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Estrogen receptor α and β subtype expression and transactivation capacity are differentially affected by receptor-, hsp90- and immunophilin-ligands in human breast cancer cells ☆

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Abstract

In MCF-7 (estrogen receptor (ER)+) and in MDA-MB-231 (ER−) cells stably transfected with either estrogen receptor α (ERα) or β (ERβ) subtype (MDA-MB-231 stably transfected with the mouse ERα cDNA (MERA) and MDA-MB-231 stably transfected with the human ERβ cDNA (HERB), respectively) N-term heat shock protein of 90 kDa (hsp90) ligands (geldanamycin and radicicol) and C-term hsp90 ligands (novobiocin) decrease the basal and estradiol (E_2)-induced transcription activity of ER on an estrogen responsive element (ERE)-LUC reporter construct concomitantly with or 1 h after E_2 treatment. All hsp90 ligands induced an E_2- and MG132-inhibited decrease of both ER cell content. However, the kinetics of these degradations are slower than those induced by the selective estrogen receptor down-regulator RU 58668 (RU). This suggests that inhibition of the hsp90 ATPase activity targets both ERs to the 26S proteasome and that hsp90 interacts with both ER subtypes. Rapamycin (Rapa) and cyclosporin A (CsA), ligands of immunophilins FK506 binding protein (FKBP52) and cyclophilin of 40 kDa (CYP40) interacting in separate ER–hsp90 complexes, both induced a proteasomal-mediated degradation of ERs but not of their cognate immunophilin. Moreover, they also decrease the E_2-induced luciferase transcription but weaker than RU and hsp90 ligands. Fluorescence activated cell sorter (FACS) analysis revealed a blockade of cell progression by RU and 4-hydroxy-tamoxifen at the G_1 phase of the cell cycle and an induction of apoptosis in MCF-7 cells. Rapa and mainly CsA (but not FK506) and hsp90 ligands promote by their own apoptosis in MCF-7, in MERA, and in HERB cells and in MDA-MB-231 ER-null cells. These data suggest that (1) hsp90, as for all steroid receptors, acts as a molecular chaperone for ERβ; (2) ER-ligands (except tamoxifen), hsp90- and immunophilin-ligands (except FK506) target the two ER subtypes to a proteasome-mediated proteolysis via different signalling pathways; (3) hsp90- and immunophilin-ligands Rapa and CsA, alone or in association with anti-estrogens such as RU, may constitute a potential therapeutic strategy for breast cancer treatment.

Keywords: Estrogen receptors; Breast cancer cells; Anti-estrogens; Proteasome; Transactivation; hsp90-Inhibitors; Immunosuppressants

Abbreviations: AP-1, activating protein 1; CsA, cyclosporin A; CYP40, cyclophilin of 40 kDa; DMEM, Dulbecco's modified eagle medium; E_2, estradiol; ER, estrogen receptor; ERE, estrogen responsive element; ERKO, Estogen Receptor Knock-Out; FCS, fetal calf serum; FK, FK506; FKBP, FK506 binding protein; G418, geneticin; GA, geldanamycin; HERB, MDA-MB-231 stably transfected with the human ERβ cDNA; hsp90, heat shock protein of 90 kDa; ICI, ICI 182,780; MAP, mitogen activating protein; MERA, MDA-MB-231 stably transfected with the mouse ERα cDNA; Nv, novobiocin; OHT, 4-hydroxy-tamoxifen; PBST, PBS–Tween; PEI, polyethylenimine; PPIase, peptidyl prolyl *cis–trans* isomerase; Rapa, rapamycin; Rd, radicicol; RU, RU 58668; SERD, selective estrogen receptor down-regulator; SERM, selective estrogen receptor modulator; TCE, total cell extract; VEGF, vascular endothelial growth factor

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1. Introduction

Estradiol (E_2) exerts a central role in both male and female physiology. These effects are mainly mediated through changing the expression profile of target genes within responsive tissues [1] which occur following E_2 binding to the estrogen receptors (ERα and ERβ). These nuclear receptors function as ligand-inducible transcription factors for genes involved in the cell growth, proliferation and differentiation [2]. They belong to the superfamily of steroid/thyroid nuclear receptors and various stimuli, in addition to estradiol itself, are capable of modifying their expression level as well as their transactivation capacity. ER ligands can be classified in three classes: (1) the pure agonists, like E_2, which induce enhancement of gene transcription when ER–E_2 complexes bind to estrogen responsive elements (ERE) contained in specific promoters of target genes; (2) the mixed agonist/antagonists (selective ER modulators (SERM)), like tamoxifen; (3) selective estrogen receptor down-regulators (SERD), previously named "pure antagonists" which block E_2-induced transcription (see [3] for a review). The ability of an ER ligand to behave as an agonist or as an antagonist depends on multiple factors like the receptor subtype, the ERE sequence to which ER–ligand complex binds [4,5] and the cellular content ([6] for a review). For example, tamoxifen and its active metabolite 4-hydroxy-tamoxifen (OHT), the archetypical anti-estrogen used for breast cancer treatment in post menopausal women, are pure antagonists in the mammary gland [7] but a full agonist in the uterus, bones, vessels and in the cardiovascular system ([3,6] for reviews). In the uterus, OHT binds to ERβ and promotes the activation of target genes containing activating protein 1 (AP-1) sites in their promoters [8]. OHT, like other SERM, is well known to promote ERα accumulation in human breast cancer cells while the "pure anti-estrogens" ICI 182,780 (ICI) and RU 58668 (RU) induce its fast delocalisation in the nucleus and its 26S proteasome-mediated degradation [9–15].

ERα and ERβ display 53% identity in their ligand-binding domain. However, despite this sequence homology, the two receptors exhibit subtle differences in ligand-binding specificities, although they are considered to have similar affinity for E_2 [16]. One of the main differences between the affinities of the two receptors resides in the strongest affinity of ERβ for phytoestrogens (such as genistein [16,17], environmental compounds produced by plants). Studies using knock-out mice for the two ERs (αEstogen Receptor Knock-Out (ERKO) and βERKO, respectively) have revealed that each subtype plays unique role in estrogen biology in a wide variety of target tissues and the lack of ER not only affect the reproductive phenotype but also induces abnormalities in the brain ([18–20,6] for a review). The αERKO phenotype, combined with the high expression level of ERα in the mammary gland, points towards ERα being the main mediator of estrogen action in this tissue [18,21]. Moreover, in the human breast cancer MCF-7 cell line which has lost ERα, cells do not proliferate under E_2 stimulation but recover this proliferation capacity once ERα is re-introduced [19]. The role of ERβ in breast cancer growth and development is not as clear as that of ERα [22]. However, several lines of evidence argue in favor of a negative dominance of ERβ versus ERα [23–27] and ERβ opposites to ERα on cyclin D_1 gene expression [28]. Recently, ERβ has been shown to inhibit human breast cancer cell proliferation by repressing c-myc, cyclin D_1 and cyclin A gene transcription and increasing the expression of $p21^{Cip1}$ and $p27^{Kip1}$ which leads to a G_2 cell cycle arrest [29]. In concert with the fact that ERβ level is highest in normal mammary tissue and decrease as tumour progresses from pre-invasive to invasive tumour [30,31], it was recently proposed that ERβ may function as a tumour suppressor [29].

Like all steroid hormone receptors, ligand-free ERα is sequestered in an inactive form associated in a large molecular complex organised around the heat shock protein of 90 kDa (hsp90) and containing a p23 protein and one immunophilin, a class of substrate proteins for immunosuppressant binding. These immunophilins can be either a FK506 (FK) binding protein (FKBP52 or FKBP51 specific for both FK506 and rapamycin (Rapa) binding) or a cyclophilin like CYP40 (cyclophilin of 40 kDa) specific for cyclosporin A (CsA) binding. Immunophilins are *peptidyl prolyl cis–trans isomerase* (PPIase) whose activity is inhibited following immunosuppressant binding ([32] for a review). hsp90 client proteins are stabilized when integrated in the molecular chaperone complex and inhibition by hsp90 ligands of its ATPase activity targets the substrate to ubiquitination and its 26S-proteasome-mediated degradation [33,34]. Since the fate of ERβ in the presence of various types of ER ligands is not as well deciphered as that of ERα, we wondered if these ligands have the same effects on the two receptors. Such a study has been hampered for a long time due to low expression of ERβ as compared to ERα in breast cancer cells and to the lack of efficient antibody against endogenously expressed ERβ isotype. We took advantage of the new breast cancer cell lines engineered after transfection of ERs cDNAs in ER null MDA-MB-231 cells [35]. In addition, we analysed the influence of various hsp90 ligands as well as that of immunosuppressants in these cells and the results were compared with the behaviour of ERα in the human breast cancer MCF-7 cells exposed to the same ligands. The influences of the drugs were carried out both on ER protein stability and on ER-mediated transactivation of a reporter gene. We also analysed the activity of these drugs on cell cycle progression and on apoptosis by flow cytometry. The results suggest that depending on the receptor, the various drugs use different pathways to contribute to the destabilization of the signals triggered by E_2 and then may constitute alone or in association with a SERD or SERM a new approach for breast cancer therapy.