Estrogen-related receptor α as a therapeutic target in cancer

R A Stein and D P McDonnell

Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina 27710, USA

Abstract

The orphan receptor estrogen-related receptor α (ERRα) is a member of the nuclear receptor superfamily of ligand-regulated transcription factors. This protein is structurally most related to the canonical estrogen receptor and has been shown to modulate estrogen signaling in some contexts. These observations have heightened interest in ERRα as a therapeutic target in both breast and ovarian cancer and in other estrogenopathies. This review details our present understanding of ERRα action with a view to highlight specific aspects of its signal-transduction pathway in breast cancer that may be amenable to pharmaceutical manipulation.

Introduction

Drugs that function by inhibiting ERα signaling have been and will continue to be an important part of pharmacotherapy in breast cancer. Foremost among these therapies are selective estrogen receptor modulators (SERMs), compounds which antagonize the mitogenic actions of estrogens in breast tissue but which function as estrogens in bone and the cardiovascular system. Of late, aromatase inhibitors have proven to be more efficacious than SERMs in certain circumstances. Although these two classes of drugs are extremely effective, it is clear that there remains a need for agents that target estrogen signaling by alternate mechanisms. Of significance in this regard is the observation that SERMs are clinically beneficial for only 50–80% of ERα-positive breast cancer patients. An additional limitation of anti-estrogen treatment is that many patients, particularly those with metastatic disease, rapidly develop resistance (Robertson et al. 1996). Indeed, in some cases it has been observed that tumors in which tamoxifen initially functions as an antagonist can ‘switch’ to recognizing this drug as an agonist. Not surprisingly, resistance to aromatase inhibitors has also emerged as a significant issue in the clinic. Resistance to anti-estrogens, SERMs, and aromatase inhibitors is multifactorial in cause, and an array of potential mechanisms to explain this phenomenon has emerged. Leading hypotheses include changes in drug metabolism, receptor mutations, inappropriate activation of growth and survival pathways, and alterations in the activity of the ERα-signaling pathway. These activities underscore the need for novel strategies to inhibit estrogen signaling in breast cancer. Our investigation into the estrogen-related receptors (ERRs) in breast cancer was prompted in part by the hypothesis that these orphan nuclear receptors may play a role in both de novo and acquired resistance to anti-hormonal agents. ERR expression, structure, and function suggest that these nuclear receptors may be intimately linked to estrogen signaling. This review will discuss the relationship between the ERRα pathway and classical estrogen signaling and evaluate the present evidence supporting a role for ERRα in breast cancer. Finally, we will identify key obstacles and questions that must be resolved if ERRα is to be utilized as a therapeutic target in breast cancer.

The estrogen receptor-signaling pathway

In the absence of hormone, estrogen receptor (ER) resides in either the cytoplasm or nucleus of the target cells associated with a large heatshock protein–chaperone complex that maintains the receptor in a transcriptionally inactive form. Upon binding a ligand, the receptor undergoes a conformational change leading
to its displacement from the chaperone complex and subsequent dimerization. In this biochemical state, the receptor can interact with target gene promoters in a direct manner through specific estrogen-response elements (EREs) or indirectly through interactions with proteins associated with the promoter. The DNA-bound receptor then nucleates the assembly of a large multiprotein co-activator complex that serves to remodel local chromatin structure, stabilize the pre-initiation complex and enhance transcriptional output. It is important to note that there are two genetically distinct ERs in target cells: ERα and ERβ. Additional complexity is introduced into the model described above as these receptor subtypes can form both homo- and heterodimeric complexes, each of which manifests distinct functional activities. Most of what we know about estrogen signaling in the breast comes from studies of ERα action. The specific roles of ERβ and the functional consequences of its expression in normal and malignant breast remain to be determined.

Until relatively recently, it was believed that estrogen binding was the only biochemical event that enabled the conversion of ER from an inactive to a transcriptionally active form in cells. However, several seminal studies published by the O’Malley group indicated that ER transcriptional activity can be activated in a ligand-independent manner by impinging signaling pathways. It is now clear that the transcriptional activity of ERα and other nuclear receptors can be altered by post-translational receptor modifications, the presence of non-hormone receptor ligands, and the particular complement of other nuclear receptors active at a given time. Since many cofactors (both co-activators and co-repressors) interact with multiple nuclear receptors and may be in limited supply, cofactor availability has also been suggested to play a role in determining the cell-specific activity of ERα (McKenna et al. 1999). The activity of ERα can also be regulated by changes in the phosphorylation state of the receptor downstream of growth factor stimulation. Increases in phosphorylation can enhance both ligand-independent and ligand-dependent activity in vitro (Kato et al. 1995). ERα phosphorylation has recently been implicated in tamoxifen resistance, which suggests new methods for predicting the occurrence of refractory tumors as well as for use in their treatment (Cui et al. 2006). Each of the signaling pathways impinging on ERα presents a potential avenue through which other nuclear receptors including the ERRs may influence ERα. More generally, the entire complicated web of ERα regulation introduces numerous opportunities for the pharmacologic regulation of its activity.

Overview of estrogen-related receptors

In 1988, Giguere et al. (1988) cloned the first orphan receptors, ERRα and ERRβ, using the DNA-binding domain (DBD) of ERα as a probe to screen recombinant DNA libraries. A third isoform, ERRγ, was later identified by Eudy et al. (1998). Sequence analysis reveals that the ERRs and the classical estrogen receptors share a high degree of homology within their DNA and ligand-binding domains (LBDs) (Laudet et al. 1992). In particular, ERRα shares with ERα approximately 68% sequence identity within the DBD and 33% within the LBD (Fig. 1). This relationship

Figure 1 Amino acid identity between the ERs and ERRs within the DNA-binding domain (DBD) and the ligand-binding domain (LBD). Although several alternative splice variants of the ERRs have been identified, the total number of amino acids in the most common variant is shown above (Adapted from Laudet 1997, reproduced with permission from the Society for Endocrinology).
provides a structural basis both for the conserved nature of DNA binding and the divergence in hormone binding between these two receptors. Each characteristic has important consequences on the functional relationship between ERRα and ERα.

The finding that ERRα and ERα bind to similar DNA-response elements in target genes is not surprising in light of the observation that the P-box region of the DNA-binding domains (which determines DNA specificity) in both the ERs and ERRs is highly conserved (Laudet et al. 1992). DNA-binding studies performed in vitro indicate that ERα and ERRα may have slightly different DNA-binding preferences. While ERα binds preferentially to the sequence AGGTCAnnnTGACCT, termed an estrogen-response element (ERE), ERRα binds with the highest affinity to the extended half-site sequence TnAAGGTCA, termed an estrogen-related response element (ERRE; Sladek et al. 1997, Vanacker et al. 1999a,b). When initially discovered, this divergence in DNA-binding preference in vitro suggested that these receptors might not display significant DNA-binding cross-reactivity. However, recently, ERα has been found to activate many of its target genes through imperfect EREs composed of multiple half-sites. This supports the hypothesis that many EREs may function as ERREs in vivo, and conversely, that a subset of ERREs may function as EREs (Vanacker et al. 1999a,b).

The transcriptional activity of several endogenous genes is regulated by both ERRα and ERα, including the pS2 breast cancer marker, osteopontin and lactoferrin (Yang et al. 1996, Vanacker et al. 1999a,b, Lu et al. 2001, Kraus et al. 2002). Early evidence that ERRα can activate ERα target genes in the absence of estrogen suggested that the ERRs might drive estrogen-independent breast tumor growth. These findings not only generated considerable interest in elucidating the role of ERRα in the development and maintenance of tumors, but also raised the possibility that ERRα antagonists might be of benefit in treating breast cancer. As yet, this hypothesis has not been formally tested. However, it has been demonstrated that in some contexts, ERRα can repress ligand-activated ERα-dependent transcriptional activity. Given the similarity in the DNA-binding specificity of these two receptors, it is possible that direct competition for promoter occupancy can explain this inhibitory activity (Fig. 2). The hypothesis that ERα and ERRα compete for binding to a shared promoter is supported by gene-expression analysis performed on breast cancer samples. In particular, Suzuki et al. (2004) found that the correlation between the expression of ERα and ERα target genes that contain an ERE within their promoters is significantly blunted when there is coincident high levels of ERRα expression. In contrast, the correlation between ERα and genes thought to be regulated by ERα binding to a non-canonical ERE is not altered by ERRα expression.

![Figure 2](image-url)

**Figure 2** (A) ERα and ERRα bind to similar promoter elements. Potentially, ERRα could substitute for ERα on an ERE, or a heterodimer formation could occur. The relative affinity for promoter binding and the transcriptional activity of ERRα are each likely to partially be determined by the entire promoter context. (B) Many nuclear receptor co-activators and co-repressors modulate the activity of both ERα and ERRα. If co-activator or co-repressors are scarce, then competition for this supply between ERα and ERRα could alter the relative activity of each receptor. (C) ERRα can induce the transcription of several enzymes in the steroidogenic pathway. Ultimately, this may lead to increased estrogen levels and ERα activity.
Importantly, gel shift assays performed by Krause et al. verified that ERα and ERRα can compete directly for promoter binding in MCF7 cells. They went on to show that this is not merely a passive process but that ERRα recruits co-repressor proteins that actively suppress the expression of ERα responsive target genes (Kraus et al. 2002). However, they note that the relationship between these two receptors seems to depend on the particular cell line tested. Furthermore, it has been shown that the phosphorylation state of ERRα can alter its activity. Of particular relevance, it has been shown that phosphorylation of ERRα, downstream of epidermal growth factor receptor (EGFR) signaling, increases its transcriptional activity on the pS2 promoter. However, this increased activity seems to be promoter selective as ERRα-mediated auto-induction is not enhanced (Yang et al. 1998, Barry & Giguerre 2005).

Insights into ERRα–ERα crosstalk from studies in bone

Bone development and maintenance is an additional potential point of convergence between ERα and ERRα as these receptors are co-expressed in osteoblasts in vivo and in vitro (Bonnelye et al. 2002). The importance of ERα in bone is supported by the finding that the cessation of ovarian estrogen production in post-menopausal women is largely responsible for the development of osteoporosis. In this setting, circulating estrogen levels track with lumbar spine bone mineral density (LS-BMD) (Felson et al. 1993, Bonnelye & Aubin 2005). ERRα is expressed throughout osteoblast development, and in vitro bone nodule formation can be inhibited by knocking down ERRα expression (Bonnelye et al. 2001). Furthermore, a polymorphism has recently been discovered within the ERRα promoter that is associated with high LS-BMD (Laflamme et al. 2005). Patients with this allelic variant, in which a portion of the promoter containing an ERRE is amplified up to four times, express increased levels of ERRα protein. Given the potential regulation of steroidogenesis by ERRα discussed above, ERRα activity may serve to enhance ERα signaling, leading to improved bone maintenance. Alternately, it may serve to activate ERα target genes in bone in the absence of estrogen as indicated by the finding that the shared ERα and ERRα target lactoferrin promotes bone formation in vivo and protects osteoblasts from apoptosis in vitro (Yang et al. 1996, Cornish et al. 2004). ERRα also may afford protection from bone loss independent of ERα as it has been shown to directly regulate several genes that are associated with osteoblast function, such as osteopontin and c-erbA1 (Bonnelye & Aubin 2005). Although these findings indicate several possible levels of interplay between ERα and ERRα, their specific roles in physiology and pathology require further investigation.

The emerging role of ERRα in cancer

In recent years, two independent clinical studies have implicated ERRα in breast cancer progression (Ariazi et al. 2002, Suzuki et al. 2004). Analysis of 102 breast cancer samples revealed that the expression of ERRα in greater than 10% of malignant cells was associated with a 20% decrease in overall disease survival at 13 years (relative risk = 5.1). Furthermore, ERRα was found to be an independent prognostic factor controlling for factors, including ERα status. Although in this study there was no correlation between ERRα and ERα
expression, an earlier study by Ariazi et al. demonstrated that high levels of ERRα mRNA correlated with ERγ-negative tumor status in the 38 tumors examined. Analysis of the other ERRs revealed that high expression of ERRγ correlates with positive outcomes for patients with breast cancer suggesting an opposing role to that of ERRα (Ariazi et al. 2002).

Following the studies implicating ERRα in breast cancer, the expression and activity of this orphan nuclear receptor have been measured in ovarian, prostate, and colorectal cancer. Sun et al. (2005) demonstrated that approximately 60% of ovarian malignancies express ERRα and postulated that ERRα may play an important role by modulating ERα signaling in this context. Measuring the expression of the ERRs in 33 ovarian cancer samples and 12 samples from normal ovaries, they demonstrated that a greater number of cancer samples had ERRα mRNA levels detectable by quantitative real-time PCR. Furthermore, a positive correlation between ERRα expression and advanced tumor stage and grade was observed. Notably, multivariate analysis implicated ERRα expression as an independent prognostic factor for poor overall patient survival. In contrast to the success of targeting ERα in breast cancer, this receptor has not been a useful target in ovarian cancer. In vitro data demonstrating the proliferative role of estrogen in ovarian cancer cell lines and the in vivo correlation between circulating estrogens and tumor development suggest that ERα likely plays an essential role in ovarian cancer. However, only 15–20% of patients with ERα-positive tumors show a clinical response to anti-estrogens (Clinton & Hua 1997). Given the potential crosstalk between the estrogen-signaling pathway and that of ERRα, it is tempting to speculate that ERRα may be part of the explanation for the common resistance of ovarian cancers to ERα blockade.

**ERRα in energy homeostasis: a potential link to cancer?**

From what is presently known about ERRα, it appears that it may have two distinct functional activities in the cell. ERRα was first described as a regulator of fatty acid oxidation, mitochondrial biogenesis, and oxidative phosphorylation (Sladek et al. 1997). More recent literature, as reviewed herein, establishes a role for ERRα as a modulator of ER signaling (Giguere 2002). How, and if, these activities are linked in the pathogenesis of cancers expressing ERRα remains an open question.

In considering the potential for targeting the ERRα to alter estrogen signaling, it is worth noting the wide range of roles that it can play in physiological and pathological settings. The tissue distribution of ERRα in the mouse provided the first clue that ERRα may regulate metabolic activity. Almost all organs express ERRα at some level. However, it is most highly expressed in kidney, heart, cerebellum, intestine, and skeletal muscle, tissues that preferentially utilize fatty acids as energy sources (Bookout & Mangelsdorf 2006). The function of ERRα as a metabolic regulator is further supported by the observation that ERRα-null mice demonstrate impaired fat metabolism and absorption (Luo et al. 2003). That the expression of ERRα is elevated in exercising muscle and fasting liver specifically implicates this receptor in β-oxidation of fatty acids, which occurs under the same conditions. On a mechanistic level, several studies have revealed that ERRα is involved in the transcriptional regulation of genes required for mitochondrial biogenesis, oxidative phosphorylation, and fatty acid oxidation (Wu et al. 1999, Yoon et al. 2001, Huss et al. 2004, Mootha et al. 2004).

Most of the metabolic studies of ERRα focus on its role as the downstream effector of PPARγ co-activator 1α (PGC-1α). PGC-1α is a promiscuous nuclear receptor co-activator expressed at low basal levels but induced by fasting and other metabolic stresses (Puigserver & Spiegelman 2003). PGC-1β, a related cofactor, may have similar functions under certain circumstances, although its expression level is not as acutely regulated by variations in energy demand (Yoon et al. 2001, Lin et al. 2003, 2005). Rather than being regulated by ligand, the magnitude of ERRα activity is thought to be largely dependent on the presence of transcriptional co-activators, such as PGC-1α and PGC-1β. Interest in the ERR–PGC-1 regulatory axis was heightened by the observation that there is a decrease in both PGC-1α and PGC-1β in the skeletal muscle of patients with diabetes and obesity (Kelley et al. 2002, Mootha et al. 2003, Oberkofler et al. 2004, Lowell & Shulman 2005). The recently identified allelic variant of the ERRα promoter thought to sensitize ERRα to PGC-1α co-activation was found to be associated not only with high bone density, but also with obesity (Kamei et al. 2005). It is essential that the effects of ERRα activity in both energy metabolism and tumor biology be understood if we are to further develop ERRα as a therapeutic target.

**Pharmacologic regulation of ERRα activity**

As of yet, the ERRs have not been shown to interact with any physiologically relevant small molecules, leading to the suggestion that these receptors manifest constitutive activity (Xie et al. 1999, Chen et al. 2001,
Crystallographic analyses of both apo-ERRα and apo-ERRγ have indicated that these receptors are in a transcriptionally active conformation (Greschik et al. 2002, 2004, Kallen et al. 2004). Furthermore, the lack of any obvious electron density in the ligand-binding pockets (LBP) indicates that the apo-receptors are indeed capable of adopting an active conformation (Greschik et al. 2002, Kallen et al. 2004). With an estimated volume of only 100 Å³, the LBP of ERRα is large enough to accommodate the binding of a small molecule agonist of only four or five carbons. It is not surprising, therefore, that the vast majority of pharmacologically active ERRα ligands act as antagonists.

The first compounds screened for activity on the ERRs were known endocrine disruptors with estrogen-like activity. Yang & Chen (1999) found that the organochlorine pesticides toxaphene and chlordane function as low-affinity ERRα antagonists in the micromolar range. The synthetic estrogen diethylstilbestrol was also found to act as a weak antagonist, disrupting co-activator–ERR interaction and inhibiting constitutive activity of all three ERRs in transfection assays (Coward et al. 2001, Tremblay et al. 2001). Recently, high throughput screening yielded an ERRα inverse agonist which was subsequently optimized to the ERRα-selective XCT790 (Busch et al. 2004). This thiazolinedione-based compound inhibits ERRα activity in transfection assays with submicromolar activity, and has been used to further define the role of ERRα in the regulation of metabolic signaling pathways (Mootha et al. 2004, Willy et al. 2004). Several other ERRα inverse agonists with submicromolar activity have been reported, including an indole, pyrazole, and thiazolidinone (Deuschle et al. 2004, Player et al. 2004, Nolte et al. 2005). Unfortunately, many of the antagonists reported cross-react with ERRβ and ERRγ as well as with a variety of other nuclear receptors. As an alternative to the small molecule approach, our lab has used phage display to develop several ERRα peptide antagonists. These peptides bind with high affinity to ERRα and block co-activator binding (S Gaillard & DP McDonnell, unpublished observations). In contrast to the numerous ERRα antagonists reported, few natural or synthetic agonists of ERRα have been identified. However, the phytoestrogens flavone and isoflavone function in transfection assays as non-selective ERR agonists (Suetsum et al. 2003). Due to the small size of the LBP, it may be necessary to design drugs to enhance ERRα activity using alternative strategies, such as altering the phosphorylation status of the receptor or targeting other upstream regulators of ERRα.

Further development of ERRα as a therapeutic target

A clear understanding of ERRα activity is likely to shed light on unresolved aspects of ER signaling and pharmacology and may validate ERRα as a useful therapeutic target in breast cancer. Ultimately, if we are to pharmacologically manipulate ERRα in the setting of metabolic disorders or cancer, we must determine if ERRα expression and activity is a cause or consequence of the underlying pathology. Regarding the role of ERRα in breast cancer, larger clinical studies as well as investigation into the molecular mechanism of ERRα function in this particular setting are essential. The extent to which ERRα signaling is intertwined with that of ERα and the extent to which ERRα function in cancer is distinct from its activity as a metabolic regulator are as yet undetermined. Answers to these compelling questions will both inform and motivate future development of ERRα as a therapeutic target within the settings of malignancy and metabolic disorders.

References

Apak TI & Duffel MW 2004 Interactions of the stereoisomers of alpha-hydroxytamoxifen with human hydroxysteroid sulfotransferase SULT2A1 and rat hydroxysteroid sulfotransferase STA. Drug Metabolism and Disposition 32 1501–1508.


Bonnelly E, Meerdad L & Aubin JE 2001 The orphan receptor, estrogen related receptor ERR alpha, is highly expressed in osteoblasts and is required for bone formation. Bone 28 S87.


proliferator-activated receptor at signaling in the trans-
scriptional control of energy metabolism in cardiac and
skeletal muscle. Molecular and Cellular Biology
24 9079–9091.

Kallen J, Schlaeppi JM, Bitsch F, Filipuzzi I, Schib A,
Riou V, Graham A, Strauss A, Geiser M & Fournier B
2004 Evidence for ligand-independent transcriptional
activation of the human estrogen-related receptor alpha
(ERR alpha) – Crystal structure of ERR alpha ligand
binding domain in complex with peroxisome prolif-
erator-activated receptor coactivator-1 alpha. Journal of
Biological Chemistry 279 49330–49337.

Kamei Y, Lwin H, Saito K, Yokoyama T, Yoshiike N,
Ezaki O & Tanaka H 2005 The 2.3 genotype of
ESRRA23 of the ERR alpha gene is associated with a
higher BMI than the 2.2 genotype. Obesity Research 13
1843–1844.

Kato S, Endoh H, Masuhiro Y, Kitamoto T, Uchiyama S,
Sasaki H, Masushige S, Gotoh Y, Nishida E &
Kawashima H 1995 Activation of the estrogen receptor
through phosphorylation by mitogen-activated protein

Kelley DE, He J, Menshikova EV & Ritov VB 2002
Dysfunction of mitochondria in human skeletal muscle in

Kraus RJ, Ariazi EA, Farrell ML & Mertz JE 2002
Estrogen-related receptor alpha 1 actively antagonizes
estrogen receptor-regulated transcription in MCF-7
mammary cells. Journal of Biological Chemistry 277
24826–24834.

Laflamme N, Giroux S, Loredo-Osti JC, Elfassili L, Dodin S,
Blanchet C, Morgan K, Giguere V & Rousseau F 2005 A
frequent regulatory variant of the estrogen-related
receptor alpha gene associated with BMD in French–
Canadian premenopausal women. Journal of Bone
Mineral Research 20 938–944.

Laudet V 1997 Evolution of the nuclear receptor superfamily:
early diversification from an ancestral orphan receptor.
Journal of Molecular Endocrinology 19 207–226.

Laudet V, Hänni C, Coll J, Catzeflis F & Stéhelin D 1992
Evolution of the nuclear receptor gene superfamily.
EMBO Journal 11 1003–1013.

Lin J, Tarr PT, Yang R, Rhee J, Puigserver P, Newgard CB &
Spiegelman BM 2003 PGC-1beta in the regulation of
hepatic glucose and energy metabolism. Journal of
Biological Chemistry 278 30843–30848.

Lin J, Yang R, Tarr PT, Wu P-H, Handschin C, Li S, Yang W,
Pei L, Uldry M, Tontonoz P et al. 2005 Hyperlipidemic
effects of dietary saturated fats mediated through PGC-1
beta coactivation of SREBP. Cell 120 261–273.

Lowell BB & Shulman GI 2005 Mitochondrial dysfunction and

Lu D, Kiriyama Y, Lee KY & Giguere V 2001 Transcrip-
tional regulation of the estrogen-inducible pS2 breast
cancer marker gene by the ERR family of orphan nuclear


Vanacker JM, Pettersson K, Gustafsson JA & Laudet V 1999b Transcriptional targets shared by estrogen receptor-related receptors (ERRs) and estrogen receptor (ER) alpha, but not by ER beta. *EMBO Journal* 18 4270–4279.


