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Review Article

Molecular Action of the Estrogen Receptor and Hormone Dependency in Breast Cancer

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The measurement of estrogen receptor (ER) α in breast cancer tissues is important to discriminate between the hormone dependent and independent tumors. Recently, a second ER, referred to as ERβ, has been identified. The DNA binding domain of ERβ is 96% conserved compared with ERα, and the ligand binding domain shows 53% conserved residues, suggesting that both receptors can bind estrogen responsive elements on target genes, and that they may also bind similar ligand. While both receptors bind to 17β-estradiol with equal affinity, other compounds bind with varying affinities to the two receptors. Since the function of ERβ in breast cancer progression is not well understood, further characterization of the function of ERβ and its isoforms in breast cancer is warranted. Various kinds of cofactors, such as steroid receptor coactivator-1 (SRC-1), transcription intermediary factor 2 (TIF2), and amplified in breast cancer 1 (AIB1), have also been reported. These coactivators interact with nuclear receptors in a ligand-dependent manner and enhance transcriptional activation by the receptor via histone acetylation/methylation and recruitment of additional coactivator, such as CREB binding protein (CBP)/p300.

Thus, action of estrogen is not as simple as thought previously, and is likely influenced by ERβ, its variants and interaction with cofactors. Improved understanding of the ER mechanism may follow from the discovery of these proteins, although their precise roles remain to be determined.

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Key words: Estrogen receptor α/β, Coactivator, Corepressor

Human breast cancer is a hormone dependent tumor, and various endocrine treatments have been employed. The measurement of estrogen receptor (ER) in breast cancer tissues is important to discriminate between the hormone dependent and independent tumors to determine whether endocrine treatments should be administrated. About 60% of ER-positive cases respond to such therapies, but less than 10% of the cases without ER also respond[1, 2]. Furthermore, ER-negative tumors are associated with early recurrence and poor patient survival, compared with ER-positive tumors.

ER belongs to a large superfamily of nuclear receptors that bind DNA at specific sites to control gene transcription[3]. The ER gene was cloned and sequenced in 1986 by Chambon's group[4]. When estrogen binds to the receptor, the receptor undergoes a conformational change and dimers of receptors recognize specific regulatory DNA sequences upstream of the target genes. The activated receptor, through interactions with coactivator proteins, directs the assembly and stabilization of a preinitiation complex that ultimately conducts the transcription of the target genes[5]. Recently, a second ER, referred to as ERβ, has been identified in the rat[6], mouse[7], and human[8]. The first identified ER has been renamed ERα. The ERβ protein is highly homologous to ERα and specifically binds estrogens with high affinity and, therefore, must influence ERα function. While ERα is

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Abbreviations:
ER, Estrogen receptor; PgR, Progesterone receptor; AF-1/-2, Activation function-1/-2 domain; LBD Ligand binding domain; ERE, Estrogen responsive element; TBP, TATA binding protein; HAT, Histone acetyltransferase; DRIP, Vitamin D receptor interacting proteins; CBP, CREB binding protein; SRC, Steroid receptor coactivator; TIF2, Transcription intermediary factor 2; GRIP1, GR-interacting protein 1; ACTR, hRARβ-stimulatory protein; P/CIP, CBP-interacting protein; RAC3, RAR-interacting protein; AIB1, Amplified in breast cancer; SMRT, Silencing mediator for retinoid and thyroid-hormone receptors; NcoR, Nuclear receptor corepressor; HDAC, Histone deacethylase; SERM, Selective estrogen receptor modulator

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mainly found in estrogen target tissues (uterus, vagina, mammary glands), ERβ has been detected in a large variety of tissues[9].

Nuclear receptor regulation of gene expression involves not only ligand and DNA binding, but also interactions with basal and general transcription factors. A number of cofactors have recently been identified that interact with the ER, and appear to act as bridging factors with the general transcriptional machinery. These proteins are called coactivators if they enhance, and corepressors if they inhibit, the transcriptional activity of nuclear receptors.

Understanding these molecular mechanisms of estrogen action is quite important for physicians to choose appropriate therapy for patients with breast cancer. In this review, the mechanism of estrogen action is discussed in terms of the transcriptional function of ERs and coactivators/corepressors.

Structure and Domain Function of Estrogen Receptor

1) Structure of Estrogen Receptor α/β

The ERα gene is located on chromosome 6q25.1[10]. The ERα protein is a 66 kDa nuclear protein and a member of the steroid hormone receptor superfamily. Its total size including introns is over 140 kb and consists of 8 exons, and its cDNA defines a sequence of 6,322 nucleotides and includes a 1,785 nucleotide coding region which is flanked by untranslated sequences of 232 nucleotides and 4,305 nucleotides at its 5′ and 3′ ends, respectively[11,12] (Fig 1).

Ten years after the discovery of ERα the existence of a second ER, ERβ, was reported[6-8]. That ERα and ERβ are true ER subtypes rather than isoforms emanating from one and the same gene through differential splicing was confirmed by their unique chromosomal locations, with the human ERα gene on chromosome 6q25.1 and the human ERβ gene on 14q22-q24[9]. Since 1996 ERβ from various species or differently sized ERβ isoforms have been reported. N-terminally extended ERβ isoforms, an inframe ligand-binding domain exon insertion, and splice variants at the extreme C-terminus with an exchange of the last exon for previously unknown 3′ exons have been identified[13-16] (Fig 1). Various alternatively spliced forms have also been described for ERα[17-19]. Whether all isofoms or differentially spliced versions of ERα and ERβ, respectively, exist as proteins or whether

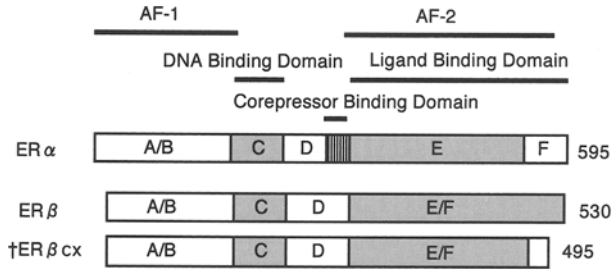

Fig 1. Schematic representation of ERα/β: The structural domains of ER (A-F) are shown with amino acid members below the figure corresponding to domain boundaries. The functional domains of the receptor are indicated in the text. ERβ shows high homology with the DNA binding domain (DBD) (96%) and ligand binding domain (LBD) (58%) of ERα.

they have any significant biological and physiological role warrants further investigation.

Additionally, ERs are subjected to phosphorylation, glycosylation, ubiquitination and other post-transcriptional conformational changes giving rise to a very high degree of molecular heterogeneity. This heterogeneity is not a biochemical artifact[20] and should be investigated further.

2) Domain Function of Estrogen Receptor

All nuclear receptors contain various domains with specific functional properties[3]. The N-terminal A/B domain encodes a ligand-independent activation function (AF-1), a site involved in coactivator binding and transcriptional activation of target genes. The C-domain contains two zinc fingers, which play an important role in DNA sequence-specific bindings and receptor dimerization. The D-domain is a hinge region, which separates these ABC domains from the ligand binding domain (LBD), and contains a nuclear localization signal. The hormone binding domain (HBD) is also involved in receptor dimerization, nuclear translocation and transactivation of target gene expression; another activation function (AF-2) localized within this domain has a ligand-dependent transcription activation function. The C-terminal part of the receptor (end of domain E and F) is involved in ER proteolysis and regulation of transcriptional activity[20,21] (Fig 1).

The estrogen-induced conformational change of ERα allows for stable association with EREs located in the promotor regions of target genes. Coactivators, through their ability to associate with AF-1/-2 sites of the receptors, participate in the transcription process[22,23]. ERs also allow the expression of genes regulated by other response elements to which ERs do not bind directly, such

Table 1. Representative Reports Concerning Estrogen Receptor β Expression in Breast Cancer

	Author	Methods	ERβ type	Characteristics
2002	Tong	RT-PCR	β 1,2,5	no association with invasiveness in breast cancer
	Omoto	RT-PCR, IHC	β 1,2,5	β1 mRNA; normal > cancer, β2 mRNA; cancer > normal, β1 positive; good prognosis, correlation with ERα
2001	Saunders	RT-PCR, IHC, Western blot	β long (59 kd) short (53 kd)	94% (48/51) positive, no association with clinicopathologic factors
	Lazennec	RT-PCR, Western blot		the loss of ERβ expression could be one of the events leading to the development of breast cancer
	Miyoshi	IHC	total β	24% positive, no association with ERα nor PgR
	Skliris	IHC	β-14C8	74% (48/65) positive, association with ERα, PgR and well-differentiated tumors
	Mann	IHC, Western blot	β (1-14 a.a.)	17% (8/47) positive, no association with prognosis, significant predictor of response to anti-hormonal treatment
	Roger	IHC	total β	positive staining; normal > cancer, inversely correlated with Ki-67 in high-grade ductal carcinoma *in situ*
2000	Jarvinen	mRNA ISH, IHC	β (467-485)	ERβ associated with negative axillary node status, low grade, low S-phase fraction, and premenopausal status
	Iwao	real-time RT-PCR	β 1,2,5	mRNA expression; normal > cancer, ERβ mRNA levels and proportions of variants; no correlation with any clinicopathologic factors
1999	Speirs	RT-PCR, IHC	β (1-19 a.a.)	91% (34/37) positive, no association ERα expression
	Fuqua	Western blot	β (1-18 a.a.)	positive almost breast cancer cell line
	Leygue	RT-PCR	β 1,2,5	ERβ2/β1 ratio, ERβ5/β1 ratio; correlated with tumor inflammation and tumor grade

as the AP-1 site that binds the Jun/Fos transcription factors[24]. Assessment of the mechanisms by which ERs increase the activity of Jun/Fos revealed two pathways depending on ER type and ligand; an AF-mediated mechanism for ERα exposed to estrogens and selective estrogen receptor modulators (SERMs), and an AF-independent mechanism for ERβ in the presence of antiestrogens only.

3) Clinical Value of Estrogen Receptor β

ERβ shows high homology with the DNA binding domain (DBD) (96%) and ligand binding domain (LBD) (58%) of ERα[25], and specifically binds estrogen with high affinity (Kd < 0.5 nM)[14,26,27]. The differences in activation of the AP-1 site between ERα and ERβ have been reported using truncated ERβ *in vitro*[28]. Many groups have assessed the expression of ERβ in human breast cancer using reverse transcription-polymerase chain reaction (RT-PCR)[15,29-32] and immunohistochemistry (IHC)[33-37]. These reports are summarized in Table 1.

Many functions have been suggested for ERβ in the breast[38-40], but its role is not fully understood. One group suggested that it contributes to the initiation and progression of chemical carcinogen-induced neoplastic transformation in breast because expression was induced in chemical carcinogen-transformed human breast epithelial cells[41]. Other groups have shown the expression mRNAs of both ERs in normal and malignant human breast tissue by RT-PCR[15,40,42]. Leygue *et al.* demonstrated that those tumors that co-expressed ERα and ERβ were node-positive and tended to be of higher grade[15]. Another study[43] found that ERβ was often co-expressed with ERα and progesterone receptor in breast cancer and that ERβ was significantly associated with negative axillary node status and low tumor grade, while a third study[44] found that expression of ERβ in more than 10% of cancer cells was associated with better survival. In one RT-PCR study there was increased expression of ERβ mRNA in tamoxifen-resistant breast cancer patients[40] and in another study[35] there was decreased expression of ERβ protein in proliferative preinvasive breast tumors. In our experience the wild-type of ERβ (ERβ1) was expressed in the patients with a good prognosis and variant ERβ (ERβcx, β5) was more frequently seen in cancer tissue than in normal tissues[45].

Because of technical diffculties with antibodies and the need for fresh or frozen samples, there have been very few studies in which ERs were measured by Western blotting[46]. From all of the

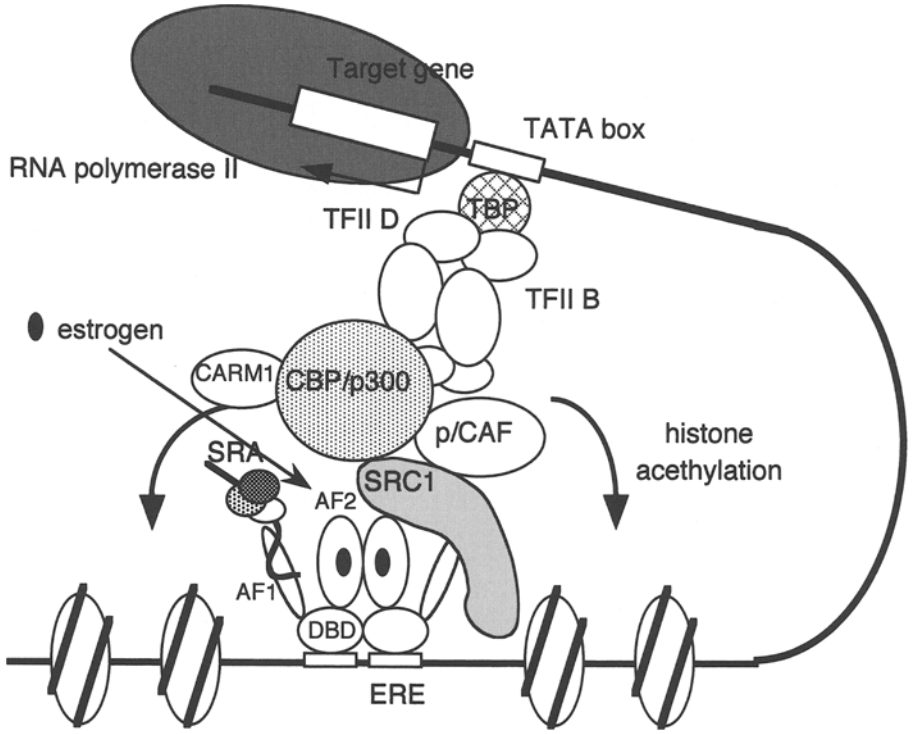

Fig 2. Models for the mechanism of action of estrogens: The estrogen/ER complex activates transcription by utilizing its DBD to bind to specific EREs and its activation functions to recruit coactivators. AF-2 contacts NR boxes (LXXLL) within the p160 molecule and AF-1 binds the p160 C-terminus and CBP/p300. The coactivator complex then enhances transcription by remodeling chromatin and direct interaction with components of the RNA polymerase II transcription complex and basal transcription factors.

data published so far, it is not yet clear how ERβ can be used as a routine prognostic indicator either independently or along with ERα[45, 47].

Regarding the use of ERβ as a potential novel target in the treatment of breast cancer, it is currently unclear whether agonists or antagonists will be useful. The potential role of ERβ splice variants to act as natural antiestrogens and repress ERα function needs further study and evaluation, as this could affect the way we currently interpret ERα immunostaining to guide treatment with antiestrogens[48].

Estrogen Receptor Regulation of Transcriptional Activation

Transcription by RNA polymerase II requires the assembly of basal and general transcription factors. In addition to interacting with each other, these proteins directly contact nuclear receptors[49]; for ER these include transcriptional factor IID (TFIID) and TFIIB. One of the first events in the assembly of the basal transcription complex involves the binding of TFIID, which is composed of the TATA box-binding protein (TBP) and TBP-associated factors (TAFIIs), to a sequence within the TATA box[50]. Following binding of TFIID to the TATA box, binding of TFIIB, RNA polymerase II, and TFIIF are required for the assembly of the minimal transcription initiation complex[51]. TFIIB makes contact with DNA sequences both upstream and downstream of the TATA box, and interacts with TBP, TFIIF, and RNA polymerase II[52], in addition to interacting with ER. Although TFIIB interacts with the ER AF-2 domain, contacts formed with other nuclear receptors involve other domains (Fig 2). The significance of these events remains to be established, but they may be important for regulating nuclear receptor function.

1) Coactivators

Factors that enhance and repress receptor activity directly, namely coactivators and corepressors, now are considered to be important for mediating steroid receptor transcriptional activity[53, 54]. These coactivators interact with nuclear receptors

Fig 3. p160 structure; The amino-terminal region of the SRC-1 family of proteins contains a PAS-A/helix-loop-helix (HLH) domain which is implicated in dimerization and DNA binding. The centrally located receptor-interacting (RID) and activation (AD) domains each contain three leucine-X-X-leucine-leucine (LXXLL) motifs that bind to nuclear receptor AF-2 functions. AD is a strong activation function that colocalizes with the region of CBP/p300 binding. The C-terminus is complex and contains a glutamine rich region and second activation function. This region binds ERα AF-1 and also contains HAT activity, a P/CAF site and a CARM1 binding site.

in a ligand-dependent manner and enhance transcriptional activation by the receptor via histone acetylation/methylation and recruitment of additional cofactors such as CBP/p300[55].

Several coactivators have been identified as a family of related proteins, which consist of steroid receptor coactivator (SRC)-1 (also termed p160/NcoA-1/ERAP-160), TIF-2 (also termed SRC-2/GRIP-1), and AIB1 (also termed SRC-3/ACTR/RAC-3/TRAM-1). The amino-terminal region of the SRC-1 family of proteins contains a PAS-A/helix-loop-helix (HLH) domain, which is a dimerization interface that has been identified in several other nuclear proteins. SRC-1, isolated in a yeast two-hybrid screen, stimulates the transcriptional activation of a number of nuclear receptors, including ER, in transient transfection analyses[56]. TIF-2 was isolated by screening an expression library with ligand-bound ER[57]. Members of the SRC family of coactivators share a common domain structure, with the highly conserved region being the N-terminal βHLH-PAS domain[58]. The βHLH region functions as a DNA-binding or dimerization surface in many transcriptional factors. The centrally located receptor-interacting (RID) and activation (AD) domains each contain three leucine-X-X-leucine-leucine (LXXLL) motifs (Fig 3). The C-terminus contains a glutamine-rich domain as well as a histone acetyltransferase (HAT) domain. Thus, the degree of recruitment of coactivator proteins seems to be a good indicator of the level of transcriptional activity, and is also thought to reflect the conformational change of ER induced by estrogens.

AIB1 is a member of the SRC-1 family of nuclear receptor coactivators, overexpressed in breast and ovarian cancer cell lines as well as in breast cancer biopsies. AIB1 interacts with ERs in a ligand-dependent fashion, and enhances estrogen-dependent transcription. It is suggested that altered AIB1 expression might contribute to the development of steroid-dependent cancers[59]. On the contrary, other investigator reported that overexpression of AIB1 mRNA in breast cancers was correlated with the absence of ER and PgR and positivity for p53 and HER2/*neu*[60]. Although these results are controversial, AIB1 expression likely plays an important role in estrogen action. Our group reported that AIB1 nuclear expression correlated with ERα status, and patients with AIB1 nuclear expression tended to be successfully treated by hormonal therapy[61]. Thus, AIB1 nuclear expression may be a predictive marker of the effectiveness of hormonal treatment for ERα-positive breast cancer.

2) Corepressors

There have been few corepressors reported until now. One corepressor, the silencing mediator of retinoic acid and thyroid hormone receptors (SMRT), was isolated and appeared to interact specifically with unliganded nuclear receptors. Recently, nuclear corepressor (N-CoR) was also isolated[62], but in a slightly different manner, using antiprogestin-occupied PgR hinge/LBD as the "bait" in two hybrid studies. To analyze the effect of corepressors on the activity of mixed antagonists, Jackson *et al.*[62] transfected SMRT with either PgR or ER, and found that both corepressors suppress the agonist activity of antagonists, but that coexpression of the PgR hinge and LBD relieves this suppression. These corepressors have disparate effects on agonist-dependent transcription.

Recent data indicates that, similar to coactivators, corepressors are components of multiprotein complexes. Furthermore, these complexes appear to repress transcription through histone deacetylation. It seems that the unoccupied receptor may interact with a corepressor complex. The ligand may result in the dissociation of the corepressor complex, which could then be replaced by a coactivator complex[63]. However, this model does not predict what the role of ligand antagonists might be, or whether all receptors, such as the ER, are involved in the same type of interactions.

3) Interaction of Cofactors with ER-Transcription

Coactivators and corepressors are found in the same cells and are involved in the regulation of

the transcriptional response to antagonists as well as agonists. It has not been determined whether mixed antagonists will have agonist or antagonist activity. Because ERα/β bind to endogenous estrogen with apparently equal affinity, their ability to activate genes differently based on promoter context and/or cell-type context might be mediated by their ability to assume different conformations upon binding to the same and/or different ligands, thereby attracting different cofactor proteins and resulting in distinct biological activities.

Tamoxifen acts like estrogen in some target tissues but like anti-estrogen in others. Estrogen-like agonist activity in bone was seen to occur simultaneously with estrogen antagonist activity in breast. An unwanted effect of tamoxifen is its estrogen-like action on the endometrium. Raloxifene has estrogen-like actions on bone, lipids and the coagulation system, and estrogen antagonist effects on the breast and uterus[64]. These compounds are called as selective estrogen receptor modulators (SERMs). Smith *et al.*[65] emphasized that the relative expression of coactivators and corepressors can modulate SERMs' regulation of ER transcriptional activity. It is suggested that they could contribute to the tissue-specific ability of mixed antieestrogens to activate or inhibit ER-mediated gene expression.

Conclusion

Estrogen action is not as simple as thought previously, and is likely to be influenced by the existence of ERβ, its variants and interaction with cofactors. Improved understanding of ER may follow from the discovery of these new proteins, although precise mechanisms remain to be determined.

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