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Saponins derived from the roots of *Platycodon grandiflorum* inhibit HT-1080 cell invasion and MMPs activities: Regulation of NF-κB activation via ROS signal pathway

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Abstract

The chemopreventive effects of saponin derived from *Platycodon grandiflorum* (Changkil saponin; CKS) on tumor invasion and migration and the possible mechanisms involved in this protection were investigated in HT-1080 tumor cells. In this study, we found that CKS reduced 12-*O*-tetradecanoylphorbol-13-acetate (PMA)-enhanced Matrix metalloproteinases (MMP)-9 and MMP-2 activation in a dose-dependant manner and further inhibited HT-1080 cell invasion and migration. In addition, CKS suppressed PMA-enhanced expression of MMP-9 protein, mRNA and transcription activity levels through suppression of nuclear factor (NF)-κB activation without changing tissue inhibitor of metalloproteinase (TIMP)-1 level. CKS also reduced PMA-enhanced MMP-2 active forms through suppression of membrane-type 1 MMP (MT1-MMP) level, but did not alter MMP-2 and TIMP-2 levels. Moreover, reactive oxygen species (ROS) production induced by PMA was partly decreased in the presence of CKS and this suppression of ROS production may be related to diminish NF-κB activity. Therefore, our results suggested that the inhibitory effects of CKS on MMP-2 and MMP-9 activation, relation of tumor invasion and migration *in vitro* possibly involve mechanisms related to its ability to suppress PMA-enhanced NF-κB activation through ROS signaling pathway. Overall, CKS may be a valuable anti-invasive drug candidate for cancer therapy.

Keywords: *Platycodon grandiflorum*; Saponins; MMP; NF-κB; Reactive oxygen species

1. Introduction

The principal mechanisms involved in cancer mortality are migration and invasion, where primary cancer cells disseminate and grow at a distant site resulting in a secondary tumor [1]. When cancer cells invade and migrate, a number of proteolytic enzymes contribute to the degradation of environmental barriers, such as the extracellular matrix (ECM) and basement membrane [2]. Matrix metalloproteinases (MMPs), a family of zinc-dependent endopeptidases, are principal enzymes in extracellular matrix degradation, which is essential in the invasive growth, metastasis and angiogenesis

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of cancer [3,4]. Among the MMPs, MMP-2 and MMP-9, which are abundantly expressed in various malignant tumors, are considered to play critical roles in tumor invasion and metastasis [5–7]. Both of MMP-2 and MMP-9 are gelatinases, but regulation of their activations is quite different between various cells [8,9]. Generally, activation of MMP-9 primarily regulated based on the balance between proenzyme activation and inhibition by tissue inhibitor of metalloproteinase (TIMP)-1 [9,10], whereas MMP-2 is constitutively expressed and secreted as a latent zymogen, pro-MMP-2. Its activation occurs on the cell membrane through the membrane-type MMP (MT1-MMP) by forming a trimolecular complex with TIMP-2 [11–13]. In addition, the highly expressed MT1-MMP leads to activation of pro-MMP-2 [14,15]. However, tissue inhibitors of metalloproteinase (TIMP)-2 play a dual role in activation of MMP-2 but inhibition of MMP-2 [16].

Changkil (CK), which is the aqueous extract made from the root of *Platycodon grandiflorum* cultivated for more than 20 years has been used as a food as well as in traditional oriental medicine to treat chronic adult diseases, such as, bronchitis, asthma and pulmonary tuberculosis, hyperlipidemia, and inflammatory diseases [17,18]. Previous studies found that CK prevented hypercholesterolemia and hyperlipidemia [18] and modulated the functions of macrophages [19]. Recently, previous data showed that CK and the Changkil sapoins (CKS) derived from CK have anti-inflammatory activity and reduced the COX-2 expression levels by inhibiting the transcription factor, nuclear factor-κB (NF-κB), as well as prostaglandin E_2 (PGE_2) production *in vitro* in lipopolysaccharide (LPS)-stimulated mouse macrophages [20]. In addition, our previous data showed that CKS have inhibitory activity on NF-κB activation in human endothelial cells [21] and CK have anti-metastatic activities *in vivo* animal model [27]. Generally, NF-κB is a key player in tumorigenesis, and inhibitors of NF-κB activation have been shown to suppress MMP-2 and MMP-9 and further tumor invasion [22,23]. Nonetheless, the physiological functions and the natures of CKS as an anti-invasive agent have not been studied. Therefore, we investigated the inhibitory effects of CKS on HT-1080 cell invasion and migration in relation to the activation and expression of MMP-2 and MMP-9 and also elucidated the mechanism(s) underlying its chemopreventive effects of HT-1080 cancer cells.

2. Materials and methods

2.1. Chemicals and materials

All the chemicals and cell culture materials were obtained from the following sources: WST-1 assay kit, and Lactate dehydrogenase (LDH) assay kit from Roche Co.; LipofectAMINE 2000, RPMI1640 medium, fetal bovine serum (FBS), and penicillin–streptomycin solution from Life Technologies, Inc.; Omniscript RT-PCR kit, pCMV-5-HA-p65, pGL3-4κB-Luc, phRG-TK (*renilla* luciferase vector) and the luciferase assay system from Promega; Mouse anti-human MMP-9, I-κBα, anti-β-actin antibodies and Matrigel from BD Pharmingen; MMP-9, MMP-2 enzymatic activity ELISA assay kit, and Western blotting detection reagents (ECL) from Amersham Pharmacia Biotech.; Mitomycin C, 12-*O*-tetradecanoylphorbol-13-acetate (PMA) and 2′,7′-dichlorofluorescin diacetate (DCF-DA) from Sigma chemical Co.; the other chemicals were of the highest commercial grade available.

2.2. Preparation of CKS

CK refers to the aqueous extract obtained from the 22-years-old roots of *P. grandiflorum*, which was supplied by Jang Saeng Doraji Co., Jinju, South Korea. The CK and CKS were prepared using the method described elsewhere and their compositions were previously published [19,21,25,26]. Briefly, CK was subjected to column chromatography over amberlite XAD-2, Diaion MCl Gel HP20 or Kogel BG4600. After removing the saccharides and amino acids with water, the column was eluted with methanol to obtain the CKS, which is the saponin fraction of CK, as described previously [26].

2.3. Cell culture and treatment

HT-1080 cells from ATCC (American Type Culture Collection, Manassas, VA) were grown in RPMI1640 supplemented with 10% FBS, 100 IU/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a 5% CO_2 humidified incubator. Cells were treated with different concentrations of CKS in the absence or presence of PMA (10 nM) for 24 h.

2.4. Cytotoxicity of CKS

Cell cytotoxicity was examined using a WST-1 assay Kit and a LDH assay kit by measuring the according to the manufacturer's instructions. Briefly, the cells (5×10^3/well) in 10% FBS-RPMI1640 were seeded into the 96-well plates. After incubation for 24 h, various concentrations of CK were added to the well, and the plates were incubated at 37 °C for an additional 24 h. After the supernatant was removed for LDH determination, the cells were used for the WST-1 assay. Relative cytotox-