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MLK3 is a direct target of biochanin A, which plays a role in solar UV-induced COX-2 expression in human keratinocytes

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ABSTRACT

Solar UV (sUV) is an important environmental carcinogen. Recent studies have shown that sUV is associated with numerous human skin disorders, such as wrinkle formation and inflammation. In this study, we found that the isoflavone, biochanin A, inhibited the expression of sUV-induced COX-2, which is a well-characterized sUV-induced enzyme, in both human HaCaT keratinocytes and JB6 P+ mouse skin epidermal cells. Several studies have demonstrated the beneficial effects of biochanin A. However, its direct molecular target is unknown. We found that biochanin A inhibited sUV-induced phosphorylation of MKK4/JNK/c-Jun and MKK3/6/p38/MSK1. Mixed-lineage kinase 3 (MLK3) is an upstream kinase of MKK4 and MKK3/6. Thus, we evaluated the effect of biochanin A on MLK3. We found that sUV-induced MLK3 phosphorylation was not affected, whereas MLK3 kinase activity was significantly suppressed by biochanin A. Furthermore, direct binding of biochanin A in the MLK3 ATP-binding pocket was detected using pull-down assays. Computer modeling supported our observation that MLK3 is a novel target of biochanin A. These results suggest that biochanin A exerts chemopreventive effects by suppressing sUV-induced COX-2 expression mediated through MLK3 inhibition.

1. Introduction

Human skin is constantly exposed to various environmental factors, such as solar UV (sUV). Many previous studies have reported that repetitive exposure of skin to sUV causes physiological changes such as sunburn [1], wrinkle formation [2], and inflammation [3]. sUV comprises three subtypes, UVA (320–400 nm), UVB (280–320 nm), and UVC (200–280 nm). UVC is blocked by the ozone layer, whereas human skin is exposed to UVA and UVB. sUV comprises approximately 95% UVA and 5% UVB. Accordingly, sUV is a suitable model for studies of physiological skin conditions.

Chronic inflammation is closely associated with several diseases, including cancer [4,5]. Thus, suppression of inflammation may be applicable as an anticancer strategy. Cyclooxygenases (COXs) are the rate-limiting enzymes for prostaglandin production from arachidonic acid and have two isoforms, COX-1 and COX-2. COX-1 is constitutively expressed, whereas COX-2 is an inducible isoform [6], which plays a critical role in carcinogenesis. Aberrant expression of COX-2 promotes cellular processes, including proliferation, angiogenesis, and differentiation [7,8]. In the skin, COX-2 is associated with skin homeostasis, but overexpression of COX-2 can result in pre-neoplastic skin phenotypes [9,10].

Previous studies indicated that COX-2 expression is regulated by inflammatory signaling pathways, such as the mitogen-activated protein kinase (MAPK) family of signaling proteins [11–13]. MAPK kinase kinase (MAP3K) phosphorylates MAP2K and subsequently activates MAPK [14]. Among the MAP3K family, mixed-lineage kinase 3 (MLK3) is well characterized and involved in many inflammatory signaling cascades, as well as cancer [15–18]. Tibbles et al. reported that MLK3 directly phosphorylates SEK1 and MKK3/6, and subsequently activates the JNKs and p38 signaling pathways, respectively [19]. Additionally, Gallo and Johnson demonstrated that MLK3 regulates the JNKs and p38 signaling pathways [20].

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Isoflavones are major components of soy. Recent studies reported that isoflavones exert chemopreventive and anticancer effects [21–24], primarily due to their antioxidative activities [25,26]. However, reports have also suggested that isoflavones have phyto-estrogenic effects [27] and function as small molecule inhibitors in cancer [28]. Biochanin A (Fig. 1A, *upper*) is an isoflavone found in red clover. Although biochanin A is known to have beneficial effects, its direct molecular target remains unknown [29–31].

Overall, we found that biochanin A inhibited sUV-induced COX-2 expression by directly targeting MLK3. Based on kinase assay data, we confirmed that biochaninA suppressed MLK3 kinase activity, and pull-down assays revealed an interaction between biochanin A and MLK3. Because several studies have indicated that

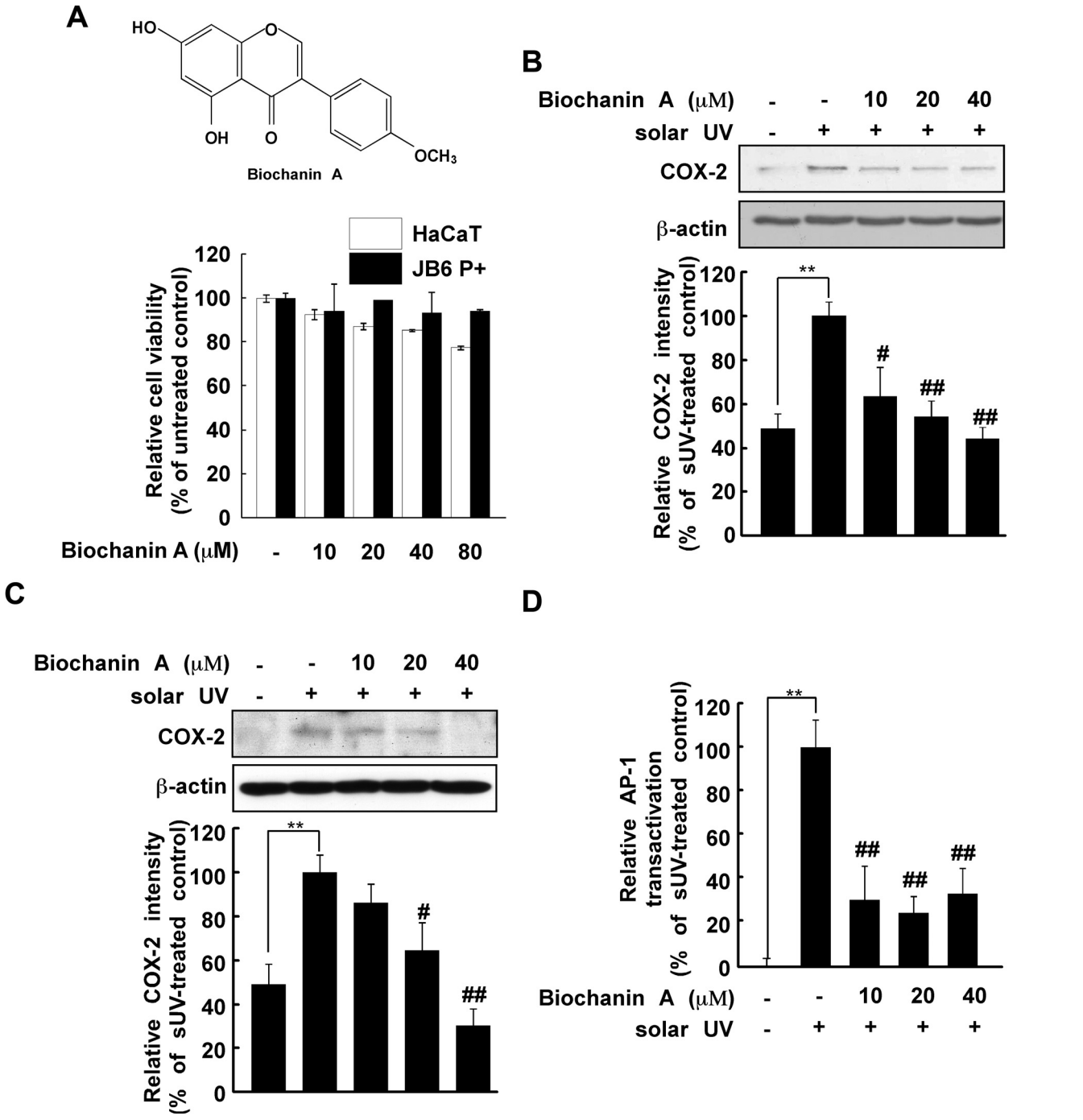

Fig. 1. Effects of biochanin A on solar UV (sUV) induced cyclooxygenase (COX)-2 expression. (A) Chemical structure of biochanin (*upper*) and cytotoxicity of biochanin A against HaCaT and JB6 P+ cells (lower). The procedure for evaluating cytotoxicity is described in Section 2. (B) and (C) Biochanin A inhibits sUV-induced COX-2 expression in human HaCaT (B) and mouse JB6 P+ (C) cells. After treatment with biochanin A (0, 10, 20, or 40 μM) for 1 h, the cells were irradiated with solar UV (sUV; 90 kJ/m^2) and harvested after 1 h. The protein levels of COX-2 and β-actin were measured by Western blotting. Data are representative of 3 independent experiments that provided similar results. The level of β-actin was detected to verify equal loading of proteins. COX-2 expression was quantified using the Image J software program. (D) Biochanin A inhibits sUV-induced AP-1 transactivation in HaCaT cells. After treatment with biochanin A (0, 10, 20, or 40 μM) for 1 h, HaCaT cells transfected with an AP-1 luciferase plasmid were irradiated with sUV. Data are normalized to the transactivation of sUV-irradiated HaCaT cells (100%). The pound ($^{\#}$ and $^{\#\#}$) signs indicate significant differences at $p < 0.01$ and 0.001, respectively, compared to the sUV-treated group. The asterisks (**) indicate a significant induction of COX-2 (B, C) and AP-1 activity (D) induced by sUV. Representative blots from triplicate experiments with similar results are shown.