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Oldenlandia diffusa suppresses metastatic potential through inhibiting matrix metalloproteinase-9 and intercellular adhesion molecule-1 expression via p38 and ERK1/2 MAPK pathways and induces apoptosis in human breast cancer MCF-7 cells

Tae-Wook Chung[a,1], Hyunju Choi[b,1], Ji-Min Lee[b], Sun-Hyung Ha[b], Choong-Hwan Kwak[b], Fukushi Abekura[b], Jun-Young Park[b], Young-Chae Chang[c], Ki-Tae Ha[a], Seung-Hak Cho[d], Hyeun Wook Chang[e], Young-Choon Lee[f], Cheorl-Ho Kim[a,*]

[a] Division of Applied Medicine, School of Korean Medicine and Research Center for Healthy Aging, Pusan National University, Yangsan City, Gyeongsangnam-Do, Korea
[b] Department of Biological Sciences, SungKyunKwan University, Seoburo 2066, Jangan-Gu, Suwon, Gyunggi-Do 16419, Korea
[c] Research Institute of Biomedical Engineering and Department of Medicine, Catholic University of Daegu School of Medicine, Daegu, Korea
[d] Division of Enteric Diseases, Center for Infectious Diseases Research, Korea National Institute of Health, Heungdeok-gu, Cheongju 363-951, Korea
[e] College of Pharmacy, Yeungnam University, Gyeongsan 712-749, Korea
[f] Department of Medicinal Biotechnology, College of Health Sciences, Dong-A University, Busan 49315, Korea

ARTICLE INFO

Chemical compounds to be constituted in Oldenlandia diffusa *in this article:*
Ursolic acid (PubChem CID: 64945)
Oleanolic acid (PubChem CID: 10494)
Ssperuloside, E-6-O-p-coumaroyl scandoside methyl ester and E-6-O-p-coumaroyl scandoside methyl ester-10-methyl ether (PubChem CID: 84298)
Stigmasterol (PubChem CID: 5280794)

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ABSTRACT

Ethnopharmacology relevance: *Oldenlandia diffusa* (OD) has long been known as an apoptotic inducer in breast tumors in ethnomedicine.
Aim of the study: To scientifically confirm the anti-breast cancer effects of water, methanol (MeOH) and butanol (BuOH) extracts of *O. diffusa* on cell apoptosis, matrix metalloproteinases (MMPs), intercellular adhesion molecule (ICAM)−1 and intracellular signaling in MCF-7 breast cancer cells.
Materials and methods: MeOH extracts (MOD) and BuOH extracts (BOD) were prepared and examined for their ability to inhibit phorbol myristate acetate (PMA)-induced matrix metalloproteinase (MMP)−9 and intercellular adhesion molecule (ICAM)−1 expressions in MCF-7 human breast cancer cells. Additionally, transwell migration, invasion and transcriptional activity were assessed. Results of immunofluorescence confocal microscopy for translocation of NF-κB and p-ERK and p-p38 were also checked. Finally, apoptotic signals including processed caspase-8, caspase-7, poly ADP-ribose polymerase, Bax and Bcl-2 were examined.
Results: MOD and BOD specifically inhibited PMA-induced MMP-9 expression as well as invasive and migration potential via ICAM-1. The inhibitory activity was also based on the suppressed transcriptional activity in MCF-7 breast cancer cells. Results of immunofluorescence confocal microscopy showed that translocation of NF-κB decreased upon BOD and MOD treatments, with a decreased level of p-ERK and p-p38 phosphorylation. In addition, treatment of MCF-7 cells with MOD and BOD activated apoptosis-linked proteins including enzymatically active forms of processed caspase-8, caspase-7 and poly ADP-ribose polymerase, together with increased expression of mitochondrial apoptotic protein, Bax and decreased expression of Bcl-2.
Conclusion: The results indicate that OD as an anti-metastatic agent suppresses the metastatic response by targeting p-ERK, p-38 and NF-κB, thus reducing the invasion capacity of MCF-7 breast cancer cells through inhibition of MMP-9 and ICAM-1 expression and plays an important role in the regulation of breast cancer cell apoptosis.

Abbreviations: OD, *Oldenlandia diffusa*; MMP, matrix metalloproteinase; ICAM-1, intercellular adhesion molecule-1; MOD, methanol extracts of OD; BOD, buthanol extracts of OD; PMA, phorbol myristate acetate; ECM, extracellular matrix; PARP, ADP-ribose polymerase; FBS, fatal bovine serum; TBS-T, Tris-Buffered Saline Tween-20; HRP, horseradish peroxidase

* Correspondence to: Department of Biological Sciences, Sungkyunkwan University, Seoburo 2066, Jangan-Gu, Suwon, Gyunggi-Do 16419, Korea.
E-mail addresses: chungtw@hanmail.net (T.-W. Chung), chj204@skku.edu (H. Choi), jml@skku.edu (J.-M. Lee), sunghyungha@skku.edu (S.-H. Ha), kwak@skku.edu (C.-H. Kwak), pokusa6@hotmail.com (F. Abekura), wnsdud2057@naver.com (J.-Y. Park), ycchang@cu.ac.kr (Y.-C. Chang), haggis@pnu.ac.kr (K.-T. Ha), skcho38@hotmail.com (S.-H. Cho), hwchang@yu.ac.kr (H.W. Chang), yclee@dau.ac.kr (Y.-C. Lee), chkimbio@skku.edu (C.-H. Kim).
[1] Equally contributed as co-first author (Tae-Wook Chung and Hyunju Choi).

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1. Introduction

Malignant cancer cells can invade tissues through degradation of the extracellular matrix (ECM) by matrix metalloproteinases (MMPs) (Aoudjit et al., 1998; Leeman et al., 2003). MMPs are associated with invasion of tumor cells through the basement membrane and stroma, blood vessel penetration, and metastasis; thus, rendering primary and metastatic tumor growth and angiogenesis in tumor promotion (Nelson et al., 2000). MMPs comprise the following three main groups: the interstitial collagenases (MMP-1), the type IV collagenases or gelatinases (MMP-2 and −9), and the stromelysins (MMP-3). Among them, gelatinases (MMP-2 and −9) degrade denatured interstitial collagens and native basement-membrane collagens (Newby and Zaltsman, 2000). The synthesis and secretion of MMP-9 can be stimulated by a variety of stimuli including cytokines and phorbol myristate acetate (PMA) during tumor invasion (Cho et al., 2007), as reported during both migration and proliferation of MCF-7 breast cancer cells (Galis et al., 2002).

Intercellular adhesion molecule (ICAM)−1 is a transmembrane glycoprotein receptor and exists as a member of the immunoglobulin super gene family (Alkhamesi et al., 2005), participating in cell–cell and cell–matrix adhesive interactions (van de Stolpe and van der Saag., 1996). Such adhesive interactions also play a major role in pathological conditions such as inflammation and tumorigenesis (Bohling et al., 1996), as mesothelial ICAM-1 is involved in tumor–mesothelial adhesion. ICAM-1 has been proven to be upregulated in most experimental models and several human inflammatory diseases associated with progress of the lesion (Ootaka et al., 1996). The up-regulation of ICAM-1 was involved in the mechanism that promotes senescence (Zhang et al., 2006). Therefore, some pathological injury could be attenuated by inhibition of ICAM-1 through the blocking antibody, antisense oligonucleotide, and gene knockout of ICAM-1. For the expression of ICAM-1 and MMP-9 genes, the nuclear factor NF-κB is a key factor (Lee et al., 2007). NF-κB is present in an IκB-bound quiescent form in the cytosolic region of non-metastatic cells, where the factors are targets for suppressing invasive diseases. MAPK signaling pathways such as p38, JNK, and ERK are important for NF-kB subunit p65 transactivation or translocation (Lee et al., 2008). Therefore, nuclear NF-κB translocation is an active invasive response.

Breast cancer is one of the most commonly found malignant tumors with a poor prognosis (Radwan et al., 2016). Due to its major impact on the population, the prognosis and specific treatment for breast cancer need to be explored. Among human breast cancer cells, the MCF-7 and SK-BR-3 cells have limited migration due to their positive expression of estrogen receptor-α (ERα). Whereas MDA-MB-468 cells are highly migratory and metastatic due to negative ERα (Goodison et al., 2003) and thus, MDA-MB-231 cells are frequently used as the ERα-negative invasion and progression model (Cazet et al., 2009; Ha et al., 2016; Goodison et al., 2003; 21–23). In contrast, ERα-positive MCF-7 cells are used as the typical model of non-invasion and non-progression of breast cancer (Yang et al., 2007). As an *in vitro* model in cancer research, MCF-7 cell line is commonly used over for the last 4 decades in studies of the molecular profile, proliferation, migration, invasion, spheroid formation, angiogenesis, lymphangiogenesis and mesenchymal stem cells (Comşa et al., 2015).

Oldenlandia diffusa (Willd.) Roxb. (OD), a member of the Rubiaceae family is a medicinal plant (Ovesna et al., 2004), which has been used for treating liver, lung, and rectal tumors (Gupta et al., 2004; Lu et al., 2016). This herb is also called *Hedyotis diffusa*, scientifically and literally translated from Chinese language as White Flower Snake Tongue Grass. OD has anti-inflammation and cancer prevention effects against tumor growth. In addition, it is used to clear heat, counteract toxins, remove of damp, treatment of boils and abscesses (Gupta et al., 2004; http://baike.baidu.com/item). OD is also known to cause apoptotic induction of cancer cells with immunomodulatory, anti-angiogenic, anti-inflammatory, anti-oxidant, and pro-apoptotic activities (Gu et al., 2012; Ovesna et al., 2004; Shan et al., 2001). More importantly, OD has been reported to exhibit anticancer activity in diverse tumor models such as breast cancer, leukemia, and small cell lung carcinoma cells (Gu et al., 2012; Willimott et al., 2007; Gupta et al., 2004; Sadava er al., 2002; Yadav and Lee, 2006). However, the inhibitory activity of OD against migration or metastasis of human cancer cells is not fully understood. The main compound of OD, ursolic acid, has anticancer effects (HS et al., 2002). Iridoid glucosides such as asperuloside, E-6-O-p-coumaroyl scandoside methyl ester and E-6-O-p-coumaroyl scandoside methyl ester-10-methyl ether has been isolated from *O. diffusa* (Liang et al., 2006). Recently, it was suggested that OD exerts antiproliferative and apoptotic effects on human breast cancer cells through ERa/Sp1-mediated p53 activation (Gu et al., 2012). However, the effect of OD on the metastatic potentials through MMP and ICAM in breast cancer cells such as MCF-7 has not yet been elucidated.

Apoptotic tumor therapy has been highlighted for the important anti-cancer mechanism [Akter et al., 2015]. Apoptotic cell death is extracellularly associated with morphological changes and various apoptotic signaling molecules including caspases, Bax, bcl-2 and poly ADP-ribose polymerase (PARP) Ha et al., 2016; Smith et al., 2016. Two functional caspases such as the initiator caspases (caspases 2, 8, 9 and 10) and the effector caspases (caspases 3, 6 and 7) play important roles in apoptotic cell death (Wang et al., 2005), where the initiator caspases activate the downstream effector caspases and cleave their substrate PARP (Chashoo et al., 2011; Ha et al., 2016).

In this study, we investigated the effects of water extracts, MOD and BOD on growth and death of human breast cancer cell line MCF-7. MOD and BOD have shown an inhibitory action against MCF-7 cells *in vitro*. We provide evidences that MOD and BOD inhibit PMA-induced MMP-9 expression in MCF-7 cells and also migration of MCF-7 cells. MOD and BOD inhibited ICAM-1 protein expression. In addition, to understand the relationship between the apoptotic potential and MOD and BOD in the above mentioned cells, we examined the effect of MOD and BOD on MCF-7 cell apoptosis.

2. Materials and methods

2.1. Cells and materials

The human breast cancer MCF-7 cells were obtained from the American Type Culture Collection (Manassas, VA). Cells were maintained in DMEM (Gibco) supplemented with 10% fetal bovine serum (FBS), 100 μ units/ml streptomycin and incubated at 37 °C in a humidified atmosphere containing 5% CO_2 in air. Herbal *Oldenlandia diffusa* (OD), as one of the voucher specimens, has been deposited in the Herbarium Deposit Unit, Medical Research Center, School of Korean Medicine, Pusan National University, Yangsan, Korea. OD was extracted with distilled water, methanol (MeOH) and butanol (BuOH), as described in our previous reports (Suh et al., 2011, Suh et al., 2012) and extracts were used for the experiments. The GAPDH monoclonal antibody was obtained from Chemicon (Temecula, CA, USA). Hoechst staining solution, Griess reagent, antibodies against β-actin, phospho-ERK, p38, phospho-p38, NF-κB, p65 and β-actin were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. XTT proliferation assay

Cell proliferation was studied using a commercial cell proliferation kit II (XTT, Boehringer Mannheim, Germany). The cells were regularly incubated in a 37 °C humidified incubator under an atmosphere of 5% CO_2 for 24 h. For detecting cytotoxicity, MCF-7 cells were plated into 96-well culture plates in 100 μl of DMEM and subcultured at a density of 1×10^5 cells per well and allowed to attach to the culture plate for 2 h. After 24 h of further incubation, media were discarded and replaced with 100 μl of fresh medium containing various concentrations of MOD