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Modulation of estrogen receptor activity by selective coregulators[☆]

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Abstract

The estrogen receptor (ER), a member of the nuclear hormone receptor superfamily, is a hormone-regulated transcription factor that mediates the effects of estrogens and antiestrogens in breast cancer and other estrogen target cells. Because of the role of estrogens in promoting the growth and progression of breast cancer, there is great interest in exploring ways to functionally inactivate the ER, thereby suppressing ER-mediated gene expression and cell proliferation. These approaches have involved the use of antiestrogens such as tamoxifen, dominant negative ERs and, more recently, the use of corepressors. Through the use of two-hybrid screening, we have recently identified a selective repressor of estrogen receptor activity (REA). This protein is recruited to the hormone-occupied ER and selectively represses its transcriptional activity but not the other steroid and non-steroid nuclear receptors. REA also interacts with a protein, prothymosin-α (PTα), that selectively enhances ER transcriptional activity by recruiting the repressive REA protein away from ER. Analysis of the mechanisms underlying the activities of these two proteins highlights a new role for REA and PTα as activity-modulating proteins that confer receptor specificity.
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1. Introduction

The estrogen receptors (ERα and ERβ) belong to the nuclear receptor hormone superfamily and are ligand-inducible transcription factors. These receptors modulate the transcription of specific genes by directly interacting with estrogen response elements located near the target gene promoter [1–4], as well as in a variety of additional modes including indirect tethering to DNA via interaction with other transcription factors [5–8]. The estrogen receptor mediates the stimulatory effects of estrogens and the inhibitory effects of antiestrogens in breast cancer and in many other target tissues [9]. Gene transcriptional activation by ER is enhanced or repressed by interaction with regulatory factors which function in a positive fashion (coactivators) or negative fashion (corepressor) and are believed to be interposed between the receptor and the basal transcription complex [10–14]. These coregulators exist as a part of large complexes that can be recruited by the ER and function as chromatin remodeling factors [13–15]. Therefore, this tripartite action of the ER, involving the receptor, its ligands and coregulator proteins [16], allows for the precise regulation of the biological effects of these hormone receptors on gene expression.

Because of the role of estrogens in promoting the growth and progression of breast cancer, there is great interest in exploring ways to functionally inactivate the ER so as to suppress ER-mediated gene expression and cell proliferation [17,18]. These approaches have involved the use of antiestrogens such as tamoxifen, as well as dominant negative ERs [19] and, more recently, the use of corepressors.

We have recently characterized a novel selective repressor of ER activity (REA) for repressor of estrogen receptor activity. REA directly interacts with the liganded ER, suppresses ER-activated gene transcription by estrogens, and competes with coactivators for binding to ER [20,21]. Furthermore, using two-hybrid screening and additional techniques, we have subsequently identified prothymosin-α (PTα) as a binding protein partner of REA [22]. PTα selectively activates ER by binding REA. This interaction of REA with PTα sequesters REA away from the ER, enabling coactivator association with ER, resulting in enhanced ER transcriptional activity. Interestingly, we have found also that PTα gene expression is rapidly increased by estrogens [23], highlighting a new role for PTα and REA as ER activity-modulating proteins that confer receptor specificity.

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2. Results and discussion

2.1. REA interaction with ER selectively suppresses its transcriptional activity

Through yeast two-hybrid screening, using either a dominant negative ER or the wild-type ER ligand binding domain as baits, we isolated a 37 kDa protein from a cDNA library we made from the human breast cancer cell line MCF-7 [20]. We named this protein REA, for repressor of estrogen receptor activity, because of its activity in cell transactivation experiments. REA potentiates the inhibitory effects of antiestrogens and, at higher concentration in cells, it represses the activity of the estrogen-occupied estrogen receptor (ERα and ERβ). It is interesting to observe (Fig. 1) that, in the presence of elevated REA, 50 times less antiestrogen would be needed for equivalent inhibitory activity. More importantly, REA inhibits gene transcription in a selective manner, acting exclusively on ER. When we tested REA on other nuclear hormone receptors such as progesterone receptor (PR), retinoic acid receptor, androgen receptor and the non-related transcription factor VP16, REA was unable either to increase the potency of antagonists or to suppress the gene transcription mediated by these proteins.

Transfection of an expression vector encoding antisense REA [21] enhanced the transcriptional response to the estrogen–ER complex, suggesting that endogenous cell REA normally suppresses the transcriptional activity of ER. We also tested the ability of REA to suppress ER activity when ER was acting at a variety of DNA-responsive elements, including non-consensus estrogen response elements (EREs). REA had a broad range of repressive activity at different promoter-enhancer sites such as the complement 3 gene and the lactoferrin gene, two genes containing non-consensus EREs [24,25], as well as when ER is tethered via other proteins to the DNA site, as with the transforming growth factor-β3 gene [26].

In contrast to other more general corepressors of the nuclear hormone receptors such as NCoR and SMRT [27–30], REA shows selectivity for ER and interacts preferentially with liganded ER while NCoR and SMRT interact with unliganded receptor and dissociate upon ligand binding. REA also suppresses the agonist activity of tamoxifen-occupied ER, while NCoR and SMRT do not [31].

Looking at the interaction region of REA with ERα, we found that REA binds only the hormone binding domain of ER whereas domains A/B, C (DNA binding), D (hinge), and F domains are not required for binding. Of interest, it is known that the F domain affects the sensitivity of ER to estrogen and antiestrogen [32]. Our studies, when we deleted the F domain or used a frame-shifted ERα with an altered F domain, revealed that the presence of the F domain prevented the recruitment of REA to the unliganded ER, suggesting its importance in maintaining the unliganded receptor in a conformation that does not recruit REA.

We also analyzed the REA interacting regions with ER: we found (Fig. 2) that the deletion of the last 125 amino acids of REA resulted in a protein showing little interaction with ER, while the regions important for repression encompass amino acids 19–49 and 150–174. Furthermore, REA contains a LXXLL motif, or nuclear receptor box, at amino acids 23–27 contained in the first repression domain. The LXXLL motif is not required for interaction with ER, although mutations of leucines with alanines reduced the repression action of REA, suggesting once again that the interaction of REA with ER is mediated in a manner very different from that of most coregulators and anticipates a new

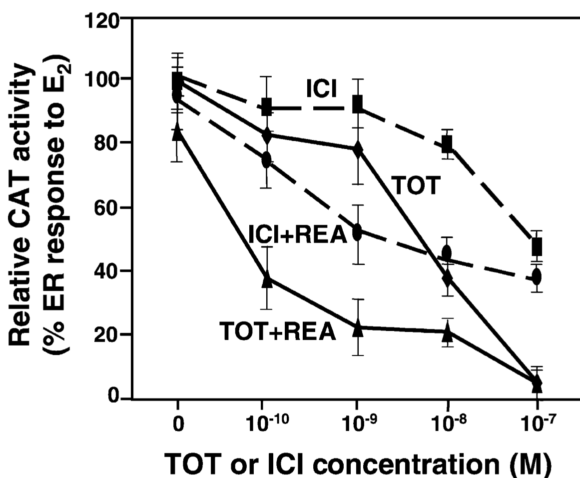

Fig. 1. REA enhances the potency of antiestrogens in cell transfection/transactivation experiments. Co-treatments with 10^{-8} M estradiol (value set at 100%) and increasing concentration of either *trans*-hydroxytamoxifen (TOT), or ICI 182,780 (ICI), in the presence of REA, shifts the dose response curve to the left, implying that less antiestrogen is required to achieve the same inhibitory activity.

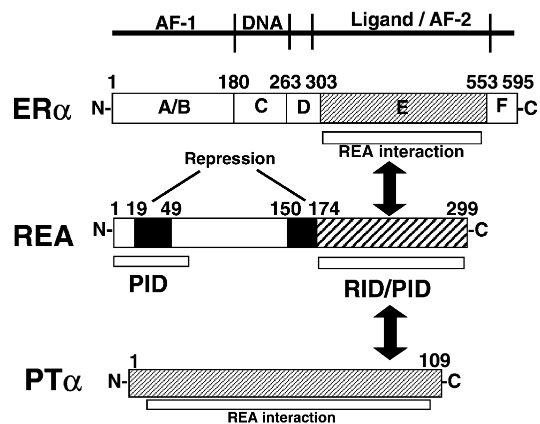

Fig. 2. REA interacts either with ER or with PTα. In mapping the interacting region of REA with ER and PTα we found that the C-terminal portion of REA interacts with the ligand binding domain of ER, the receptor interaction domain (RID). Interestingly, the same portion of REA is involved in the interaction with PTα in cooperation with a small N-terminal portion of REA, the prothymosin-α interaction domain (PID), whereas PTα does not interact with ER. See text for details.

molecular mechanism by which REA acts to modulate the activity of ER.

2.2. Prothymosin-α interacts with REA and enhances ER transcriptional activity

To better understand the mechanism by which REA works, we used REA as bait in a two-hybrid interaction screening with a cDNA library from MCF-7 human breast cancer cells. In this way, we identified the nuclear protein prothymosin-α, a 12.5 kDa protein with chromatin remodeling functions, to be a protein with which REA directly interacts [33–39]. Cell transfection of PTα [22] was found to increase the transcriptional activity of ERα and ERβ but to have no effect on the progesterone receptor or glucocorticoid receptor (GR) (Fig. 3). This ability of PTα to increase ER transcriptional activity was observed with both consensus and non-consensus EREs and with different promoters in several cell types. Interestingly, in co-transfection experiments, this increased transcription activation of ER stimulated by PTα was suppressed by REA, whereas PTα, in combination with antisense REA, further stimulated the activity of the hormone-occupied ER, suggesting that PTα and REA are both important factors in modulating ER activity. Thus, binding up and neutralizing the inhibitory activity of REA, as observed with PTα, and eliminating REA through the use of antisense REA, allows the greatest magnitude of response to estrogens.

In addition to interacting directly in in vitro GST-pulldown assays, endogenous PTα and REA interacted also in cell extracts where REA was co-immunoprecipitated with PTα using a specific PTα antibody; in contrast, ER was not co-immunoprecipitated with PTα. GST-pulldown competition assays show that ERα binds to REA in the presence of estrogen, and increasing the amount of PTα prevents ER from binding to REA, implying that REA binding to ER and to PTα is mutually competitive. We know also that there is functional competition between REA and the steroid receptor coactivator 1 (SRC-1) [20,21] for regulation of ER transcription. REA competitively reduces the binding of SRC-1 to the ER in in vitro GST-pulldown assays, while increasing the level of PTα has no effect on the in vitro interaction of SRC-1 with the ER. But when we incubate a fixed amount of REA in the presence of SRC-1 with estrogen-occupied ER, increasing the amount of PTα increases the ER–SRC-1 interaction, indicating that REA and SRC-1 mutually compete for binding to ER whereas PTα does not compete with SRC-1. This suggests that PTα can recruit REA away from the receptor, thereby allowing increased binding of SRC-1 to ER (Fig. 4).

We mapped the interacting region of REA with PTα. Of interest, while the full length PTα is required for its interaction with REA, we found that the strength of this interaction is determined by both the N- and C-terminal portions of REA that may collaborate in promoting optimal interaction with PTα. More important is that the C-terminal half of REA is the portion most crucial for interaction with ERα (Fig. 2). Therefore, REA binding to the ER is mutually competitive with its binding to PTα. Thus, as cellular levels of PTα increase, this protein will increasingly bind up REA and sequester it away from ER.

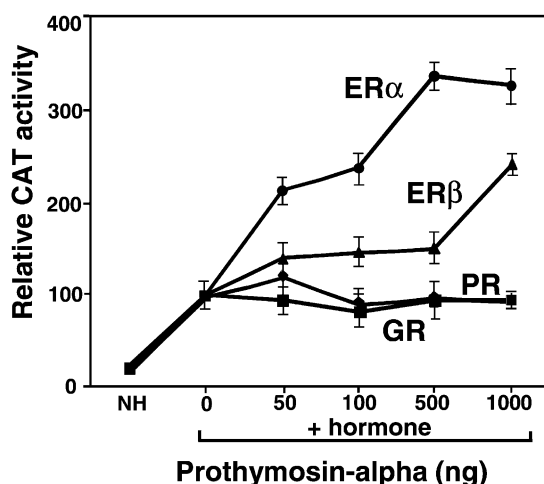

Fig. 3. PTα increases transcriptional activity of ERα and ERβ but has no effect on progesterone receptor (PR) or glucocorticoid receptor (GR) activity. The indicated nuclear receptor was co-transfected along with the appropriate hormone-responsive reporter gene construct with increasing amount of PTα expression plasmid in the presence of either 10^{-8} M estradiol for ERs, 10^{-8} M R5020 for PR or 10^{-8} M dexamethasone for GR. Cell extract CAT activity values, normalized for β-galactosidase activity, are the mean ± S.D. from three separate experiments. See text for details.

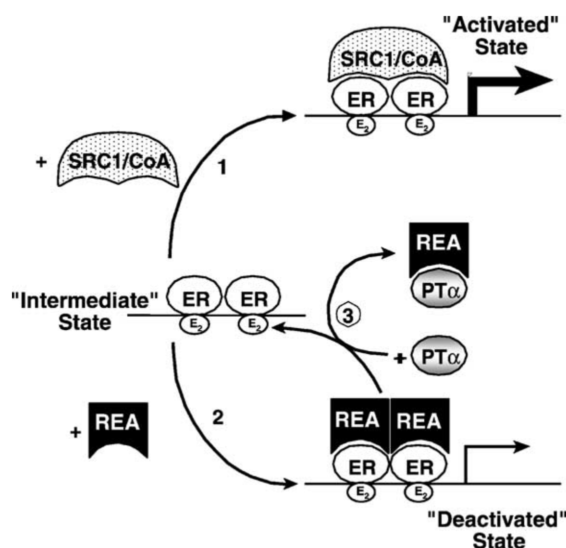

Fig. 4. Schematic model of a novel ER mechanism of action. In the presence of ligand, the selective coregulator REA and the coactivator SRC-1 compete for binding to the ER; REA blocks SRC-1 receptor binding. When intracellular PTα level rises upon estrogen stimulation, PTα binds REA and sequesters it away from the receptor enabling the receptor to interact with SRC-1 or other p160 coactivators and enhance gene transcription.

2.3. The PTα gene is up-regulated by estrogens through direct binding with ER

Previous studies showed that PTα levels were increased in rapidly proliferating cells [33–35,37–39] and in response to estrogens in an ER-positive neuroblastoma cell line [40]. Therefore, we investigated the role of estrogens in stimulating PTα gene transcription. In addition to its association with cell proliferation, PTα has been proposed as a breast tumor prognostic marker [33,38,39]. Estrogens stimulate the proliferation of ER-containing breast cancer cells [41] and, as we have demonstrated above, PTα selectively increases ER transcription via its interaction with REA. We asked whether PTα might be up-regulated by estrogens in ER-positive breast cancer cells. We found that the level of PTα mRNA rapidly increases, as early as 1–2 h, following estrogen treatment in ER-containing breast cancer cells, and this increase is a primary response not dependent on prior protein synthesis. Substantial elevation of PTα protein level is also observed by Western blot analysis by 12–24 h and continues to increase to 72 h.

To understand the mechanism of this hormonal regulation, we analyzed the 5 kb promoter region of the PTα gene [42]. By sequence analysis we found three half-estrogen response elements at positions −750, −1051 and −1437. Two of the half-EREs are consensus (−750, −1051) while the one at the position −1437 is not. Estrogen induction of PTα gene expression is mediated only via the two half-palindromic EREs (−750, −1051), with the proximal motif (−750) being of somewhat greater importance than the distal (−1051), similar to the situation in the ovalbumin gene [43]. The non-consensus half-ERE (−1437) was not involved in estrogen regulation of the PTα gene and mutation [44] of the active half-consensus EREs completely abolished estrogen induction. Furthermore, this induction required a functional ER with an intact DNA binding domain confirming that good activation of PTα transcription by the ER can be mediated through direct DNA binding domain interaction with the two half-consensus EREs of the PTα gene, as demonstrated in our electrophoretic mobility gel-shift studies [42].

Interestingly, the PTα promoter region contains multiple Sp1 sites responsive to the Sp1 transcriptional activator, in addition to the half-EREs. Previous studies on cathepsin D, retinoic acid receptor α, heat shock protein 27, IGF binding protein 4 [5], c-myc, creatine kinase B and progesterone receptor A [6,7], demonstrate that Sp1 and half-ERE sites play a role in the regulation of estrogen inducible genes [8] when direct DNA binding by the ER is also not involved. We used Drosophila SL cells, deficient in Sp1, and we found that Sp1 plays a role in regulating the PTα gene independent of the presence of estrogen–ER complex, suggesting that regulation by the ER or Sp1 is determined independently by the relative levels of ER, estrogen and Sp1 modulating via its own ERE or Sp1 sites.

In summary, PTα is a chromatin remodeling protein [35,45] that modulates histone acetyltransferase activity [46] and interaction of histone H1 with chromatin [36,47]. More recent studies have shown also PTα association with CREB-binding protein in potentiating transcription [48]. Because PTα is a marker of cell proliferation and cell cycle regulation [38], it has been proposed as a marker of prognostic value in breast cancer [49,50]. Therefore, estrogen regulation of PTα expression is perhaps not surprising, knowing that estrogen is a major stimulator of proliferation of ER positive breast cancer cells [41]. In accordance with our studies described above, PTα, induced by estrogens, interacts with REA and selectively enhances ER transcriptional activity by binding to REA, thereby keeping REA away from ER and allowing coactivator association with the receptor. We can now propose a schematic model that represents an intriguing mechanism for regulation of the actions of the ER (Fig. 4). In this model ER interacts either with SRC-1 (or related p160 coactivators) promoting gene transcription, or with REA which blocks gene transcription. PTα does not bind either ER or SRC-1 whereas REA binding with ER is mutually competitive with its binding to PTα. Thus, after estrogen stimulation, PTα level raises in cells and PTα will increasingly bind up REA and sequester it away from ER. This will allow ER to bind to SRC-1 or other coactivators, resulting in enhanced gene transcription. More importantly, because REA does not interact with other nuclear receptors nor repress their transcriptional activity, and PTα, which interacts with REA, is increased by estrogen, this mechanism of action provides for a very selective and novel one that is unique for the estrogen receptor.

3. Conclusions

In the studies described above, we reveal additional dimensions to this aspect of the regulation of receptor activity as we have identified a new role for REA and PTα in modulating ER transcriptional activity. Neither of these two proteins has an intrinsic activation or repression function, yet they have important effects on ER activity. They act by either interfering with (REA) or enabling (PTα) interaction with other coactivator complexes. Moreover, changes in the level of PTα by estrogens, have the potential of magnifying the effectiveness of estrogens in stimulating ER transcriptional activity in rapidly proliferating cells.

In addition, these observations suggest interrelationships between cell proliferation and gene transcriptional activity and indicate a positive mechanism by which PTα,which increases ER transcriptional effectiveness, is itself up-regulated by the estrogen–ER complex.

Continuing studies should provide further understanding of how the activity of the ER is influenced not only by ligands but also by interaction of the receptor with these activity-modulating proteins.

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