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Regulation of estrogen receptor (ER) levels in MCF-7 cells by progesterone metabolites

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Abstract

Estradiol-17β (E2) may participate in carcinoma of mammary cells containing estradiol receptors (ER) at sufficient levels. Hence, the regulation of ER levels may be important for the progression of estrogen-dependent mammary carcinomas. Our previous findings that the progesterone metabolite, 5α-pregnane-3,20-dione (5αP), exhibits marked mitogenic and metastatic properties, whereas the progesterone metabolites, 4-pregnen-3α-ol-20-one (3αHP) and 4-pregnen-20α-ol-3-one (20αHP), oppose these actions, prompted examination of the possible effects of these progesterone metabolites on ER concentration in MCF-7 breast cancer cells. Cells were exposed for 24 h to 0 (control) or 10^{-10} to 10^{-6} M E2, 5αP, 3αHP, 20αHP or combinations of these steroids, and ER concentrations were determined for intracellular estrogen receptors by specific binding of [^{3}H]E2. The total ER number (nuclear plus cytosolic) in control samples was 2551 ± 164 per cell. E2 and 5αP resulted in significant dose-dependent increases in total ER numbers (∼1.6-fold and ∼2.2-fold at 10^{-6} M, respectively). In combination, E2 + 5αP resulted in additive increases in ER numbers. Individually, 3αHP and 20αHP each resulted in dose-dependent decreases (43% and 54% at 10^{-6} M, respectively) in total ER numbers and inhibited the E2- or 5αP-induced increases in ER levels. In combination, 3αHP + 20αHP resulted in dose-dependent additive suppression of ER levels. Treatment with cycloheximide or actinomycin D indicated that both transcription and translation are involved in 5αP and 3αHP action on ER numbers. Real time RT-PCR showed increases in expression of ERα transcripts due to 5αP and increases in expression of ERβ due to 3αHP; expression levels of either ERα or ERβ were not significantly altered when cells were treated with 5αP + 3αHP. The results are the first to show that the pro- and anti-cancer progesterone metabolites also have marked selective (up or down) regulatory effects on ER levels in MCF-7 breast cancer cells.

Keywords: Breast cancer; Progesterone metabolites; Estradiol (E2); Progesterone; 5α-Dihydroprogesterone (5αP); 3α-Dihydroprogesterone (3αHP); 20α-Dihydroprogesterone (20αHP); MCF-7; Estrogen receptors (ER)

1. Introduction

The main estrogenic hormone, estradiol-17β (E2), regulates a wide variety of biological processes including reproduction, cell proliferation, differentiation, apoptosis, metabolism, and brain function [1]. The primary mechanism of E2 action is generally considered to be mediated by the transcriptional ability of the nuclear estrogen receptors, ERα and ERβ [2]. ERα and ERβ are members of a large family of highly specialized transcription factors which include receptors for all steroid hormones [3]. The concept is that in response to estradiol binding, ERα and ERβ are released from inactive complexes containing heat shock proteins [4], and then homodimerize or heterodimerize [5] and bind to a specific DNA estrogen response element located in the promoter of target genes [2,6].

The ER concentration is not constant within cells, and is substantially modified by a number of factors including cell density [7], growth rate [8], tumor promoters [9], differentiation inducing agents [10], cytokines [11] and various steroids and other hormones [6]. One regulator of ER that has shown varying effects is E2 itself. Depending on experimental conditions and cell types, E2 can cause either decreases [6,12–17] or increases [15] in ER levels. Also, ER levels may be regulated by androgens, progesterone and various growth

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factors [6,18–20]. Progesterone, growth factors and 1α,25-dihydroxyvitamin D_3 (the active metabolite of vitamin D) have been shown to down-regulate [6,19–21] whereas EGF and IGF-1 have been shown to up-regulate ER numbers [22]. 1α,25-dihydroxyvitamin D_3 down-regulates ER by exerting a direct effect on ER gene transcription instead of altering ER mRNA half-life which occurs when E2 regulates its own receptor [21]. EGF and IGF-1 both up-regulate ER by stimulating phosphorylation at serine 118 via the mitogen-activated protein kinase (MAPK) signaling pathway [22].

Recent studies indicate that progesterone is readily metabolized by normal and tumorous breast tissues [23] and various (including MCF-7) human breast cell lines [24] into 5α-pregnanes and 4-pregnenes. Tumorous breast tissues and tumorigenic breast cell lines produce significantly higher levels of 5α-pregnanes, especially 5α-pregnane-3,20-dione (5αP), whereas normal breast tissues and non-tumorigenic breast cell lines produce more 4-pregnenes, especially 4-pregnen-3α-ol-20-one (3αHP) and 4-pregnen-20α-ol-3-one (20αHP) [23,24]. 5α-Pregnanes (e.g., 5αP) increase breast cancer cell proliferation and detachment while 4-pregnenes (e.g., 3αHP and 20αHP) decrease breast cancer cell proliferation and detachment [23]. Progesterone metabolites and E2 have also been shown to affect 5αP receptor levels [25,26] and these changes were correlated with changes in proliferation and adhesion [26]. The findings that progesterone metabolites and E2 can both influence proliferation in ER positive mammary cells and interact with each other to regulate 5αP-R numbers, led us to investigate the role of the progesterone metabolites, 5αP, 3αHP and 20αHP on ER levels in MCF-7 cells. The results presented here show for the first time that ER levels can be up- or down-regulated by progesterone metabolites, formed in situ and that the regulation of ER numbers is dependent upon transcription and translation.

2. Materials and methods

2.1. Chemicals

3αHP was synthesized and purified according to published procedures [27]. [2,4,6,7-^{3}H]-Estradiol-17β (71 Ci/mmol) was purchased from Perkin Elmer Life Sciences (New England Nuclear; Woodbridge, Ontario). Other steroids as well as bacitracin, leupeptin, Tris–HCl and Bradford Reagent were obtained from Sigma Chemical Co. (Oakville, Ontario). Sucrose and EDTA were purchased from BDH (Toronto, Ontario).

2.2. Cell culture

MCF-7 cells were grown as previously described [25,26] in Dulbecco's Modified Essential Medium (DMEM): F12 HAM in a 1:1 ratio (Sigma Chemical Co.). The medium was supplemented with 5% calf serum (Gibco BRL, Burlington, Ont.), penicillin (100 units/ml), streptomycin (100 μg/ml), insulin (10 μg/ml) and sodium bicarbonate (1.2 mg/ml). The cells designated for experimental usage were grown in culture medium containing serum that had been stripped with dextran-coated charcoal (DCC) to remove steroids and other low-molecular weight factors. The cells were grown and passaged in T-75 flasks and maintained in a humidified incubator at 37 °C, with a 5% CO_2 atmosphere.

2.3. Cell treatments

Cells were rinsed with balanced salt solution (BSS) and steroids (final concentrations of 10^{-10} to 10^{-6} M) and/or protein synthesis inhibitors were added to media containing DCC-stripped serum for 24 h. The steroids used were E2, 3αHP, 5αP, 20αHP or selected combinations as indicated under results. The concentrations of protein synthesis inhibitors (cycloheximide (50 μM) and actinomycin D (4 μM)) were used in accordance with previously published values [13,26,28].

2.4. Cell collection and fractionation

Cell collection and fractionation procedures were similar to those previously described [25,26]. Cells were harvested when the T-75 flasks reached 80–90% confluency and contained approximately 1.2×10^7 MCF-7 cells per flask. Medium was removed, cells were rinsed with BSS, removed with a cell scraper and suspended in homogenization buffer (0.25 M sucrose, 25 mM Tris–HCl, 20 μg/ml bacitracin, 5 μg/ml leupeptin, pH 7.4, 0 °C). Homogenization in a tight fitting ground-glass homogenizer (Wheaton) with 25–30 manual strokes resulted in disruption of >90% of the cells, as confirmed by microscopy. The homogenates were centrifuged at 800 × *g* for 15 min to form a nuclear pellet. The supernatant was centrifuged at 16,000 × *g* in an Eppendorf 5415 microcentrifuge for 30 min. The resulting supernatant was designated as the cytosolic ER fraction. The nuclear pellet was suspended in incubation buffer (25 mM Tris–HCl, 1.5 mM $MgCl_2$ pH 7.5) and brought to a final 0.6 M NaCl concentration and after a 30 min swelling at 4 °C was homogenized in a tight fitting ground-glass homogenizer (Wheaton) with 20 manual strokes. Samples were allowed to sit for 30 min at 4 °C then centrifuged at 16,000 × *g* for 30 min. The resulting supernatant was designated as the nuclear ER fraction [29].

2.5. Enzyme assays and DNA quantitation

The relative activities/amounts of lactate dehydrogenase and DNA as markers of cytosol and nuclear fractions, respectively, were determined as previously described [25,26].

2.6. Radioligand binding assays

Nuclear and cytosolic ER: To determine ER levels, nuclear and cytosolic ER fractions were incubated separately for 1 h