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Differential cross-talk of estrogen and growth factor receptors in two human mammary tumor cell lines

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Abstract

Cultured human mammary MCF7 and T47D tumor cell lines were used to test the interference of the partial antiestrogen 4′-hydroxytamoxifen (4-OH-TAM) and the pure antiestrogen ZM 182780 with growth factor (IGF-I, heregulin) signaling pathways. Growth of both cell lines was stimulated by IGF-I (20 ng/ml) or heregulin (3 nM). ZM 182780 effectively blocked growth factor induced as well as basal proliferation of MCF7 cells while the compound was ineffective in interfering with growth factor mitogenic activity in T47D cells. On both cell lines the IGF-I or heregulin- induced proliferation was enhanced further by 4-OH-TAM. This synergism could be inhibited dose-dependently by ZM 182780. When cells were grown in the presence of estradiol plus growth factors, the antiestrogenic potencies of both compounds and the efficacy of ZM 182780 were unaffected, while the efficacy of 4-OH-TAM was reduced. Our data show cell type specific cross-talk between the receptor for estrogen and that for IGF-I or heregulin, which is different in MCF7 and T47D cells, respectively. In MCF7 cells with demonstrable cross-talk, a clear superiority exists for a pure antiestrogen over a partial agonist in interfering with growth factor mitogenic activity.

1. Introduction

In the treatment of estrogen receptor (ER) positive human breast cancers the antiestrogen tamoxifen is widely used clinically. Despite initial response, the cancer eventually develops acquired resistance to tamoxifen [1]. Potential cause of treatment failure might be, among others, the partial agonistic activity of this compound [2]. Compounds with pure antagonistic activity like ZM 182780 exhibit superiority with respect to inhibition of cell proliferation and E_2-induced gene expression on cultured tumor cells. In a phase II clinical study, anti-tumor effects of ZM 182780 could be demonstrated in women with advanced breast cancer relapsed under tamoxifen therapy [3,4].

While estradiol (E_2) is the most potent mitogen known for mammary carcinoma cells, these cells also respond to a variety of autocrine or paracrine growth factors [5]. First indications for the potential of antiestrogens to block peptide growth factor stimulated cell growth came from Vignon et al. [6] and Koga and Sutherland [7] and have been extended subsequently [8–10]. Experimental evidence for an intracellular cross-talk between the nuclear ER and membrane bound peptide growth factor receptor was demonstrated in the normal uterus of mice [11]. Recombinant cell systems were generated in order to test the direct effects of growth factors on transcriptional activity of ER. Thus, transfection of either a reporter gene into immature ER-positive rat uterine cells [12] or of the ER gene into neuroblastoma [13] or pituitary GH3 tumor cells [14] resulted in cells in which considerable increase in reporter gene activity upon stimulation with IGF-I or other growth factors was observed. However, transcriptional activation of ER by growth factors and interference of antiestrogens is highly

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dependent on the transfection state, e.g. if genes are transfected stably or transiently [15].

The communication between a cell surface peptide hormone receptor and an intracellular steroid hormone receptor can take various routes as dictated by the physiology of a particular cell type. It could even be different in individual cells within a given solid tumor as a consequence of tumor progression and subsequent development of antihormone resistance. In this study we used two different cultured human mammary tumor cell lines (T47D and MCF7) expressing endogenously receptors for E_2 and various growth factors. Growth factors were selected to which the cells are mitogenically responsive and represent those most extensively studied. Thus, the impact of the antiestrogens 4-OH-TAM and ZM 182780 on cell proliferation was investigated in the presence of IGF-I [12–14] and heregulin [16,17].

2. Materials and methods

2.1. Material

4′-Hydroxytamoxifen (4-OH-TAM), ZM 182780 and E_2 were synthesized in the laboratories of Schering AG. The compounds were solubilised in ethanol (10^{-3} M) and stored at −20°C. They were always freshly diluted to the desired concentrations in PBS (containing 8 g of NaCl, 0.2 g of KCl, 1.14 g of Na_2HPO_4 and 0.2 g of KH_2PO_4/l, pH 7.4). IGF-I, EGF and HGF were acquired from Sigma (Munich, Germany). The ER-enzymatic immunoassay (ER-EIA) ELISA kit for determination of ER protein levels was obtained from Abbott (Wiesbaden, Germany). Recombinant heregulin was prepared as described below.

2.2. Cell lines and culture conditions

Human mammary carcinoma MCF7 and T47D cells were grown routinely in phenol free RPMI (Roswell Park Memorial Institute) medium supplemented with 10% fetal calf serum, 200 mU/ml insulin plus 10^{-10} M E_2. At 60–70% confluency, the tumor cells were harvested using 0.05% trypsin plus 0.02% EDTA in PBS. Cells were stored in frozen stocks in liquid nitrogen and were only used for up to 10 passages. Prior to experiments cells were exposed for three days to charcoal treated serum (CCS) at the indicated concentrations without E_2.

2.3. Recombinant heregulin

The β_2-subform of heregulin was produced by the baculovirus expression system and was purified on Heparin Sepharose using a NaCl gradient [18].

2.4. Growth studies

Tumor cells were detached with trypsin/EDTA and seeded in 200 μl at 5000 cells/well in 96 well plates. Cells were allowed to adhere for 4 or 24 h, respectively, and then fresh medium plus 5 or 10% CCS (as indicated) plus compounds were added and exchanged after three days. On day seven cells were fixed with glutaraldehyde, stained with crystal violet and the absorbance recorded [19]. Values were normalised to the absorbance of untreated cells. Experiments have been performed at least twice and in the result section one representative experiment with mean ± SD from six wells is shown.

2.5. Determination of ER protein level by ER-EIA

MCF7 and T47D cells were plated in 10 cm dishes (1×10^6 cells) and cultivated for three days in medium containing 10% CCS and insulin (200 mU/ml). The cells were then treated for 24 h with E_2 and antiestrogens at the indicated concentrations. After detachment and centrifugation cell pellets were resuspended in high salt buffer (400 mM KCl, 20 mM Tris-HCl pH 7.5, 10% glycerol, 1.5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM dithiothreitol (DTT)) and homogenised with a Polytron (PT 1200) mixer (Kinematica AG). After centrifugation (100,000 *g*, 1 h, 4°C) cell homogenates were adjusted for their protein levels and the amount of ER was determined by ER-EIA according to the manufacturer's instruction.

3. Results

Only a few different ER-positive breast cancer cell lines are available to study the effects of antiestrogens on growth and gene expression. Here we used the standard cell line MCF7 in comparison with the less used but also well known T47D cell line. In order to initially characterise the cells used in this study we tested the responses of both cell lines to inhibition of E_2-dependent growth by antiestrogens as well as changes of steady state levels of ER protein and mRNA following exposure to antiestrogens. Both cell lines are reported to respond mitogenically to IGF-I and heregulin [20]. While IGF-I binds mainly to the IGF-IR, the situation is more complex for heregulin which is a ligand for several of the four members of the erbB family [21]. Thus, it has to be stated that the precise molecular composition of the receptors binding heregulin is not known and might be different between MCF7 and T47D cells.

3.1. Inhibition of E_2-induced growth of MCF7 and T47D cells by antiestrogens

Following steroid depletion and replating into medium containing CCS, cells were allowed to attach overnight followed by addition of fresh medium plus compounds. Human mammary tumor cell lines MCF7 and T47D were growth stimulated 1.7- (MCF7) or 2.5-(T47D) fold by 10^{-10} M E_2 after seven days of culture (data not shown). The antiestrogens 4-OH-TAM and ZM 182780 counteracted the E_2-stimulated growth of MCF7 and T47D cells in a dose-dependent manner, the pure antiestrogen ZM 182780 ($IC_{50}=4$ and 2.6×10^{-9} M, respectively) being five times more potent than 4-OH-TAM ($IC_{50}=23$ and 10×10^{-9} M, respectively). Cells were also cultivated without E_2 and increasing concentrations of antiestrogens. There was no growth stimulation by the partial agonist 4-OH-TAM while the pure antagonist ZM 182780 suppressed growth of both cell lines below control levels at concentrations $>3 \times 10^{-9}$ M (data not shown).

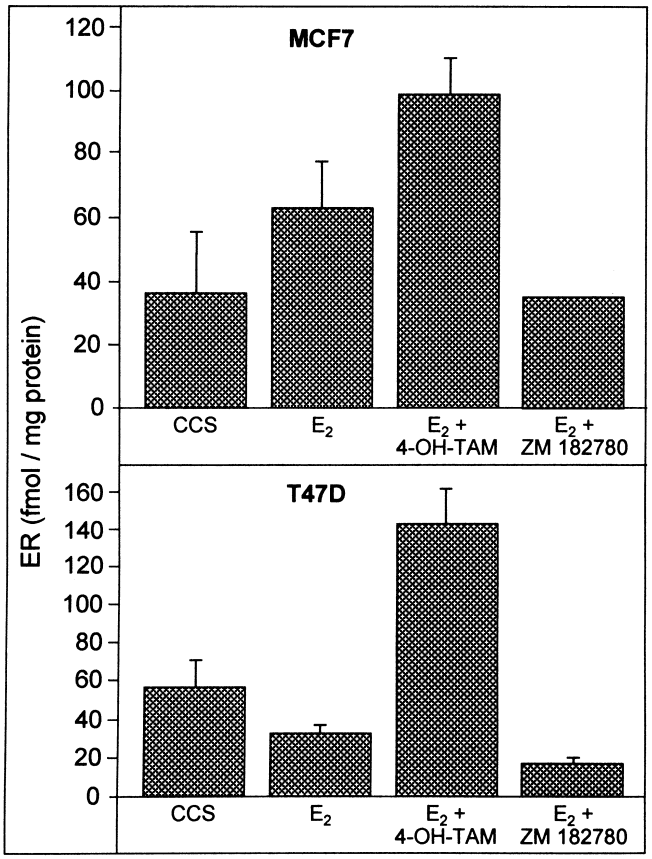

Fig. 1. ER protein expression and antiestrogens. MCF7 and T47D cells were grown in medium with 10% CCS and 200 mU/ml insulin, than 10^{-10} M E_2 plus or minus 4-OH-TAM or ZM 182780 (10^{-8} M), respectively, were added for 24 h. Cells were lysed, protein levels adjusted and the amount of ER was determined by ER-EIA.

3.2. Effects of antiestrogens on levels of ER protein in MCF7 and T47D cells

Antiestrogens not only compete with ligand binding to the ER, they also influence steady state levels of ER protein with no effects on ER mRNA [22]. In order to verify this well known effect in the two cell lines used here, we tested the consequences of 4-OH-TAM and ZM 182780 exposure on the amounts of ER protein in both cell lines and mRNA in T47D cells. Cells starved for three days in 10% CCS plus insulin (200 mU/ml) were incubated for 24 h with 10^{-10} M E_2 plus 10^{-8} M of the antiestrogens and the ER protein amount was quantified using an ELISA (Fig. 1). Both cell lines expressed comparable ER protein levels (36–56 fmol/mg protien) in the absence of E_2. In MCF7 cells, addition of E_2 or E_2 plus 4-OH-TAM resulted in an increase of the ER protein level while addition of E_2 plus ZM 182780 decreased the ER protein level to that

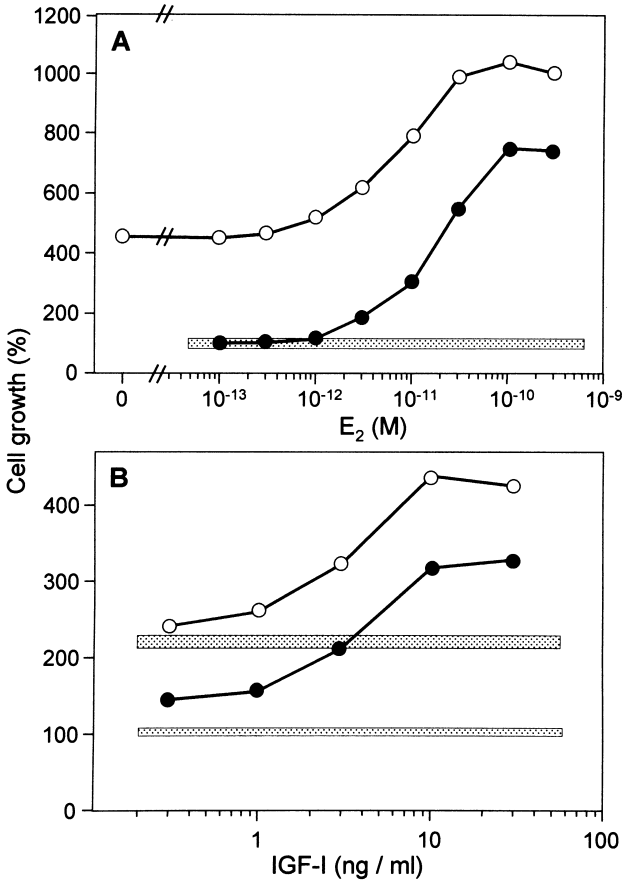

Fig. 2. Responsiveness to growth factors. MCF7 cells were grown in medium with 5% CCS without insulin. (A): shaded bar represents cells grown in the absence of E_2, cells were grown with increasing concentrations of E_2 in the presence (open symbols) or absence (filled symbols) of 3 nM heregulin. (B) Upper and lower shaded bars represent cells grown in the presence or absence, respectively of 10^{-11} M E_2, plus increasing concentrations of IGF-I in the presence (open symbols) or absence (filled symbols) of 10^{-11} M E_2.

of the CCS control. In T47D cells, addition of E_2 plus 4-OH-TAM resulted in a 3-fold increase of the ER protein amount, while E_2 alone or E_2 plus ZM 182789 reduced ER protein levels below that of the CCS control. In T47D cells the changes in ER protein levels were not reflected on the level of ER mRNA (data not shown).

3.3. Effects of heregulin and IGF-I on growth of MCF7 cells

Four growth factors (HGF, EGF, IGF-I, heregulin) were tested for their mitogenic activity on MCF7 and T47D cells grown in medium with 5% CCS and in the absence of insulin. While both cell lines did not respond to HGF (1 U/ml), only T47D cells were growth-stimulated by EGF (10 ng/ml) (data not shown). However, the proliferation of both cell lines was stimulated by heregulin and IGF-I and these factors were used for the subsequent experiments. Growth and differentiation of mammary epithelium is induced by heregulin and this is potentiated by E_2 [23,24]. Thus, we cultivated MCF7 cells in medium with 5% CCS without insulin, supplemented with 3 nM of heregulin and increasing concentrations of E_2 (10^{-13} to 10^{-10} M) to investigate the interference of both mitogens (Fig. 2A). Starting from 3×10^{-12} M, E_2 dose-dependently stimulated growth of cells with a maximum at 10^{-10} M. Addition of 3 nM heregulin stimulated growth of cells 4–5-fold in the absence of E_2 and this growth was additive in the presence of the hormone. No synergy was evident between these two mitogens over a wide range of E_2 concentrations. In order to find the optimal IGF-I concentration for our further studies, we cultivated MCF7 cells in the presence of increasing concentrations of IGF-I (0.3–30 ng/ml) with or without a suboptimal concentration of E_2 (10^{-11} M). Fig. 2B demonstrates, that IGF-I dose-dependently stimulated growth of cells with a maximal 2.5-fold increase in cell proliferation at 10 ng/ml. In the presence of 10^{-11} M E_2 , the growth stimulatory effects were additive indicating efficient growth stimulation by the growth factor. Again, there was no synergy with E_2 over a wide range of IGF-I concentrations. For our subsequent studies we chose 3 nM heregulin, 20 ng/ml IGF-I in medium with 5% CCS without insulin and E_2 was used at 10^{-10} M when indicated.

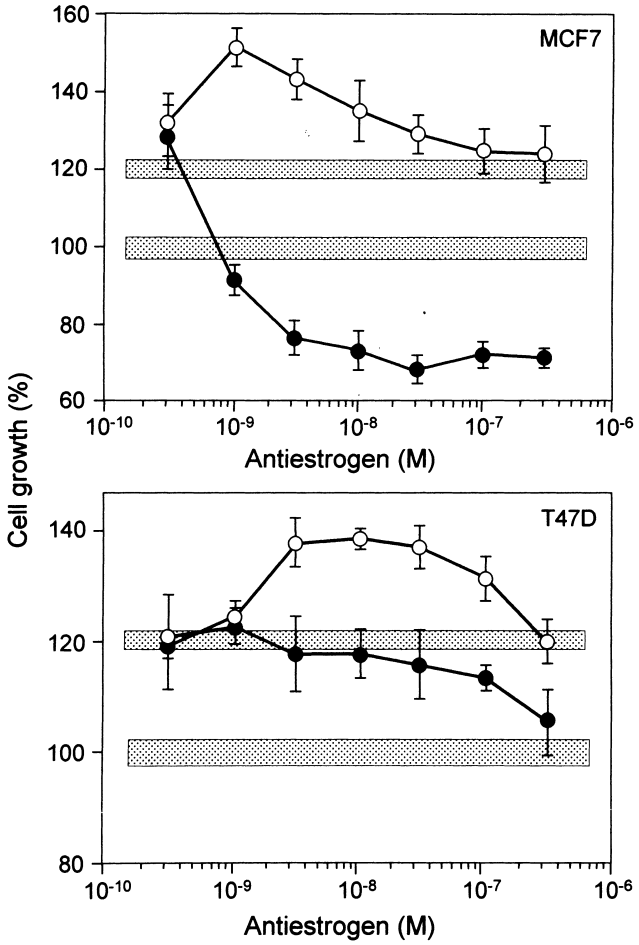

Fig. 3. IGF-I-stimulated growth and antiestrogens. MCF7 and T47D cells were grown in medium with 5% CCS without insulin. Upper and lower shaded bars represent cells grown in the presence or absence, respectively, of 20 ng/ml IGF-I, plus increasing concentrations of 4-OH-TAM (○) or ZM 182780 (●).

3.4. Effects of antiestrogens on IGF-I stimulated growth of MCF7 and T47D cells

Next we tested the effects of antiestrogens on IGF-I-stimulated growth of cells in the absence of E_2. Fig. 3 demonstrates a 20% increase in MCF7 cell proliferation by 20 ng/ml of IGF-I, ZM 182780 dose-dependently interfered with the IGF-I growth effect and even inhibited basal growth. In contrast, the partial agonist 4-OH-TAM synergized at low concentrations (10^{-9}–10^{-8} M) with the IGF-I dependent growth of MCF7 cells. Using cultured T47D cells, ZM 182780 only interfered with the IGF-I-induced proliferation at high concentrations ($>10^{-7}$ M), while 4-OH-TAM again synergized at low concentrations (3×10^{-9}–10^{-7} M) with the IGF-I dependent growth. In these experiments we found much lower growth stimulatory effects of the growth factors compared with Fig. 2. Due to the addition of antiestrogens, the alcohol concentration had to be raised up to 0.03%, which might have interfered with the growth factors' mitogenic activities.

3.5. Effects of antiestrogens on heregulin-stimulated growth of MCF7 and T47D cells

A similar experiment was performed using heregulin (data not shown). The marginal increase in MCF7 cell proliferation by 3 nM of heregulin was dose-dependently inhibited by ZM 182780 and even below basal growth. Again, 4-OH-TAM synergized at low concentrations (10^{-9}–10^{-8} M) with the growth factor induced proliferation of MCF7 cells. Growth stimulation of T47D cells by 3 nM of heregulin was 50%. ZM 182780 did not interfere with the heregulin-induced proliferation, while 4-OH-TAM again synergized at low concentrations (3×10^{-9}–10^{-8} M) with the heregulin dependent growth.

3.6. Inhibition of synergism in growth stimulation of 4-OH-TAM with IFG-I by the pure antiestrogen

In contrast to the pure antiestrogen the partial agonist 4-OH-TAM had shown synergism with both growth factors in two cell lines concerning mitogenic activity (Fig. 3 and data not shown) while given alone it was ineffective (data not shown). In order to test if this synergism was due to the estrogenic activity of 4-OH-TAM, T47D cells were cultivated with concentrations of IGF-I and 4-OH-TAM that had resulted in maximal synergism, 20 ng/ml and 10^{-8} M, respectively, and increasing concentrations of ZM 182780. In the absence of E_2 the growth of T47D cells was stimulated marginally (20%) by IGF-I or 4-OH-TAM alone (Fig. 4). The concomitant exposure of cells to IGF-I and 4-OH-TAM resulted in a doubling of cell proliferation indicating synergistic growth stimulation. Addition of the pure antiestrogen ZM 182780 inhibited this synergism dose-dependently.

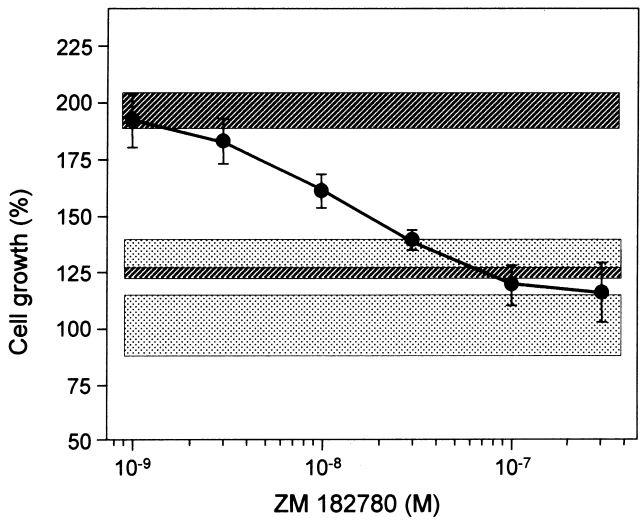

Fig. 4. Synergism of IGF-I with 4-OH-TAM inhibited by pure antiestrogen. T47D cells were grown in medium with 5% CCS without insulin. Upper and lower shaded bars represent cells grown in the presence or absence, respectively of 10^{-8} M 4-OH-TAM, while hatched bars indicate the presence of 20 ng/ml IGF-I alone (lower bar) or plus 10^{-8} M 4-OH-TAM (upper bar), symbol indicates growth in increasing concentrations of ZM 182780.

3.7. Effects of antiestrogens on IGF-I and E_2-stimulated growth of MCF7 and T47D cells

The synergism of growth factors and 4-OH-TAM prompted us to test the antiestrogenic potency of the two compounds in the presence of growth factors plus E_2. Thus, MCF7 and T47D cells were cultivated in medium without insulin, supplemented with 5% CCS and 10^{-10} M E_2, plus or minus 20 ng/ml of IGF-I and increasing concentrations of 4-OH-TAM or ZM 182780 (Fig. 5). In the absence of IGF-I, the antiestrogens dose-dependently inhibited the E_2-induced growth, with 4-OH-TAM being equipotent with ZM

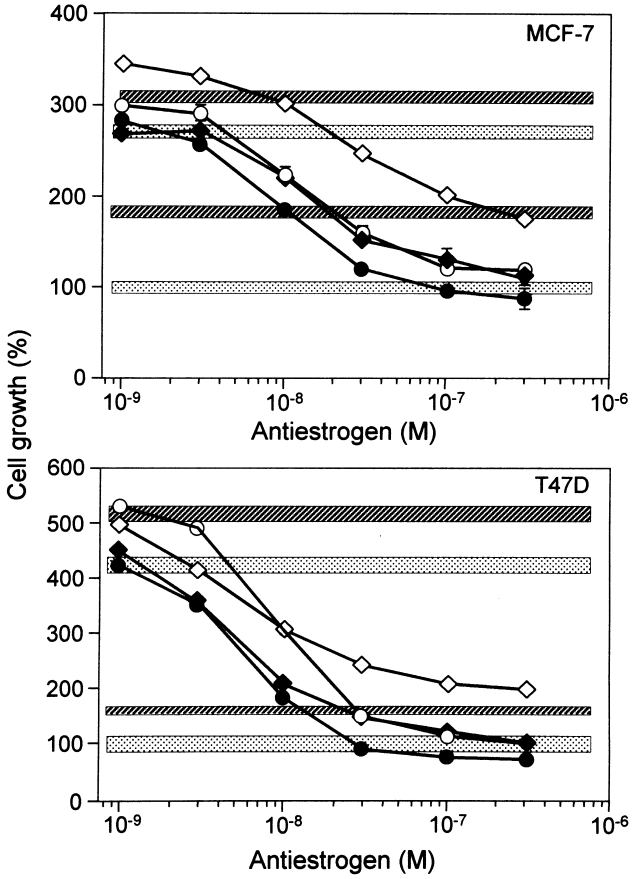

Fig. 5. E_2- and IGF-I-stimulated growth and antiestrogens. MCF7 and T47D cells were grown in medium with 5% CCS without insulin. Upper and lower shaded bars represent cells grown in the presence or absence, respectively of 10^{-10} M E_2, while hatched bars indicate the presence of 20 ng/ml IGF-I alone (lower bar) or plus 10^{-10} M E_2 (upper bar) plus increasing concentrations of 4-OH-TAM (◇) and ZM 182780 (○) in the absence (filled symbols) or presence (open symbols) of 20 ng/ml IGF-I.

182780 at this serum concentration and without insulin. Both cell lines showed an additive increase in their growth response when exposed to optimal concentrations of IGF-I and E_2. Addition of both antiestrogens dose-dependently inhibited growth of cells. Regarding E_2-stimulated growth, the potency of both antiestrogens was unaffected while the efficacy of the pure antiestrogen was unchanged, and that of 4-OH-TAM reduced by the presence of IGF-I. Both cell lines reacted similarly.

3.8. Effects of antiestrogens on heregulin and E_2-stimulated growth of MCF7 and T47D cells

The same experimental protocol was applied to test the interference of heregulin with the antiestrogenic effects of the compounds (data not shown). In the absence of heregulin, the antiestrogens dose-dependently inhibited the E_2-induced growth as discussed above. Addition of heregulin to E_2 additively stimulated growth of both cell lines and further addition of both antiestrogens dose-dependently interfered with the growth. Regarding E_2-stimulated growth, the potency of both antiestrogens was unaffected, while the efficacy of the pure antiestrogen was unchanged and that of 4-OH-TAM reduced by the presence of heregulin. Both cell lines reacted similarly.

4. Discussion

Cross-talk of nuclear steroid and membrane-bound peptide hormone receptors can occur on the level of rapid signal transduction and/or on the level of gene transcription control which takes longer time periods to show an effect. Cross-talk on the signal transduction pathways is indicated by the observations that the ER is regulated by phosphorylation at functionally significant sites by specific protein kinases [23]. It has been shown that serine-118 is phosphorylated by mitogen-activated protein kinase, and that this phosphorylation is involved in receptor activation by growth factors [24–26]. The EGF effects on the reporter system via the ER were completely abolished by a pure antiestrogen and were blocked by inhibitors of EGFR signaling. Furthermore, phosphorylation of ER on tyrosine-537 by c-scr kinase was shown to be necessary for receptor-DNA binding [27]. The fastest effects of steroids on growth factor signal transduction pathways (within min) have been demonstrated recently, suggesting the existence of non-genomic short term effects of E_2 and antiestrogens via direct activation of c-src in MCF7 cells [28]. The cytoplasmic tyrosine kinase c-scr can be activated by several growth factor receptors including erbB2, IGF-IR and EGFR and thus direct attenuation of signaling of several growth factor receptors by E_2 could be envisaged.

Here we report on functional cross-talk between growth factor and estrogen receptor pathways assayed several days following coexposure. Thus, the consequences of rapid interference as well as control of gene transcription are superimposed. MCF7 and T47D cells were mitogenically responsive to IGF-I or heregulin and this was not inhibited by the partial agonist 4-OH-TAM. Rather, there was synergistic stimulation of cell proliferation by 4-OH-TAM with either growth factor (see below). These data are in agreement [8,9] or at variance with others [6] who demonstrated complete blockade of IGF-I-stimulated growth of MCF7 cells at 50 nM 4-OH-TAM in E_2-free conditions. Also Guvakova and Surmacz [29] using MCF7 derivatives genetically engineered to overexpress IGF-IR or the insulin-receptor-substrate-I (IRS-I) demonstrate complete blockade of cellular growth by tamoxifen. In both studies very low concentrations or even no CCS was present, possibly explaining the strong sensitivity of cells to tamoxifen. Under the culture conditions applied in this study, cells exhibited similar sensitivity to IGF-I compared with other investigations, in which IGF-I could elicit a proliferative response at concentrations ranging from 1 to 50 ng/ml [30,31]. For both cell lines a bell shaped dose-dependent synergistic response curve of cellular proliferation was obtained in the presence of growth factors plus 4-OH-TAM. The synergism could be blocked completely by the pure antiestrogen and is therefore indicative of the estrogenic participation of 4-OH-TAM. Using insulin, similar data have been reported by Wakeling et al. [9].

In hormone-dependent mammary carcinoma cells the response to IGF-I is influenced by estrogens via regulation of the expression of IGF-IR and several IGF-binding proteins. A positive correlation in control of gene transcription between ER and the IGF-IR could be established in MCF7 cells, demonstrating that estrogens markedly induce the IGF-IR content and the levels of IGF-IR mRNA [10,32]. Pure antiestrogens are reported to reduce the level of IGF-IR [33,34] while tamoxifen had no effect on IGF-IR protein or mRNA levels in a recent study [29]. In this study IGF-IR levels were not estimated experimentally; however, the data indicate sufficient amounts of receptor in 4-OH-TAM treated cells to be sensitized to the mitogenic effect of IGF-I [33]. Furthermore, in clinical samples a positive relationship between ER and IGFR expression has been reported [35].

Besides influencing IGF-IR levels antiestrogens also can affect availability of the ligand in MCF7 cells [20,34]. Pronounced upregulation of IGF-I binding proteins IFGBP-3 and IGFBP-5 has been described for pure antiestrogens [36,37]. In nuclear run-off assays it was revealed that E_2 decreased and ZM 182780

increased the rate of IGFBP-5 gene transcription relative to controls [37]. It is not clear yet if IGFBP-5 acts as a growth inhibitor simply by reducing the availability of IGFs for binding to IGF receptors. Recent data suggest that IGFBPs in the extracellular environment may have direct (IGF-independent) actions on target cells [38]. In MCF7 cells the pure antiestrogens not only increase levels of IGFBPs but concomitantly reduce the level of the IGF-I receptor [33]. Thus, pure antiestrogens stimulate an autocrine inhibitory loop from two sides and very effectively abolish the IGF-IR/IGF-I signaling pathway in MCF7 cells.

Involvement of the IGF-IR/IGF-I pathway in tamoxifen resistance is suggested by several studies. Thus, in a TAM-resistant subline of human ZR-75-1 breast tumor cells the expression of IGF-IR was decreased compared with the wild type cells [39]. Sustained activation of the IGF-IR by the overexpression of IGF-II in MCF7 cells has been found to contribute to the development of the estrogen-independent phenotype in these cells [30,40]. In derivatives of MCF7 genetically engineered to overexpress several elements of the IGF-IR signaling (IGF-IR, IRS-I) reduced E_2 growth requirements were observed while sensitivity to tamoxifen was retained [39].

Heregulin was originally described as a ligand for erbB2 [41]. Now it is established as a ligand for several of the four members of the erbB family (erbB3, erbB4 and erbB2 in heterodimers) [21]. Both MCF7 and T47D cells are reported to express erbB2, erbB3 and erbB4 although at different stoichiometries [42]. Receptor homo- or heterodimer formation and thus affinity for heregulin follows a strong hierarchy dependent on monomer stoichiometries in a particular cell [43]. Thus, the precise molecular composition of the receptor homo- or heterodimers binding heregulin in MCF7 and T47D cells is not known and could be different between the two cell types. Furthermore, heregulin-binding receptor composition most probably changed during the course of the study due to cell exposure to E_2 and antiestrogens. Mutual negative control of gene transcription has been demonstrated for ER and erbB2 in multiple studies while this has not been established yet convincingly for erbB3 and erbB4. Thus, treatment of MCF7 and T47D cells with E_2 decreased and with tamoxifen or pure antiestrogens increased erbB2 mRNA and protein expression [16,44–46; data not shown]. Conversely, ligand-induced activation of the erbB signaling pathways by heregulin led to downregulation of ER and dose-dependently interfered with E_2-stimulated growth of cells [16,17].

Epidemiologically, increased erbB2 expression of breast tumor patients is associated with a lack of response to endocrine therapy on relapse [47]. Experimentally, upregulation of the erbB/heregulin pathway in MCF7 cells by either transfection of erbB2 [17,48,49] or of heregulin [50] unequivocally led to E_2-insensitive or even -independent cells. They were always resistant to tamoxifen when grown in tissue culture or exhibited increased tumorigenicity as xenotransplants in nude mice. These data led to the hypothesis that blocking the ER pathway by tamoxifen might lead to increased proliferation through the erbB2 pathway while blocking the erbB2 pathway could cause enhanced growth through the ER pathway [51]. In fact, the combined use of tamoxifen and the erbB2-blocking antibody 4D5 led to greater growth inhibition of BT-474 cells than that achieved by either agent alone [52].

In conclusion, this study shows synergism between E_2- and growth factor-induced mitogenicity in two hormone-dependent mammary carcinoma cell lines. In the presence of E_2 the antiestrogenic component of 4-OH-TAM was apparent, while the partial agonism came into prominence when combined with growth factors in the absence of E_2. Consequently, addition of growth factors reduced potency and efficacy of 4-OH-TAM on E_2-stimulated proliferation of both tumor cell lines. Thus, since synergism of 4-OH-TAM was observed with two growth factors stimulating different receptors, cross-talk of signal transduction components downstream of the respective receptors with the ER signal transduction pathway should be involved and be similar in both cell lines. However, this study indicates also differences in the downstream signal transduction components in the two cell lines based on the data obtained with the pure antiestrogen. ZM 182780 was very effective in inhibiting cell proliferation in the presence of E_2 as expected and was also interfering with growth factor action in the standard cell line MCF7, but it did not interfere with growth factor action in T47D cells. This indicates differential cross-talk between ER and growth factor receptors in two mammary tumor cell lines and might reflect different potentials to develop antihormone resistance of individual tumor cells within a given solid breast tumor.

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