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Stable transfection of an estrogen receptor beta cDNA isoform into MDA-MB-231 breast cancer cells

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Abstract

We previously reported stable transfection of estrogen receptor alpha (ERα) into the ER-negative MDA-MB-231 cells (S30) as a tool to examine the mechanism of action of estrogen and antiestrogens [J. Natl. Cancer Inst. 84 (1992) 580]. To examine the mechanism of ERβ action directly, we have similarly created ERβ stable transfectants in MDA-MB-231 cells. MDA-MB-231 cells were stably transfected with ERβ cDNA and clones were screened by estrogen response element (ERE)-luciferase assay and ERβ mRNA expression was quantified by real-time RT-PCR. Three stable MDA-MB-231/ERβ clones were compared with S30 cells with respect to their growth properties, ability to activate ERE- and activating protein-1 (AP-1) luciferase reporter constructs, and the ability to activate the endogenous ER-regulated transforming growth factor alpha (TGFα) gene. ERβ6 and ERβ27 clones express 300–400-fold and the ERβ41 clone express 1600-fold higher ERβ mRNA levels compared with untransfected MDA-MB-231 cells. Unlike S30 cells, 17β-estradiol (E2) does not inhibit ERβ41 cell growth. ERE-luciferase activity is induced six-fold by E2 whereas neither 4-hydroxytamoxifen (4-OHT) nor ICI 182, 780 activated an AP-1-luciferase reporter. TGFα mRNA is induced in response to E2, but not in response to 4-OHT. MDA-MB-231/ERβ clones exhibit distinct characteristics from S30 cells including growth properties and the ability to induce TGFα gene expression. Furthermore, ERβ, at least in the context of the MDA-MB-231 cellular milieu, does not enhance AP-1 activity in the presence of antiestrogens. In summary, the availability of both ERα and ERβ stable breast cancer cell lines now allows us to compare and contrast the long-term consequences of individual signal transduction pathways.

Keywords: Estrogen receptor beta; Breast cancer; AP-1; TGF alpha; Stable clones; Tamoxifen; MDA-MB-231

1. Introduction

The role of estrogen receptor alpha (ERα) in breast cancer has been examined and clearly established over the past 35 years. The identity of another form of the receptor, ERβ, was discovered more recently [1–3]. Human ERβ was originally cloned from testis and reported to encode a 477 amino acid protein [2]. Subsequently, longer forms of the receptor encoding 485, 530 and 548 amino acids were reported [4–7]. In addition to the expression of wild-type ERβ of various lengths due to the use of alternative start sites, a number of ERβ variants have been identified arising from alternative splicing [8–12]. The ERβ variants exhibit altered response to 17β-estradiol (E2), therefore the relative expression levels of the wild-type versus variant ERβ subtypes is of potential clinical significance and is the subject of active investigation [13–15].

The regulatory role of ERβ in breast cancer has not yet been fully described, but there is an ongoing debate in the literature regarding the role of ERβ as a predictive marker for breast cancer [16]. The studies of ERβ and its variants conducted thus far have examined RNA levels via RT-PCR, because there were few reliable antibodies to detect ERβ proteins. However, the recent emergence of suitable antibodies has allowed the examination of ERβ expression by immunohistochemical staining and Western blot [17–19]. Although it is suggested that ERβ expression is associated with a good prognosis, many others report the opposite. These conflicting reports may be due in part to the use of PCR-based analyses since the measurement of RNA is indirect and less reliable. Studies in support of the notion that ERβ expression is a good prognostic indicator include the association of ERβ with decreased proliferation and

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invasion [20], down-regulation of ERβ during breast cancer carcinogenesis [13] and progression [21], correlation of ERβ positivity with disease-free survival [22] and likely response to hormonal therapy [23,24]. In contrast, ERβ has also been correlated with tamoxifen-resistance [25,26] and increased proliferation [27].

One mechanism whereby ERβ may be associated with tamoxifen-resistance is attributed to the opposite activation of activating protein-1 (AP-1) transcription compared with ERα. In the presence of E2, ERβ inhibits and ERα activates AP-1-mediated transcription [28]. Furthermore, in breast cancer cell lines, the antiestrogen/ERα complex inhibits AP-1 transcription [29], whereas the antiestrogen/ERβ complex activates AP-1 transcription [28].

ERα and ERβ have a high degree of structural similarity in the DNA binding domain (96% amino acid identity) and slightly less in the ligand binding domain (58% amino acid identity), suggesting that the receptors are capable of binding to identical DNA sites [30], with both similar and distinct ligand preferences. The transcriptional activity of ERα is mediated by two activation functions, AF-1 and AF-2, that can function independently, but usually act in concert. AF-1 exhibits ligand-independent activity and in certain cellular and promoter contexts, is responsible for the agonist activity of tamoxifen [31]. ERβ is lacking an intact and functional AF-1 and therefore does not exhibit ligand-independent transcriptional activity nor the agonist activity of tamoxifen [32,33]. Specific interactions between co-activators and co-repressors occur at the AF-1 and AF-2 domains of the receptor to affect gene transcription, therefore ERα and ERβ are predicted to exhibit distinct transcriptional regulation.

We previously reported stable transfection of ERα into the ER-negative MDA-MB-231 cells (S30) as a tool to examine the mechanism of action of estrogen and antiestrogens [34]. To examine the mechanism of ERβ action directly, we have similarly created ERβ stable transfectants in MDA-MB-231 cells. A comparison between the ERα and ERβ stable clones was examined with respect to growth response and activation of estrogen response element (ERE)- and AP-1-mediated transcriptional activation. The MDA-MB-231/ERβ stable clone was utilized to determine the requirement of an intact AF-1 for the agonist effect of tamoxifen on endogenous transforming growth factor alpha (TGFα) gene expression as we previously reported [35].

2. Materials and methods

2.1. Cell lines and culture conditions

The MDA-MB-231 cells were obtained from the American Type Culture Collection (Rockville, MD), and the single cell clone, clone 10 A was previously described [34]. S30 cells are MDA-MB-231 clone 10 A stably transfected with ERα cDNA [34]. Cell lines were maintained in phenol-red free minimal essential media (MEM) containing 5% dextran coated charcoal (DCC)-stripped calf serum as previously described [34].

2.2. ERβ stable transfection

An expression plasmid containing ERβ cDNA was obtained from Dr. Laird Madison (Center for Endocrinology, Metabolism and Molecular Medicine, Northwestern University). The expression plasmid was constructed partially from a human testis λgt10 cDNA library (Clontech), and PCR generated fragments from human testis cDNA were cloned into the eukaryotic pcDNA3.1 expression plasmid (Invitrogen, Carlsbad, CA). This plasmid expresses a short form of the ERβ protein (485 aa) that is identical to that previously reported [6]. MDA-MB-231 clone 10A was stably transfected with the ERβ expression plasmid using Lipofectin (Gibco BRL). Forty-eight hours post-transfection, the media was replaced with G418-containing media (500 mg/ml media). Individual colonies were picked following 2 weeks of selection and were screened for ERβ-dependent transactivation of an ERE-luciferase reporter plasmid. Clones ERβ6, ERβ27 and ERβ41 were chosen for further characterization.

2.3. Infection of adenoviral ERE-luciferase reporter

The AdERELuc adenovirus was generously provided by Dr. Eun Jig Lee (Division of Endocrinology, Metabolism and Molecular Medicine, Northwestern University) and carries two EREs, the minimal thymidine kinase promoter and the luciferase gene [36]. Briefly, 4×10^5 cells/ml (in 6 ml) were mixed with 1 μl AdERELuc adenovirus (6×10^6 pfu/μl, 2.5 pfu/cell), and incubated overnight followed by treatment with either vehicle control (ethanol) or E2 (10^{-9} M). Luciferase activity was measured 24 h later as previously described [37].

2.4. Transient transfection of an AP-1 reporter plasmid

A TRE-TK-Luc reporter plasmid and the β-galactosidase (β-gal) expression plasmid pCMVβ (for the purpose of transfection efficiency normalization) was transiently transfected by electroporation. The following day, cells were treated as follows: control (ethanol vehicle); E2 (10^{-9} M); 4-OHT (10^{-7} M); ICI 182,780 (10^{-7} M); or phorbol 12-myristate 13-acetate (PMA) (10^{-7} M). Luciferase activity was measured 24 h later as previously described [37].

2.5. Detection of ERβ protein by Western blot

Whole cell extracts were prepared from MDA-MB-231, ERβ6, ERβ27 and ERβ41 clones when the cells reached 70–80% confluence. The cells were resuspended in 200 μl protein extraction buffer (150 mM NaCl; 50 mM Tris, pH 7.4; 5 mM EDTA, 0.5% Nonidet P-40 (IGEPAL) containing 1 mM PMSF, and protease inhibitor cocktail (Sigma–Aldrich, St. Louis, MO)). Samples were kept on ice

with intermittent vortexing for 30 min and then centrufuged. The supernatant was collected and stored at −80 °C. Protein concentration was measured using the BioRad protein microassay and equal amounts of protein were run in a standard Western blot protocol using the ECL chemiluminescent detection system (Amersham). The ERβ CWK-F12 monoclonal antibody (1:7000 dilution) was a generous gift from Dr. Benita Katzenellenbogen (University of Illinois, Champaign, Urbana) [18]. The β-actin antibody, AC-15 (Sigma) was used to standardize protein loading.

2.6. Immunocytochemistry

All reagents were purchased from Sigma unless otherwise indicated. MDA-MB-231 and ERβ41 cells were grown on chamber slides, the culture medium was removed, and cells were washed for 5 min in PBS. Cells were fixed for 10 min in 10% neutral buffered formalin, rinsed twice in PBS (5 min/rinse) and incubated in 5% normal horse serum in 0.25% Triton X-100 for 2 h. The solution was drained off, and slides were incubated overnight at room temperature with the ERβ primary antibody ERβ-14C8 (Gene Tex, San Antonio, TX) at a concentration of 5 μg/ml. ERβ-14C8 was produced by immunizing mice with a recombinant protein encoding 1–153 amino acids of the human ERβ sequence. Appropriate positive control (MCF-7 cell line) and negative control (non-immune rabbit IgG) were also included. Slides were rinsed twice in PBS (5 min/rinse) and incubated in biotinylated anti-mouse solution, 1:2000 dilution in 1% normal horse serum in 0.25% Triton X-100 in PBS, for 2 h at room temperature. Slides were incubated for 20 min with the Vectastain® Elite ABC reagent (Vector Laboratories, Burlingame, CA), rinsed twice in PBS and incubated in DAB (DAKO Corporation, Carpinteria, CA) for 5–10 min. Cells were then rinsed with distilled water, counterstained with hematoxylin for 30 s, washed twice with distilled water, dehydrated and cover-slipped. Slides were observed under a Nikon light/epifluorescence microscope and images captured using software from Media Cybernetics (Silver Spring, MD).

2.7. Real-time RT-PCR

The primer/probe pairs to detect the ERβ target gene were obtained from MegaBases Inc. (Evanston, IL). Primer pairs were located within different exons (exons 5 and 7) to prevent amplification from the contaminated genomic DNA. The primer sequences were ERβ forward primer: 5′-TGTATGCGGAACCTCAAAAGAGT-3′; ERβ reverse primer: 5′-CCTTCCTTTTCAGTGTCTCTCTGTTT-3′; and the ERβ probe containing the FAM-QSY7 dye pair: 5′-GTGAAGCAAGATCGCTAGAACACACCTTACC-3′. PCR reactions were performed using the ABI Prism 7700 Sequence Detection System (Perkin-Elmer Applied Biosystems). Each reaction was normalized by co-amplification of a human GAPDH transcript (Perkin-Elmer Applied Biosystems).

2.8. Proliferation assays

The cell lines S30, MDA-MB-231, ERβ6, ERβ27 and ERβ41 were seeded at 3×10^4 cells/ml in estrogen-free MEM supplemented with 500 μg/ml G418 (except MDA-MB-231) into T25 tissue culture flasks. Media containing compound was added the following day, control (0.01% ethanol) or E2 (10^{-9} M). Cells were counted on days 4–8.

2.9. Northern blot to detect TGFα transcripts

ERβ41 and S30 cells were treated with either E2 (10^{-7} to 10^{-11} M); 4-OHT (10^{-7} to 10^{-11} M); or ICI 182,780 (10^{-7} M); or combinations of E2 (10^{-9} M) + 4-OHT (10^{-7} M); or E2 (10^{-9} M) + ICI 182,780 (10^{-7} M) for 24 h. RNA isolation and detection of TGFα transcripts was performed as previously described [38].

3. Results

3.1. Stable transfection of MDA-MB-231 cells with ERβ

ERβ cDNA was stably transfected into MDA-MB-231 cells and clones were screened on the basis of E2-induced ERE-luciferase activity. Three clones were selected, ERβ6, ERβ27 and ERβ41 exhibiting 2–3.5-fold E2-induced ERE transactivation (Fig. 1). The level of ERβ protein expression was determined by Western blot and indicates that both ERβ6 and ERβ27 express lower levels of ERβ protein than the ERβ41 clone, which exhibits the highest level of expression (Fig. 2). Real-time RT-PCR analysis confirms that

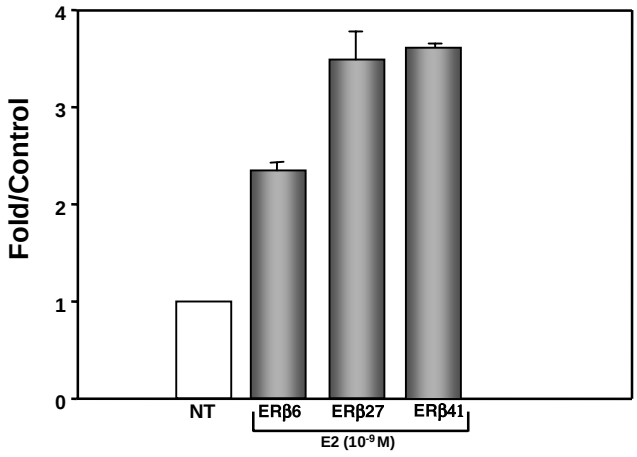


Fig. 1. MDA-MB-231/ERβ clones exhibit estradiol-induced transcriptional activation of an ERE-luciferase reporter. The AdERELuc adenovirus was used to infect into MDA-MB-231 or MDA-MB-231/ERβ stable clones as described in Section 2. Results are expressed as fold E2-induced luciferase activity relative to untreated control (fold/control) and presented as the mean ± S.E. of three independent experiments performed in triplicate.

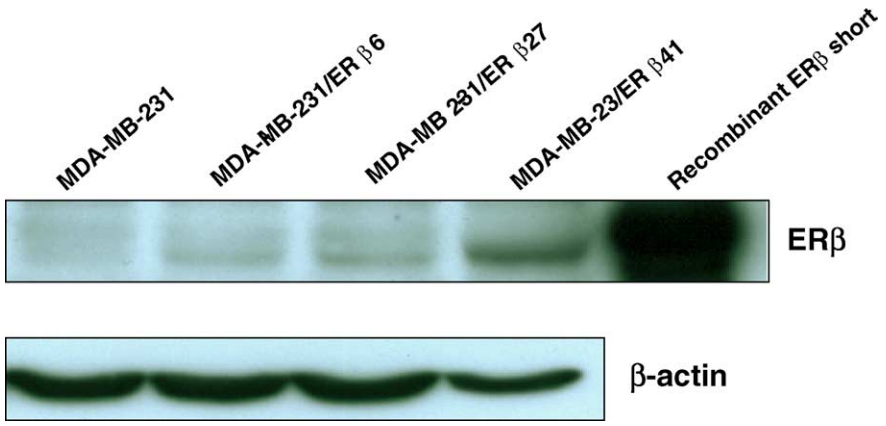


Fig. 2. ERβ expression in MDA-MB-231/ERβ clones. Whole cell extracts were prepared as described in Section 2. ERβ Primary antibody: CWK-F12 monoclonal (1:7000). The β-actin antibody is shown to reflect total protein loaded per well.

ERβ41 cells express the highest level of ERβ (1600-fold) relative to the parental MDA-MB-231 cells, compared with ERβ6 and ERβ27 cells that express 418- and 322-fold mRNA levels, respectively (Fig. 3). Immunohistochemical staining demonstrates nuclear and diffuse cytoplasmic localization of ERβ in ERβ41 (Fig. 4). All three clones exhibited stable ERβ expression over at least 30 passages, and represent low level (ERβ6 and ERβ27) and high level (ERβ41) ERβ protein expression. We characterized the ERβ6, ERβ27 and ERβ41 clones representing low and high ERβ expression.

3.2. Growth characteristics of MDA-MB-231/ERβ stable clones

The growth characteristics of the MDA-MB-231/ERβ stable clones were examined by performing proliferation assays. The ERβ41 clone, expressing the highest level of ERβ protein, exhibits the fastest proliferation rate relative to the ERβ6 and ERβ27 which represent low ERβ expression, and to both parental MDA-MB-231 and S30 cells (Fig. 5A and C). The ERβ6 and ERβ27 clones, expressing lower levels of ERβ, shows an intermediate rate of proliferation; faster than the parental MDA-MB-231,

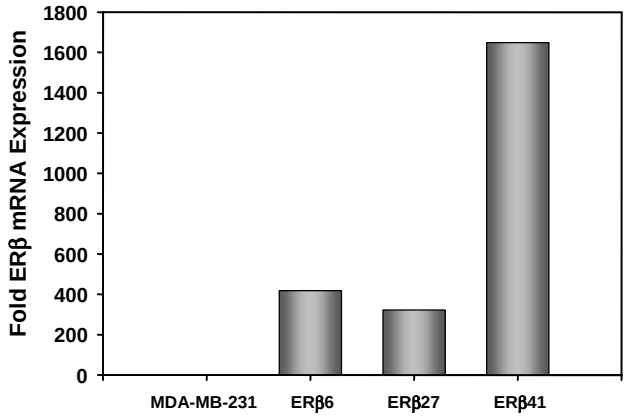


Fig. 3. ERβ mRNA quantification by real-time RT-PCR. Relative expression of ERβmRNA normalized to GAPDH. The level of ERβ mRNA in each clone is expressed relative to ERβ expression in MDA-MB-231 set = 1.

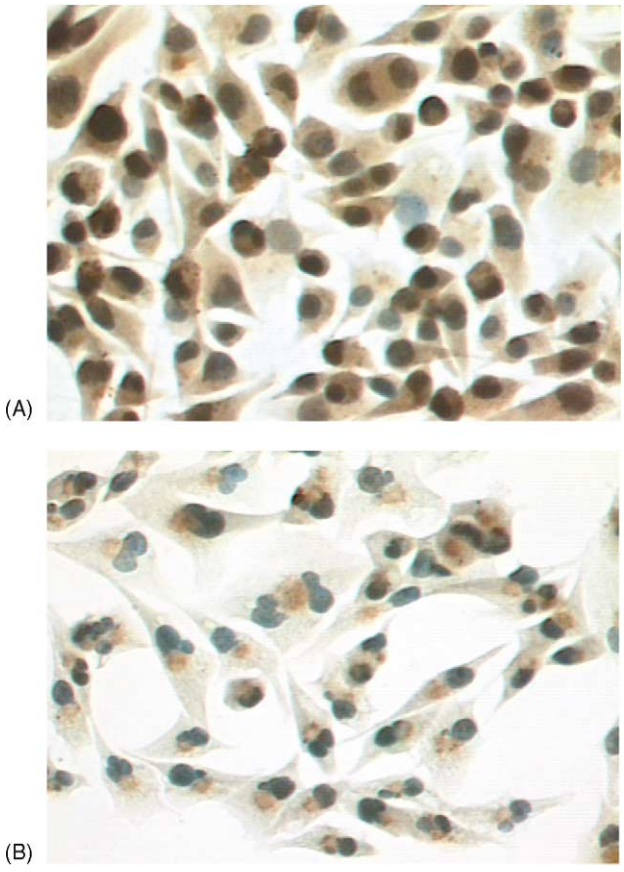
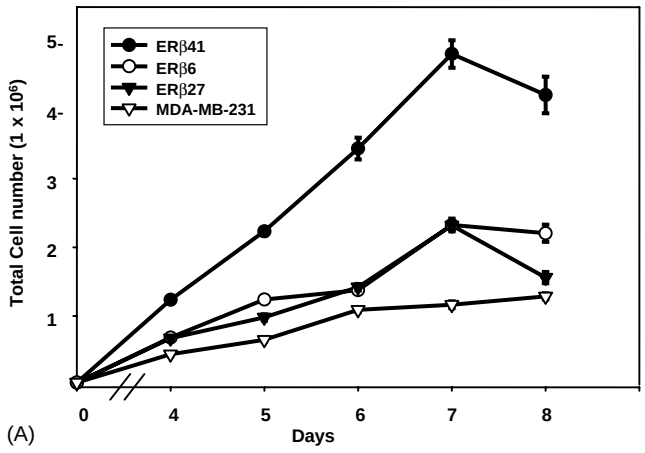
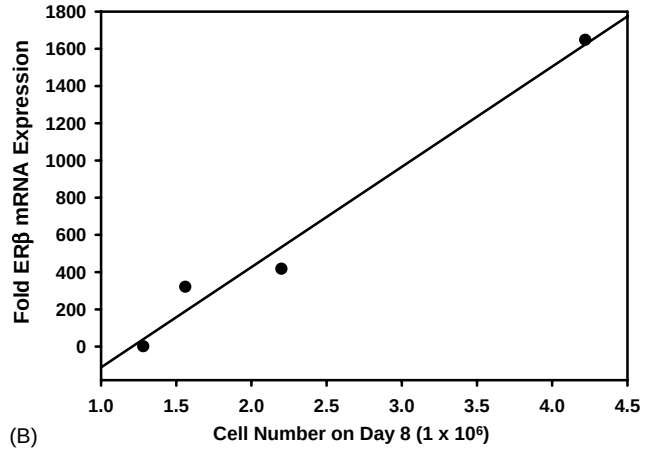


Fig. 4. Immunohistochemical analysis of ERβ expression. ERβ expression was detected by immunohistochemical staining with the ERβ-14C8 primary antibody as described in Section 2. (A) ERβ41 cells; (B) MDA-MB-231 cells. Magnification 40×.





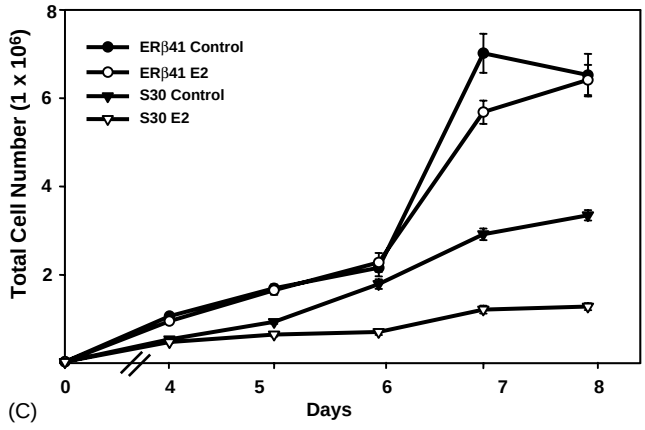


Fig. 5. Growth characteristics of MDA-MB-231, MDA-MB-231/ERβ and S30 cells. Proliferation assays were performed as described in Section 2. Error bars represent S.E. ($n = 3$). Comparable results were achieved in three independent experiments. (A) Comparison of proliferation rate of ERβ clones with the parental MDA-MB-231 cells. (B) Correlation of mean cell number achieved after 8 days (data presented in (A)) with relative ERβ mRNA expression level as determined by real-time RT-PCR (original data presented in Fig. 2). (C) Proliferation of ERβ41 and S30 cells in the presence and absence of E2 (10^{-9} M).

yet slower than ERβ41 (Fig. 5A). The proliferation rate positively correlates with the level of ERβ expression as quantified by real-time RT-PCR (Fig. 5B). It is a well-known phenomenon that E2 causes growth inhibition in ERα stable transfectants [39], however E2 does not appear to alter the growth rate of ERβ41 cells (Fig. 5C) or ERβ27 (results not shown). It was reported that transient overexpression of ERβ in MDA-MB-231 cells results in ligand-independent inhibition of proliferation [20]. However our results using stable ERβ clones indicate that proliferation is increased as ERβ expression is increased (Fig. 5A and B).

3.3. Antiestrogens do not transactivate an AP-1 luciferase reporter

It was previously reported that the antiestrogens tamoxifen, raloxifene and ICI 182,780 when bound to ERβ are potent activators of transcription at an AP-1 site in cells transiently transfected with ERβ [28]. To determine the ability of antiestrogens to activate AP-1 transcription in cells stably transfected with ERβ, luciferase assays were performed with ERβ41 cells transiently transfected with the AP-1-luciferase reporter plasmid, TRE-tk-luc. Neither 4-OHT nor ICI 182,780 was capable of activating AP-1 luciferase activity in ERβ41 cells, whereas treatment with PMA verifies the functionality of the reporter plasmid (Fig. 6). Antiestrogen-mediated AP-1 activation was also not observed with MDA-MB-231 cells transiently co-transfected with ERβ and TRE-tk-Luc plasmids

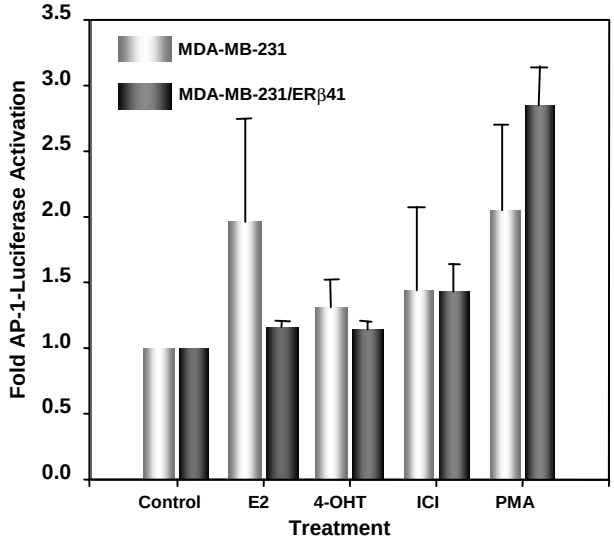


Fig. 6. Induction of AP-1 luciferase activity in ERβ41 cells. ERβ41 cells were transiently transfected with the TRE-tk-Luc reporter plasmid. Luciferase activity was measured 24 h post-treatment. Control (EtOH vehicle); E2 (10^{-9} M); 4-OHT (10^{7} M); ICI 182,780 (10^{-7} M); PMA (10^{-7} M). Results are presented as fold induction relative to the untreated control set = 1, and expressed as the mean ± S.E. of four independent experiments performed in triplicate.

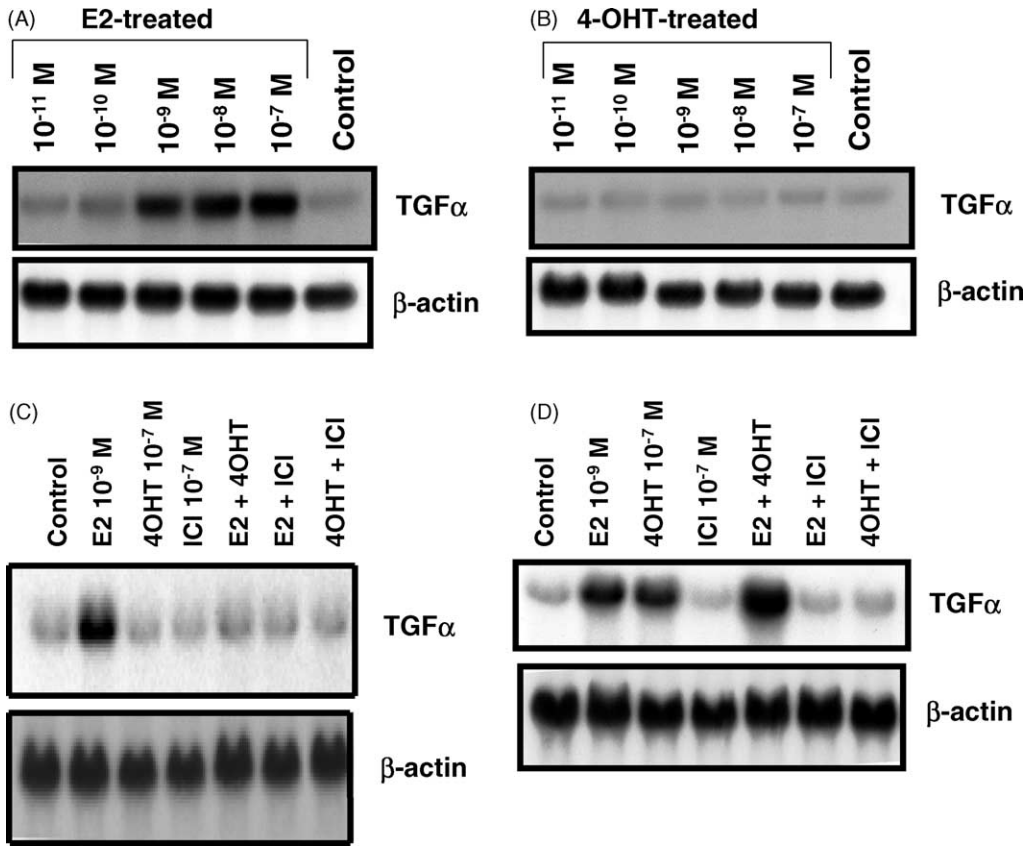


Fig. 7. Induction of TGFα gene expression by E2 and antiestrogens. RNA was extracted from MDA-MB-231/ERβ41 (A–C) and S30 cells (D) following 24 h of treatment with the compound indicated. Northern blots were performed to detect TGFα and β-actin as described in Section 2.

(results not shown). These results suggest that antiestrogens complexed with ERβ are incapable of activating AP-1 sites in the MDA-MB-231 cellular context.

3.4. Differential transcriptional activation of TGFα by ERα and ERβ

We previously reported that 4-OHT when bound to wild-type ERα acts as an agonist of TGFα transcript expression [38]. This agonist activity is dependent upon an intact AF-1 and helix 12 along with a negative charge at amino acid 351 [40]. Since ERβ lacks an AF-1 domain [32], we tested the hypothesis that the 4-OHT/ERβ complex could not activate TGFα mRNA expression. To address this question, Northern blot analysis was conducted to monitor the induction of TGFα mRNA in ERβ41 cells. In dose-response experiments, ERβ41 cells were treated with either E2 (10^{-11} to 10^{-8} M) or 4-OHT (10^{-11} to 10^{-8} M) (Fig. 7). Whereas E2-dose-dependent expression of TGFα was observed in ERβ41 cells, 4-OHT at any dose tested did not induce TGFα. This can be compared with the ability of both E2 and 4-OHT to elicit TGFα mRNA expression mediated by ERα as we have previously reported in S30 cells (Fig. 7D) [38].

4. Discussion

This is the first report of stable expression of an ERβ isoform in a human breast cancer cell line. This MDA-MB-231 cell line was chosen since it is devoid of both ERα and ERβ expression [41] and we had previously established and characterized ERα stable expression (S30 cells) [34]. Clones were established expressing both low and high levels of ERβ protein expression as represented by ERβ6, ERβ27 and ERβ41 and it was demonstrated that increased ERβ protein expression is correlated with accelerated proliferation rate. Whereas it is well documented that E2 causes growth inhibition in cell lines stably expressing exogenous ERα [20,34], E2 has no effect on the growth of ERβ stable clones. Despite reports of antiestrogen-mediated activation of AP-1 transcriptional activation through ERβ [28,29], we find that antiestrogens are incapable of activating AP-1 in an ERβ stable transfectant. Finally, we provide additional evidence that an intact and functional AF-1 is required for tamoxifen to act as an agonist with respect to endogenous TGFα gene expression. The creation of stable ERβ clones in MDA-MB-231 has allowed us to compare and contrast ERα and ERβ-mediated signaling events on cell growth characteristics and gene expression.

The majority of studies examining the effect of ERβ expression on proliferation has utilized transient transfection of ERβ or has correlated decreased ERβ expression with neoplastic transformation. Transient infection of ERβ into MDA-MB-231 cells resulted in decreased proliferation rate and invasion [20]. The only other stable transfection of ERβ was reported in a rat fibroblast cell line and no effect of ERβ expression on growth was reported [42]. Other indirect evidence linking decreased proliferation with ERβ expression include the observation of prostatic hyperplasia in ERβ knock-out mice [43] and the ability of ERβ to act as a dominant regulator of ERα signaling [44,45]. Our results clearly demonstrate that as ERβ expression increases, proliferation rate increases (Fig. 5A and B). It is interesting to note that whereas E2 classically causes growth inhibition in cells expressing exogenous ERα [39], we find that E2 has no effect on the growth of ERβ stable clones (Fig. 5C). ERβ has a lower affinity for E2 than ERα, and ERβ exhibits reduced transcriptional activity compared with ERα [31,46]. However the difference in affinity for E2 between the receptors is not that great ($K_d = 0.1$ nM versus 0.4 nM) [30], and it is more likely the pronounced difference in amino acid identity in the amino termini of the receptors is responsible for the differential growth characteristics in response to E2.

A potential mechanism of tamoxifen-resistance is hypothesized to be the activation of the growth-promoting AP-1 pathway mediated by ERβ [47]. ERα and ERβ signal in opposite ways at the AP-1 site; the E2/ERα complex activates AP-1 transcription, whereas the E2/ERβ complex causes AP-1 inhibition. However, antiestrogens such as tamoxifen, raloxifene and the pure antiestrogen ICI 164,384 in conjunction with ERβ activate AP-1 transcription both using complex collagenase and minimal AP-1 consensus motifs [28,29,48]. Thus far, evidence of activation of this pathway by antiestrogens through ERβ has only been obtained using transiently transfected receptors. Our results using the MDA-MB-231/ERβ41 stable clone indicate antiestrogens are incapable of activating an AP-1 site cloned into the luciferase reporter plasmid, TRE-TK-luc. This result is in contrast to the activation of AP-1 in ERβ transiently transfected MCF-7 and MDA-453 cells by antiestrogens [28,48]. These conflicting results may be due in part to the difference between stable and transient expression of the receptors.

We have reported that tamoxifen acts as an agonist on endogenous TGFα gene expression [38]. Recently we reported the requirement of AF-1, an intact helix 12, more specifically amino acid D538, and an appropriately positioned negative charge at amino acid 351 to allow 4-OHT to act as an agonist with respect to TGFα gene transcription [40,49]. Since ERβ does not have a functional AF-1 [32], we were able to substantiate the requirement of a functional AF-1 for tamoxifen to act as an agonist on TGFα gene expression. We demonstrated that E2 treatment of ERβ41 cells induced TGFα expression, indicating the functional expression of ERβ in this clone. However, 4-OHT was incapable of inducing TGFα expression, illustrating that an intact AF-1 is required for 4-OHT to act as an agonist (Fig. 7).

The ERβ cDNA we used to create the stable transfectants is not the longest form that has been identified, and it is speculated that since the longer ERβ cDNAs encode additional amino acids in the A/B region, that perhaps the longer forms possess a more functional AF-1. However, Hall and McDonnell [44] report indistinguishable activities between the ERβ-long and ERβ-short versions with respect to response to ER agonists and antagonists. Furthermore, all isoforms of ERβ are expressed in the breast [9]. We have attempted to compare and contrast the long and short forms of ERβ transfectants by introducing the ERβ long form into MDA-MB-231. Despite selecting many clones; none were stable beyond three to four passages. This finding implies that the additional N-terminal amino acids may confer the growth inhibitory effects observed by Lazennec et al. [20], however in their report it was not clearly stated which form of ERβ cDNA was transiently infected into MDA-MB-231 cells.

In summary, we have established the first reported stable transfection of an ERβ isoform into human breast cancer cells. The resulting clones have allowed us to make direct comparisons of the growth characteristics of exogenous expression of ERα and ERβ in MDA-MB-231 cells and also to compare the effects of stable versus transient transfection on cell growth and gene expression. Our results highlight the important differences between the N-termini of the ERα and ERβ proteins on the growth response to estrogen and the ability of antiestrogens to transactivate TGFα gene expression. The inability of the ERβ41 clone to activate AP-1 transcription by antiestrogens suggests that transient versus stable expression of ERβ may produce profoundly different results. Lastly, although our findings do not support or dispute the role of ERβ as a good prognostic indicator for breast cancer, they emphasize the need to characterize and quantify all isoforms of ERβ expressed and determine the relative ratio of ERα/ERβ in breast cancer. As the ability to detect all isoforms of the receptor proteins reliably improve, and as a result we have a more complete understanding of the functional characteristics of each isoform, these data will become essential to decipher the complexity of competing signal transduction pathways.

Acknowledgements

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