograft treated with matrine at 21 days after cell implantation was significantly less than that of control group. At the end of the experiment, the xenograft tumors were dissected and weighed. Matrine significantly decreased the solid tumor mass and the inhibitory rate of tumor weight was 58.33% (Fig. 6B). Moreover, we assessed the levels of MMP-2, MMP-9, p38, p-p38, Akt, p-Akt, p65 and p-p65 in the tumors dissected from nude mice. Western blot analysis showed that matrine treatment led to significantly reduced levels of MMP-2, MMP-9 and p-p38, but had weaker effects on the levels of p-Akt and p-p65 (Fig. 6C). Taken together, these findings suggest that matrine inhibits cervical cancer growth through specific suppression of p38 signaling.

Discussion

In the present study, we confirmed that matrine significantly inhibited the growth of human cervical cancer HeLa and C33A cells *in vitro* in a dose- and time-dependent manner by inducing apoptosis. In addition, we demonstrated that matrine significantly inhibited the invasive and metastatic ability of cervical cancer cells *in vitro* by the downregulation of MMPs through inhibiting the p38 signaling pathway.

Recently, natural products have opened up a new avenue for successful cancer treatment because of their pharmacological activity and therapeutic possibilities (41,42). Matrine has been shown as a new antitumor natural product (43). However, the anticancer efficacy of matrine and the underlying mechanisms in cervical cancer are not clear. To explore the role of matrine in cervical cancer, we first performed MTT assay and found that matrine significantly inhibited cervical cancer cell growth in a dose- and time-dependent manner. To rule out the cytotoxicity of matrine, we chose concentrations of 0, 25, 50 and 100 μg/ml matrine for subsequent experiments.

A failure of cancer cells to undergo apoptosis is a common feature of many cancers. Thus, many anticancer drugs activate apoptotic pathways to eliminate cells that harbor genetic damage or divide inappropriately, which is a predominant antitumor mechanism (36). It has been reported that matrine exerts its antitumor effects by inhibiting the proliferation and inducing the apoptosis of gastric cancer cells as well as

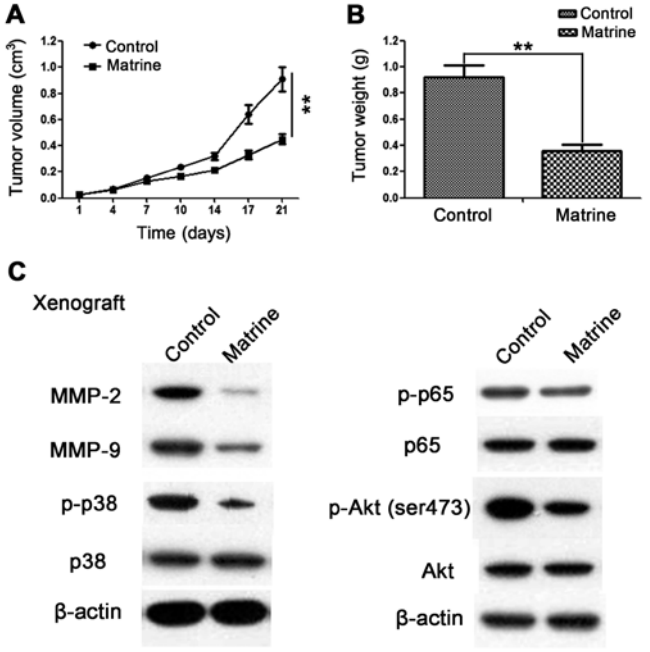

Figure 6. Matrine inhibits tumorigenesis of HeLa cells *in vivo*. HeLa cells were implanted subcutaneously into nude mice. Mice were treated with matrine (50 mg/kg/day) or saline as control. (A) The relative tumor volume and (B) final tumor weight of xenografts in saline or matrine treated Balb/c nude mice. Data are presented as the mean ± SD of at least three independent experiments. $^{**}P<0.01$ vs. saline treatment. (C) Xenografts were dissected and the levels of MMP-2, MMP-9, p38, p-p38, Akt, p-Akt, ERK1/2 and p-ERK1/2 were detected by western blot analysis. Representative blots from three independent experiments with similar results are shown. β-actin is a loading control.

leukemic and glioma cells (10,17,44). Consistent with these previous studies, we confirmed that matrine induced cervical cancer cell apoptosis in a dose-dependent manner in the range of 25-100 μg/ml. Taken together, these data indicate that matrine inhibits the growth of human cervical cancer cells by inducing apoptosis.

Metastasis is one of the leading causes of cancer-related death among cervical cancer patients. To further examine the effects of matrine on cervical cancer cell migration and invasion, we performed cell migration and invasion assay. The results showed that matrine could significantly inhibit the migration and invasion of cervical cancer cells at non-toxic doses (no more than 100 μg/ml). Zhang *et al* (33) demonstrated that matrine could suppress HeLa cell adhesion and migration, and this was correlated to decreased VASP phosphorylation. However, the development of metastasis is a complex series of steps (45,46). Successful implantation and invasion are closely linked to the degradation of ECM by MMPs. MMPs, especially MMP-2 and MMP-9, play critical roles in the degradation of type IV collagen, a major constituent of the ECM, and are closely related to the invasion and metastasis of various cancer cells (21-23). Consistent with previous studies (14,47), we found that matrine suppressed the expression and activity of MMP-2 and MMP-9 in cervical cancer cells. These results indicate that the anti-metastatic effect of matrine on HeLa cells is correlated with the down-regulation of MMP-2 and MMP-9.

The expression of proteinases is regulated by multiple signaling cascades, including p38 signaling pathway (27,48-50). p38 is relatively inactive in the unphosphorylated form and can be activated rapidly by MAPK kinase-3 and -6 upon exposure to cellular stress or inflammatory cytokines (51-53). p38 signaling pathway induces the expression of MMPs and thereby promotes the degradation of ECM proteins, leading to cell invasion (54). p38 has also been reported as a downstream target of matrine in many cells (34,47). Therefore, we hypothesized that the anti-invasion effect of matrine may be associated with the inhibition of p38 signaling pathway. To confirm it, we determined the level of phosphorylated (activated) p38 and demonstrated that the phosphorylation (activation) of p38 was significantly reduced in HeLa cells treated with matrine, compared to control cells. Furthermore, we employed specific p38 inhibitor (SB203580) and activator (anisomycin). We found that treatment with SB203580 significantly reduced cell invasion, accompanied by decreased MMP-2 and MMP-9 protein expression. Anisomycin could partly abolish the inhibition effects of matrine on the invasion of HeLa cells and the expression and activity of MMP-2/-9. These results indicate that matrine inhibits cell invasion and decreases the expression and activity of MMP-2 and MMP-9 via suppressing the activation of p38 signaling pathway in HeLa cells. The mechanisms of action of matrine against cancer cell invasion have been shown to be associated with EGF/VEGF-VEGFR1-Akt-NF-κB signaling (55). We also noted that matrine produced suppressing effect on activity of Akt and p65, even though weaker. Previous studies demonstrated that S100A8/9 promoted gastric cancer cell migration and invasion through p38 MAPK-dependent NF-κB activation (56). Thus, we presumed that there might be a potential link between p38 and Akt-NF-κB in cervical cancer cells treated with matrine. Therefore, it is necessary to further investigate whether pro-inflammatory cytokines or other signaling pathways contribute to the inhibitory effects of matrine on cervical cancer invasion and metastasis.

In conclusion, we demonstrated the inhibitory effects of matrine on the growth, invasion and metastastic capabilities of cervical cancer cells. Mechanistically, we found that matrine decreased the expression and activity of MMP-2 and MMP-9 via the suppression of p38 signaling pathway. Taken together, these findings suggest that matrine inhibits the growth and metastasis of cervical cancer through downregulating the p38 signaling pathway, and provide evidence that matrine has potential application in treating cervical cancer progression and metastasis.

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