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Communication

Molecules and Cells

Melittin Suppresses PMA-Induced Tumor Cell Invasion by Inhibiting NF-κB and AP-1-Dependent MMP-9 Expression

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Matrix metalloproteinase-9 (MMP-9) plays an important role in the invasion and metastasis of cancer cells. In this study, we examined the inhibitory effect of bee venom (BV) and its major peptides, melittin and apamin, on PMA-induced invasion induced by MMP-9 expression in Caki-1 renal cancer cells. BV and melittin, but not apamin, significantly suppressed PMA-induced invasion by inhibiting MMP-9 expression in Caki-1 cells. Furthermore, as evidenced by MMP-9 promoter assays, melittin inhibited MMP-9 gene expression by blocking the PMA-stimulated activations of activator protein-1 (AP-1) and nuclear factor-kappa B (NF-κB). In addition, melittin suppressed the PMA-induced phosphorylations of ERK and JNK mitogen-activated protein kinases, upstream factors involved in Ap-1 and NF-κB. These results suggest that the suppression of MMP-9 expression contributes to the anti-tumor properties of melittin.

INTRODUCTION

Regulation of metastasis and invasion represent important therapeutic targets, as our inability to control metastasis and cancer invasion remains the most formidable obstacle to successful treatment. The biological processes of invasion and metastasis require the destruction of extracellular matrix (ECM), to include of mesenchymal collagen and endothelial basement membrane. Therefore, ECM degradation enzymes, such as matrix metalloproteinases (MMPs) are important pharmaceutical targets for tumor invasion and metastasis regulation (Nabeshima et al., 2002; Son et al., 2004; Stetler-Stevenson et al., 1996).

The MMPs are a family of highly homologous protein-degrading zinc dependent enzymes endopeptidases. Currently, 28 members of this family have been identified, which include collagenases, gelatinases, stromelysins, matrilysins, and membrane-type MMPs (Chambers and Matrisian, 1997; Stetler-Stevenson et al., 1996). MMP family members are important components of many normal biological processes, which include embryonic development, angiogenesis, and wound healing. They also participate in many pathological processes, such as ECM degradation, which facilitates the migration of cancer cells (Nabeshima et al., 2002). MMP-2 (gelatinase-A) and MMP-9 (gelatinase-B) are known to be involved in the degradation of type IV collagen (Lakka et al., 2004; Zucker et al., 1993), and to have structural and catalytic similarities. However, their transcriptions are independently regulated due to the presence of distinct regulatory elements in gene promoters (Nabeshima et al., 2002; Stetler-Stevenson et al., 1996). Furthermore, MMP-2 is constitutively expressed and always overexpressed in highly metastatic tumors, whereas MMP-9 can be induced by cytokines, growth factors, or phorbol esters. MMP-9 is also known to be activated by transcription factors, such as nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1) (Cho et al., 2007; Woo et al., 2005).

Bee venom is a natural toxin produced by the honey bee (*Apis mellifera*), and has been used in traditional medicine to treat various diseases, to include arthritis, rheumatism, cancerous tumors, and various skin diseases (Hider, 1988). Bee venom contains a large number of biologically active peptides, including melittin (which constitutes 50% of whole bee venom), apamin, adolapin, and mast cell-degranulating peptide (MCCP) (Kwon et al., 2002). Several previous studies have examined the biological and pharmacological activities of bee venom, and it has been shown to have anti-inflammatory, anti-rheumatoid arthritis (Kwon et al., 2002), and pain-relieving effects (Kwon et al., 2001). Bee venom has been found to inhibit cyclooxygenase-2 (COX-2) expression in human cancer cells (Jang et al., 2003), and to suppress the growths and proliferations of leukemia (Moon et al., 2006), osteosarcoma (Chu et al., 2007), and mammary carcinoma cells (Orsolic et al., 2003). Moreover, recent studies have reported that bee venom induces the apop-

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tosis of hepatocellular carcinoma cells by activating Ca^{2+}/calmodulin-dependent protein kinase, TAK1, and JNK/p38 (Wang et al., 2009). However, no information is available concerning its ability to regulate MMP-9 expression or its effects on related molecular entities.

In the present study, the effects of bee venom and of its major peptides, melittin and apamin on PMA-induced MMP-9 expression were examined in Caki-1 cells (a human renal carcinoma cell-line). Additionally, we predict that inhibition of tumor invasion by melittin is related with MMP-9 expression via the transcriptional activities of AP-1 and NF-κB.

MATERIALS AND METHODS

Cells and materials

Caki-1 (renal carcinoma), Caski (cervical cancer), SK-BR-3 (breast carcinoma cancer) cells were obtained from the American Type Culture Collection (USA). Cells were cultured in DMEM (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) and 5% CO_2 at 37°C. Bee venom was obtained from the National Institute of Agricultural Science and Technology (NIAST) of Korea. All chemicals were obtained from Sigma (USA), unless otherwise indicated. Luciferase and β-galactosidase assay systems were from Promega (USA).

Cell viability assays

Cells were plated in 96-well culture plates at 1×10^4 cells/well in DMEM culture medium and allowed to attach for 24 h. Media were then discarded and replaced with 100 μl of new medium containing various concentrations of melittin and cultured for 24 h. The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetra-zolium bromide (MTT) (Roche Molecular Biochemicals, USA) was added to each well. The amount of formazan deposits was quantified according to the supplier's protocol after 4 h of incubation with MTT test solution in a 37°C and 5% CO_2 incubator.

Cell invasion assays

Cell invasion assays were carried out as previously reported (Cho et al., 2005) with slight modification; 1×10^5 cells per chamber were seeded for each invasion assay. Briefly, the upper chamber of a transwell insert (Corning Costar, USA) was coated with 30 μl of a 1:2 mixture of matrigel:PBS. Cells were then plated on the matrigel-coated upper chamber. The lower chamber was filled with culture media containing various concentrations of melittin. Cells in the chamber were incubated for 24 h at 37°C and cells that invaded the lower membrane surface were fixed with methanol and stained with hematoxylin and eosin. The cells that passed through the matrigel and were located on the underside of the filter were counted. Random fields were counted by light microscopy under a high power field (X400).

Wound-healing assays

These assays were performed using the procedure described by Lin et al. (2008) with minor modification. Caki-1 cells were seeded at 5×10^5 cells/well in six-well plates and incubated until they reached 80% confluence. Monolayers were scratched with a 200 μl pipette tip to create a wound, and cells were then washed twice with serum-free culture media to remove floating cells. Media were then replaced with fresh serum-free medium. Cells were subjected to the indicated treatment for 24 h, and cells migrating from the leading edge were photographed at 0 and 24 h.

Gelatin zymography assays

Zymography was performed using the procedure described by Cho et al. (2005) with minor modification. Caki-1 cells were plated at 3×10^5 cells in 35 mm-diameter dishes and incubated until they reached 80% confluence. Fresh serum-free medium was then added to each dish, and further cultured for 24 h. Conditioned media, so obtained, were electrophoresed in polyacrylamide gels containing 0.1% (w/v) gelatin. Gels were washed at room temperature for 30 min with 2.5% Triton X-100 and then incubated at 37°C for 24 h in a buffer containing 10 mM $CaCl_2$, 0.01% NaN_3, and 50 mM Tris-HCl (pH 7.5). Gels were then stained with 0.2% Coomassie brilliant blue and photographed on a light box. Proteolysis was detected as a white zone in a dark blue field.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted using TRIzol (Invitrogen, USA), according to the manufacturer's instructions. For RT-PCR, a cDNA was synthesized from 1 μg of total RNA using AMV RNA PCR Kit (Takara, Japan) according to the manufacturer's protocol. The cDNA was amplified by PCR with the following primers: MMP-9 (537 bp), 5′-CGGAGCACGGAGACGGGTAT-3′ (sense) and 5′-TGAAGGGGAAGACGCACAGC-3′ (antisense); TIMP-1 (481 bp), 5′-CTGTTGTTGCTGTGGCTGATA-3′ (sense) and 5′-CCGTCCACAAGCAATGAGT-3′ (antisense); TIMP-2 (416 bp), 5′-GTAGTGATCAGGGCCAAAG-3´ (sense) and 5′-TTCTCTGTGACCCAGTCCAT-3′ (antisense); β-actin (247 bp), 5′-CAAGAGATGGCCACGGCTGCT-3′ (sense) and 5′-TCCTTCTGCATCCTGTCGGCA-3′ (antisense). PCR products were analyzed by agarose gel electrophoresis and visualized by treatment with ethidium bromide.

Plasmid transfections and luciferase gene assays

MMP-9 wild-type (pGL2-MMP-9WT), AP-1 site-mutated MMP-9 (pGL2 MMP-9mAP-1-1 and pGL2-MMP-9mAP-1-2), NF-κB site-mutated MMP-9 luciferase promoter constructs (pGL2-MMP-9mNF-κB) (Chae et al., 2004; Cho et al., 2007) were used in transient transfection assays, as described previously. Cells were plated onto 12-well plates at 1×10^5 cells/well and grown overnight. They were then cotransfected with 1 μg of the respective plasmids and 0.5 μg of pCMV-β-galactosidase reporter plasmid for 5 h using Lipofectamine reagent (Invitrogen, USA). After transfection, cells were cultured in 10% FBS medium and incubated with drugs for 24 h. Luciferase and β-galactosidase activities were assayed using luciferase and β-galactosidase enzyme assay systems (Promega). Luciferase activities were normalized versus β-galactosidase activities in cell lysates. Experiments were performed in triplicate and results were averaged.

Western blot

Cells lysates, SDS-PAGE, transfer to an Immonobilon-p-membrane (Millipore, USA), and immunoblotting were performed as described previously (Hong et al., 2005). To determine the activations of NF-κB and AP-1, nuclear extracts of cells were subjected to described as follows. Cells were suspended in tubes with 0.4 ml of lysis buffer containing 10 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 0.5 mM PMSF, 2.0 μg/ml leupeptin, and 2.0 μg/ml aprotinin. Cells were then allowed to swell on ice for 15 min, and 25 μl of 10% Nonidet P-40 was added. Homogenates were centrifuged at 4°C for 2 min at 13,000 rpm. The nuclear pellets were resuspended in 50 μl of ice-cold nuclear extraction buffer containing 20 mM HEPES (pH 7.9), 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM PMSF, 2.0 μg/ml leupeptin, and 2.0 μg/ml aprotinin, and incubated on ice for 15 min with intermittent mixing. Nuclear extracts were then centrifuged at 4°C for 5

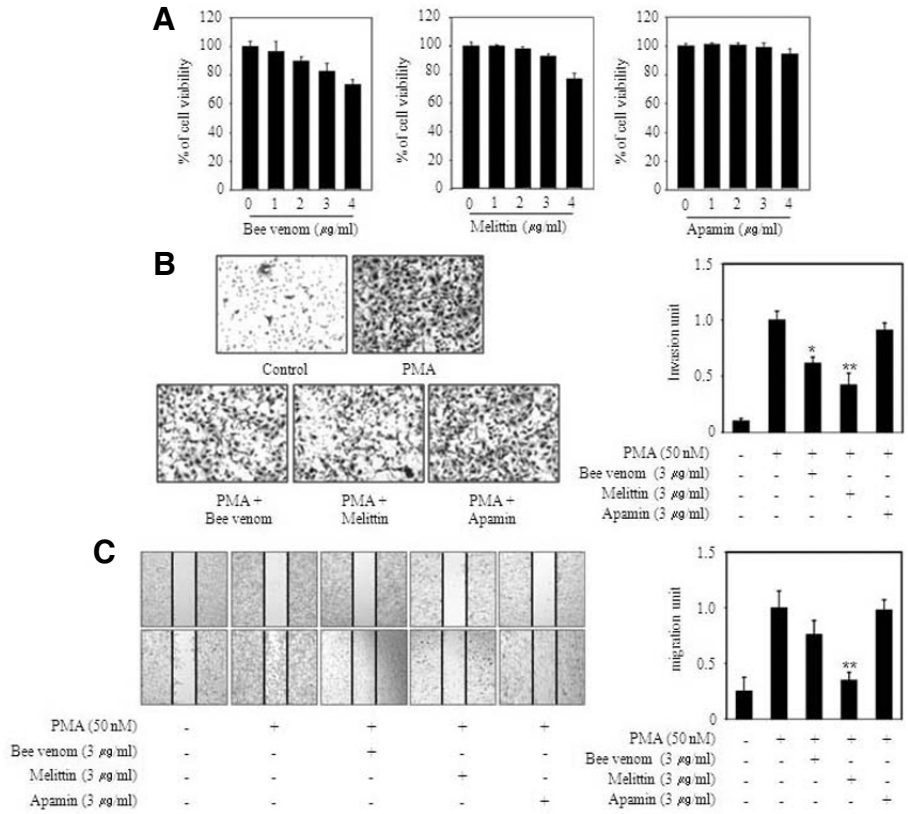

Fig. 1. Effects of bee venom, melittin, and apamin on the PMA-induced invasion and migration of Caki-1 cells. (A) Caki-1 cells were treated with the indicated concentrations of test samples for 24 h. Cell viabilities were determined using MTT assays. (B) Matrigel invasion assays were carried out using bee venom, apamin, or melittin (3 μg/ml) in the presence of PMA (50 nM). After incubating for 24 h, cells filter bases were fixed, stained, and counted. (C) Confluent attached cell layers were scratched with a pipette tip and then co-treated with bee venom, apamin, or melittin (3 μg/ml) and PMA (50 nM) for 24 h. Migrated cells were photographed under a phase contrast microscope. The invasion and migration unit were determined as the number of each condition cells divided by the control cell number counted (right panels of B and C). Values represent means ± SE of 3 independent experiments and are expressed relative to controls; **P* < 0.05 vs PMA, ***P* < 0.001 vs PMA.

min at 13,000 rpm and supernatants were either used immediately. Specific antibodies for MAPKs and phospho-MAPKs were purchased from Santa Cruz (USA).

Statistical analysis

All *in vitro*-results presented here derived from at least three independent experiments performed in triplicate. The significances of differences between experimental and control values were calculated using analysis of variance with the Newman-Keuls multi-comparison test. *P* values of < 0.05 were deemed to be significantly different.

RESULTS

Bee venom and melittin, not apamin, suppressed PMA-induced invasion and migration in Caki-1 cells

Prior to investigating the pharmacologic potential of bee venom, melittin, and apamin on PMA-induced Caki-1 cell invasion, we first examined the cytotoxic effects of three substances using MTT assays. Bee venom and melittin induced a decrease in cell viability by 20% at 3 μg/ml and 4 μg/ml, whereas apamin at concentrations lower than 4 μg/ml had little effect on cells (Fig. 1A). Based on these results bee venom at 3 μg/ml was used in subsequent experiments. Cell invasion and wound-healing assays were used to investigate the inhibitory effects of bee venom, melittin, and apamin on the invasive natures of Caki-1 cells. As illustrated in Fig. 1B, cell invasion was increased by treating cells with PMA, and cell invasion induced by PMA was inhibited by treating cells with bee venom or melittin, whereas apamin had no effect (Fig. 1B). Furthermore, wound-healing assays indicated that migration of Caki-1 cells was increased by PMA, while inhibited by bee venom or melittin, but not by apamin (Fig. 1C).

Melittin inhibited PMA-induced MMP-9 enzyme activity

The up-regulation of MMP-9 expression has been previously shown in the invasion of Caki-1 cells (Hong et al., 2005). Thus, we used gelatin zymography assays to investigate the effects of bee venom, melittin, or apamin on PMA-induced MMP-9 secretion. The cultured media of Caki-1 cells contained a weakly proteolytic protein at 92 kDa, corresponding to MMP-9, and a highly proteolytic protein at 72 kDa, corresponding to MMP-2. Treatment of PMA for 24 h induced MMP-9 secretion by Caki-1 cells, while the level of MMP-2 secretion was not affected by PMA. Furthermore, this induction of MMP-9 secretion by PMA was dramatically inhibited in the presence of bee venom or melittin in a dose-dependent manner in the concentration range of 1 to 3 μg/ml. However, apamin did not show any inhibitory effects on PMA-induced MMP-9 secretion (Fig. 2A). In addition, melittin showed similar inhibitory effects on MMP-9 secretion in Caski and SK-BR-3 cells, indicating that the inhibitory effect of melittin on MMP-9 secretion is not cell type-specific (Fig. 2B).

Melittin suppressed MMP-9 secretion by inhibiting its protein and mRNA expression in Caki-1 cells

In order to investigate the inhibitory effect of melittin on PMA-induced MMP-9 protein and mRNA, we used western blot analysis and RT-PCR (Fig. 2C). The PMA-induced MMP-9 protein and mRNA expression were inhibited in the presence of melittin in a dose-dependent manner, indicating that reduced MMP-9 enzyme activity was the result of decreased amounts of MMP-9 gene expression. On the other hand, because MMP-9 enzyme activity is tightly regulated by endogenous inhibitors, such as tissue inhibitor of metalloproteinases (TIMPs) (De Clerck et al., 1994; Stetler-Stevenson et al., 1990), we examined the transcription levels of TIMP-1 and TIMP-2 by RT-PCR.

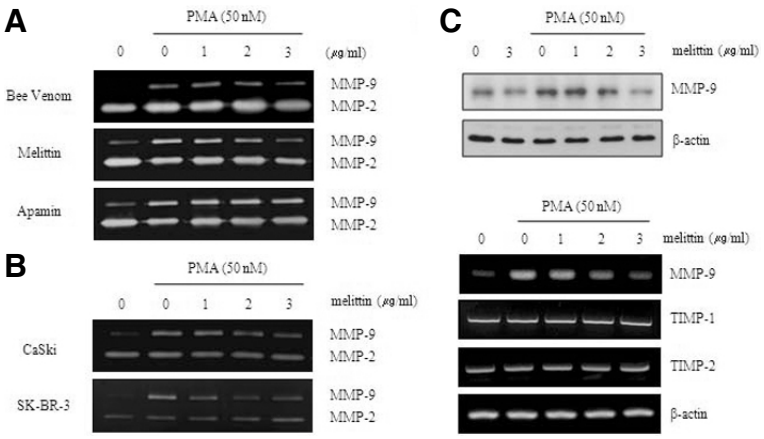

Fig. 2. Effect of melittin on the PMA-induced MMP-9 expression. (A) Caki-1 cells were cotreated with various concentrations of bee venom, apamin, or melittin and PMA (50 nM) for 24 h. (B) CaSki and SK-BR-3 cells were also both cotreated with various concentrations of melittin and PMA for 24 h. Conditioned media was analyzed by gelatin zymography. (C) Caki-1 cells were treated with melittin and PMA for 12 h, and MMP-9 protein expressions was analyzed by western blotting and mRNA levels by RT-PCR. β-actin was used as an internal control.

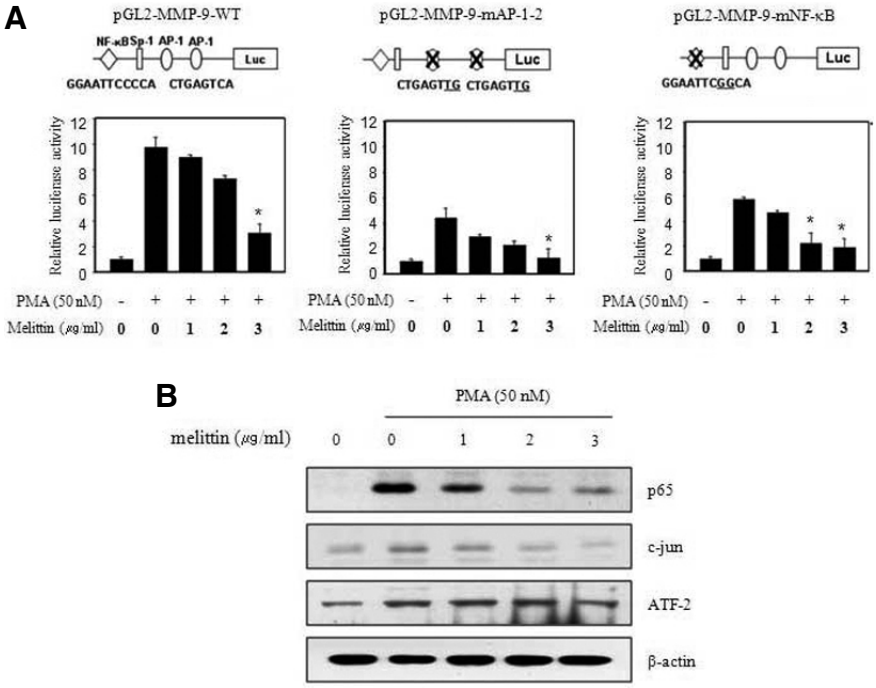

Fig. 3. Effects of melittin on AP-1 and NF-κB activation by PMA. (A) Mutations were introduced in the NF-κB or AP-1 binding sites of pGL2-MMP-9WT by introducing 2-bp changes. Caki-1 cells were transfected with pGL2-MMP-9WT, pGL2-MMP-9mAP-1-2, or pGL2-MMP-9mNF-κB reporter plasmids. Caki-1 cells transfected with the indicated reporters were cultured with PMA (50 nM) and melittin (1, 2 or 3 μg/ml) for 24 h, and then luciferase activities in cell extracts was determined. The values shown represent means ± SE of three independent experiments and are expressed relative to controls; $^*P < 0.05$ vs PMA. (B) Nuclear extracts prepared from control or Caki-1 cells treated for 24 h with PMA (50 nM) and melittin (1, 2 or 3 μg/ml) were examined for the expressions of p65, c-Jun, and ATF-2 protein by Western blotting. β-actin expression was used as an internal control.

Melittin did not appear to affect their mRNA levels (Fig. 2C). These results indicated that melittin suppresses PMA-induced MMP-9 secretion through inhibition of its transcriptional activity in Caki-1 cells but not through induction of endogenous inhibitors of MMP-9.

Melittin inhibited PMA-induced MMP-9 expression by blocking the activations of AP-1 and NF-κB

To determine whether the inhibition of MMP-9 secretion by melittin was due to a decrease in a transcription factor, we performed a promoter assay using transiently transfected cells with a luciferase reporter gene linked to the MMP-9 promoter sequence. As shown in Fig. 3A, luciferase gene expression was activated up to 10-fold in cells that had been treated with PMA compared with untreated cells, and treatment of cells with melittin dose-dependently decreased PMA-stimulated luciferase activity (Fig. 3A, left panel). These results suggested that melittin suppresses PMA-induced MMP-9 secretion through inhibition of its transcriptional activity in Caki-1 cells.

The MMP-9 promoter contains two important transcriptional elements, binding sites of AP-1 (located at -79 bp and -533 bp) and NF-κB (located at -600 bp). The AP-1 and the NF-κB elements are centrally involved in the induction of the MMP-9 gene by PMA (Cho et al., 2007; Hong et al., 2005). The effect of melittin on MMP-9 promoter activity was investigated using Caki-1 cells that had been transiently transfected with a luciferase reporter gene linked to the MMP-9 promoter with mutations at the AP-1 and the NF-κB site. As illustrated in Fig. 3A, luciferase gene expression of the AP-1 and NF-κB mutation was activated up to 4-fold and 6-fold in cells that had been treated with PMA compared with untreated cells, respectively. The melittin treatment in conjunction with PMA presence decreased the transcription activity of the reporter gene containing the AP-1 or NF-κB mutations. These results suggested melittin targets both NF-κB and AP-1.

We also confirmed the effect of melittin on the PMA-stimulated nuclear translocation of p65 (a major unit of NF-κB), c-Jun (a major subunit of AP-1), and ATF-2, which are all re-

quired for transcriptional activity as determined by Western blotting (Fig. 3B). The nuclear translocations of p65 and c-Jun were significantly found to be dose-dependently reduced by melittin, whereas melittin did not affect the expression of ATF-2. These findings suggest that reduced c-Jun levels contribute to the decrease in AP-1 binding activity induced by melittin.

Melittin blocked ERK and JNK signaling pathway activation induced by PMA

MMP-9 gene expression can be activated by a number of signal transduction pathways, including mitogen-activated protein kinase (MAPK) pathways, which are the upstream modulators of AP-1 and NF-κB (Eberhardt et al., 2000; Nakshatri et al., 1997). In a previous study, we confirmed that PMA-induced MMP-9 activation is inhibited by PD98059 (an ERK1/2 inhibitor) and by SP600125 (a JNK inhibitor), but not by SB203580 (a p38 MAPK inhibitor) in Caki-1 cells (Hong et al., 2005). We then investigated whether melittin inhibited the activations of ERK1/2, JNK or p38 MAPK pathways. PMA induced the phosphorylation of all three members of these MAPKs within 5 min, and their phosphorylations peaked at 15 min (data not shown). Melittin also dose-dependently inhibited the phosphorylations of ERK1/2 and JNK at 10 min after PMA treatment, but did not inhibit the phosphorylation of p38 (Fig. 4). We also confirmed that the inhibitor concentrations used in these studies effectively reduced the phosphorylations of the corresponding target kinases. These results suggest that specific inhibitions of ERK 1/2 and JNK signaling pathways are directly involved in the regulation of PMA-induced MMP-9 expression by melittin in Caki-1 cells.

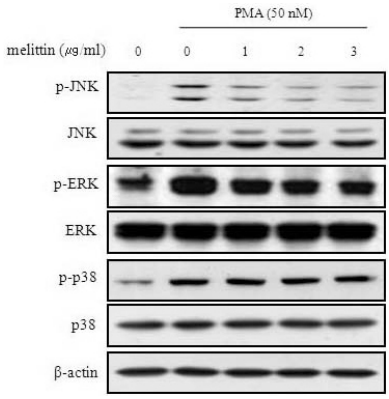

Fig. 4. Effect of Melittin on the PMA-induced activation of MAP kinase pathways in Caki-1 cells. Caki-1 cells were incubated with melittin and PMA as indicated, and levels of phospho-JNK, phospho-ERK1/2 and phospho-p38 were determined by Western blotting using phosphor-specific antibodies. Protein expression levels of β-actin in cell lysates were used as controls.

DISCUSSION

MMPs are known to process a broad spectrum of cell surface molecules, and to function in several important biological processes. MMPs are also collectively capable of cleaving virtually all extracellular matrix (ECM) substrates, and play an important role in some physiological and pathological processes (Ray and Stetler-Stevenson, 1994; Zucker et al., 2000). In particular, the over expression of MMP-9 has been shown to be associated with the progression and invasion of renal carcinoma cells (Lein et al., 2000), inhibitors of MMP-9 have been demonstrated to block endothelial cell activities, which are essential for new vessel development, and leading to motility and invasion (Benelli et al., 1994; Park et al., 2008).

Melittin is a small protein containing 26 amino acid residues, and represents the principal toxic compound in bee venom (Gevod and Birdi, 1984). Melittin has been shown to possess detergent-like action. Because melittin shares its amphipathic properties with a series of peptides, it is able to disturb cell membrane bilayer integrity through creation of defects, cell membrane disruption or formation of pores. Although melittin is a toxic peptide, it has long ago been investigated because of its potential therapeutic effects (Dempsey, 1990; Gevod and Birdi, 1984). Previous studies have shown that melittin has therapeutic potential for the treatment of atherosclerosis and arthritis. Furthermore, it has been reported that melittin suppresses the proliferation of vascular smooth muscle cells (Son et al., 2006) and inhibits LPS-activated MMP-3 production in human arthritic chondrocytes (Nah et al., 2007). In addition, melittin has been shown to cause apoptosis in several cancer cells, inducing osteosarcoma and human leukemic cells (Chu et al., 2007; Moon et al., 2008). However, the effects of melittin on the expression of MMP-9 in Caki-1 cells and on cancer cell invasion have not been previously examined. Thus, we examined the effect of melittin on PMA-induced MMP-9 expression and sought to identify the mechanism involved.

Our results indicate that bee venom and melittin, but not apamin, inhibit the PMA-induced invasion and migration of Caki-1 cells (Fig. 1). Bee venom and melittin also both inhibited the enzymatic activity of MMP-9 secreted by PMA-induced Caki-1 cells (Fig. 2). Apamin on the other hand failed to affect MMP-9 expression even at 10 μg/ml, the highest concentration examined (data not shown), suggesting that melittin, among the main compound of bee venom, is correlated inhibition of cell invasion and migration by decreased MMP-9 activity. Although both melittin and apamin are major peptides of bee venom, there are differences in their pharmacological character, as well as in their chemical structure (Son et al., 2007). In addition, the signaling pathways effect by melittin and apamin are distinct. For example melittin inhibits MAP kinase (Moon et al., 2006) while apamin regulates small conductance calcium-activated potassium channels (SK channels) (Kohler et al., 1996). As a result, we suggest that the different inhibitory effects of melittin and apamin on MMP-9 might be due to the differences in their chemical structure and by their distinct signaling pathways.

Previous studies have analyzed MMP-9 associated AP-1, NF-κB and SP-1 sites in tumor cell lines in response to PMA (Sato and Seiki, 1993). However, we reported that SP-1 binding activity was not stimulated by PMA in the human renal carcinoma cell-line (Hong et al., 2005). Therefore, to determine the inhibitory effect of melittin on MMP-9 gene transcription through suppression of AP-1 and NF-κB, except by SP-1, we carried out reporter gene assays. Melittin blocked the activations of NF-κB and AP-1 by suppressing the transcription activity of the reporter containing the AP-1 and NF-κB mutations (Fig. 3A). Furthermore, melittin has been reported to be involved in the suppression of p65 and c-Jun protein expressions (Lee et al., 2003). The abolishment of p65 and c-Jun genes expression levels compared the NF-κB and the AP-1 with decrease of transcriptional activity by melittin is consistent (Fig. 3B). Our data demonstrate that melittin blocks PMA-induced MMP-9 production by inhibiting both NF-κB and AP-1-mediated MMP-9 induction.

The regulation of MMP-9 gene expression is mediated by various signal transduction pathways that involve the transcription factors, NF-κB and AP-1 (Gum et al., 1996; Simon et al.,

1998). These signal pathways are known to involve the MAPK family members ERK1/2 and JNK/SAPK, which up-regulate MMP-9 in mammalian cells (Gum et al., 1997; Shishodia et al., 2003). PMA stimulation of MMP-9 expression has also been reported to involve the p38 pathway, because SB 203580 (a p38 inhibitor) was found to suppress MMP-9 expression (Simon et al., 1998). In a previous study, we investigated the effects of various kinase inhibitors on MMP-9 expression and found that PMA-induced MMP-9 activation was reduced by ERK1/2 and JNK inhibitors, but not by p38 inhibitor. Furthermore, we found that ERK1/2 is a major regulator of MMP-9 expression (Hong et al., 2005). Congruent with these results, it is known that ERK1/2 activation plays a major role in MMP-9 expression by PMA in various cell types (Lee et al., 2007). Data obtained during the present study shows that the ERK1/2 and JNK pathways are involved in the melittin-mediated inhibition of PMA-induced MMP-9 expression in Caki-1 cells (Fig. 4). From these findings, NF-κB and AP-1 activation through the ERK1/2 and JNK pathway appears to be required for the induction of MMP-9 expression in Caki-1 cells.

In summary, melittin-inhibited level of phospho-ERK and phospho-JNK led to affect AP-1 and NF-κB, which led to suppression of MMP-9 expression. This is the first study to show that melittin effectively suppresses PMA-stimulated cancer cell invasion by inhibiting MMP-9 expression. These findings suggest that melittin may suppress cancer cell invasion and migration by preventing ECM degradation.

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