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Estrogen receptor-α36 is involved in icaritin induced growth inhibition of triple-negative breast cancer cells

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ABSTRACT

A sub-class of ER-negative breast cancer that is negative for ER, PR and HER2 expression known as triple-negative breast cancer (TNBC) is highly malignant and lacks effective treatment. Recently, it has been reported that an isoform of estrogen receptor-alpha ER-α36 is expressed and plays a critical role in development of TNBC. ER-α36 forms a positive regulatory loop with epidermal growth factor receptor (EGFR), which promotes malignant growth of TNBC cells. Thus, ER-α36 has been proposed as an important target for development of novel drugs for TNBC. In this study, we evaluated the effects of icaritin, a prenylflavonoid derivant purified from *Epimedium Genus*, on growth of TNBC cells and examined the possible underlying mechanisms. Our study demonstrated that icartin decreased both ER-α36 and EGFR protein expression, and induced apoptosis in TNBC MDA-MB-231 and MDA-MB-453 cells. We also found that icaritin inhibited ER-α36-mediated MAPK/ERK pathway and cyclin D1 induction by estrogen. Our results thus indicated that icaritin has a potential to be developed into a novel therapeutic agent for human TNBC.

1. Introduction

Triple-negative breast cancer is one subtype of breast cancer that lacks expression of estrogen receptor (ER) and progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2), and that identifies approximately 15% of breast cancers with a feature of higher relapse rates and shorter overall survival compared to other subtypes of breast cancer [1–3]. Since lack of necessary targets, TNBC is refractory to HER2-targeted therapies such as trastuzumab as well as hormonal therapies such as tamoxifen and aromatase inhibitors [4,5]. Considering the severe cell toxicity, side effects and poor responding of conventional chemotherapies, a more effective and less toxic therapeutic agent is urgently needed.

In 2005, Wang's laboratory identified and cloned a 36 kDa variant of ER-α known as ER-α36 that is mainly located in the cytoplasm and at the plasma membrane. ER-α36 lacks the activation function (AF)-1 and AF-2 domains but retained the DNA-binding domain, and partial dimerization and ligand-binding domains of ER-α. ER-α36 mediates the non-classic estrogen pathway, such as a rapid activation of the MAPK/ERK and the PI3K/AKT signaling pathways [6–9]. Both ER-positive/-negative breast cancer [10] and triple-negative breast cancer [9] are expressing ER-α36. ER-α36 and EGFR forms a positive regulatory loop that regulates each other's expression in TNBC cells, which promotes malignant growth of TNBC cells [9]. Recent study further indicated that ER-α36 mediated estrogen-induced cyclin D1 promoter activity through the Src/EGFR/STAT5 pathway [11,12], which lead to increased proliferation of TNBC cells. Fulvestrant, a 'pure' anti-estrogen that is able to induce degradation of ER-α protein failed to influence ER-α36 expression [13]. Thus, it is necessary to find a novel agent that targets ER-α36 in TNBC cells.

Icaritin, a prenylflavonoid compound, derived from Herba Epimedium stems and leaves [14]. Previous studies indicated that icaritin has immunomodulatory and neuroprotection activities [14–16], and protects the cardiovascular system [17] and prevents steroid-associated osteonecrosis [18,19]. Recently, icaritin has been reported to exhibit anti-cancer activity in different cancer cells including hepta carcinoma [20,21], renal cancer [22], breast cancer [23], endometrial cancer [24], lymphoma [25], and glioblastoma [26]. Icaritin inhibited growth and promoted apoptosis in these cancer cells through inhibition of the JAK/STAT3 and the MAPK/ERK signal pathways [22] as well as cytotoxic effects. However, the exact mechanisms underlying these activities are currently unclear.

In this study, we examined the effects of icaritin on growth of TNBC cells and found that icaritin disrupted the positive regulatory loop

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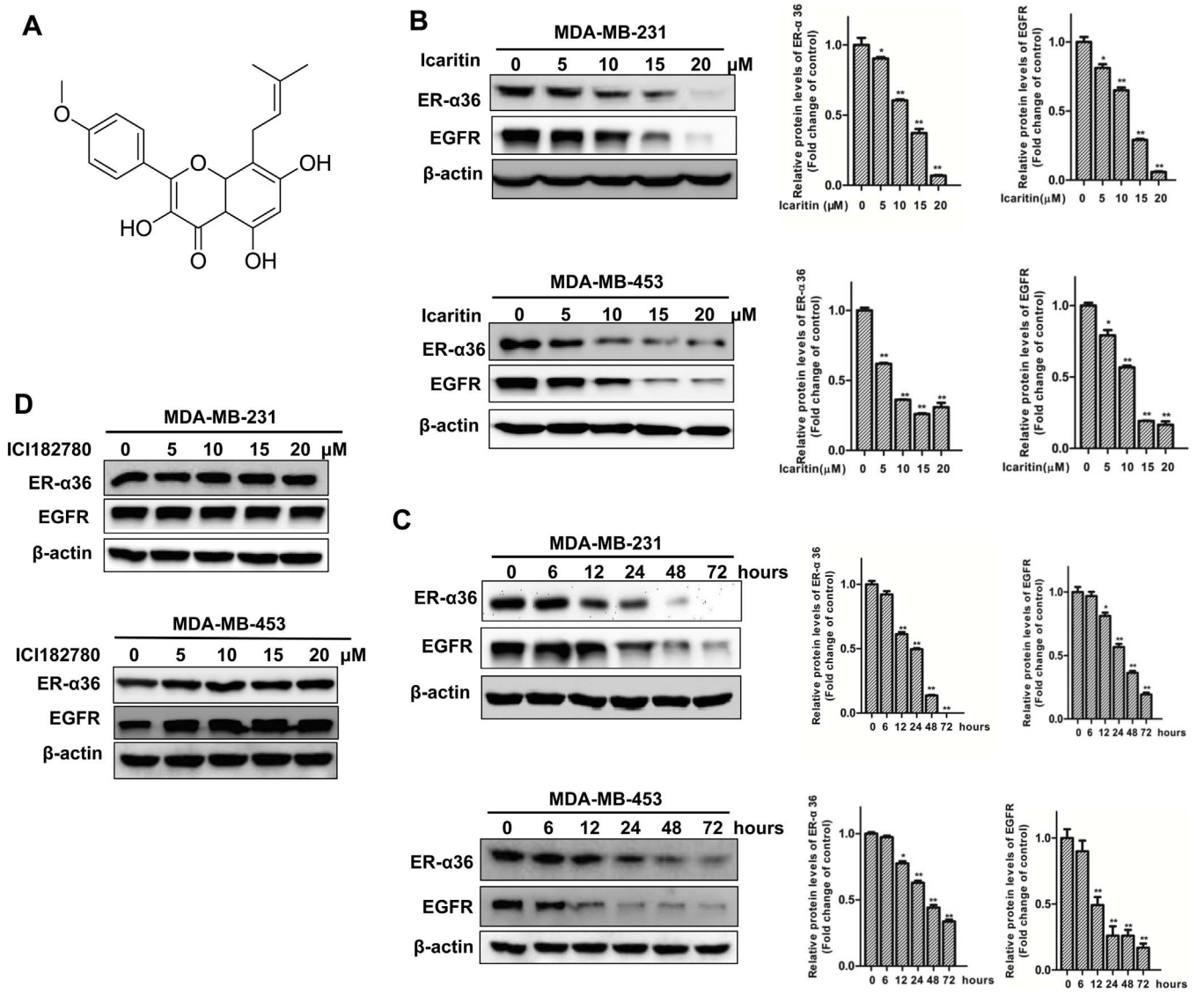

Fig. 1. Icaritin treatment downregulates the expression ER-α36 and EGFR expression in TNBC cells.
(A) The chemical structure of icaritin. MDA-MB-231 and MDA-MB-453 cells were treated with DMSO (0), or indicated concentrations of icaritin for 24 h (B) or 5 μM of icaritin for indicated time periods (C). (D) MDA-MB-231 and MDA-MB-453 cells were treated with DMSO (0), and indicated concentrations of ICI182780 for 24 h. Cell lysates were subjected to Western blot analysis with antibodies for ER-α36 and EGFR. β-actin was the internal control. The columns represent the means of three independent experiments; bars, SEM. Differences with $p < 0.05$(*) or $p < 0.01$(**) are considered statistically significant.

between ER-α36 and EGFR and induced apoptosis in TNBC cells. Our results thus suggested a possibility to develop Icaritin into a novel therapeutic agent for TNBC.

2. Results

2.1. Icaritin down-regulates the expression levels of ER-α36 and EGFR protein in TNBC cells

To examine the effects of icaritin on TNBC cells, we used the well-characterized TNBC MDA-MB-231 and MDA-MB-453 cells. Since ER-α36 plays an important role in malignant growth of TNBC cells [27,28], we first determined whether icaritin influences ER-α36 expression. Western blot analysis indicated that icaritin treatment potently reduced ER-α36 expression in a dose- and time-dependent manner (Fig. 1B & C) in both MDA-MB-231 and MDA-MB-453 cells. The estrogen-receptor disruptor ICI 182,780 was used as a negative control which slightly increased the ER-α36 protein level in MDA-MB-453 while without obvious effect in MDA-MB-231 cells (Fig. 1D). The exact mechanism underlying this discrepancy is unclear. It seems to be a cell context dependent event. Previously, Zhang et al. reported that ER-α36 and EGFR formed one positive regulatory loop in TNBC cells [9]. Thus we decided to further test whether icaritin also down-regulates EGFR and found that icaritin indeed attenuated EGFR expression (Fig. 1B–C). These results thus suggested that icaritin was able to disrupt the positive regulatory loop of ER-α36/EGFR.

2.2. Icaritin inhibits growth of TNBC cells

Since the positive regulatory loop of ER-α36 and EGFR is important for malignant growth of TNBC cells, we further examined whether icaritin influences the growth of TNBC cells. MDA-MB-231 and MDA-MB-453 cells were treated with increasing concentrations of icaritin for seven days, and then the cell numbers were examined. Our data indicated that icaritin strongly inhibited growth of these TNBC cells, mainly at low dosages 2 μM and 5 μM (MDA-MB-231:$p = 2.8 \times 10^{-8}$ at 2 μM and $p = 5.6 \times 10^{-10}$ at 5 μM; MDA-MB-453: $p = 1.6 \times 10^{-9}$ at 2 μM and $p = 4.9 \times 10^{-13}$ at 5 μM)(Fig. 2A). We also confirmed