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TNF-α induces expression of urokinase-type plasminogen activator and β-catenin activation through generation of ROS in human breast epithelial cells

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ABSTRACT

Malignant tumors have a capability to degrade the extracellular matrix (ECM) by controlled proteolysis. One of the important components of the proteolysis system involved in such process is urokinase-type plasminogen activator (uPA). Tumor necrosis factor (TNF)-α was found to stimulate uPA. TNF-α impaired the ability of cells to aggregate and to attain compaction. Dyscohesion (cell–cell dissociation) induced by TNF-α was associated with the disordered expression of cadherin/β-catenin at the sites of cell–cell contact. We observed that human breast epithelial (MCF-10A) cells treated with TNF-α transiently up-regulated expression of uPA and its mRNA transcript. In addition, TNF-α induced activation of β-catenin in MCF-10A cells. Based on these findings, we attempted to examine the role of β-catenin and its partner, Tcf-4 in upregulation of uPA. siRNA knock down of β-catenin abrogated TNF-α-induced uPA expression as well as Tcf-4/β-catenin DNA binding. TNF-α-stimulated MCF-10A cells exhibited increased intracellular accumulation of reactive oxygen species (ROS). TNF-α-induced expression of uPA and activation of β-catenin signaling appear to be mediated by ROS in MCF-10A cells, as both events were blocked by the antioxidant N-acetylcysteine.

Eupatilin (5,7-dihydroxy-3′,4′,6-tri-methoxy-flavone), a pharmacologically active flavone derived from *Artemisia asiatica*, has been shown to possess strong antioxidative activity. Eupatilin inhibited TNF-α-induced intracellular ROS accumulation, expression of uPA and β-catenin activation. Moreover, eupatilin inhibited the TNF-α-induced invasion of MCF-10A cells. Taken together, the above results suggest that eupatilin has chemopreventive effects on mammary tumorigenesis by targeting the β-catenin-uPA axis stimulated by TNF-α.

1. Introduction

Metastasis, one of the most detrimental causes associated with malignant progression, is a complex cascade of events involving tumor dissemination from the primary site of growth to distant organs. Metastasis is multistep events involving degradation or rearrangement of the extracellular matrix (ECM), local invasion, angiogenesis, intravasation, survival of malignant cells during the circulation, extravasation and the growth at a secondary site [1]. One of the key mediators causally involved in the aforementioned processes is the serine protease, urokinase-type plasminogen activator (uPA) [2]. Members of the plasminogen activator system, including urokinase plasminogen activated receptor (uPAR), have been found to be over-expressed in a large number of tumors, particularly breast cancer, which is associated with a poor prognosis [2,3]. uPA catalyzes the formation of plasmin from plasminogen to generate the proteolytic cascade that contributes to the breakdown of ECM, a key step in cancer metastasis [4,5].

During the metastatic cascade, many pathogenic changes occur. These include inflammation mediated by over-secretion of cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-8 [6,7]. TNF-α is a pleiotropic pro-inflammatory cytokine that has a wide range of biological activities. TNF-α can mediate tumor progression by inducing the cellular transformation, promotion, survival, proliferation, invasion, angiogenesis, and metastasis [8–10]. Many of TNF-α-induced cellular responses and alterations have been associated with accumulation of reactive oxygen species

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(ROS) [11,12]. ROS are relatively harmless, but when produced excessively or in the absence of sufficient antioxidant defense, the balance between oxidants and antioxidants is disturbed. This may lead to the initiation and promotion of cancer [13–15]. ROS produced by TNF-α activates several intracellular signaling molecules, such as β-catenin and its partner T-cell factor (Tcf)-4 [16,17]. β-Catenin has been shown to have a dual role as a major structural component of cell–cell adherence junctions and also as a transcription activator. In addition, β-catenin is involved in tumorigenesis by transactivating the lymphoid enhancer factor/T-cell factor (Lef/Tcf) transcription factor. Some of the genes whose expression is up-regulated by β-catenin/Tcf signaling are *c-jun*, *c-myc*, *fibronectin*, *cyclin D1*, *mmp* and *uPA* [18,19].

In the present study, we found that treatment of immortalized human breast epithelial (MCF-10A) cells with TNF-α resulted in the increased expression of uPA with concurrent enhancement of invasive capacity. Interestingly, TNF-α-induced uPA up-regulation and cell invasion were mediated by ROS that triggered the activation of β-catenin signaling. As TNF-α is a typical pro-inflammatory cytokine, its overactivation of β-catenin signaling may contribute to the inflammation-associated carcinogenesis. In this context, it is noticeable that β-catenin-mediated signaling has recently been recognized as an important molecular target for chemoprevention with anti-inflammatory substances [19]. *Artemisia asiatica* (Asteracease), widespread in nature, has been frequently used in traditional oriental medicine for the treatment of inflammation, cancer, and microbial infections [20–22]. Our previous studies have shown that eupatilin (5,7-dihydoxy-3′,4′,6-tri-methoxy-flavone), one of the pharmacologically active ingredients of *A. asiatica*, possesses anti-inflammatory and anti-tumor promoting activities [21]. This prompted us to examine the ability of this flavonoid to inhibit TNF-α-induced expression of uPA, to activate β-catenin and to cause invasion of MCF-10A cells.

2. Materials and methods

2.1. Materials

Euaptilin was supplied from Dong-A Pharmaceutical Co. Ltd. (Yong-In Si, South Korea) and dissolved in DMSO for treatment. TNF-α, sodium dodecylsulfate (SDS), N-acetylcysteine (NAC), cholera toxin, hydrocortisone, insulin and human recombinant epidermal growth factor (h-EGF) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM)/Ham's nutrient mixture F-12 (1:1) and horse serum were obtained from Gibco BRL (Grand Island, NY, USA). Dichlorofluorescein diacetate (DCF-DA) was purchased from Molecular Probes, Inc. (Eugene, OR, USA). Primers for *uPA* and *glyceraldehydes-3-phosphate dehydrogenase* (*GAPDH*) were synthesized by Bionics (Seoul, South Korea). Negative control and β-catenin short interfering RNA (siRNA) were purchased from Ambion (Austin, TX, USA).

2.2. Cell culture

MCF-10A cells were kindly supplied by Prof. Aree Moon (Duksung Women's University, South Korea). The cells were cultured in DMEM/F-12 medium supplemented with 5% heat-inactivated horse serum, 10 μg/ml insulin, 100 ng/ml cholera toxin, 0.5 μg/ml hydrocortisone, 20 ng/ml h-EGF, 2 mmol/l L-glutamine and 100 units/ml penicillin/streptomycin at 37 °C in a humidified atmosphere of 5% CO_2/95% air.

2.3. Western blot analysis

MCF-10A cells were lysed in RIPA lysis buffer [150 mM NaCl, 0.5% Triton X100, 50 mM Tris–HCl (pH 7.4), 25 mM NaF, 20 mM EGTA, 1 mM dithiothreitol (DTT), 1 mM Na_3VO_4, protease inhibitor cocktail tablets] for 15 min on ice followed by centrifugation at 12,000 × *g*

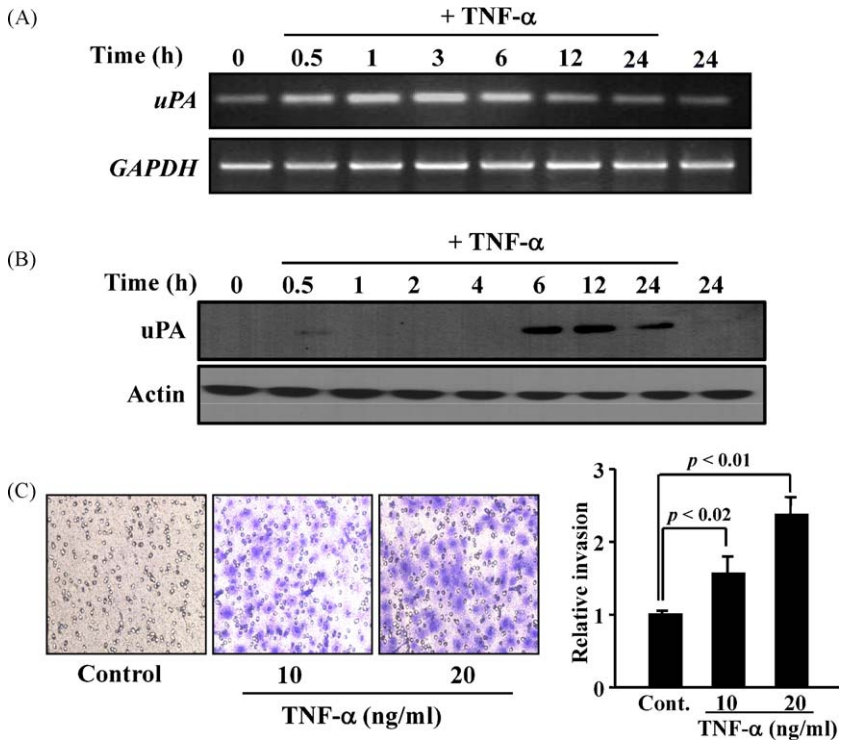

Fig. 1. TNF-α-induced invasiveness and uPA expression in MCF-10A cells. (A) The expression of uPA mRNA was determined by semi-quantitative RT-PCR at various time intervals after treatment with 10 ng/ml TNF-α. (B) TNF-α-induced uPA protein expression was assessed by Western blot analysis using anti-uPA antibody after treatment with 10 ng/ml TNF-α for indicated times. (C) After treatment of MCF-10A cells with TNF-α for 24 h, an *in vitro* invasion assay was performed as describe in Section 2.