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Hormone Receptors in Breast Cancer

edited by
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Hormone Receptors in Breast Cancer

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Preface

Since radiolabeled estrogens were first observed in the early 1960s to be preferentially concentrated in estrogen target organs — observations that gave rise to the concept of an "estrogen receptor (ER)," it has become clear that many human breast cancers are dependent on estrogen for their growth. Estrogens' mitogenic effects are mediated through ERs α and β, which is the therapeutic target for hormonal therapies. The purpose of the book is to provide an up-to-date resource on the role of hormone receptors in breast cancer. Since approximately 1 of 8 women in the United States and 1 of 12 women in European countries are affected by breast cancer, there has been a massive effort to understand the mechanisms of hormone action. This explosion of information has led to exciting new areas of gene-specific targeting of the disease and breast cancer prevention. Paradigm shifts in treatment options and sequencing of hormonal therapies have recently occurred in breast cancer management, necessitating close cooperation and communication between translational scientists and physicians. This book is focused on providing this communication.

The 11 chapters of this book examine many aspects of hormone receptors, including basic and translational information on the molecular biology of the ERs, the utility of the ERs for the clinical management of breast cancer as it relates to assessing clinical outcome and selecting appropriate therapy, a review on the biology of ER and its role in the diagnosis and treatment of breast cancer, the importance of non-nuclear ER expression in breast cancer and other endocrine target tissues, the importance of ERs α and β inaggressive breast tumors of African-American women, cross-talk between BRCA1 and ER, and a detailed discussion of the role of ER in metastasis of breast cancer. We have included the latest clinical information on sequencing of hormonal therapies in breast cancer, the use of biomarkers in presurgical neoadjuvant trials, the problem of clinical hormone resistance, strategies to utilize hormonal prevention in high-risk patients, and the elucidation of hormone-responsive phenotypes as defined by state-of-the-art molecular expression profiling.

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Houston, TX Suzanne A.W. Fuqua

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Hormone Action and Clinical Significance of the Estrogen Receptor α

Matthew H. Herynk, Jennifer Selever, Janagi Thirugnanasampanthan, Yukun Cui, and Suzanne A.W. Fuqua

Clinical Relevance of ERα

ERα expression in breast cancer has many functions, including tumor growth enhancement, serving as an efficacious therapeutic target, and being a prognostic and predictive factor. Thus, a great deal of research has attempted to delineate the roles of ERα in human breast cancer. It has long been known that approximately two-thirds of human breast cancers express ERα and that estrogen drives tumor growth through its receptor. Because of its role in tumor growth, the ERα signaling pathway is a highly useful axis for hormonal manipulation. Several types of drugs have been developed for this purpose, including SERMs (selective estrogen receptor modulators), aromatase inhibitors, and pure antagonists. These agents will be discussed in greater detail in subsequent chapters.

Several assays have been developed for the detection of ERα in breast cancer patients. The dextran-coated charcoal (DCC) assay utilizes radiolabeled steroid ligand to detect ERα (reviewed in [1]). Since cutoff values for defining ERα status vary among different laboratories using this assay, there can be ambiguity in the definition of certain tumors. However, using this assay can be advantageous in that it can provide reproducible quantitation of ERα under proper conditions. Another method that detects ERα is the use of antibodies directed against specific epitopes of the receptor [2, 3]. This method also has a disadvantage in that there are procedural variations among different laboratories [4]. However, if this assay can be standardized, then the subjective nature of the assay will not pose a significant problem. The detection of ERα in patients can be carried out in different ways with assays that have problematic disadvantages but still serve important roles in the treatment of these patients.

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ERα has utility as both a prognostic and a predictive factor. The former indicates the inherent biologic aggressiveness of the disease if left untreated, whereas the latter indicates the likelihood of a response to treatment. In terms of prognostic factors, positive ERα expression correlates with a better outcome [5]. However, prognostic evaluations can change at the time of first relapse, and this is partly based on ERα status at the time of diagnosis as well as the time interval between primary treatment and relapse [6]. ERα expression also correlates with other factors indicative of better prognosis such as greater differentiation, diploidy, lower number of dividing cells, and lower mutation rates of breast cancer-associated genes.

As a predictive factor, ERα expression generally reflects that the patient is likely to respond to hormonal therapy, including second-line therapies [7]. On the other hand, lack of ERα expression predicts that the patient may not respond to hormone-based therapies [8]. The intensity of ERα expression also directly correlates with the degree of responsiveness to hormonal manipulation. While the ERα status of metastases may not always be consistent with that of the primary tumor, the ERα status of metastases is more predictive of response to hormonal therapy [9]. Thus, the ERα status of a patient is useful in determining the most appropriate method of treatment.

ERα Activation Domains

Transcription of estrogen-responsive genes is stimulated predominantly via two transactivation domains, activation function 1 (AF-1) at the amino terminus and activation function 2 (AF-2) at the carboxyl terminus of ERα (Fig. 1). These two domains span large areas of the receptor, and both are necessary for maximal ERα transcriptional activity. AF-1 and AF-2 bind various receptor co-regulatory proteins leading to different transcriptional outcomes (for a

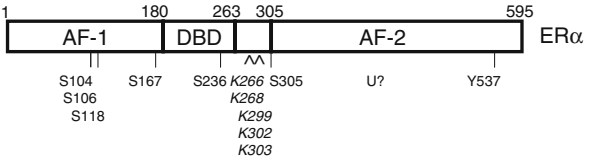

Fig. 1 ERα is divided into four important functional domains: the amino-terminal transactivation domain containing the AF-1 motif spanning amino acids 1–180, the DNA-binding domain spanning amino acids 181–263, the hinge domain spanning amino acids 264–305, and the ligand-binding domain containing the AF-2 motif spanning amino acids 306–595. AF-2a is located between amino acids 282 and 351 (not shown). The post-translational modified residues are depicted in the figure: phosphorylated residues are marked with a vertical line, ^^ indicates the region containing the known acetylation and/or sumoylation sites. Ubiquitination is depicted as a black U? because the exact residue within the ligand-binding domain is not known

complete review, see Hall and McDonnell [10]). Transcription can also be stimulated to a lesser extent by a less-described transactivation domain referred to as AF-2a [11], and the significance of this domain is less understood.

AF-1 and AF-2 each function in distinct ways, and depending on the nature of the cell and the promoter type, one or both can affect signaling. AF-1 functions in a ligand-independent manner to exert transcriptional activity [12]. AF-1 can be differentially phosphorylated by a number of important signaling molecules, such as AKT2 (also known as protein kinase B or PKB) and Erk1/2 (extracellular regulated kinase 1/2), resulting in diverse responses to SERMs. For example, phosphorylation of serine 167 by AKT2 leads to insensitivity to tamoxifen, whereas phosphorylation of serine 118 by Erk1/2 leads to sensitivity to tamoxifen [12]. AF-2, on the other hand, stimulates transcription in a ligand-dependent manner [13]. Thus, transcription of ERα-regulated genes depends on these two main transactivation domains which function in a highly regulated manner.

Crystal Structure of ERα

To date, the three-dimensional structure of full-length ERα has not yet been solved. However, due to ERα's similarity with other nuclear hormone receptors and molecular modeling, we can infer a broad model of ERα structure. Crystallization efforts have focused on the DNA-binding and the ligand-binding domains, which have revealed the mechanism of action for several ERα agonists as well as antagonists. Estradiol binds ERα within a carboxy-terminal hydrophobic pocket, and upon ligand binding, helix 12 repositions itself over this pocket [14]. This new confirmation stabilizes helix 12 in the receptor, allowing it to recruit transcriptional receptor coactivators [15]. The large side chains of the antagonists tamoxifen, faslodex, or raloxifene prevent helix 12 from adopting an agonist-bound confirmation, thus antagonizing coactivator binding to the receptor. In contrast, compounds without large side chains, such as genistein or 5,11-*cis*-diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol (THC), inhibit ER activation by stabilizing nonproductive conformations of the ligand-binding pocket [16, 17]. Recently, a number of groups have utilized the crystal structure and molecular modeling in an attempt to identify better, more specific drugs for disrupting estrogen receptor signaling [18, 19], an effort which is currently underway.

Formation of the Transcriptome

Stimulation of transcription by ERα occurs via a number of distinct molecular events in the nucleus. ERα homo- or heterodimerizes with other nuclear receptors such as estrogen receptor β (ERβ) or androgen receptor (AR) and binds,

via the DNA-binding domain (DBD), to estrogen response elements (EREs) located on the promoters of estrogen-responsive genes [20]. This allows interaction with other components of the transcription factor complex, including receptor co-regulatory proteins which will be discussed in the following sections of this chapter and the basal transcription machinery (for a complete review, see Klein and Hitpass [21]). ERα also has the ability to dimerize with proteins such as stimulating protein 1 (Sp1) and activating protein 1 (AP1) and affects transcription through the binding of these proteins to non-ERE-containing sites [22, 23]. Thus, the regulation of ERα transcriptional activity is complex and involves a myriad of proteins from those specific to nuclear hormone receptors to components of the basal transcription machinery.

Estrogen Receptor Cofactors

It was well known that ER's function is tissue specific and ligand dependent, indicating that ERα alone could not account for its diversified functions, thus requiring additional signaling factors [24]. This concept led to the discovery of the first ER cofactors in 1995 [25]. Using techniques such as yeast two-hybrid and protein library screening, a growing body of proteins and RNAs affecting ERα transcriptional activity, either directly and/or indirectly, have been identified [26]. To date, the Nuclear Receptor Signaling Atlas (NURSA) website (www.nursa.org) lists over 170 known nuclear cofactors. These factors are generally categorized as coactivators (enhance ER transcriptional activity) or corepressors (reduce ER transcriptional activity). In general, these cofactors do not bind to DNA directly but rather through association with sequence-specific DNA-binding proteins, including but not limited to nuclear receptors. Upon recruitment to the promoter complex, these factors may affect transcription directly or via recruiting additional cofactors. In this section, we will focus on the fundamentals of ER cofactors and some of the latest findings in this field.

Coactivators

The first subcloned steroid receptor coactivator, SRC-1 or NcoA1, enhanced the transcriptional activity of ERα when cells were treated with estrogen [25]. Additionally, SRC-1 also has been shown to be involved in ligand-independent activation of ERα. The second member of this coactivator family, SRC-2, also known as GRIP1 in mice or TIF2 in human tissues, can only activate ERα transcriptional activity in the presence of estrogen [27, 28]. Like SRC-1, SRC-3 (also called RAC3, p/CIP, AIB1, or ACTR) activated both ligand-dependent and ligand-independent ERα transcriptional activity [29, 30]. Sequence analysis of these family members elucidated an LxxLL nuclear receptor-binding motif (the so-called NR box, L = leucine, isoleucine, or other large hydrophobic amino

acid residues) that is conserved among other coactivators such as CBP/p300 and TRAP220 [31]. While the coactivators mentioned above act in a ligand-dependent manner, additional coactivators directly interact with the ligand-independent AF1 domain (e. g., p68 RNA helicase) [32], hinge domain (e.g., PGC-1α) [33], or the DBD (e.g., Ciz1) [34]. In addition to the coactivators that directly interact with ERα, additional cofactors such as protein arginine methyl transferase, CARM1, and PRMT2 [35] affect ER transcriptional activity through indirect association with ERα mediated by the SRC family of coactivators. Coactivator regulation of ERα is a complex process that leads to enhanced transcriptional activity in both a ligand-dependent and -independent manner.

Corepressors

Compared with coactivators, there are far fewer corepressors identified so far. Corepressors inhibit transcription of ERα target genes through directly or indirectly interacting with steroid receptors. Sequence analysis between nuclear corepressors, including NcoR1 and SMRT, identified an LxxxI/HIxxxI/L conserved nuclear corepressor-binding motif (the so-called CoNR box), which has been demonstrated to mediate either ligand-independent or anti-estrogen-stimulated association with the AF2 domain of ERα [36]. Similar to coactivators, corepressors have been shown to interact with other domains of ERα, including the AF1 (HDAC4) [37] and hinge domains (SAFB and MTA2) [38, 39]. It has been reported that overexpression of the nuclear corepressors NCoR and SMRT enhances tamoxifen antagonist activity without interfering with estrogen-stimulated gene expression [40]. This is consistent with a later discovery that reduced levels of NCoR correlate with hormone resistance in breast cancer cells [41]. Furthermore, we have recently shown that overexpression of the MTA2 corepressor resulted in hormone-independent and anti-estrogen-resistant cell growth [39]. These findings, in combination with many additional corepressor studies, suggest that corepressors may be involved in the processes of anti-estrogen function and the development of resistance as well.

Transcriptional Cofactor or Transcriptional Factor?

Some ERα cofactors also contain specific DNA-binding domains (e.g., NcoR, MTA1/2, or Ciz1), raising the possibility that they may affect gene transcription directly. One study demonstrated that MTA1, an ERα corepressor, could activate breast cancer amplified sequence 3 (BCAS3) promoter activity, probably through direct interaction and recruitment of the p300 coactivator [42]. To date, the majority of studies have analyzed the ability of these proteins to alter transcriptional activity as cofactors, however, it is clear that some may directly effect the transcriptional activity of their target genes.

Chromatin Remodeling and the Cyclical Occupancy of ERα Cofactors

Acetylation and/or methylation of histones promote decondensation of chromatin structure, thereby favoring gene transcription. In contrast, deacetylation and/or demethylation lead to chromatin condensation, thus abrogating transcription. A large number of steroid receptor cofactors are implicated in these chromatin remodeling processes by either directly modifying histones (e.g., CBP/p300, P/CAF, SRC-1, CARM1, and HDAC1) or indirectly deacetylating histones through interaction with histone deacetylases (e.g., MTA1 and 2 or SIN3; for a review, see [26]). The importance of these co-regulatory proteins in controlling gene activity is further emphasized by the findings that these cofactors or cofactor complexes are recruited to estrogen-responsive promoters in an ordered, cyclical manner. There is some evidence suggesting that histone premodification is essential to direct the recruitment of individual cofactors. For example, the recruitment of histone methyl transferase PRMT1 to the pS2 promoter requires the SET (patient SE translocation) protein [43], which demethylates histone H4 arginine 3 and provides a target for the histone methyl transferase activity of PRMT1. In addition, ERα and cofactors are also modified during transcriptional activation. These modifications may represent a signal to release these cofactors from the promoter. For example, acetylation of ERα results from agonist-induced interactions with certain coactivators that leads to decreased transcriptional activity [44]. SRC-3, an ERα coactivator with intrinsic histone acetyl transferase activity, loses its coactivator ability upon acetylation by p300 [45]. In addition, the presence of SRC-3 enhances ERα recruitment to the promoter, however, SRC-3 also helps to direct agonist-induced ERα degradation [46]. Collectively, these studies suggest that a common physiologic network exists controlling both the "ON" and "OFF" signals for ERα action.

Alternative Exons in the 5′UTR

One mechanism of regulating ERα protein expression is through differential usage of upstream untranslated exons. As many as eight exons have been identified, and this review will use the nomenclature suggested by Flouriot et al. [47], as modified by Kos et al. [48]. ERα exon 1 contains an acceptor splice site at +163 permitting the splicing of several different exons encoding various 5′UTRs. At least seven different promoters have been described that show relative tissue specificity (for a complete review, see Kos et al. [48]). Promoter A in exon 1 is the most common promoter expressed in tissues and cell lines. Promoter C was first described in 1991 [49], but a longer version of promoter C was described in subsequent years [50]. Additional exons A–E have been described and have also been shown to affect reporter gene expression

levels [51]. One hypothesis is that the numerous AUG start codons found in the ERα 5′UTRs inhibit scanning ribosomes from reaching the start codon, thereby reducing ERα protein expression [51]. Promoters within 2 kilobase pairs of the acceptor splice site (generally A, B, and C) are utilized in cell lines and tissues that express high levels of ERα. The more distal promoters, E and F, are found in tissues where ERα expression is less abundant, such as the liver and human osteoblasts [52]. Additionally, promoters T1 and T2 are expressed predominately in the testis and epididymis [53]. While these alternative promoters can account for the tissue-specific expression of ERα, they may also play a role in the regulation of ERα levels. In vitro studies analyzing promoter usage have demonstrated increased use of promoter A in breast cancer cells when compared with normal mammary epithelium [54]. Additionally, in breast tumor cell lines, Weigel et al. have shown activation of promoters not normally activated in breast epithelium [55].

Epigenetic and Post-translational Regulation of ERα

Epigenetic information on the genome provides directions on when, where, and how the genetic information should be used. Post-translational regulation of nuclear steroid receptors is an exciting field of study, which is comprised of events encompassing methylation, phosphorylation, acetylation, ubiquitination, and most recently protein sumoylation [56]. Post-translational regulation of the nuclear receptor family is dynamic, with member proteins being differentially affected by modifications either singly or in combination, thereby influencing receptor conformation, ligand binding, DNA binding, and coactivator interactions [57]. It has been postulated that post-translational modifications of ERα play a key role in the regulation of its functions.

Methylation

DNA methylation is one of the most important forms of post-translational modifications in which a methyl group is covalently bonded to the 5-carbon on the cytosine base by DNA methyltransferases [58]. Methylation of the estrogen receptor occurs on cytosine within the CpG islands associated with the promoter [59]. CpG islands are regions close to the promoter of genes that contain cytosine (C) and guanine (G) residues at a greater than 50% frequency. Hypermethylation of the ERα promoter silences the gene by repressing transcription and in some cases is associated with malignant transformation of cells, whereas hypomethylation of ERα is associated with gene activation indicating an inverse relationship between promoter methylation and transcriptional activity [60].

Acetylation

ERα is known to be acetylated on lysines, and the conserved acetylated amino acids in ERα are lysines (K) 266, K268, K299, K302, and K303 (Fig. 1). The acetylation of K266 and K268 has opposite effects compared to the acetylation of K302 and K303. K266 and K268 induce DNA-binding and ligand-dependent activation, whereas K302 and K303 inhibit ERα ligand-dependent activation [61]. Our recent experiments using ERα deletion constructs suggest that the phosphorylation status of S305 within the hinge domain of ERα coordinately regulates the acetylation of lysines 302 and 303 [44]. Although mass spectrometry has previously identified these same two lysines as sites of acetylations [62], Kim et al. have recently shown that these two lysine residues may not be acetylated in the full-length protein, although these results need to be validated [63]. Thus, the hinge domain of the receptor is replete with post-translational modifications having the potential for important functional consequences.

Phosphorylation

ERα is phosphorylated on multiple residues and a complete list of phosphorylation sites and their respective kinases is found in Table 1. The diversity of kinases and responses to phosphorylation illustrate the range of effector pathways that are utilized in the complex regulation of ERα or amplification of its signal. For instance, phosphorylation of S305 ERα can be mediated by both the protein kinase A (PKA) and p21-activated kinase 1 (PAK-1) signaling networks [44, 64, 65]. PKA-mediated phosphorylation of ERα does not alter its DNA-binding abilities but instead enhances ligand-binding affinity [64]. Additionally,

Table 1 ERα phosphorylation sites

Amino acid	Modification	Effect	References
S104	Phosphorylation by Cyclin A-CDK	Enhanced transcriptional activity	[86]
S106	Phosphorylation by Cyclin A-CDK	Enhanced transcriptional activity	[87]
S118	Phosphorylation by MAPK	Enhanced transcriptional activity	[88]
S167	Phosphorylation by Akt2	Enhanced transcriptional activity	[89]
S236	Phosphorylation by PKA	Enhanced ER dimerization and DNA binding	[64, 90]
S305	Phosphorylation by PKA or PAK1	Enhanced ligand-binding affinity, tamoxifen resistance	[64, 65]
Y537	Phosphorylation by Src kinase	Enhanced transcriptional activity	[74, 90, 91]

the PKA-mediated phosphorylation of S305 allows tamoxifen to act as an agonist of ERα, and PKA is known to be frequently overexpressed in breast tumors [44, 64, 66]. Clearly, ERα phosphorylation has a variety of effects in the physiologic actions of ERα and is an emerging area of study.

Ubiquitination

The tight regulation of ERα function is partially due to the ubiquitin–proteasome pathway regulating the levels of protein and the receptor's response to ligand [67]. Ubiquitination is the reversible covalent bonding of the highly conserved 76 amino acid ubiquitin to lysine residues on target proteins. Upon ligand binding to ERα, ubiquitin binds the receptor on lysine residues within the AD core region of the ligand-binding domain inducing the protein to undergo ubiquitin-mediated proteasomal degradation. This has been shown to be an important step in the transactivation of ERα, and transactivation can be inhibited by proteasome inhibitors [67–69]. While ubiquitination and proteasomal degradation are important mechanisms of regulating ERα protein levels, the ubiquitination of ERα may play an important role in the transactivation of ERα.

Sumoylation

SUMO-1, a small ubiquitin-like modifier, covalently and reversibly bonds to target proteins with the assistance of conjugating enzymes. Recent experiments by Sentis et al. reveal that ligand-dependent sumoylation occurs on lysine residues within the hinge domain of ERα and that sumoylation regulates transcriptional activity of this nuclear receptor [70]. The same lysine residues that are acetylated can also be sumoylated including K266, K268, K302, and K303 (Fig. 1), suggesting a tight regulatory pathway governing the occupation of these residues and subsequent downstream effects.

ER*α* Mutations

A number of mutations and polymorphisms have been identified in ERα from numerous diseases including psychiatric diseases, precocious puberty, and many cancers (for a complete review, see Herynk and Fuqua [2]). While over 20 different mutations have been identified, rarely has any independent mutation been found in more than one sample, in contrast are the A86V, K303R, and Y537S/N ERα mutations. The A86V mutation was found in 12% of the breast cancer specimens analyzed and has been associated with lower levels of ERα protein and spontaneous abortions [71, 72]. The tyrosine at 537 is the only site

that has been found to be mutated to two different residues, serine and asparagine [73, 74]. This residue lies at the amino-cap of H12, therefore it is not surprising that mutations at this site would significantly affect the activity of ERα [74–76].

We originally identified the K303R ERα mutation in 34% of premalignant breast hyperplasias [77]. More recently, utilizing a sensitive primer extension sequencing technique, we have demonstrated that this mutation was present in invasive breast cancer specimens and the presence of the K303R ERα mutation correlated with older age, larger tumor size, and lymph node-positive disease [78]. In comparison, Conway et al. have identified this mutation in only 5.7% of breast cancers utilizing a different gel electrophoresis detection method [79]. Therefore, we propose that while the absolute frequency of this mutation remains to be validated, it is clearly present in a significant number of breast cancer samples.

Analysis of the K303R ERα mutation has shown that this mutated receptor exhibits hypersensitive growth to low concentrations of estrogen [77]. Additionally, the mutated ERα has increased binding to the coactivator TIF2, and the corepressor MTA2 was unable to repress the activity of the mutant receptor [39]. The presence of an arginine at the 303 position removes a key acetylation site and allows ERα to be more highly phosphorylated by PKA signaling [44]. Collectively, these data indicate that this residue plays a key role in ERα signaling, and whether or not this mutation will affect other epigenetic regulatory mechanisms of ERα remains to be determined. While identification of mutations has been rare, the role of mutations in breast cancer may be underappreciated, and is an underexplored field, which might effect future breast cancer therapeutic decisions with hormone-based therapies. The use of alternative sequencing strategies, employing accurate primer extension sequencing to replace standard dye terminator approaches, may be warranted in this regard.

Mouse Modeling of ERα

Mice lacking ERα expression are viable and demonstrate a wide range of phenotypes altering normal functions including effects on sexual organs and function, bone, brain, and cardiovascular, to name a few (for a complete review, see Couse and Korach [80]). Additionally, mice deficient in ERα exhibit normal early development of mammary glands, however, these glands never develop beyond the newborn stage [81]. In contrast, ERβ knockout (KO) mice develop normal ductal structures with reduced side branching [82], thereby demonstrating that ERα has a central role and is the predominant receptor involved in mammary gland development.

While ERα has a vital role in normal mammary gland development, aberrant ERα signaling has been shown to function in the development of preneoplastic mammary lesions and breast cancer development and progression.

Ninety-five percent of mice conditionally overexpressing wild-type ERα displayed abnormal ductal structures at 4 months of age [83]. While 52 and 36% of 4-month-old virgin mice had lobular and ductal hyperplasias, respectively, 21% of 4-month-old virgin mice displayed DCIS [84]. Earlier, the same group reported 37% of mice overexpressing T antigen – ERα had developed adenocarcinomas by 11 months of age [83]. While exogenous estrogen stimulation did not alter the incidence of hyperplasias or DCIS in the wild-type receptor system [84], aromatase overexpression was sufficient to cause preneoplastic changes within the mammary gland [85]. These data demonstrate that increased ERα can lead to preneoplastic changes contributing to mammary tumorigenesis.

Conclusions

The role of ERα in the human breast has been extensively studied over the past several decades. The development of transgenic mice overexpressing or lacking ERα expression has greatly aided in defining the roles of ERα in both normal mammary gland development and breast cancer development and progression. Laboratory studies have clearly shown that ERα is a highly regulated molecule demonstrating complex, multilayered regulation including organ-specific alternate promoters, epigenetics, cofactor levels and interactions, and a highly regulated degradation. Additionally, disruption of this complex regulation can drastically effect the physiologic regulation and homeostasis of the body leading to a variety of disease states. The presence of ERα in human breast cancer has proven to be clinically useful, both as a prognostic indicator to suggest the inherent biologic aggressiveness of the disease and as a predictive factor to guide therapies for the treatment of this widespread disease. Clearly, ERα has proven to be an important molecule in breast cancer and will further demonstrate its important roles in the future.

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Role of ERβ in Clinical Breast Cancer

Valerie Speirs and Abeer M. Shaaban

Introduction

A second estrogen receptor (ER), ERβ, was cloned from rat in 1996 by Jan-Ake Gustafsson [1] and soon afterward human and murine isoforms were identified [2, 3]. Although unexpected, the discovery of ERβ was not totally surprising as other members of the steroid receptor superfamily, to which ER belongs, had multiple family members, and up to this point ER was an exception in this regard. As shown in Fig. 1, ERβ is structurally and genetically distinct from its sib ERα: mature full-length ERα is 595 amino acids and located on chromosome 6q while ERβ comprises 530 amino acids and resides on chromosome 14q22-25 [4, 5]. Because of the recognized importance of ERα in the breast, it follows that ERβ may also fulfill an important role. In this chapter we review the current understanding of ERβ in clinical breast cancer and discuss the potential role it may play in the future management of this disease.

ERβ Isoforms and Their Function

ERβ exists as five distinct isoforms, termed ERβ1–5, each distinguished by a unique exon 8 sequence. Moreover, in breast cancer, these variants are usually found in greater abundance than wtERβ (ERβ1) at least in terms of RNA expression [6–8]. Ethnic differences in expression of ERβ isoforms have been reported with ERβ1 and in particular, ERβ5 expressed at significantly higher levels in African Americans compared to Caucasians [9]. Tumors from African

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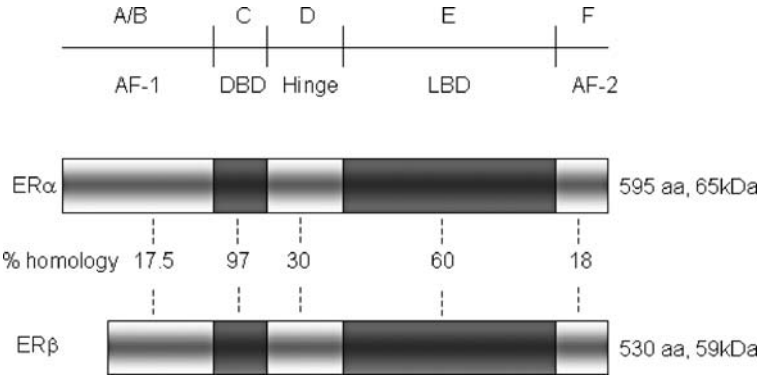

Fig. 1 Schematic illustration of human ERα and ERβ

Americans are often ERα negative with poorer survival [10]; so the high expression of ERβ isoforms suggests that these patients may well benefit from specific ERβ-targeted therapies (discussed later). These isoforms are schematically illustrated in Fig. 2 and described in detail below.

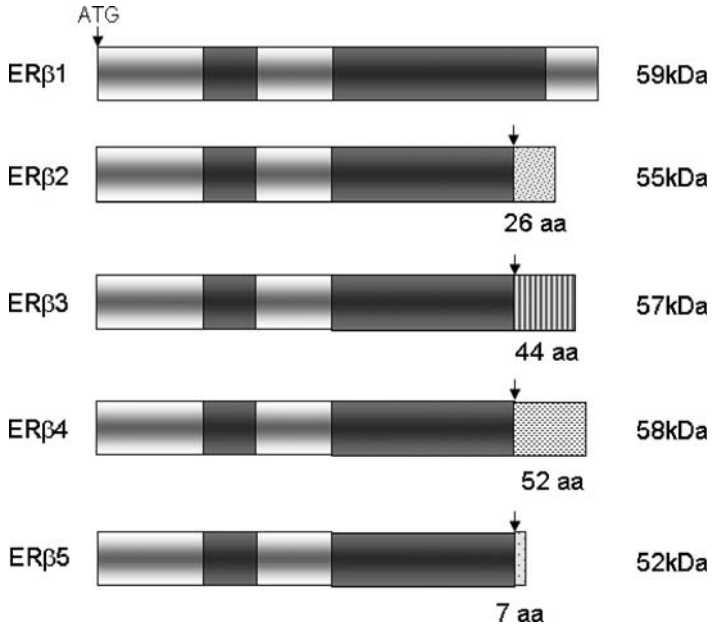

Fig. 2 Structure of ERβ1–5. All five isoforms are identical in structure through exons 1–7 but have a unique exon 8 sequence