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17-β-estradiol inhibits transforming-growth-factor-β-induced MCF-7 cell migration by Smad3-repression

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Abstract

Motility of malignant cells plays a crucial role for the metastasis of tumours. Both, 17-β-estradiol and transforming growth factor-β (TGF-β), induce migration of MCF-7 breast cancer cells and simultaneous treatment resulted in an additive effect of the migratory response. But most interestingly, when cells were preincubated with 17-β-estradiol, the ability of TGF-β to evoke chemotaxis was drastically diminished. Abrogation of Smad signalling indicated that this pathway is essential for TGF-β-mediated MCF-7 cell migration. In agreement, pretreatment of MCF-7 cells with 17-β-estradiol resulted in a reduced phosphorylation of Smad2 and Smad3 as well as a diminished Smad2 and Smad3 gene reporter activity in response to TGF-β. Thus, these results indicate a controversial role of 17-β-estradiol on MCF-7 cell migration. 17-β-estradiol potently increases the migratory potency of MCF-7 cells, but inhibits TGF-β-induced migration by an interaction between estrogen receptors and Smad proteins.

Keywords: Chemotaxis; 17-β-estradiol; Estrogen receptor; Transforming growth factor-β; Smad proteins; Breast cancer cell

1. Introduction

The metastatic spread of cancer cells is based on their ability to induce proliferation, motility and degradation of extracellular matrix proteins. Especially, in breast cancer it is well established that hormones may contribute to tumour progression (Platet et al., 2004). Thus, the tumourigenic effect of estrogens within breast cancer cells is dependent on their ability to bind to specific intracellular receptors (estrogen receptors α and β) which directly interact with estrogen response elements in the promoters of target genes and recruit various coactivators to mediate transcriptional regulation (Iwase, 2003; Osborne et al., 2000). Besides this classical models, estrogen receptors also interact with heterologous transcription factors like Sp1, c-Jun and AP-1, thus also influencing transcriptional activity of genes apart from estrogen response elements (Shang and Brown, 2002). Moreover, in 1983 Rambo and Szego (Rambo and Szego, 1983) described an immediate non-genomic effect of estrogens and now it has been well established that this effect occurs via signalling pathways normally aligned with activated growth factor receptors at the cell surface (Osborne and Schiff, 2003; Osborne et al., 2005). The influence of estrogens on cell-cycle progression has been well characterized as 17-β-estradiol positively modulates the transcriptional activation of cyclins, the regulatory phosphorylation of cyclin-dependent kinases and the induction of c-*myc* (Doisneau-Sixou et al., 2003; Foster et al., 2001).

An opposed role on proliferation of breast cancer cells has been contributed to transforming growth factor-β (TGF-β) as it causes cell-cycle arrest by inhibition of cyclin-dependent kinase activity and the repression of c-*myc* expression (Donovan and Slingerland, 2000). Indeed, estrogen receptor-expressing breast cancer cells have been postulated to inhibit TGF-β-mediated growth arrest due to a reduced expression of TGF-β-receptors (Wu et al., 2003). Moreover, antiestrogens mediate their growth inhibitory properties at least in part under involvement of TGF-β (Brattain et al., 1996; Buck et al., 2004; Pouliot and Labrie, 1999).

TGF-β exerts its multiple biological effects through binding to specific TGF-β-receptors with serine/threonine kinase activity resulting in the formation of a heteromeric activated receptor complex, which then phosphorylates intracellular effector molecules, the so-called receptor-Smad proteins (R-Smads) (Massague, 1998; Massague et al., 2000). Upon activation, R-Smads

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heteromerize with their common partner, Smad4, and the complex translocates into the nucleus to modify the activity of target genes. Although the Smad pathway is the main mediator of TGF-β-responses, a variety of studies have also implicated other pathways such as extracellular signal-regulated kinases (ERK), mitogen activated protein kinases (p38 MAPK) or phosphatidylinositol 3-kinase (PI-3 kinase).

The genes encoding TGF-β-receptors and Smad proteins have been shown to be altered in some types of cancer but it is not known whether these changes lead to a loss of sensitivity to growth inhibition by TGF-β (Kim et al., 2000). Besides its growth inhibitory and therefore tumour suppressing role, TGF-β has also been identified to act as a pro-metastatic agent in late stages of cancer progression. Thus, the cytokine is considered as an autocrine regulator of breast cancer cell motility as it is secreted by several tumours and significantly enhances migration rates of several breast cancer cell lines (Muraoka-Cook et al., 2005; Muraoka et al., 2002; Tong et al., 2002).

In breast cancer cells it is not well defined whether Smad signalling contributes to the migratory response to TGF-β, but in a variety of cells, Smad-phosphorylation has been indicated to be the initiation point of chemotaxis (Ashcroft et al., 1999; Hjelmeland et al., 2004). In detail, keratinocytes, monocytes or dermal fibroblasts show no migratory response to a treatment with TGF-β, when they were isolated from Smad3-deficient mice (Ashcroft and Roberts, 2000; Flanders, 2004; Sauer et al., 2004).

Smad3 has also been shown to cross-communicate with estrogen receptors as TGF-β-induced activation of Smad3 responsive genes is significantly suppressed in the presence of the activated estrogen receptor α (Matsuda et al., 2001). There exists evidence that a direct physical interaction between Smad3 and estrogen receptor α occurs, which provides a molecular mechanism for the opposing effects of estrogens and TGF-β (Matsuda et al., 2001).

The influence of estrogens on TGF-β migration in breast cancer cells is not well characterized. To prove an antagonistic role of TGF-β and estrogens on the invasiveness of breast cancer cells, which would be in accordance with their opposed effect on cell cycle progression, we measured chemotaxis of breast cancer cells in response to a single or cotreatment with both agents. Here we report a bimodal effect of estrogens on chemotaxis. Thus, both TGF-β and estrogens promote migration of MCF-7 cells and treatment to a gradient of both agents resulted in an additive migratory response. But, when cells were sensitised with 17-β-estradiol the chemotactic activity of TGF-β was drastically diminished due to an interaction between estrogen receptors and Smad signalling.

2. Materials and methods

2.1. Chemicals

17-β-estradiol was purchased from Fluka (Buchs, Switzerland) and ICI 182,780 (7-α-[9-(4,4,5,5-penta fluoropentylsulphinyl) nonyl] estra-1,3,5-(10)-triene-3,17-β-diol) was from Tocris Cookson (Avonmouth, UK). For cell culture experiments 17-β-estradiol and ICI 182,780 were solved in ethanol (stock solution 5×10^{-3} M). Protein G plus agarose, molecular weight markers, sodium dodecyl sulfate (SDS)-sample buffer and dithiothreitol were from Calbiochem (Bad Soden, Germany), charcoal from Serva (Heidelberg, Germany). Goat polyclonal anti-Smad3-, mouse monoclonal anti-Smad4-antibodies, anti-goat and anti-rabbit IgG-horseradish peroxidase (HRP) as well as siRNA were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Phospho-Smad3-antibodies were a generous gift from Dr. Anita Roberts (NIH, Bethesda MA). Phospho-Smad2-antibodies were from Cell Signalling Technology (Beverly, MA), LumiGlo reagent and peroxide from New England Biolabs (Beverly, MA). Fugene6™ was from Roche Diagnostics (Mannheim, Germany). Polyvinylidene fluoride immobilon transfer membranes were purchased from Millipore (Schwalbach, Germany). TGF-β, aprotinin, bovine serum albumin (BSA), deoxycholic acid, Eagle's Minimal Essential Medium (EMEM), McCoy's 5A medium, EDTA, fetal calf serum, fibronectin, Giemsa, leupeptin, pepstatin, phenylmethylsulfonylfluoride, SDS, sodium fluoride, sodium orthovanadate, ethanol, dimethyl sulfoxide, Tris/HCl, sodium pyruvate, sodium chloride, β-glycerophosphate, L-glutamine, Tween 20, Nonidet P40 and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (Taufkirchen, Germany). Calcium- and magnesium-free phosphate-buffered saline (PBS) were purchased from Invitrogen (Karlsruhe, Germany), sodium bicarbonate from Merck, gentamycine sulfate from PAN Biotechology (Aidenbach, Germany) and the dual luciferase assay system from Promega (Mannheim, Germany).

2.2. Cell culture

MCF-7 cells were maintained as a monolayer culture in EMEM with L-glutamine (2 mM) and 10% fetal calf serum. MDA-MB-231 cells were cultured in McCoy's 5A medium with L-glutamine (1.5 mM) and 5% fetal calf serum. Both media were supplemented with sodium bicarbonate (25 mM), sodium pyruvate (1 mM), and gentamycine sulfate (50 mg/l). In all cell culture experiments the ethanol concentration did not exceed 0.1%.

2.3. Cell viability assay

Cell viability was measured by the MTT dye reduction assay. Cells, seeded into 24-well plates for 24 h, were incubated with test substances for 24 h at 37 °C in 5% CO_2. After addition of 100 µl MTT solution (5 mg/ml) per well, the plates were incubated for another 4 h. The supernatants were removed and the formazan crystals were solubilised in 1 ml of dimethyl sulfoxide. The optical density was determined at 540 nm using a scanning microplate spectrophotometer (Multiscan® Plus, Labsystems, Finland).

2.4. Chemotaxis and chemokinesis assays

Migration of cells was measured as recently described (Vogler et al., 2003). Cells (10^6/well) were seeded in the upper well of a modified Boyden chamber with 8 µm pore width (Neuroprobe, Gaithersburg, MD). The lower chamber, separated by a