Emodin inhibits tumor necrosis factor-α-induced migration and inflammatory responses in rat aortic smooth muscle cells

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Abstract. Emodin, a naturally occurring anthraquinone derivative in oriental herbal medicine, has been shown to exert a variety of pharmacological activities. The goal of this study was to determine the effects of emodin on the modulation of cell proliferation, migration, inflammatory responses, and matrix metalloproteinase (MMP)-2 and MMP-9 expression in tumor necrosis factor (TNF)-α-induced rat aortic smooth muscle cells (RASMCs). Cell proliferation and migration were measured using the MTT assay and the transwell chamber assay, respectively. Quantitative real-time PCR and western blot analysis were used to detect MMP expression. Gel shift was used for analysis of nuclear factor (NF)-κB activation. In addition, the expression of several inflammatory genes was also analyzed. Treatment of RASMCs with emodin significantly and dose-dependently attenuated TNF-α-induced proliferation, migration, mRNA and protein expression of MMP-2 and MMP-9, and NF-κB activation. Furthermore, emodin significantly inhibited TNF-α-evoked inflammatory responses, as demonstrated by the reduction in the expression of inflammatory genes. These results suggest that emodin inhibits TNF-α-induced proliferation, migration, MMP-2 and MMP-9 expression as well as inflammatory responses in cultured RASMCs, supporting the notion that emodin may have potential application in clinical atherosclerosis disease.

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Introduction

Atherosclerosis is a chronic inflammatory disease characterized by the presence of atherosclerotic lesions in the arterial intima, leading to hardening and narrowing of the major arteries (1,2). It is widely recognized that abnormal proliferation and migration of vascular smooth muscle cells (VSMCs) play fundamental roles in the formation of atherosclerotic lesions. Generally, VSMCs in the tunica media have a low mitogenic activity level. However, during the early stages of arterial-wall injury or atherosclerosis, VSMCs may undergo a series of phenotypic changes and migrate to the arterial intima where they begin proliferating in response to various growth factors or cytokines, such as tumor necrosis factor-α (TNF-α) and platelet derived growth factor (PDGF) (1,3). In addition to growth factor stimulation, the proliferation and migration of VSMCs may require the degradation or remodelling of the extracellular matrix (ECM) surrounding the cells. An imbalance between the accumulation and degradation of ECM may be an important determinant for the development of intimal hyperplasia following vascular wall intrusions (3).

Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes that are believed to contribute to the development and progression of atherosclerosis by facilitating the proliferation and migration of VSMCs (4). Among MMP sub-species, MMP-2 and MMP-9 are indispensable for the degradation of type IV collagen, a major component of the basement membrane (5). Previous studies have clearly demonstrated that MMP-2 and MMP-9 are expressed abundantly in atherosclerotic lesions in both human and animal models, and knockout of MMP-2 and MMP-9 or inhibition of their activities by synthetic compounds greatly reduces neo-intima formation (6-8). In addition, recent experimental data indicate that inflammatory cytokines, including TNF-α, can stimulate the production of MMPs in VSMCs via the activation of intracellular signalling pathways, such as redox-sensitive transcription factor nuclear factor-κB (NF-κB) and the Ras/ERK1/2 pathway (9,10).

Emodin (1,3,8-trihydroxy-6-methylanthraquinone), the major compound in the root of rhubarb (*Rheum palmatum* L.), has been shown to display various biological and pharmacological properties, such as anti-viral (11), anti-cancer (12), anti-inflammatory (13), and anti-oxidant activities (14). Emodin has also been reported to induce growth arrest and death of human VSMCs through reactive oxygen species and p53 (15). In addition, Kumar *et al* showed that emodin inhibits TNF-induced NF-κB activation and IκB degradation in human vascular endothelial cells (16). A previous study demonstrated that emodin inhibits TNF-α-induced human aortic smooth muscle cell (ASMC) proliferation via a caspase- and mitochondrial-dependent apoptotic pathway (17). However, information is still lacking on the effects of emodin on migration, MMP-2 and MMP-9 expression in TNF-α-induced VSMCs. Therefore, the aim of this study was to investigate the effects of emodin on the migration and inflammatory responses in TNF-α-induced rat ASMCs (RASMCs).

Materials and methods

Reagents and antibodies. Emodin, dimethyl sulfoxide (DMSO), and 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma (St. Louis, MO). Recombinant rat TNF-α was obtained from Invitrogen (Carlsbad, CA). Polyclonal antibodies to MMP-2, MMP-9, NF-κB p65 and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Cell culture. Eight-week-old male Sprague-Dawley rats were obtained from the Experimental Animal Center of China Medical University. Primary RASMCs were prepared from the aortas of rats by the method previously described (18) and cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS, Hyclone, Logan, UT), 2 mmol/l glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in a humidified 5% CO_2 atmosphere. The purity of the cultured RASMCs was verified by immunostaining with a monoclonal antibody against smooth muscle-specific α-actin. The study protocol was reviewed and approved by the Animal Experimental Committee of Shenyang Medical College.

MTT assay. Cell viability was assessed using the MTT cell proliferation assay. In brief, cells were seeded in 96-well plates and incubated with various concentrations of agents for different intervals. At the end of the incubation period, 100 μl MTT solution (0.5 mg/ml) was added to each well and incubation continued at 37°C for an additional 4 h. The supernatant was then carefully removed by aspiration and the converted dye was dissolved with 100 μl DMSO. The absorbance of the suspension was measured at 570 nm using a microplate reader.

Quantitative real-time PCR. Total RNA was extracted from cells with TRizol reagent (Life Technologies, Inc., Rockville, MD), according to the manufacturer's instructions. The concentration and purity of the RNA in each sample were determined by measuring the absorbance at 260 and 280 nm. RNA integrity was confirmed by electrophoresis on a 1% agarose gel. cDNA was synthesized from 1 μg of total RNA using a PrimeScript RT reagent kit (Takara, Dalian, China). Quantitative real-time PCR was performed using SYBR-Green (Takara, Dalian, China) on a Real-Time Quantitative Thermal Block (Biometra, Göttingen, Germany). PCR primers were designed using the Primer Express 2.0 software (Applied Biosystems, Foster, CA) and are listed in Table I. The specificity of the amplified products was analyzed through dissociation curves generated by the equipment yielding single peaks. β-actin was used as an internal control to normalize samples. PCR reactions of each sample were conducted in triplicate. Data were analyzed through the comparative threshold cycle (C_T) method.

Western blot analysis. Cells were washed with ice-cold phosphate buffer saline (PBS) and then harvested by the addition of 1 ml lysis buffer (containing 10 mM Tris, pH 7.2, 150 mM NaCl, 5 mM EDTA, pH 8.0, 0.1% Triton X-100, 1 mM DTT, and 0.1 mM PMSF). Following incubation on ice for 30 min, the supernatant was collected by centrifugation at 12,000 g for 10 min at 4°C, and the protein concentration was determined using the Bradford assay. Subsequently, proteins were denatured in sample buffer containing 2-mercaptoethanol and bromophenol blue for 10 min at 100°C. Equal amounts of total proteins were loaded onto each lane of a 10% sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE), separated electrophoretically, and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA). The membranes were blocked with 5% non-fat dry milk, and then probed with anti-MMP-2, anti-MMP-9, or anti-β-actin antibodies at 4°C overnight. Densitometric analysis was conducted with ChemiImager™ 5500 (Alpha Innotech Inc, Santa Clara, CA) to semi-quantify western blot data.

Cell migration assay. In vitro migration assays were performed using 24-well transwells (8 mm pore size; Costar, Cambridge, MA, USA) coated with matrigel (1 mg/ml, BD Sciences, San Jose, CA). Cells ($5x10^4$ cells/well) were detached from tissue culture plates, washed with PBS, and re-suspended in conditioned medium. Cells (200 μl) suspended in conditioned medium was added to the upper compartment of the invasion chamber, then 500 μl of the same conditioned medium was added to the lower compartment of the invasion chamber. Following incubation at 37°C for 6 h in 5% CO_2, cells remaining in the chamber were removed with a cotton swab, and the migrated cells situated on the lower side of membranes were fixed with 4% formaldehyde, stained with hematoxylin and eosin, and examined under a microscope. Cells in at least six random microscopic fields (x200) were counted.

Preparation of nuclear extracts. Nuclear extracts from cells were prepared by hypotonic lysis followed by high salt extraction. In brief, after washing with PBS, cells were homogenized in 0.5 ml of ice-cold cell lysis buffer composed of 10 mM HEPES (pH 7.9), 10 mM KCl, 2 mM $MgCl_2$, 0.1 mM EDTA, 1 mM dithiothreitol (DTT), and 0.5 mM phenylmethylsulfonyl fluoride (all from Sigma). Following centrifugation at 500 g for 30 sec at 4°C, the supernatants were incubated on ice for 20 min and then 50 μl of 10% Nonidet P-40 (Sigma) was added; the mixture was vortexed for 30 sec and then centrifuged at 5,000 g for 1 min at 4°C. The nuclear pellet was

Table I. Specific primers used for quantitative real-time PCR analysis.

Gene symbol	Sequences	Product size (bp)
MMP-2	F: 5'-TTGTAGGAGGTGCCCTGGAA-3' R: 5'-CCCCATGAAGCCTTGTTTACC-3'	150
MMP-9	F: 5'-CCAGATGATGGGAGAGAAGC-3' R: 5'-GGCCTTTAGTGTCTCGCTGT-3'	105
IL-1β	F: 5'-GGATGATGACGACCTGC-3' R: 5'-CTTGTTGGCTTATGTTCTG-3'	146
IL-6	F: 5'-CAAAGCCAGAGTCCATTCAGAGC-3' R: 5'-GGTCCTTAGCCACTCCTTCTGT-3'	151
ICAM-1	F: 5'-CAAACGGGAGATGAATGG-3' R: 5'-TGGCGGTAATAGGTGTAAAT-3'	183
VCAM-1	F: 5'-GGGGATTCCGTTGTTCT-3' R: 5'-CAGGGCTCAGCGTCAGT-3'	136
MCP-1	F: 5'-ATGCAGGTCTCTGTCACGCT-3' R: 5'-GGTGCTGAAGTCCTTAGGGT-3'	345
CINC-2β	F: 5'-TCAGGGACTGTTGTGG-3' R: 5'-TGACTTCTGTCTGGGTG-3'	127
β-actin	F: 5'-ATGGATGACGATATCGCTGCG-3' R: 5'-CAGGGTCAGGATGCCTCTCTT-3'	200

F, forward; R, reverse.

resuspended in nuclear lysis buffer (20 mM HEPES pH 7.9, 420 mM NaCl, 1.5 mM MgCl$_2$, 0.1 mM EDTA, 1 mM DTT, 0.5 mM phenylmethylsulfonyl fluoride, and 25% glycerol), then incubated on ice for 30 min, followed by centrifugation at 12,000 g for 15 min at 4˚C. The resulting supernatants were stored at -70˚C as nuclear extracts. The protein concentrations were determined using the Bradford assay.

Electrophoretic mobility shift assay (EMSA). EMSA was carried out with a commercially available kit (Gel Shift Assay System; Promega, Madison, WI), according to the recommended protocol. Briefly, nuclear extracts (6-8 µg) were incubated with γ-^{32}P-labeled double-stranded oligo-nucleotide probes for NF-κB containing a consensus NF-κB sequence (5'-AGTTGAGGGGACTTTCCCAGGC-3'; Free Biotech, Beijing, China) in a reaction mixture containing 10 mM Tris-HCl (pH 7.5), 4% glycerol, 1 mM MgCl$_2$, 0.5 mM EDTA, 0.5 mM DTT, 50 mM NaCl, and 0.05 mg/ml poly(di-dc)·poly(di-dc) for 30 min at 37˚C. The reaction mixtures were loaded on a 4% non-denaturing polyacrylamide gel and run until the free probe reached at the end of the gel. The gel was vacuum-dried and exposed to radiograph film. The specificity of the complex was checked using an anti-NF-κB p65 antibody. NF-κB activity was quantified by densitometric analysis with the Gel pro 3.0.

Statistical analysis. The experimental results are expressed as the mean ± standard deviation (SD), and data were analyzed using the one-way analysis of variance (ANOVA) with the Bonferroni test as the post-hoc for significant difference. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Emodin inhibits TNF-α-induced proliferation of RASMCs. We first investigated the proliferation of RASMCs in response to 1, 10 and 100 ng/ml of TNF-α using the MTT assay. As shown in Fig. 1A, TNF-α treatment at all concentrations (1, 10 and 100 ng/ml) for 24 h significantly increased cell proliferation with the maximum effect occurring at 10 ng/ml ($P < 0.01$). Herein, we chose 10 ng/ml TNF-α as the working concentration for subsequent experiments in this study.

Next, we determined the dose-response for emodin-mediated effects on the TNF-α-induced proliferation of RASMCs. RASMCs were pre-treated in the presence of emodin (0.1-10 µM) in serum-depleted medium for 30 min and then stimulated with 10 ng/ml TNF-α for 24 h. Fig. 1B shows that emodin significantly and dose-dependently inhibited the proliferation of RASMCs (38.5, 27.5 and 21.9% of TNF-α alone at final concentrations of 0.1, 1 and 10 µM emodin, respectively, $P < 0.01$); however, emodin alone (10 µM) had no effect on the proliferation of RASMCs. These data suggest that emodin significantly suppressed proliferation of RASMCs stimulated by TNF-α.

Emodin inhibits TNF-α-induced cell migration and the MMP expression in RASMCs. VSMC migration is known to be a key event in the pathogenesis of atherosclerosis (19). In this study, we investigated the effect of emodin on the cell migra-
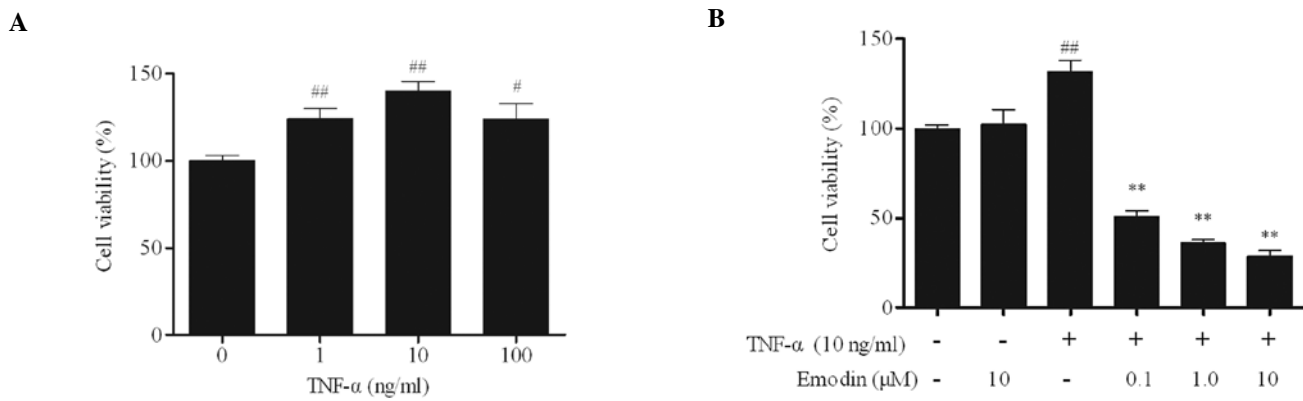
A

B

Figure 1. Effect of RASMC proliferation induced by TNF-α and emodin. RASMCs were stimulated with 0.1, 1 or 10 μM emodin for 30 min prior to treatment (A) without or (B) with 10 ng/ml TNF-α for 24 h. RASMC proliferation was determined by the MTT assay. Data represent the mean ± SD. [#]$P<0.05$, [##]$P<0.01$ vs. control (0 ng/ml TNF-α) group; [**]$P<0.01$ vs TNF-α alone group.

A

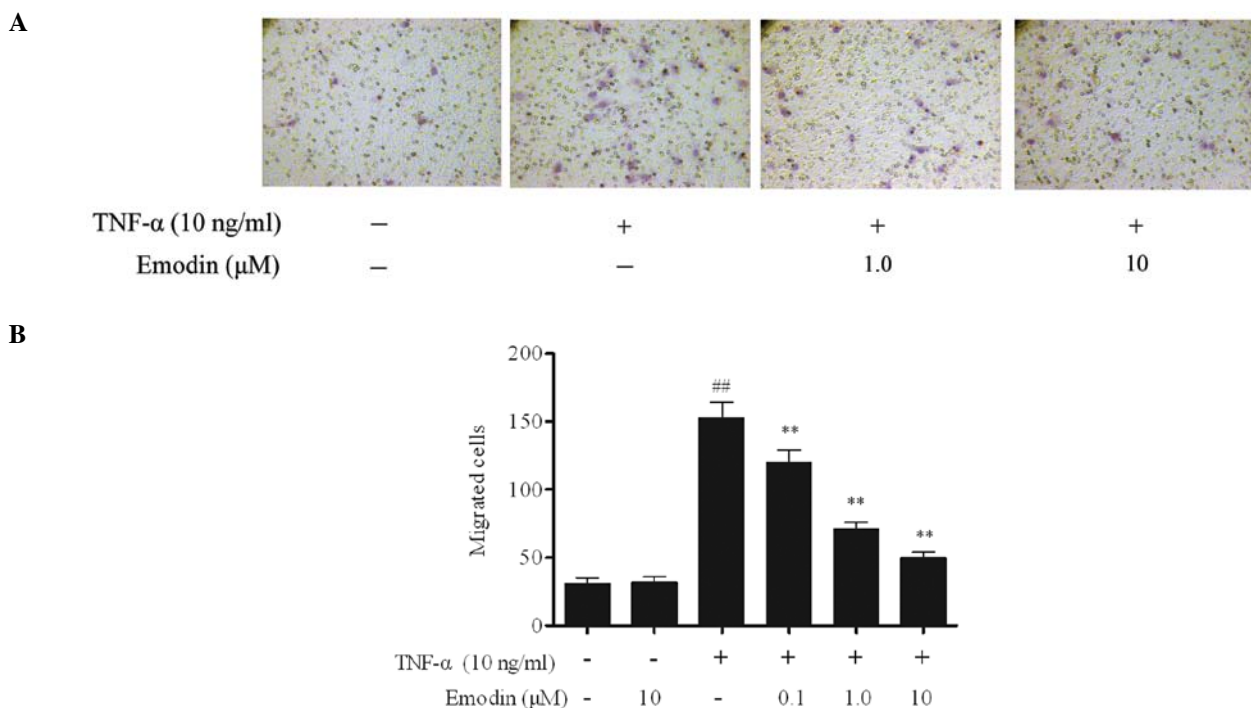| TNF-α (10 ng/ml) | − | + | + | + |
| Emodin (μM) | − | − | 1.0 | 10 |

B

Figure 2. Effect of emodin on RASMC migration. Cells were seeded in the upper chambers with the indicated concentrations of emodin and then allowed to migrate to the lower chambers in the presence of TNF-α for 6 h. (A) Hematoxylin and eosin staining of migrated cells; (B) total number of cells from three filters was averaged, and results are expressed as the mean ± SD. [##]$P<0.01$ vs. control (0 ng/ml TNF-α) group; [**]$P<0.01$ vs. TNF-α alone group.

tion of TNF-α-stimulated RASMCs by using the transwell systems. The RASMCs were seeded in the upper chambers with various final concentrations of emodin (0.1, 1 and 10 μM) and then allowed to migrate in the lower chambers in the presence of TNF-α for 6 h. Cells without TNF-α treatment served as control. It was found that TNF-α-induced RASMC migration was diminished by emodin treatment in a dose-dependent manner (Fig. 2, $P<0.01$).

Several studies have demonstrated that MMPs are important for VSMC proliferation and migration into the intima, and TNF-α stimulates the induction of MMPs in VSMCs (1). Therefore, to determine the efficacy of emodin in inhibiting MMP-2 and MMP-9 expression induced by TNF-α, RASMCs were cultured with TNF-α (10 ng/ml)

in the absence or presence of varying concentrations of emodin. After 24 h incubation, cell lysates were harvested for analysis of MMP-2 and MMP-9 mRNA and protein expression by quantitative real-time PCR and western blot analysis, respectively. As shown in Fig. 3A, treatment with TNF-α (10 ng/ml) significantly increased the mRNA expression of MMP-2 and MMP-9 ($P<0.01$). By contrast, this induction of MMP mRNA expression by TNF-α was inhibited in the presence of emodin in a dose-dependent manner, and similar results were found in western blot analysis (Fig. 3B and C). These findings suggest that treatment with emodin inhibits the TNF-α-stimulated increase in MMP-2 and MMP-9 expression at both the mRNA and protein levels.
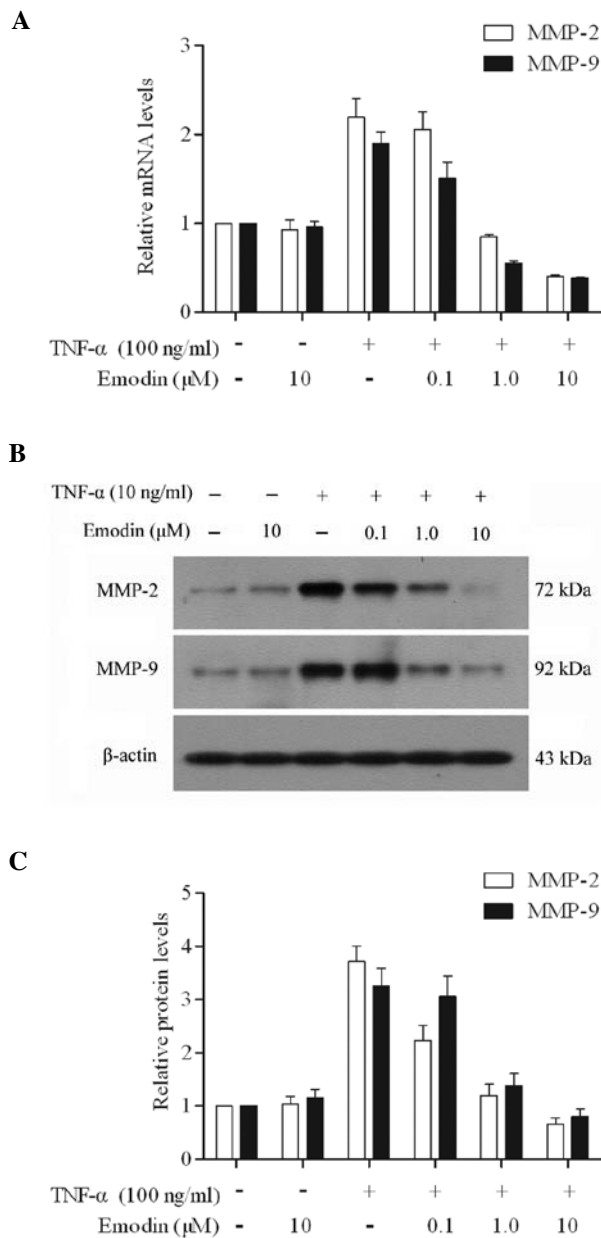
Figure 3. Effect of emodin on the expression of MMP-2 and MMP-9 in TNF-α treated RASMCs. RASMCs were cultured with TNF-α (10 ng/ml) in the absence or presence of varying concentrations of emodin. After 24 h incubation, cell lysates were harvested for analysis of MMP-2 and MMP-9 mRNA and protein expression by (A) quantitative real-time PCR and (B) western blot analysis, respectively. (A) Data represent the mean ± SD from three experiments with duplicates; (B) Representative blots are shown, and protein size is expressed in kDa; (C) Protein levels were normalized with β-actin, and data were subjected to densitometric quantification.

Figure 4. Effect of emodin on TNF-α-induced mRNA expression of (A) cytokines, (B) adhesion molecules, and (C) chemokines in RASMCs. Data (mean ± SD) are from quantitative real-time RT-PCR assays and are normalized by β-actin. ##P<0.01 vs. control (0 ng/ml TNF-α) group; **P<0.01 vs. TNF-α alone group.

Emodin downregulates mRNA levels of inflammatory mediators induced by TNF-α in RASMCs. Inflammation is considered to play a vital role in the pathogenesis of atherosclerosis. Previous studies have shown that VSMCs are able to produce inflammatory mediators, such as adhesion molecules and chemokines, when stimulated with cytokines (20). To determine whether emodin has anti-inflammatory actions in RASMCs *in vitro*, we examined the effects of emodin on TNF-α-stimulated expression of cytokines [interleukin-1β (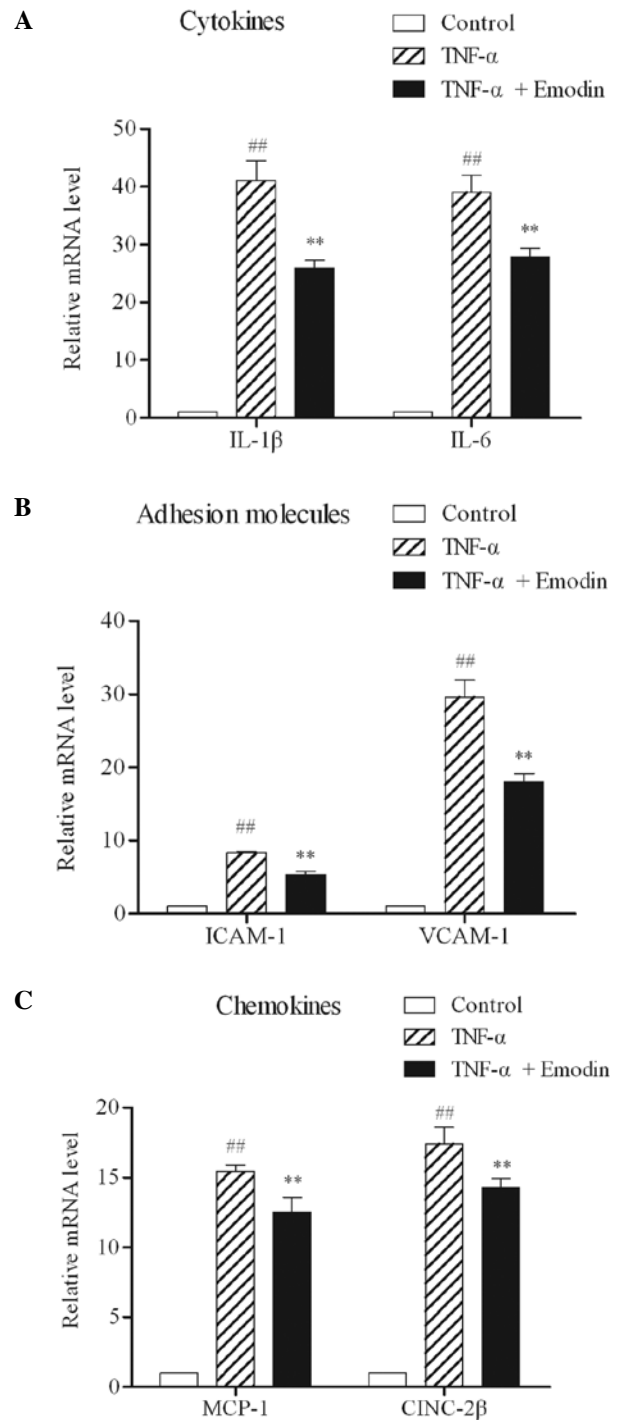IL-1β) and IL-6], adhesion molecules [intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)] and pro-inflammatory chemokines [neutrophil chemoattractant-2β (CINC-2β) and monocyte chemotactic protein-1 (MCP-1)] using quantitative real-time PCR. All mediators were expressed at low levels in unstimulated RASMCs. However, treatment with TNF-α significantly increased these levels, which were markedly attenuated by pre-treatment with 10 μM emodin (Fig. 4, P<0.01). These results indicate that
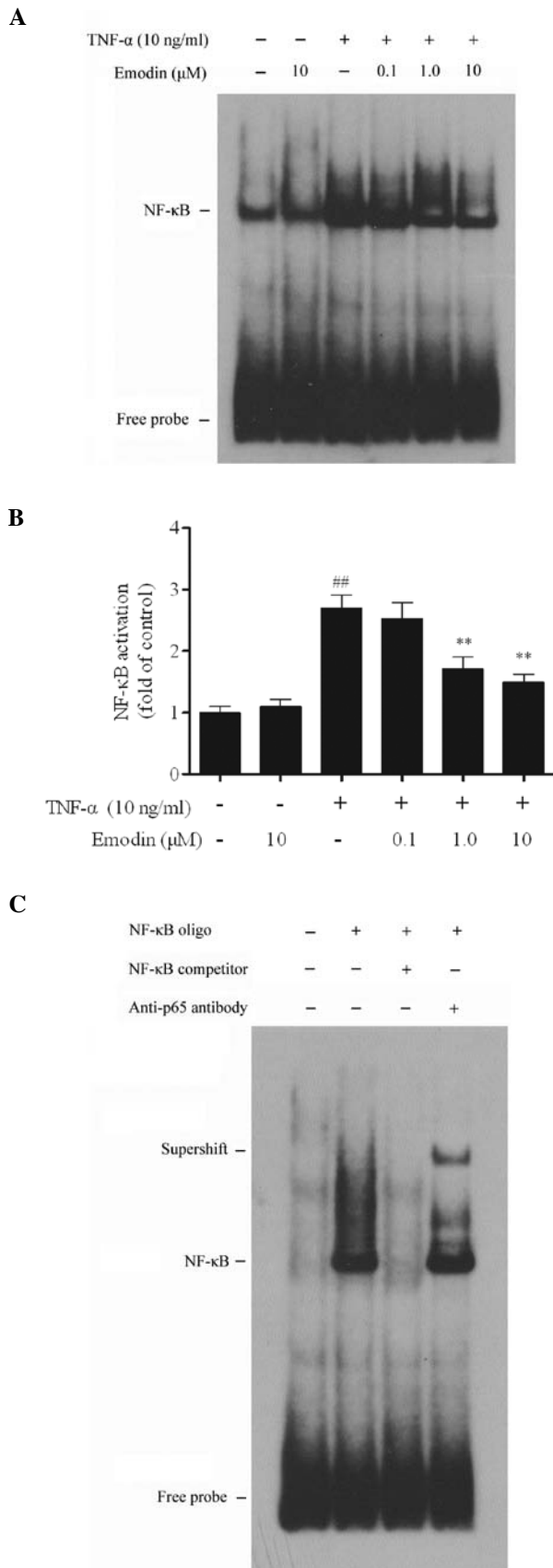
A

B

C

Figure 5. EMSA for NF-κB activation in cultured RASMCs. (A) Nuclear protein extracts were prepared and gel shift assay was performed using γ-^{32}P-labeled oligonucleotide containing a consensus NF-κB sequence. (B) Densitometric analysis was conducted to semi-quantify EMSA data. $^{##}$P<0.01 vs. control (0 ng/ml TNF-α) group; **P<0.01 vs. TNF-α alone group. (C) To confirm that the presence of bands is specific to NF-κB, unlabelled oligonucleotide and anti-p65 antibody controls were also performed in separate studies.

emodin has anti-inflammatory effects in RASMCs stimulated with TNF-α.

Emodin inhibits TNF-α-induced activation of NF-κB. Transcriptional regulation involving NF-κB activation has been implicated in TNF-α-induced MMP expression and inflammatory responses in VSMCs (21,22). To examine whether emodin inhibits NF-κB activation, EMSA was performed with consensus NF-κB binding sequence. As shown in Fig. 5, incubation of RASMCs with TNF-α (10 ng/ml) caused strong activation of NF-κB at 1 h. The activation of NF-κB induced by TNF-α could be dose-dependently inhibited by emodin, as detected by DNA binding activity. These results suggest that emodin inhibits NF-κB activation in TNF-α treated RASMCs.

Discussion

Emodin is a naturally occurring anthraquinone present in rhubarb and many other plants. Several studies have described the beneficial effects of emodin on cardiovascular disease either *in vitro* or *in vivo* (14,15,23,24). To date, little is known about the anti-migration and anti-inflammatory roles of emodin in VSMCs. In this study, we demonstrated for the first time that emodin treatment effectively attenuates TNF-α-induced migration, inflammatory responses, and mRNA and protein expressions of MMP-2 and MMP-9 in RASMCs. It was also found that this inhibition of MMPs may be associated with the suppression of the NF-κB signaling pathway.

Proliferation and migration of VSMCs are key contributors to the pathogenesis of atherosclerosis, and the inhibition of VSMC proliferation and migration is important for the treatment of cardiovascular diseases (3). The aim of this study was to investigate the anti-atherogenic mechanism of emodin in RASMCs against TNF-α treatment, as TNF-α functions as a stimulator in the pathogenesis of vascular lesions, such as atherosclerosis. We found that 10 ng/ml TNF-α was maximally effective at promoting RASMC proliferation, and this induction of proliferation was significantly and dose-dependently inhibited by emodin. This result is consistent with a previous study from Heo *et al* who showed that emodin inhibits TNF-α-induced human ASMC proliferation via a caspase- and mitochondrial-dependent apoptotic pathway (17). In addition, we also found that emodin attenuated the TNF-α-induced ASMC migration in a dose-dependent manner, as evidenced by transwell systems. Our results, together with previous findings, suggest that emodin may be useful for the treatment of vascular diseases and restenosis.

It is evident that initial degradation of the ECM is an essential event for vascular cell hypertrophy, proliferation and migration, and this degradation is mediated by VSMC secreted MMPs that can selectively digest the individual components of the ECM (25). Among MMPs, MMP-2 and MMP-9 represent the gelatinase group and are of particular interest in atherosclerosis due to their significant roles in the regulation of VSMC proliferation and migration. It has been shown that MMP-2 is constitutively expressed in VSMCs in normal arteries in addition to increased MMP-2 expression, and MMP-9 expression is induced in VSMCs and macrophages in atherosclerotic arteries (26). In this study, we found that TNF-α enhanced MMP-2 and MMP-9 mRNA and protein expression in

cultured RASMCs. These observations are in agreement with the results from Lin *et al* in human ASMCs, suggesting that TNF-α may contribute to the development of atherosclerotic vascular remodeling at multiple steps mediated by MMP-2 and MMP-9 (27). We also examined whether emodin treatment inhibits TNF-α-stimulated MMP-2 and MMP-9 expression in RASMCs. Of considerable interest in this study was the marked reduction by emodin of the secretion of MMP-2 and MMP-9 from TNF-α-stimulated RASMCs, as evidenced by quantitative real-time PCR and western blot analysis. These results indicate that emodin may have anti-atherogenic effects on RASMCs through the inhibition of MMP-2 and MMP-9 expression, which have been linked to the progression of plaque rupture and intimal formation in arterial lesions.

Recent results have indicated that the regulation of MMPs in response to TNF-α is involved in NF-κB activation in VSMCs (28,29). Consistent with previous studies, we demonstrated that TNF-α activates NF-κB in RASMCs, suggesting that the upregulation of MMP expression in response to TNF-α is mediated by this transcriptional factor. However, emodin dose-dependently inhibited the activation of NF-κB, which indicates that emodin suppresses MMP expression by the inhibition of NF-κB activation in TNF-α-treated RASMCs. Moreover, it is well-known that NF-κB activation is typically activated by proteasomal degradation of IκB-α and nuclear translocation of NF-κB (30). Heo *et al* have shown that emodin dose-dependently inhibited the phosphorylation of IκB-α in TNF-α-treated human ASMC (17). Based on these findings, it seems reasonable to conclude that the inhibitory effect of emodin on NF-κB activation is IκB-α-dependent. Additionally, reactive oxygen species (ROS) are important intercellular second messengers to regulate many downstream signaling molecules including NF-κB, and thus mediate VSMC growth/migration and expression of proinflammatory molecules (31). Emodin has been shown to be a potent ROS scavenger (32). Therefore, emodin may also decrease the NF-κB activation by inhibition of oxidative stress in TNF-α-stimulated RASMCs. Furthermore, NF-κB activation in cells controls the expression of many inflammatory genes, including adhesion molecules, cytokines and chemokines. In the present study, the effect of emodin on TNF-α-induced adhesion molecules (ICAM-1 and VCAM-1), cytokines (IL-1β and IL-6), and pro-inflammatory chemokines (CINC-2β and MCP-1) was further investigated. We found that emodin significantly inhibits the TNF-α-induced expression of these inflammatory genes, suggesting that emodin is capable of inhibiting TNF-α-evoked inflammatory responses in RASMCs. Our results are similar to those in previous studies showing that emodin exerts anti-inflammatory effects via inhibition of the NF-κB pathway (33-35). However, the precise mechanisms by which emodin exerts its anti-inflammatory actions in TNF-α-stimulated RASMCs need to be further elucidated.

In summary, the present study demonstrates that emodin effectively inhibits TNF-α-induced proliferation, migration, MMP-2 and MMP-9 expression, and inflammatory responses in cultured RASMCs. In addition, the effects exerted by emodin may be associated with the inhibition of the NF-κB pathway. The findings presented here also imply that emodin may have therapeutic potential in the prevention of cardiovascular diseases.

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