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MCF-7 유방암 세포에서 PMA 유도 MMP-9 발현과 침윤에 대한 혈통령의 억제효과

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Inhibitory effects of Hyul-Tong-Ryung on PMA- induced MMP-9 expression and invasiveness in MCF-7 human breast carcinoma cells

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Objectives

To elucidate molecular mechanism involved in transcriptional suppression of MMP-9 by Hyul-Tong-Ryung (HTR), a traditional Chinese formulation, we investigated on MMP-9 expression, invasion and transcriptional regulation in the PMA-induced MCF-7 human breast carcinoma cells by using the methanol extract of Hyul-Tong-Ryung (HM).

Materials and Methods

○ Materials

- Cell line : MCF-7 (ATCC, Manassas, VA, USA)
- Hyul-Tong-Ryung : Da Lian Han Bin Healthy Food Corproation, China.
- PMA(phorbol myristate acetate) : Sigma

○ Methods

- XTT cytotoxicity assay, Gelatin zymography assay
- Matrigel invasion assay, Western blot analysis
- Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis
- Transient transfection and luciferase reporter assay
- Electrophoretic mobility shift assay (EMSA)

Results and Discussion

From gelatin zymography and invasion assay, we found that HM inhibits invasion and MMP-9 protein expression. Report gene promoter assay, electrophoretic mobility shift assay (EMSA) and specific kinase inhibitors assay showed that HM specifically inhibits MMP-9 gene transcriptional activity by blocking PMA-induced activation of activator protein-1(AP-1). In addition, HM suppressed PMA-stimulated phosphorylation of extracellular signal regulated kinase1/2 (ERK1/2), upstream modulators involved in AP-1 activation, whereas it did not affect the phosphorylation of either c-Jun N-terminal kinase (JNK) or p38 mitogen-activated protein kinase (MAPK). In this study, therefore, we provide evidences showing that HM suppresses PMA-induced MMP-9 expression by blocking the AP-1 activation via ERK 1/2 signaling pathway.

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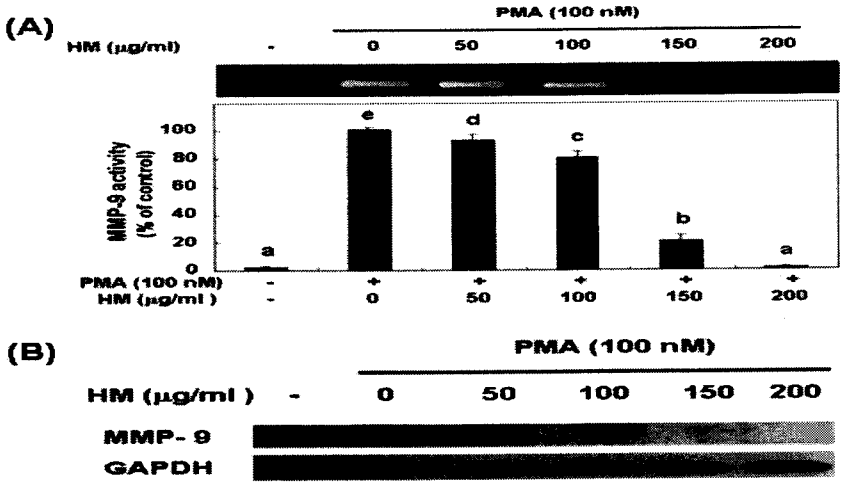

Fig. 1. Effects of HM on the PMA-induced MMP-9 activity and production in MCF-7 cells. Cells were treated with various concentrations of HM in the presence or absence of PMA (100 nM). The conditioned media were collected after 24 h incubation and subjected to zymography (A). Western blot analysis was performed with antibody specific for MMP-9 and GAPDH was used as an internal control (B).

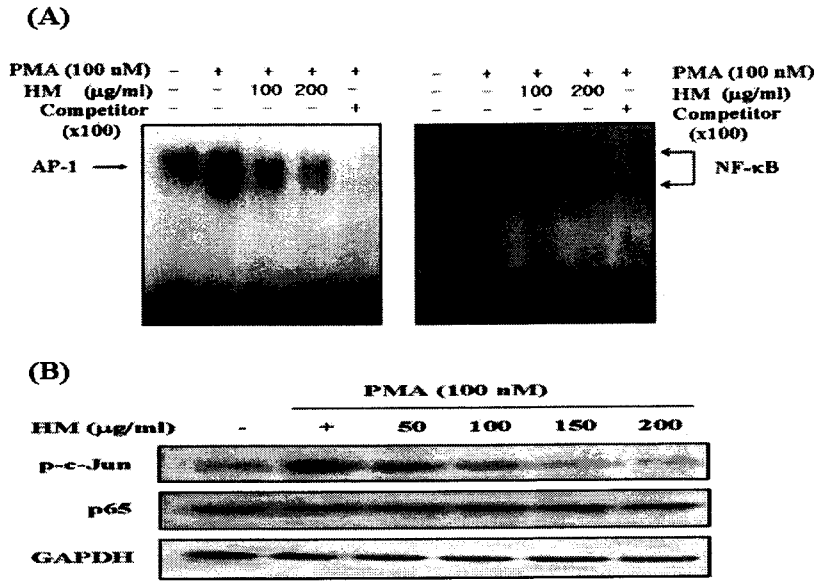

Figure 2. Effects of HM on the PMA-induced AP-1 and NF-κB activations in MCF-7 cells. Cells were treated with the indicated concentrations of HM in the presence or absence of PMA (100 nM). Nuclear extracts were prepared from MCF-7 cells and examined for AP-1 and or NF-κB activations by EMSA. Competition was performed using an unlabeled AP-1 and or NF-κB consensus oligonucleotides (A). The nuclear extracts were also examined for phospho-c-Jun and p65 protein expressions by Western blot analysis. GAPDH was used as an internal control (B).