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8-Prenylnaringenin, inhibits estrogen receptor-α mediated cell growth and induces apoptosis in MCF-7 breast cancer cells

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Abstract

The discovery that the hop constituent 8-prenylnaringenin (8PN) shows potent estrogenic activity, higher than that of the known phytoestrogens coumestrol, genistein and daidzein, has spurred an intense activity aimed at elucidating its biological profile and its dietary relevance connected with the consumption of beer. We have investigated if 8PN can induce signal transduction pathways via rapid estrogen receptor (ER) activation. Under conditions of estrogen-dependent growth, treatment of MCF-7 human breast cancer cells with 8PN induced a rapid and transient activation of the MAP kinase Erk-1 and Erk-2, with kinetics similar to those induced by 17β-estradiol (E2). 8PN could trigger the MAP kinase pathway via dual c-Src kinase activation and association with ERα. Co-treatment with the ER antagonist ICI 182,780 blocked each step of this transduction pathway, confirming its ER dependence. However, and in striking contrast with E2, 8PN could not induce the PI3K/Akt pathway, resulting in altered kinetics and levels of cyclin D1 expression. In accordance with these observations, flow cytometric and biochemical analysis showed that 8PN inhibited cell cycle progression and induced apoptosis in MCF-7 cells. Interference with an ER associated PI3K pathway is proposed as a possible mechanism underlying the inhibition of survival and proliferation of estrogen responsive cells by 8PN. Taken together, our finding show that 8PN is an interesting new chemotype to explore the biology of ERs.

Keywords: 8-Prenylnaringenin; Estrogen receptor; Breast cancer; Signaling; Apoptosis

1. Introduction

Hops (*Humus lupulus* L.) has long been known to contain an estrogenic principle [1], only recently identified as the flavonoid 8-prenylnaringenin (8PN) [2]. 8PN is not only one of the strongest plant-derived ERα ligand identified to date, but also shows a hormonal profile different from soy isoflavones, the archetypal phytoestrogens, being over three times more powerful at ERα than ERβ activation [3]. The concentration of 8PN in beer is too low to affect human health, but the concentration of its 5O-methylderivative (isoxanthohumol) is over one order of magnitude higher. Since conversion of xanthohumol into 8PN by the intestinal flora has been demonstrated [4], pharmacologically active doses of 8PN might be reached in people consuming large amounts of beer.

While the biological effects of the dietary exposure to 8PN are substantially unknown [5], several intriguing observations have been done regarding its activity at the cellular level. Thus, apart from ERs, 8PN can also interact with the androgen receptor, exhibiting antiandrogenic activity [6]. Since naringenin is devoid of significant hormonal activity [7], prenylation alone is capable of inducing activity on estrogen receptors, a remarkable observation so far unexplained in molecular terms. Of particular interest for cancer research is the powerful anti-angiogenic activity displayed by 8PN in in vitro and in vivo assays [8]. This activity is comparable to that of the soy phytoestrogen genistein, and might be mediated by a pleiothropic mechanism that involves inhibition of the urokinase-plasminogen activator (uPA) as well as interference with the activity of Na(+) channel and the Na(+)/H(+) exchanger. Taken together, these observations

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make 8PN an interesting probe to explore the biology of ERs and steroid hormones in general, but also highlight the need of a better mechanistic knowledge of its pleiotropic pattern of bioactivity.

Novel, non-genomic actions of estradiol depending on the ability of the ERα or β to interact with c-Src and activate the c-Src/Shc/Ras/Erk pathway have recently been identified [9–14]. Interference with this pathway activation abolishes the hormone-dependent cell growth [11]. Furthermore, estradiol can also activate the PI3-kinase/AKT pathway, a critical component of the cell growth regulation while the finding that Δp85α and Akt k- expression in MCF-7 cells suppresses estradiol activation of the cyclin D1 promoter suggests that estradiol can increase cyclin D1 expression by stimulating cyclin D1 transcription via PI3-kinase/Akt pathway [11,15].

In this work, we have analyzed the ability of 8PN to activate both the ER mediated c-Src/Ras/Erk and PI3kinase/Akt pathways in human hormone responsive breast cancer cells, investigating the significance of these pathways in the control of cyclin D1 and E expression and cell cycle progression.

2. Results

2.1. 8PN induced ER dependent Erk 1/2 MAPK activation in MCF-7 cells

MAP kinase signaling pathway is rapidly activated by E2 treatment in different cell types. In order to study the effect of 8PN on the MAP kinase pathway, we used hormone sensitive MCF-7 mammary carcinoma cells. To reduce the levels of activated ER, the cells were maintained for 3 days in the presence of charcoal-treated serum and in the absence of phenol red, a substance with a weak estrogenic activity. The Erk 1/2 activity in MCF-7 cells treated for different times with 10 μM of 8PN was evaluated by immunoblot of cell lysates with anti phospho-Erk 1/2 antibody, followed by anti Erk

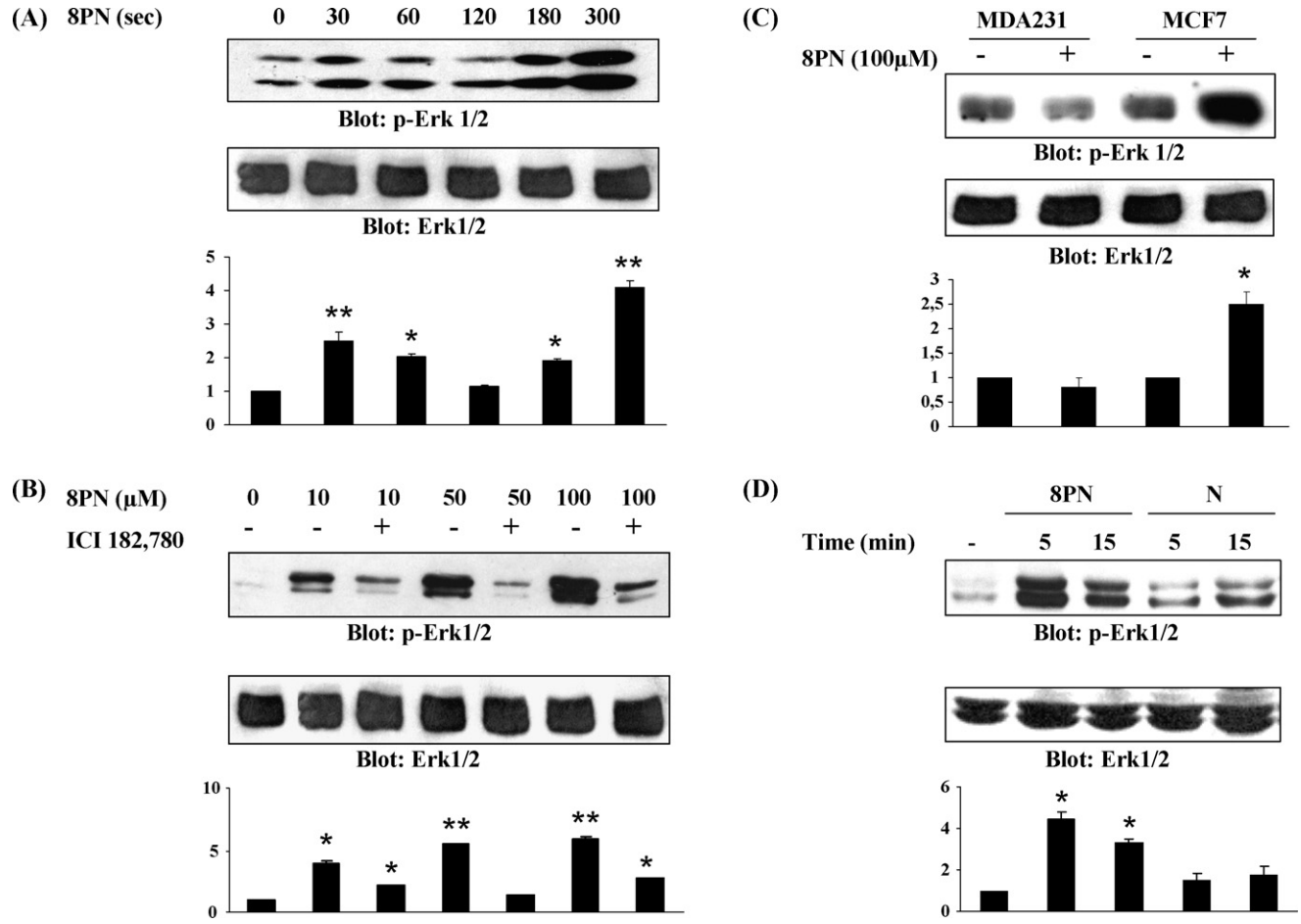

Fig. 1. 8PN-dependent Erk 1/2 MAPK activation. (A) MCF-7 cells grown at confluence and made quiescent as indicated in Section 4 for 24 h, left untreated or treated with 10 μM 8PN for the indicated times and detergent extracted. (B) MCF-7 cells in the same conditions as in (A) were treated with 10, 50 or 100 μM 8PN in the presence of 1 mM ICI 182,780 for 300 s. (C) Alternatively MDA- MB-231 and MCF-7 cells were treated with 100 μM 8PN for 300 s. or (D) MCF-7 cells were treated 5 or 15 min with 100 μM 8PN. (A–D) Cell extracts were run on 8% SDS-PAGE, transferred to nitrocellulose, blotted with anti-phospho Erk 1/2 antibodies (upper panels) and re-blotted with polyclonal antibodies to Erk 1/2 (lower panels). The relative Erk 1/2 phosphorylation levels normalized with the total protein and converted to fold induction above the control value are shown in the adjacent bar graphs. Each column represents the mean ± SEM of triplicate determinations ($^{*}P < 0.05$ and $^{**}P < 0.001$, compared with control).