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Prenylation inhibitors stimulate both estrogen receptor α transcriptional activity through AF-1 and AF-2 and estrogen receptor β transcriptional activity

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Abstract

Introduction We showed in a previous study that prenylated proteins play a role in estradiol stimulation of proliferation. However, these proteins antagonize the ability of estrogen receptor (ER) α to stimulate estrogen response element (ERE)-dependent transcriptional activity, potentially through the formation of a co-regulator complex. The present study investigates, in further detail, how prenylated proteins modulate the transcriptional activities mediated by ERα and by ERβ.

Methods The ERE-β-globin-Luc-SV-Neo plasmid was either stably transfected into MCF-7 cells or HeLa cells (MELN cells and HELN cells, respectively) or transiently transfected into MCF-7 cells using polyethylenimine. Cells deprived of estradiol were analyzed for ERE-dependent luciferase activity 16 hours after estradiol stimulation and treatment with FTI-277 (a farnesyltransferase inhibitor) or with GGTI-298 (a geranylgeranyltransferase I inhibitor). In HELN cells, the effect of prenyltransferase inhibitors on luciferase activity was compared after transient transfection of plasmids coding either the full-length ERα, the full-length ERβ, the AF-1-deleted ERα or the AF-2-deleted ERα. The presence of ERα was then detected by immunocytochemistry in either the nuclei or the cytoplasms of MCF-7 cells. Finally, *Clostridium botulinum* C3 exoenzyme treatment was used to determine the involvement of Rho proteins in ERE-dependent luciferase activity.

Results FTI-277 and GGTI-298 only stimulate ERE-dependent luciferase activity in stably transfected MCF-7 cells. They stimulate both ERα-mediated and ERβ-mediated ERE-dependent luciferase activity in HELN cells, in the presence of and in the absence of estradiol. The roles of both AF-1 and AF-2 are significant in this effect. Nuclear ERα is decreased in the presence of prenyltransferase inhibitors in MCF-7 cells, again in the presence of and in the absence of estradiol. By contrast, cytoplasmic ERα is mainly decreased after treatment with FTI-277, in the presence of and in the absence of estradiol. The involvement of Rho proteins in ERE-dependent luciferase activity in MELN cells is clearly established.

Conclusions Together, these results demonstrate that prenylated proteins (at least RhoA, RhoB and/or RhoC) antagonize the ability of ERα and ERβ to stimulate ERE-dependent transcriptional activity, potentially acting through both AF-1 and AF-2 transcriptional activities.

Keywords: estrogen receptor, farnesyltransferase inhibitor, geranylgeranyltransferase inhibitor, Rho proteins, transcription

Introduction

Both estrogen receptor (ER) subtypes, ERα and ERβ, are ligand-activated transcription factors. ERα is the major ER in mammary epithelium and is an important regulator of cell growth, differentiation and malignant transformation. After binding to estrogen, the receptors associate with specific estrogen response elements (EREs) within the promoters of estrogen-regulated genes or the receptors affect the activity of other transcription factor complexes such as AP-1 (Jun–Fos). The two ER subtypes share affinity for the

DCC-FCS = dextran-coated charcoal treated fetal calf serum; DMEM = Dulbecco's modified Eagle's medium; E2 = estradiol; ER = estrogen receptor; ERE = estrogen response element; FCS = fetal calf serum; FTI-277 = farnesyltransferase inhibitor; GGTI-298 = geranylgeranyltransferase inhibitor; MNAR = modulator of non-genomic activity of estrogen receptor; PBS = phosphate-buffered saline.