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JNP3, a new compound, suppresses PMA-induced tumor cell invasion via NF-κB down regulation in MCF-7 breast cancer cells

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ABSTRACT

The expression of matrix metalloproteinase (MMPs)-9 is critical for cell migration and can lead to invasion and metastasis of cancer cells. In the present study, we examined the inhibitory effects of JNP3, a new compound which was isolated from traditional Chinese medicine, on cell invasion and MMP-9 activation in phorbol myristate acetate (PMA)-induced MCF-7 cells. Treatment with JNP3 significantly and selectively inhibited PMA-induced MMP-9 secretion, mRNA expression and protein levels, and these results led to reduction of cell invasion and migration in PMA-induced MCF-7 cells. The results of MMP-9 promoter assay and EMSA showed that JNP3 specifically inhibited PMA-induced MMP-9 gene expression by blocking NF-κB-dependent transcriptional activity. In addition, PMA-induced phosphorylation of ERK1/2 and JNK were suppressed by JNP3 treatment, whereas the phosphorylation of p38 MAPK was not affected by JNP3. These results suggest that JNP3 can be potential anti-cancer agents through specific inhibition of NF-κB-dependent MMP-9 gene expression.

1. Introduction

MMPs play an important role in normal and pathological processes, including embryogenesis, angiogenesis, wound healing, rheumatoid arthritis and cancer progression [1,2]. The MMPs are multigene family of zinc-dependent proteolytic enzymes and divided into four sub classes including collagenases, gelatinases, stromelysins and membrane-associated MMPs according to substrate specificity and domain homologies. MMP activity is regulated at least at three levels including transcription, activation of the precursor zymogens, and inhibition by tissue inhibitors of metalloproteinases (TIMPs) [3]. Among the previously reported MMPs, both MMP-2 and MMP-9 are the key enzymes for degradating ECM and type IV collagen which is a major component of basement membrane. Although these two gelatinases have similar substrate specificities, their gene expression is independently regulated due to the distinct regulatory elements in the promoter region of their genes. Furthermore, MMP-2 and MMP-9 are abundantly expressed in tumors and highly correlated with the metastatic potential [4,5]. Generally, MMP-2 is constitutively present in tissues, and it is maximally expressed in malignant neoplasms rather than an initial response to invasion. MMP-9 is strongly correlated with malignant phenotype in various cancers and its expression can be stimulated by a variety of stimuli, including inflammatory cytokines and growth factors during pathological processes and by agents such as PMA [3,6]. In addition, the expression of PMA-induced MMP-9 is modulated by the activation of ranscription factors such as AP-1 and NF-κB through MAPK signaling pathways [6–8].

Several studies to detect new anti-cancer drugs have demonstrated that natural compounds with chemo-preventive potential inhibit the invasiveness of several types of cancer by suppressing MMP-9 expression [6–8]. Hyul-Tong-Ryung (HTR), which is composed of 12 herbal materials, has been used for the treatment of inflammation, vascular disorders, atherosclerosis-related disorders and neuronal diseases. In our previous studies, the methanol extract of HTR was found to inhibit PMA-induced cell invasion and MMP-9 expression in MCF-7 cells [9,10]. Thus, it was suggested that HTR may have anti-cancer potential inhibiting cancer invasion. Very recently we have isolated an active single compound (JNP3), showing a strong inhibition of MMP-9 activity, from the ethyl acetate fraction of HTR. The present study was undertaken to investigate the molecular mechanisms involved in inhibitory effects of JNP3 on MMP-9 activity. Here we provide evidence showing thatJNP3 reduces PMA-induced cell invasion and metastasis and suppresses MMP-9 expression by inhibiting activation of NF-κB via ERK1/2 and JNK signaling pathways.

2. Materials and methods

2.1. Materials and compound isolation

HTR was obtained from Da Lian Han Bin Healthy Food Cooperation Da Lian Han China. The air-dried plant material (180 g) was

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finely ground and extracted at room temperature with MeOH for 24 h. The resulting MeOH extract (17.1 g) was suspended in H_2O (500 ml) and partitioned with *n*-hexane, ethylacetate and *n*-butanol, successively, to give ethyl acetate (1.57 g)-soluble fractions. The ethyl acetate fraction was applied to a silica gel column and eluted with chloroform–methanol mixtures of increasing polarity to give ten subfractions (HTRE-1 ~ 10) whose main subfraction (HTRE-6; 130 mg) was chromatographed with silica gel eluted with chloroform/methanol (19:1) followed by chloroform/methanol (9:1) to give HTRE-6-3 (6 mg) was purified by Lobar A (Merck; CH_2Cl_2/EtOAc, 20:1 ~ 9:1) to yield JNP-3 (1.5 mg). JNP-3: Oils, HRESIMS m/z 565.2739 (calcdfor $C_{30}H_{38}O_9Na$, 565.2726); ^{1}H NMR(600 MHz, $CDCl_3$, δppm): 2.46(dd, *J* = 3.3, *J* = 7.6,H-1), 4.88(brs, H-5), 3.43(brs, H-7), 3.11(brd, *J* = 2.7, H-8), 3.03(d, *J* = 7.6, H-10), 2.02(m, H-11), 1.72(d, *J* = 13.0, H-12), 2.15(dd, *J* = 7.3, *J* = 13.5, H-12), 4.25(d, *J* = 2.3, H-14), 4.86(brs H-16), 4.96(brs H-16), 1.73(brs, H-17), 1.23(d, *J* = 6.4, H-18), 1.73(brs, H-19), 3.61(d, *J* = 12.6, H-20), 4.05(d, *J* = 12.6, H-20), 2.34(m. H-2′), 2.45(m. H-3′), 5.36(m. H-4′), 5.39(m. H-5′),2.78(t,*J* = 7.6,H-6′), 5.27(m. H-7′), 5.35(m. H-8′), 2.35(m. H-9′), 1.13(t, *J* = 6.5, H-10′);13C NMR(150 MHz, $CDCl_3$, δppm): 54.2(C-1), 112.5(C-1), 172.8(C-3),86.5(C-4),68.8(C-5),59.0(C-6),59.3(C-7),35.2(C-8),81.1 (C-9),49.3(C-10),37.1(C-11),36.8(C-12),83.7(C-13),81.2(C-14), 146.3(C-15),112.7(C-16),18.5(C-17),21.2(C-18),19.1(C-19),63.9C-20), 121.6(C-21),33.1(C-22),173.5(C-1′),35.5(C-2′),23.6(C-3′),127.5(C-4′), 129.6(C-5′),25.7(C-6′),126.8(C-7′),132.9(C-8′),31.0(C-9′),19.1(C-10′).

2.2. Cell culture and cell viability assay

MCF-7 cells were obtained from the American Type Culture Collection (Manassas, VA) and cultured in DMEM (Gibco-BRL, Rockville, MD) supplemented with 10% fatal bovine serum (FBS), penicillin (100 U/ml)and streptomycin (100 μg/ml), as previously described [10]. Cell viability was determined by XTT assays. All chemicals containing PMA and dimethyl sulfoxide were purchased from Sigma Chemical (St. louis, MO). Polyclonal antibodies to MMP-9, total MAPK family (ERK1/2, JNK and p38 MAPK) and phospho-MAPK family were purchased from Cell Signaling Technologies (Beverly, MA). Polyclonal NF-κB (p65) and phospho c-Jun antibodies were purchased from Santa Cruz Biotechnology (California, USA).

2.3. Zymography assay

Zymography was performed as described previously [9,10]. Briefly, cells were cultured for 24 h, and then starved in serum-free medium for 24 h and treated with various concentrations of JNP3 in the presence or absence of 100 nM PMA. After 24 h incubation, conditioned media were collected, centrifuged, and electrophoresed in a polyacrylamide gel containing 1% gelatin (w/v) at 4 °C. The gelatinolytic activity of MMP-9 was detected as a white zone in a dark blue field.

2.4. Western blot analysis

After treatment with various concentrations of JNP3 in the presence or absence of 100 nM PMA, the cells were analyzed by immunoblotting as described previously [9,10]. Primary antibodies that recognize MMP-9, the phospho- or the total forms of ERK1/2, JNK, p38MAPK, p-c-Jun and p65 were used. The detection of specific proteins was carried out by enhanced chemiluminescence kit (Amersham, Piscataway, NJ). Equal loading were assessed using GAPDH and hnRNP antibodies (Chemicon, El Segundo, CA) to normalize the amounts of total protein.

2.5. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from MCF-7 cells using the Trizol reagents (Invitrogen, Carlsbad, CA) 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 instructions. The cDNA was amplified by PCR using MMP-9, TIMP-1 and β-actin primers as described previously [9]. PCR products were analyzed by agarose gel electrophoresis and visualized by treatment with ethidium bromide.

2.6. Transient transfection and luciferase reporter assay

pGL2-MMP-WT, pGL2-MMP-9-mAP-1-2 and pGL2-MMP-9-mNF-κB were used in transient transfection assays as described previously [12].The AP-1 and NF-κB reporter constructs were purchased from Clontech (Palo Alto, CA). Cells were cultured and grown overnight and cotransfected with 1 μg of various plasmid constructs and 0.5 μg of the pCMV-β-galactosidase reporter plasmid for 5 h using Lipofectamine reagent (Invitrogen, San Diego, CA). After transfection, the cells were starved in serum-free media for 6 h. JNP3 was added to fresh serum-free media at 10-20 μg/ml concentration in the presence of PMA (100 nM) and then cells were incubated for 24 h. The enzyme activities of luciferase and β-galactosidase were determined using commercial kits (Promega, Madison, WI). Luciferase activity was normalized with the β-galactosidase activity in each cell lysate.

2.7. Electrophoretic mobility shift assay

Nuclear extracts were prepared as described previously [13]. EMSA was performed using a gel shift assay system kit (Promega, Madison, WI) as described previously [10]. Double-stranded oligonucleotides containing the consensus sequences for AP-1 and NF-κB were end-labeled with [γ^{32}-P] ATP using T4 polynucleotide kinase and used as probes for EMSA. Competition was performed using either 100-fold excess of an unlabeled AP-1 or NF-κB consensus oligonucleotides. The reaction mixture was electrophoresed in a 4% nondenaturing polyacrylamide gel in 0.5 XTBE running buffer at 300 V for 15 min. The gel was dried and exposed to X-ray film overnight.

2.8. Matrigel invasion assay

Invasion of tumor cells was analyzed in Transwell chambers (Corning Coster, Cambridge, MA), which were coated with 1 mg/ml Matrigel matrix as described previously [9]. Cells were suspended in conditioned medium with or without JNP3 in the presence or absence of PMA, and plated on the Matrigel-coated upper chamber. Cells that had invaded Matrigel were located on the lower surface of the membrane filter. Non-invading cells, remained the upper side of the membrane, were removed by wiping. The filter was excised, fixed with methanol, stained with hematoxylin and eosin, and then mounted according to the manufacturer's instructions (Becton–Dickinson). Random fields were counted by light microscopy. The values obtained were calculated by averaging the total number of cells from three filters.

2.9. Wound-healing assay

Cells were seeded in a six-well plate and incubated until they reached 80% confluence. The monolayer cells were scratched with a 200 μl pipette tip to create a wound, and cells were washed twice with serum-free DMEM to remove floating cells and then replaced with fresh medium without serum. Cells were subjected to the indicated treatment for 24 h, and cells migrating from the leading

edge were photographed at 0 and 24 h. Each value was derived from three randomly selected fields.

2.10. Statistical analysis

Differences between means for two groups were determined by unpaired Student's *t* test and all of data were expressed as the means ± S.E. The differences were considered as a significant at $p < 0.05$.

3. Results

3.1. Structure identification, and the effect of JNP3 on cell viability in MCF-7 cells

To identify some bioactive molecules that have anti-cancer activity, we tested the effect of several fractions of HTR onMCF-7 cells using an XTT assay. We found that PMA-induced MMP-9 activity was dramatically inhibited by the ethyl acetate fraction of HTR (data not shown). We fractionated the ethyl acetate fraction into 10 different subfractions and tested their effects on MCF-7 cells by an XTT assay. We also found that the active subfraction (HTRE-6) contained almost pure (>96%) compound by analyzing HPLC. The active molecule isolated from HTR was identified by the ^{1}H, ^{13}C, Dept, HSQC and HMBC NMR spectra in $CDCl_3$. From the molecular ion signal at *m*/*z* 565, the elemental composition of $C_{32}H_{44}O_8Na$ was ascertained. The NMR spectra of JNP3 were almost identical to those of Pimelotides C [11], with the presence of two extra double carbon signals in the upfield region of the ^{13}C NMR spectrum. This was also consistent with the molecular formula obtained by HRESIMS. JNP3 had the decrease of 4 in molecular weight relative to Pimelotides C, and similar patterns in the NMR spectra for the two compounds suggested that JNP3 had a skeleton similar to Pimelotides C with an extra double carbon group. The structure of JNP3 was identified as Fig 1A. Full characterization has been carried out in this work for the first time, and NMR data are presented in materials and compound isolation. We first examined the effect of JNP3 on the viability of MCF-7 cells by an XTT assay. JNP3 at concentrations lower than 40 μM had modest cytotoxic effect on the cells, but JNP3 at >40 μM significantly reduced cell viability (Fig. 1B). Thus, we used JNP3 at concentrations lower than 20 μM in the present study.

3.2. JNP3 inhibits PMA-induced MMP-9 activity andgene expression

We next investigated the inhibitory effect of JNP3 on PMA-induced MMP-9 activity using a zymography in the conditioned medium. As shown in the upper panel of Fig. 2A, the gelatinolytic activity of MMP-9 was significantly inhibited by JNP3 treatment in a dose-dependent manner, and almost completely suppressed at 20 μM. To determine whether the decreased MMP-9 activity by JNP3 treatment was due to a decrease in the amount of protein, we performed Western blot analysis using specific MMP-9 antibody. As shown in the middle panel of Fig. 2A, JNP3 inhibited the PMA-induced MMP-9 protein expression in a dose-dependent manner, indicating that the reduced MMP-9 enzyme activity is result of decreased amount of MMP-9 protein. Because activity of MMP-9 is tightly regulated by its endogenous inhibitor, TIMP-1, we further examined the both mRNA levels of TIMP-1 and MMP-9 by RT-PCR. The treatment of cells with JNP3 significantly decreased the level of PMA-induced MMP-9 mRNA expression, whereas the level of TIMP-1mRNA remained unchanged by JNP3 (Fig. 2A).

3.3. JNP3 suppresses PMA-induced MMP-9 activity through inhibition of its transcriptional activity

To evaluate whether the inhibition of MMP-9 activity by JNP3 was resulted from the decreased level of transcription, we performed promoter assay using transiently transfected cells with a luciferase reporter gene linked to the 0.7 kb fragment of MMP-9 promoter sequence. The MMP-9 promoter contains *cis*-acting regulatory elements for transcription factors including two AP-1 sites and an NF-κB site, which are pivotally involved in the induction of MMP-9 gene by PMA [12]. To confirm which of these transcription factors may regulate the MMP-9 gene expressions, MCF-7 cells were transiently transfected with luciferase reporter genes includingMMP-9-WT promoter or promoters with mutations in two AP-1sites or an NF-κB site. When the cells were transiently transfected with reporter plasmid including the MMP-9-WT promoter, luciferase reporter gene activity was increased up to 15-fold in cells treated with PMA as compared with control cells. Treatment with JNP3 significantly inhibited PMA-stimulated luciferase reporter gene activity (Fig. 2B). On the other hand, the mutations of AP-1 and NF-κB binding sites markedly decreased the response to PMA compared with WT, indicating increase to 9-fold in cells treated with PMA as compared with control cells. Treatment with JNP3 significantly decreased the luciferase reporter gene activity in the cells transfected with MMP-9-mAP1-2 reporter plasmid, whereas the luciferase activity of the cells transfected with MMP-9-mNF-κB reporter plasmid was not affected by JNP3, suggesting that the major target of is the NF-κB transcription factor (Fig. 2B).

3.4. JNP3 inhibits PMA-induced MMP-9 activity by blocking the NF-κB activity

Based on the results of promoter assay, to confirm whether JNP3 inhibits PMA-induced MMP-9 expression by blocking NF-κB

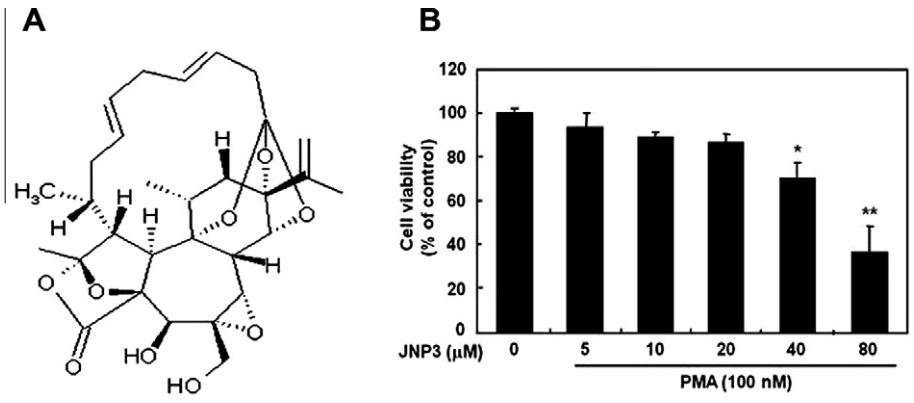

Fig. 1. Effect of JNP3 on PMA-induced cell viability. (A) Chemical structure of JNP3. (B) To determine the appropriate concentration not toxic to cell, cells were treated with the indicated concentrations of JNP3 in the presence of PMA (100 nM) for 24 h. Cell viability was determined by an XTT assay. $^{*}P < 0.05$ vs PMA, $^{**}P < 0.01$ vs PMA.

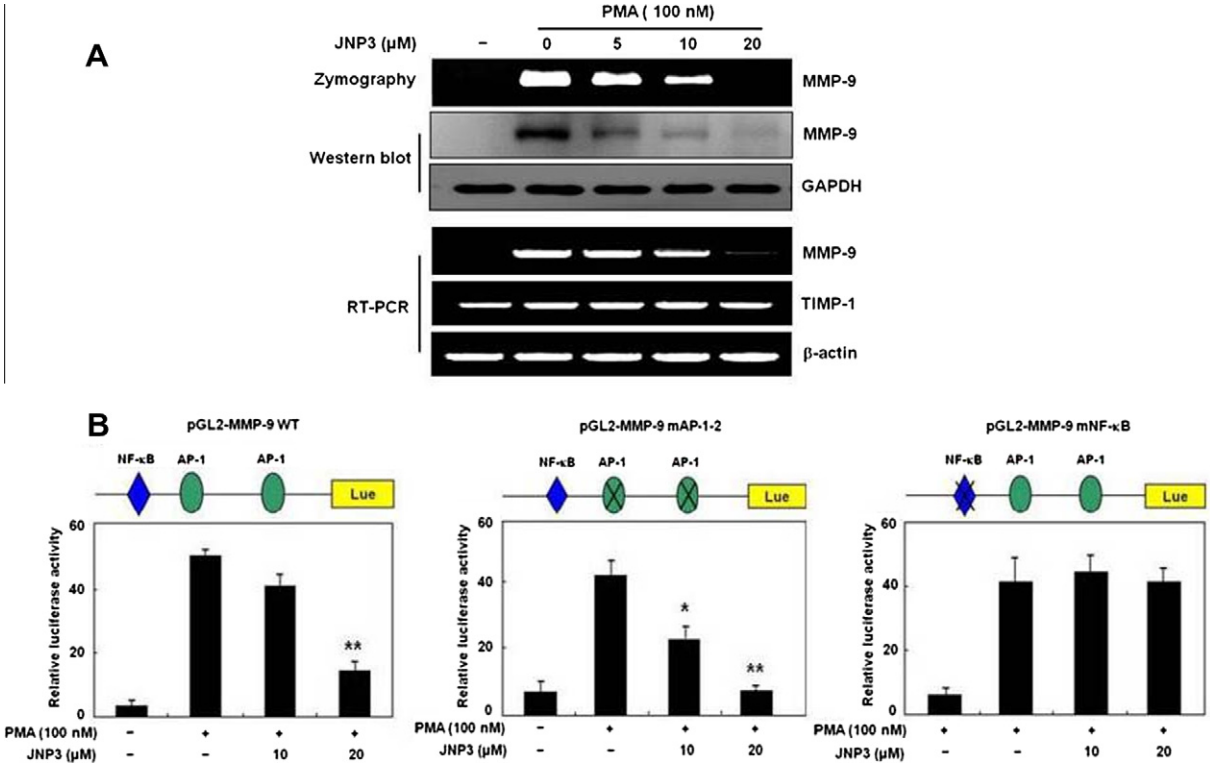

Fig. 2. Effects of JNP3 on the PMA-induced MMP-9 activity and expression. (A) Cells were treated with various concentrations of JNP3 and PMA (100 nM) for 24 h. Conditioned media was analyzed by zymography. MMP-9 expression was measured by Western blotting and GAPDH was used as an internal control. The mRNA levels of MMP-9 and TIMP-1 were analyzed by RT-PCR and β-actin was used as an internal control. (B) Mutations were induced in the AP-1 or NF-κB binding sites of pGL2-MMP-9WT. Cells were transfected with pGL2-MMP-9WT, pGL2-MMP-9-mAP-1-2 and pGL2-MMP-9-mNF-κB reporter plasmids. Cells were cultured with JNP3 (10 and 20 μM) and/or PMA (100 nM) for 24 h, and then the relative luciferase activity in the cell extract was determined. Data represent the mean ± SE of three-independent experiments; $^{*}P < 0.05$ vs PMA, $^{**}P < 0.01$ vs PMA.

activation, we performed EMSA using double-stranded ^{32}P-labeled oligonucleotide fragments containing the consensus sequences for Ap-1 and NF-κB as probes. As shown in Fig 3A, PMA significantly increased DNA binding activity of NF-κB, and JNP3 dramatically decreased the binding activity, whereas PMA-induced binding activity of AP-1 was not affected by JNP3.These data were consistent with the results of promoter assay (Fig. 3A). To confirm these data furthermore, in subsequent experiment, we examined the effect of JNP3 on the PMA-induced phosphorylation of c-Jun, a major subunit of AP-1, and PMA-induced nuclear translocation of phospho-p65, a major subunit of NF-κB, which are required for the transcriptional activities, by Western blot analysis. As shown in Fig. 3B, PMA induced the nuclear translocation of phospho-p65 and phosphorylation of c-Jun and JNP3 inhibited nuclear translocation of phospho-p65 in a dose-dependent manner. As expected, however, the phosphorylation level of c-Jun was not affected by JNP3. These data clearly suggest that JNP3 regulates the transcriptional activation of MMP-9 by inhibiting PMA-induced NF-κB activity, but AP-1 activity.

3.5. JNP3 inhibits the activation of ERK and JNK signaling pathways induced by PMA

MAPK pathways are the most important signaling molecules involved in regulating NF-κB and/or AP-1-dependent MMP-9 expression by various PMA-induced cell lines [6–8]. We investigated the effects of JNP3 on MAPK pathways using antibodies against the phosphorylated forms of the three MAPKs including ERK1/2, JNK, and p38 MAPK pathway. Treatment with PMA induced the phosphorylation of all of three members of the MAPKs as early as 5 min, with a maximal phosphorylation at 15 min (data not shown). JNP3specifically suppressed phosphorylation of ERK1/2 and JNK pathways, but p38MAPK phosphorylation was not significantly affected by JNP3 (Fig. 3C). These results suggest that the specific inhibitions of ERK1/2 and JNK signaling pathways are directly involved in the regulation of PMA-induced MMP-9 expression by JNP3.

3.6. JNP3 inhibits PMA-induced cell invasion and migration of MCF-7 cells

Since MMPs, including MMP-9, play an important role in cellular invasion and migration, we further examined the effect of JNP3 on invasive and migratory potency of PMA-induced MCF-7 cells using Matrigel invasion and wound-healing assays (Fig. 4A and B). Our results showed that the invasion and migration of MCF-7 cells were significantly increased by treatment with PMA as compared with PMA-untreated control cells, and treatment with JNP3 (20 μM) significantly inhibited the PMA-induced invasion (Fig. 4A) and migration (Fig. 4B).

4. Discussion

In our previous studies, although we have demonstrated that the methanol extract of HTR, inhibits PMA-induced MMP-9 expression and cellular invasion in MCF-7 cells [9,10], we did not verify which components of HTR possess the inhibitory effects that were exerted on the PMA-stimulated MMP-9 expression and cellular invasion. Thus, understanding the molecular mechanisms involved in inhibitory effects of natural compounds from HTR on the MMP-9 expression and cellular invasion is important in exploring its

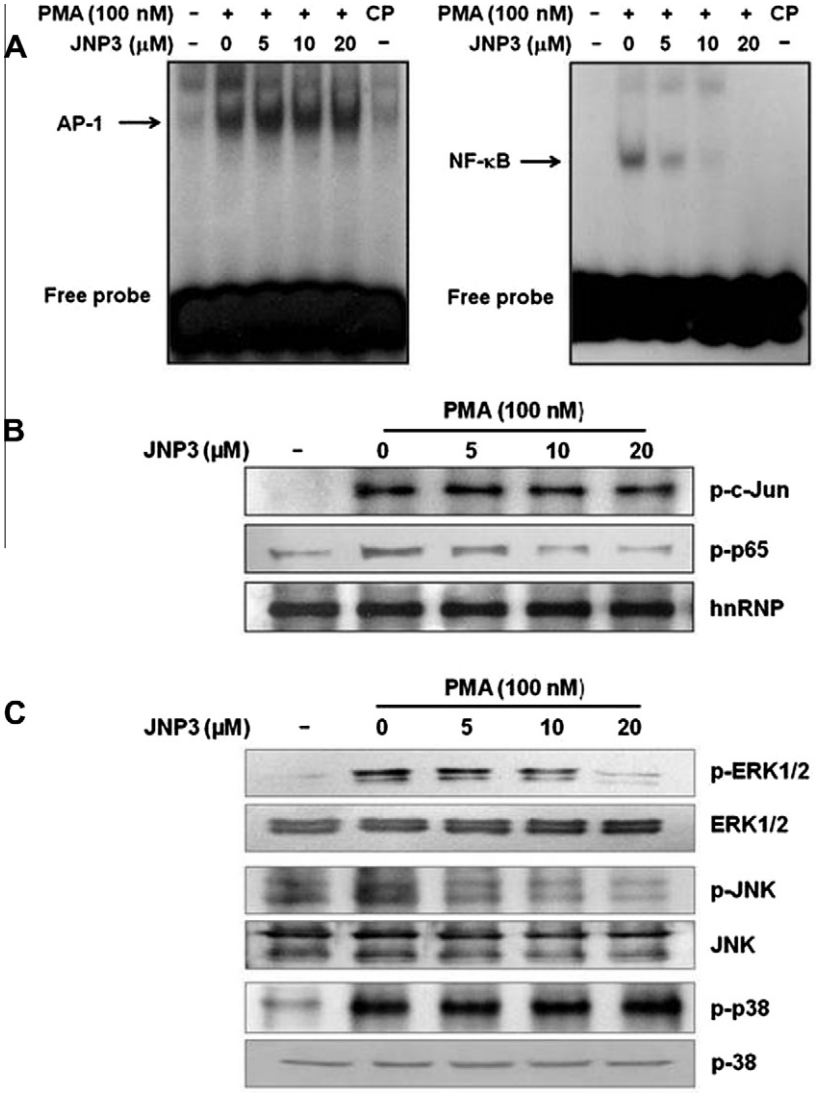

Fig. 3. Effects of JNP3 on PMA-induced AP-1 and NF-κB activations and activation of MAPK signaling pathways. Cells were pretreated with various concentrations of JNP3 for 1 h and then stimulated with PMA (100 nM) for 1 h. (A) Nuclear extracts were prepared and analyzed for AP-1 and NF-κB binding by EMSA, and (B) examined for phospho-c-Jun and phospho-p65protein expression by Western blotting. The hnRNP was used as an internal control. To elucidate MAPK signaling pathways, cells were pretreated with indicated concentrations of JNP3 for 1 h and then stimulated with PMA (100 nM) for 15 min and the levels of total/phospho-ERK1/2, JNK and p38 MAPKs were determined by Western blotting (C).

properties for cancer treatment. Very recently we have succeeded in isolating a new compound, JNP3, from the ethyl acetate fraction of HTR. In the present study, we firstly demonstrated that JNP3 effectively inhibits the invasive and migratory abilities of PMA-induced MCF-7 cells through the suppression of MMP-9 expression and its activity at both the mRNA and protein level without abolishing TIMP-1 expression, implying that JNP3 has the possibility of using an agent that inhibits MMP-9 expression and activity as an anti-cancer therapy.

The expression of MMP-9 is largely controlled at the transcriptional level. There are two AP-1 binding sites and one NF-κB binding site in the promoter region of MMP-9gene [4]. The stimulation of MMP-9 promoter activity by PMA is regulated by modulating the activation of transcription factors such as AP-1 and NF-κB [3–8,13,14]. It has been reported that PMA-induced MMP-9 expression is regulated through suppressing both AP-1 and NF-κB signaling pathways [14].In this study, we found thatJNP3 markedly suppressed the PMA-induced NF-κB transcriptional activity and NF-κBDNA-binding activity, whereas it did not affect on these activities of AP-1. Moreover, JNP3 effectively inhibited the phosphorylation of p65, a main subunit of NF-κB, leading to its translocation into nucleus, whereas it did not affect on phosphorylation level of c-Jun, a major component of AP-1. Under normal conditions, NF-κB is present in the cytoplasm as an inactive heterotrimer consisting of p50, p65 and IκBα subunits. Activation by stimulator such as PMA leads to the dissociation of IκBα from NF-κB through the ubiquitin/proteasome-dependent pathway following its phosphorylation. The released NF-κB translocates into the nucleus and binds to the promoter region of MMP-9, leading to gene expression [15].In the present study, our results showed that JNP3 inhibits the transcriptional activation of MMP-9 mainly through the specific inhibitions of PMA-induced NF-κB activity by blocking the translocation of p65 to nucleus.

Several studies identified that NF-κB- and/orAP-1-dependent MMP-9 expression is regulated by MAPKs including ERK, JNK, p38, PKC and by PI3K/Akt signaling pathway, depending on the cell type and on the type of stimuli [6–9,13,14].In this study, JNP3 dramatically inhibited the PMA-induced phosphorylation of ERK and JNK, but not p38 MAPK. Previous studies have shown that activation of JNK and/or ERK can induce cancer invasion

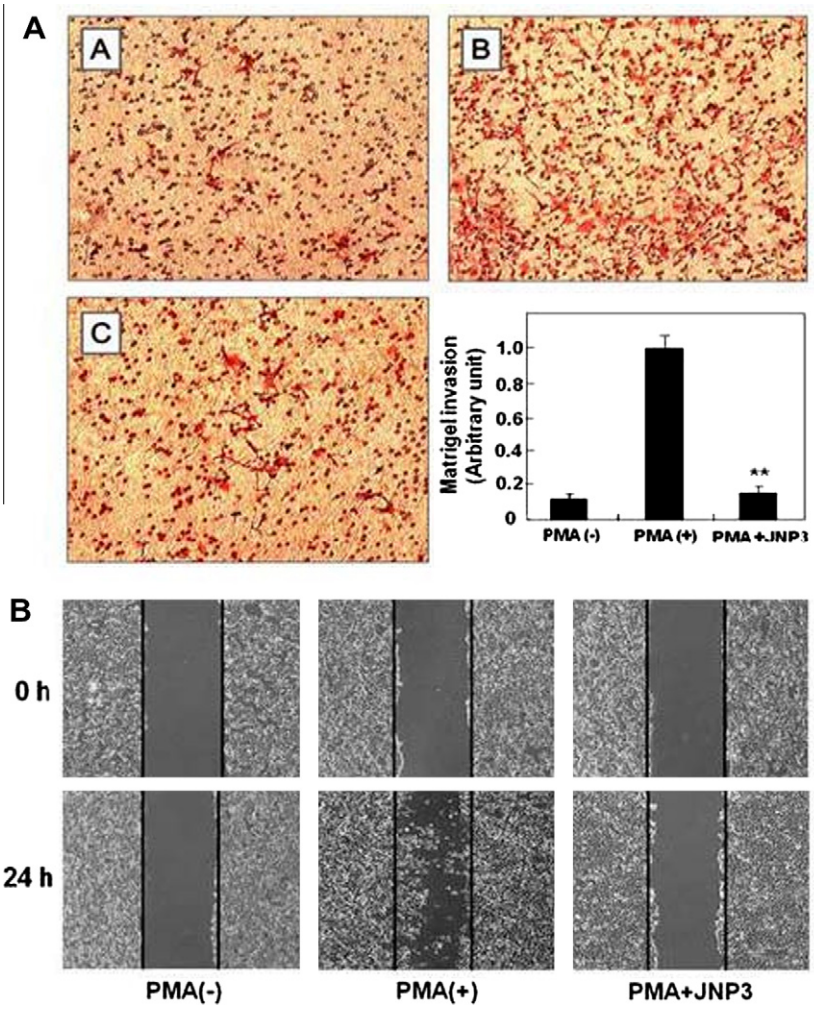

Fig. 4. Effects of JNP3 on PMA-induced invasion and migration of MCF-7 cells. (A) The invasive ability was evaluated by a Matrigel invasion assay with JNP3 (20 μM) in the presence of PMA (100 nM) as described in Section 2. After incubation of 24 h, invasive cells located on the lower surface of the membrane filter were fixed, stained, and counted. Data represent the mean ± SE of three-independent experiments; $^{**}p < 0.01$ vs PMA. (B) Cells were scratched with a pipette tip and then co-treated with JNP3 (20 μM)and PMA (100 nM) for 24 h. Migrating cells were photographed under phase contrast microscopy.

through c-Jun and AP-1 signaling pathway [16,17]. It has also reported that JNK activity is extremely increased in colonic neoplasm and the suppression of JNK activity lead to decrease tumor metastases [17,18]. In line with these reports, we observed that JNP3 significantly suppressed invasion and migration in PMA-induced MCF-7 cells. Taken together, it is feasible that anti-invasive effects of JNP3 in PMA-induced MCF-7 cells may be through inhibiting NF-κB activation via downregulation of JNK and ERK pathways.

A number of MMP inhibitors have been developed for the treatment of cancer and most of these MMP inhibitors including synthetic compounds are reported to exert side effects such as musculoskeletal pain in tendons and joints [19,20]. Thus, natural compounds with minimal or few side effects pay considerable attention as a new source of MMP inhibitors. In this light, the present study suggests that JNP3 could be a strong candidate for treatment of tumor invasion and metastasis via dual inhibition of MMP-9 activity and expression by transcriptional repression of the MMP-9 promoter.

In conclusion, we have demonstrated here for the first time that JNP3 inhibited PMA-induced MMP-9 expression and activity by blocking the activation of NF-κB via ERK1/2 and JNK signaling pathways in MCF-7 cells. Considering the strong evidence for anti-invasive effect of JNP3, our results indicate that JNP3 might act as a chemotherapeutic agent in the suppression of tumor invasion and metastasis, and inhibitory effect of JNP3 on MMP-9 activity may extend future clinical research on the anti-cancer properties of JNP3 *in vivo*.

Acknowledgments

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