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PMA 유도 MMP-9 발현과 침윤에 대한 상황지탕의 효과

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Effects of Sang-Hwang-Ji-Tang on PMA- induced MMP-9 expression and invasiveness

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Objectives

We investigated the potential inhibitory effects of Sang-Hwang-Ji-Tang (SHJT), a traditional Chinese formulation, on MMP-9 activity and expression, and the cellular invasion in the PMA-induced various cell lines including MCF-7 human breast carcinoma cells by using the methanol extract of Sang-Hwang-Ji-Tang (SM).

Materials and Methods

○ Materials

- Cell lines : American Type Culture Collection (ATCC, Manassas, VA, USA)
- Sang-Hwang-Ji-Tang : Da Lian Han Bin Healthy Food Corproation, Da Lian, China.
- PMA(phorbol myristate acetate) : Sigma

○ Methods

- Methanol extract of Sang-Hwang-Ji-Tang (SM)
- XTT cytotoxicity assay
- Gelatin zymography assay
- Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis
- Matrigel invasion assay
- Promoter assay

Results and Discussion

The result from gelatin zymography showed that SM dramatically inhibited MMP-9 activity in PMA-induced various cells. In addition, treatment of various cells with SM significantly decreased both the levels of PMA-induced MMP-9 mRNA expression and protein in a concentration-dependent manner. Moreover, the Matrigel invasion assay showed that SM strongly inhibited PMA-induced invasion of MCF-7 cells as compared with the control, in a dose-dependent manner. As evidenced by MMP-9 promoter assay, SM also remarkably inhibited the transcriptional activity of the MMP-9 gene in PMA-induced MCF-7 cells. These results suggest that SM could be used as potential anti-metastatic agent

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for anti-tumor suppressing PMA-induced cancer cell invasion through the inhibition of MMP-9 gene expression level.

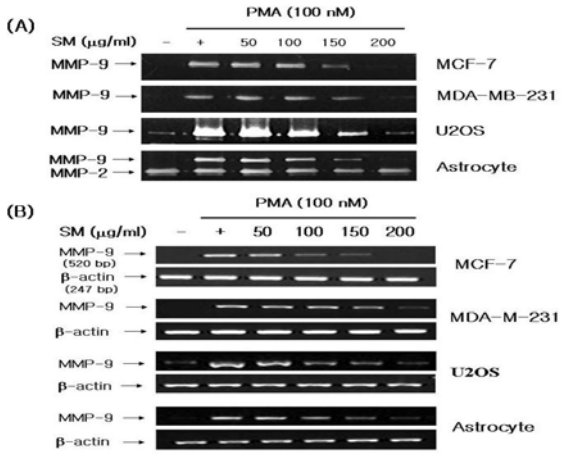

Fig. 1. Effect of SM on the PMA-induced MMP-9 expression in various cell lines.
MCF-7, MDA-MB-231, U2OS, and Astrocyte cells were treated with various concentrations of SM (0, 10, 50, 100, 150 and 200 μg/ml) in the presence or absence of PMA (100 nM). The conditional media were collected and subjected to gelatin zymography (A). The MMP-9 mRNA expression in various cells was analyzed by RT-PCR, β-actin expression was included as an internal control. MMP-9 and β-actin signals were shown as a PCR bands of 520 bp and 247 bp, respectively.

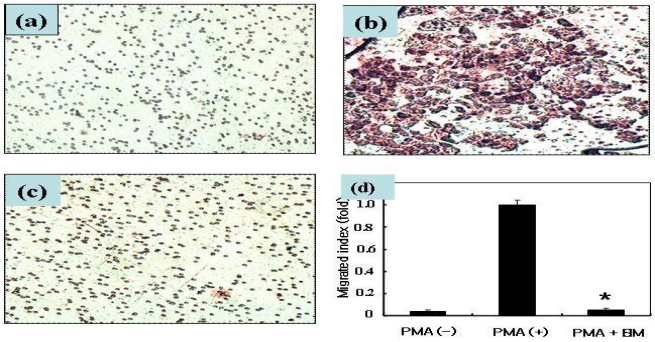

Fig. 2. Effect of SM on PMA-mediated Matrigel invasion by MCF-7 cells.
Cells were plated (5 x 10^4 cells per well) into upper chamber of Matrigel invasion chamber. MCF-7 cells were cultured in the presence or absence of either PMA or SM (200 μg/mL). (a) untreated PMA as control; (b) treated PMA; (c) treated PMA with SM (200 μg/ml); (d) Data represent the mean ± SD of three-independent experiments and is expressed relative to a control. Statistically significant (*P < 0.001).