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Review Article

Estrogen-related receptor α as a therapeutic target

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Abstract

The utility of nuclear receptors (NRs) as targets for drug discovery is well recognized. Growing evidence suggests that ERRα, an orphan NR whose physiological and pathological roles remain under investigation, may have utility in the treatment of various cancer types as well as metabolic and osteopenic disorders. In this article, we review the literature on ERRα as a potential therapeutic target. Within this context we identify several key challenges in progressing small molecules which target ERRα, including the separation of efficacy and liability.

Introduction

The nuclear receptor (NR) superfamily comprises 48 ligand-activated transcription factors which respond to a wide variety of signaling events to regulate processes such as differentiation, development and cellular growth. The identification of several endogenous and synthetic NR ligands has facilitated the elucidation of the physiological roles of many NRs and, in several instances, has led to the development of marketed drugs, affirming the tractability of the NRs as viable therapeutic targets. The function of several NRs remains to be fully elucidated, and for many of these NRs, the endogenous ligands have not yet been identified; these NRs have accordingly been classified as orphan NRs. The first orphan NRs to be identified, two estrogen receptor-related receptors, ERRα (NR3B1) and ERRβ (NR2B2), were discovered in a screen using a probe derived from the DNA binding domain of the classical estrogen receptor ERα (Fig. 1) (1). A third member of the ERR subfamily, ERRγ (NR3B3), was subsequently identified. ERRα is a constitutively active NR which binds to DNA as a homodimer and is broadly expressed in adult tissues. Although the ERRs do not bind natural estrogens, the similarity in DNA-binding sequence preference between the classical estrogen receptors and the ERRs has prompted investigations into the potential interplay between these NRs in physiological and pathological settings. Herein we review the current ERRα literature that has generated interest in further developing ERRα as a therapeutic target.

ERRα and cancer

Although estrogens play a critical role in the pathophysiology of breast cancer, the exact mechanisms of estrogen action are not fully understood. A guiding principle in the study of estrogen action is that estrogen promotes tumor growth through estrogen receptors ERα and ERβ. ERα status is the most accurate predictor of both survival and response to estrogen antagonism in the treatment of breast cancer (2). Although hormone deprivation is an initially successful treatment in the majority of ERα-positive breast cancers, this approach eventually fails in at least 20% of patients (3). For the 25% of patients with ERα-negative breast cancer, the overall prognosis is significantly worse, as the success of existing therapies is limited (4). The discovery of the closely related but constitutively active ERRs has provided a novel pathway that may contribute to the acquired ability of some breast tumors to grow in the absence of estrogen stimulated ERα signaling. ERRα signaling may be particularly relevant to breast cancer since, unlike ERRβ, ERRα has been detected in all breast cancer cell lines and the majority of tumor samples examined to date (5, 6). Moreover, ERRα is expressed more highly in breast cancer tissue than in surrounding normal tissue (7).

In the first study to link ERRα to clinical and pathological characteristics of breast cancer, Ariazi *et al.* focused on comparing mRNA levels of the ERRs to that of well-established prognostic markers. In this study of 38

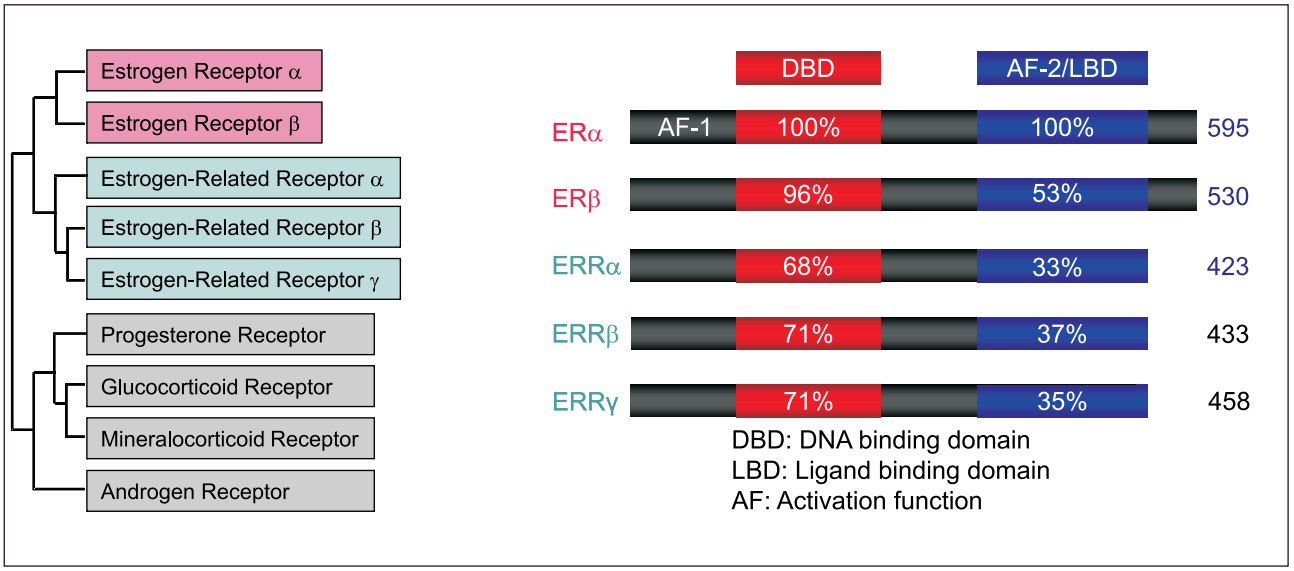

Fig. 1. Schematic representation of the structural relationship between the ERs and the ERR orphan nuclear receptors. The phylogenetic tree of subfamily III nuclear receptors illustrates the close kinship between the ERs and the three members of the ERR subfamily: ERRα (NR3B1), ERRβ (NR3B2) and ERRγ (NR3B3). All of the nuclear receptors have shown a common domain structure with the salient domains illustrated above. Percent sequence identity with ERα is shown within the DBD and LBD. Although several alternative splice variants of the ERRs have been identified, the total number of amino acids in the most common is shown above. (Adapted from Laudet *et al.* Journal of Molecular Endocrinology 1997, 19: 207-26.)

primary breast cancer samples, the authors found that ERRα expression is significantly associated with ERα-negative and progesterone receptor-negative tumor status. In addition, ERRα expression was shown to correlate with that of Her2, an epidermal growth factor (EGF) receptor which has been implicated in the development of antiestrogen resistance. Further exploring the relationship between ERRα and Her2, Barry *et al.* demonstrated that ERRα transcriptional activity can be enhanced by phosphorylation events downstream of Her2 activated by EGF (8). The specific role of ERRα-regulated transcription in the Her2-initiated events, however, remains to be elucidated.

Building on the association between ERRα and negative prognostic biomarkers, Suzuki *et al*. demonstrated a direct correlation between ERRα expression and unfavorable breast cancer patient outcomes (7). The study of 102 specimens of invasive ductal carcinoma revealed that positive immunohistochemical staining for ERRα is significantly associated with an increased risk of tumor recurrence and a decreased overall survival rate. Importantly, the predictive value of ERRα expression was shown to be independent of ERα status, confirming that targeting the ERRα pathway may be of therapeutic benefit in patients with either ERα-positive or ERα-negative breast cancer.

The structural and functional similarity between the ERs and ERRs suggests that there may be multiple complex interactions among these receptors. Because of their significant homology within the DNA binding domain, ERRα and ERα bind to similar DNA response elements in target genes (9). Transcription of several endogenous genes can be activated by both ERRα and ERα, including the pS2 breast cancer marker, osteopontin and lactoferrin (6, 10-12). This 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 inverse agonists might be of benefit in treating breast cancer (6).

A more complex relationship between ERα and ERRα is suggested, however, by the fact that ERRα can repress ligand-stimulated ERα activity in certain cell lines (10). This finding has been variously attributed to (i) inactive ERα/ERRα heterodimerization, (ii) direct competition for promoter occupancy and (iii) indirect competition for coactivators shared by both receptors (Fig. 2) (12, 13). Suzuki *et al*. were among the first to provide clinical data to support the theory that ERα and ERRα compete for binding to a shared promoter (7). They demonstrated that the correlation between ERα expression and expression of several ERα target genes that contain an estrogen response element (ERE) within their promoters is significantly blunted in breast cancer tissues with high ERRα expression levels. In contrast, ERRα expression has no effect on the correlation between ERα and genes thought to be regulated by ERα binding to a non-canonical ERE.

The potential for cross-talk between ERRα and ERα is further illustrated by the discovery that ERRα can stimulate the transcription of aromatase, as well as several other key enzymes in steroidogenesis (14, 15). The clinical efficacy of aromatase inhibitors underscores the importance of this enzyme in breast cancer biology. Whereas estrogen synthesis in premenopausal women

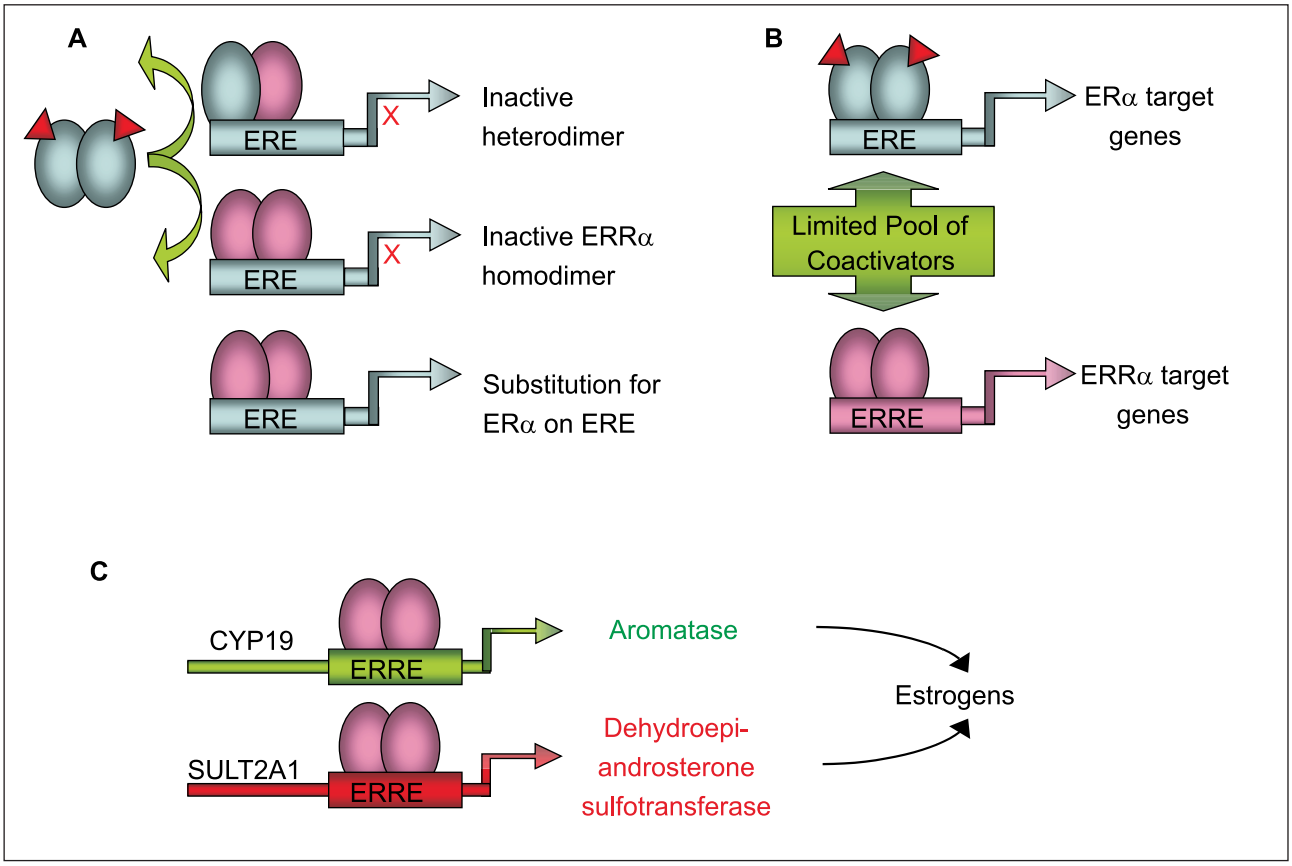

Fig. 2. Potential impact of ERRα on ERα signaling. (A) Due to the similar DNA binding sequence preference of ERα and ERRα, it has been suggested that they compete for occupancy of specific promoters. ERRα homodimer and ERRα-ERα heterodimer binding of EREs has been proposed, resulting in estrogen-independent gene transcription or active gene repression. (B) Several nuclear receptor coactivators bind to and recruit transcription factors to both ERα and ERRα. It is possible that ERα and ERRα compete for coactivators within a limited pool. (C) By inducing enzymes within the steroidogenic pathway, ERRα can affect the amount of estrogens present, thereby altering ERα activity.

occurs predominantly in the ovaries, local steroid biosynthesis in postmenopausal women is thought to be essential for breast cancer progression (16). In postmenopausal women, ERRα-stimulated aromatase transcription in the breast may enhance local production of estrogen, which would in turn stimulate breast cancer progression. Recent data suggests that ERRα induces transcription of the steroid sulfotransferase SULT2A1 within the adrenal glands (15). This sulfotransferase maintains high levels of peripheral DHEAS, which in turn is thought to be required for estrogen synthesis in certain peripheral tissues. Although these intriguing findings indicate several possible levels of interplay between ERα and ERRα, their specific roles in physiology and pathology require further investigation.

ERRα expression and activity have also been measured in a variety of other types of cancer, including ovarian, prostate and colorectal cancer. The finding that approximately 60% of ovarian malignancies express ERα and the association between high levels of circulating estrogens and tumor development prompted Sun *et al*. to postulate that the cross-talk between ERRα and ERα may have a role in ovarian cancer (17). Despite encouraging *in vitro* data demonstrating the proliferative role of estrogen in ovarian cancer cell lines, only 15-20% of patients with ERα-positive tumors show a clinical response to antiestrogens (18). The elucidation of the molecular mechanism behind this finding, as well as identification of patients most likely to respond to antiestrogen therapy, would significantly advance the treatment of ovarian cancer. Could ERRα activity serve to circumvent the potential therapeutic consequences of ERα blockade in certain ovarian malignancies? To begin to address this question and to determine if the ERRs are associated with malignant ovarian tumors, Sun *et al*. studied the expression of the ERRs in several ovarian cell lines, 33 ovarian cancer samples and 12 samples from normal ovaries. First, they demonstrated that, compared to normal ovary samples, a significantly higher percentage of ovarian cancer samples had ERRα mRNA levels detectable by quantitative real time polymerase chain reaction. Furthermore, a positive correlation between ERRα expression and advanced tumor stage and grade was observed. Notably, ERRα expression was shown to be an independent prognostic factor for poor overall patient survival (17). The authors suggest that determin-

ing ERRα status might be a useful criterion in selecting patients for whom hormonal therapies are most likely to succeed, as well as in establishing accurate patient prognoses.

Perhaps more surprising than a role for ERRα in breast and ovarian cancer is the possibility that ERRα is involved in colorectal cancer. Analysis of 80 colorectal tumor samples demonstrated that higher levels of ERRα mRNA are expressed in tumor tissue in comparison to the surrounding normal mucosa. Furthermore, tumor tissue ERRα mRNA levels positively correlated with increased tumor stage and histological grade. These findings point toward a potential role of ERRα in tumor biology and suggest its possible utility as a biomarker for the progression of colorectal cancer (19). Finally, Cheung *et al*. investigated the expression patterns of ERR mRNA and protein in normal and malignant human prostate epithelial cells and cell lines (20). The authors also characterized ERR protein expression and localization in normal, dysplastic and malignant prostate tissue. They concluded that ERR protein expression is reduced in neoplastic prostatic cells compared to their nonmalignant counterparts and suggested that the ERRs are downregulated in the progression of prostate cancer. The authors went on to measure the effect of transiently overexpressing the ERRs on the proliferation of an immortalized prostate cell line and a prostate cancer cell line. They concluded that ERRα, β or γ activity can inhibit proliferation in cells derived from normal and malignant prostate epithelium. Their preliminary experiments with stably transfected cell lines indicated that this is due to a decreased growth rate rather than an increased rate of apoptosis. While no correlation with prostate cancer prognosis has yet been demonstrated, it is tempting to speculate how ERR expression may affect prostate tumor growth and progression.

Although available therapies for cancer have not been designed specifically to target the ERRs, it has been demonstrated that diethylstilbesterol, tamoxifen and 4-hydroxytamoxifen are inverse agonists of both ERRα and ERRβ (20, 21). The extent to which the actions of these drugs are dependent on ERR signaling remains to be determined. Interest in generating an ERRα inverse agonist has been sparked by the well-documented role of PGC-1α-coactivated ERRα in regulating metabolic processes such as mitochondrial biogenesis and adaptive thermogenesis. As such, published data regarding the ERRα inverse agonist XCT-790 have thus far been limited to the effect on PGC-1α-coactivated ERRα in liver and muscle cell lines (22). Determining the effects of this compound on the metabolic and growth parameters of cancer cell lines would provide insight into the specific role of ERRα in these processes. Although most closely linked to the development of disorders of lipid homeostasis, the metabolic changes resulting from ERRα transcriptional activity may have important implications in the setting of malignancy as well. If we are to further develop ERRα as a potential therapeutic target in cancer – breast, ovarian, colorectal or prostate – it is essential to determine the mechanism of ERRα activity and to elucidate the underlying relationship between this activity, tumor behavior and, ultimately, patient outcomes.

ERRα as a metabolic regulator

Among the first indications of a biological role for ERRα was the identification of a consensus ERRE (ERR response element: TnAAGGTCA) within the medium-chain acyl Co-A dehydrogenase (MCAD) promoter. MCAD is the key enzyme that catalyzes the initial step in mitochondrial β-oxidation of fatty acids. Gel-shift experiments from whole-cell extracts derived from ERRα-expressing COS-7 cells demonstrated ERRα binding to this promoter element in a sequence-specific manner. Additionally, a constitutive active chimeric ERRα-VP16 construct was capable of transactivating this element in both HIB and COS-7 cells (23). Identification of *MCAD* as an ERRα target gene suggested a role for this NR in lipid metabolism. Further evidence implicating ERRα in the regulation of lipid metabolism is the induction of ERRα gene expression during metabolic states of high energy demand such as exercise and fasting (24). Likewise, ERRα is most highly expressed in tissues that primarily utilize fatty acids as their energy substrate. These observations suggest that ERRα may be important in regulating lipid metabolism at basal levels as well as under conditions of increased metabolic energy consumption.

A clearer picture of the biological role ERRα plays *in vivo* began to emerge following studies undertaken using an ERRα-null mouse model. These animals displayed altered expression of several genes involved in lipid synthesis, adipogenesis and energy metabolism in adipose tissue (25). Additionally, expression of genes important in oxidative phosphorylation, lipid digestion and absorption were all found to be downregulated in the intestine of ERRα-null mice. Consistent with these findings, isolated intestinal enterocytes from ERRα-null animals had a reduced capacity for fatty acid oxidation. Metabolic studies demonstrated that ERRα knockout animals have reduced fat mass and are resistant to diet-induced obesity, an effect likely due to lipid malabsorption defects in the intestine (26). These results further demonstrated a role for ERRα in lipid metabolism and energy homeostasis.

ERRα and co-regulator signaling

Structural and functional studies have demonstrated that the ERRs exhibit varying levels of activity in the absence of a ligand-occupied binding pocket (27, 28). However, like many ligand-activated NRs as well as non-NR transcription factors, the functional activity of ERRα can be regulated through its interaction with multiple transcriptional regulators. Both coactivator and co-repressor proteins, as well as other NRs, have been shown to regulate ERRα activity (6, 29-31). Studies examining the functional consequences of ERRα/co-regulator interactions have greatly enhanced our understanding of ERRα activation and the downstream signaling mechanisms. For example, the ligand-dependant co-repressor receptor-

interacting protein 140 (RIP-140) has been shown to regulate ERRα activity, in addition to that of other NRs (32, 33). In a recent study by Powelka *et al*., depletion of RIP-140 transcripts in 3T3-L1 adipocytes by siRNA suggested that RIP-140 may have a role in the regulation of energy metabolism. In particular, RIP-140 was implicated in the suppression of several gene clusters from pathways involved in energy metabolism, including glucose uptake, fatty acid oxidation, mitochondrial biogenesis and oxidative phosphorylation. These investigators demonstrated the requirement of ERRα in RIP-140-mediated regulation of several genes including *GLUT4* and the mitochondrial gene succinate dehydrogenase subunit B. Importantly, depletion of ERRα expression by siRNA in the same cells abrogated both the uptake of 2-deoxyglucose and the induction of genes seen in RIP-140-depleted cells alone (34). These data demonstrate a role for ERRα in regulating adipocyte *GLUT4* and mitochondrial gene expression, as well as glucose uptake, and provide evidence that ERRα can regulate energy balance in adipocytes via its association with the co-repressor RIP-140.

The PPARγ coactivator 1 proteins (PGC-1α and PGC-1β) interact with and increase the transcriptional activity of several NRs and non-NR transcription factors, including peroxisome proliferator-activated receptors (PPARs), hepatocyte nuclear factor 4 (HNF4), estrogen receptor α (ERα) and nuclear respiratory factor 1 (NRF-1) (35). Studies from several laboratories have demonstrated that PGC-1α regulates lipid metabolism, mitochondrial biogenesis, oxidative metabolism, hepatic gluconeogenesis and thermogenesis. These processes are initiated in response to a variety of stimuli including fasting, exercise and cold exposure (36-43). These studies have firmly established PGC-1α as a key mediator in regulating cellular energy balance through an array of NR and non-NR transcription factors.

The initial observation that ERRα and PGC-1α interact came from a yeast two-hybrid screen for PGC-1α-interacting proteins (24, 44). Detailed expression studies in adult animals have demonstrated a striking overlap in the spatial pattern of ERRα and PGC-1α expression. These genes are most highly expressed in tissues with high energy demand and enhanced need for fatty acid oxidation, including skeletal muscle, kidney, heart and brown adipose tissue (23, 35, 36, 45-47). The temporal pattern of expression for ERRα mirrors that of both PGC-1α and *MCAD* in the pre- to postnatal murine heart. In the murine heart, levels of these genes dramatically increase immediately following birth, coincident with a pre- to postnatal switch in energy substrate utilization from glucose to fatty acids (44).

Which PGC-1α-regulated pathways involve ERRα? What mechanism(s) define the PGC-1α/ERRα-mediated signaling events? The initial clues to these questions have come from functional studies demonstrating that overexpression of PGC-1α strongly coactivates both ERRα and ERRγ in reporter assays and transcription of target genes including *MCAD* in several cell types (46, 48). Studies in SAOS2 osteoblast progenitor cells established that ERRα is downstream of PGC-1α activation in a pathway leading to increased mitochondrial biogenesis and the expression of several genes encoding mitochondrial proteins including mtTFA, IDH3A, Cyt c and ATPsynβ. Importantly, ERRα induction and activation was demonstrated to be required for these PGC-1α-mediated effects (46).

Coincident expression of PGC-1α and ERRα has also been found in the liver, where PGC-1α plays a major role in coordinating the expression of phosphoenolpyruvate carboxykinase (PEPCK) in response to fasting. PGC-1α regulates PEPCK expression through its interactions with other transcription factors including HNF-4α, glucocorticoid receptor and FOXO1 (40, 43, 49, 50). The observation that ERRα expression is also induced by fasting suggested the possibility that ERRα is involved in PGC-1α regulation of gluconeogenesis. While some studies have suggested that ERRα represses PGC-1α activity in the liver, others were unable to demonstrate an effect of ERRα on PGC-1α-mediated expression of PEPCK (22, 24). Interestingly, a recent study suggests that ERRα may confer opposing activities on different PGC-1α targets in the liver (51). Herzog and colleagues demonstrated that ERRα overexpression repressed the induction of PEPCK by PGC-1α in Hep G2 cells. Moreover, ERRα-null animals expressed elevated hepatic PEPCK mRNA, while levels of message for ERRα target genes involved in oxidative phosphorylation were moderately decreased. Interestingly, the ERRα-null animals expressed higher levels of hepatic PGC-1α, providing a potential mechanism for the induction of PEPCK expression in the ERRα-null animals and suggesting that ERRα may normally suppress PGC-1α expression in the fed liver. The complexity of ERRα activity suggested by these findings underscores the need for continued investigation into the role of ERRα in the liver.

PGC-1α overexpression studies have shown that this co-factor can induce the expression of ERRα in HeLa, SAOS2 and C2C12 cells (22, 46, 52). In a series of elegant experiments aimed at identifying *cis*-regulatory elements based on PGC-1α-mediated transcriptional responses over time, Mootha *et al*. identified a binding site in PGC-1α-induced transcripts corresponding to the published sequence motif of an ERRE (5'-TGACCTTG-3') (9). This motif was shown to be present in a subset of PGC-1α-regulated oxidative phosphorylation genes that are downregulated in human diabetes (53, 54). A second PGC-1α-responsive gene motif was identified as the binding site for GABPα (55). GABPα and GABPβ heterodimerize to form the transcription factor nuclear respiratory factor 2 (NRF-2) (56), which regulates the expression of several mitochondrial proteins involved in oxidative phosphorylation (36, 57). In addition, both ERRα and GABPα expression were induced by PGC-1α, and remarkably, these genes were found to contain mutual functional response elements. This finding suggests that ERRα and GABPα can regulate the transcription of their own and each others' genes from a PGC-1α-responsive signaling event. This regulation can affect down-

stream target genes important for mitochondrial oxidative phosphorylation and ATP synthesis. Indeed, PGC-1α-induced increases in several ERRE-containing OXPHOS genes, as well as total and uncoupled mitochondrial respiration, were inhibited by the ERRα inverse agonist XCT-790, thereby demonstrating a functional role for an ERRα/PGC-1α complex in mediating mitochondrial respiration (22). Consistent with this finding, Huss *et al*. demonstrated that overexpression of ERRα in cardiac myocytes led to increased fatty acid uptake and palmitate oxidation, reflected by changes in expression of genes involved in lipid metabolism and mitochondrial oxidative respiration (48). This result correlated well with previous studies using PGC-1α and PPARα and was consistent with known effects of PPARα in cardiac myocytes. Intriguingly, further analysis demonstrated that ERRα overexpression could induce PPARα expression in C2C12 muscle cells and cardiac myocytes, where a functional ERRE was identified within the PPARα promoter (48). Importantly, ERRα overexpression had no effect on several PPARα target genes in PPARα-null fibroblasts, but did activate these targets in PPARα-expressing cells. These studies provided strong evidence that ERRα activation of PPARα in tissues such as skeletal muscle and heart is an important mechanism to regulate cellular fatty acid metabolism (44).

In a third independent study, Laganiere and colleagues identified a polymorphic region in the ERRα promoter. This polymorphism, termed ESSRA23, is present with variable copy (one to four copies) 682 bases upstream of the ERRα transcriptional start site (52). Functional analysis of this element in HeLa cells confirmed that a constitutively active ERRα-VP16 chimera is capable of inducing transcription from ESSRA23, and co-expression of ERRα and PGC-1α synergistically activated this element in a copy dosage-dependent manner. Furthermore, in ERRα-null fibroblasts, reduced PGC-1α-mediated, ESSRA23-dependent reporter activity was restored by co-expressing ERRα, again demonstrating feed-forward regulation by an ERRα-PGC-1α complex. These results further suggested that an ERRα promoter polymorphism may determine the extent to which PGC-1α and ERRα regulate target gene transcription in individuals and could have consequences in human metabolic disorders. In support of this finding, a single polymorphic variant of ESSRA23, found at a frequency of 18.5% within a study from 703 Japanese individuals, was shown to be associated with higher body mass index, suggesting that polymorphisms in the promoter of human ERRα may be an independent genetic factor in human obesity (22, 58). Taken together, these findings have led investigators to propose a model in which transcriptional responses to changing energy demand regulate lipid metabolism, mitochondrial oxidative capacity and biogenesis in tissues with high energy demand. This model proposes a signaling cascade involving PGC-1α and several transcription factors including ERRα, PPARα and NRFs (Fig. 3). Within this cascade, evidence suggests that ERRα and GABPα, through a positive feed-forward mechanism, can activate downstream target genes directly or indirectly via PPARα and NRF-1 (22, 48).

ERRα and bone

The role of ERRα in bone has recently been reviewed (59). Despite the observation of no anomalous bone effects in the ERRα knockout mouse, mounting evidence suggests roles for ERRα in bone formation, maintenance and turnover. ERRα is highly expressed in bone cells, and its functional activity may be in concert with, or independent of, the classical estrogen receptors. Several known ERRα target genes have established roles in bone physiology, including osteopontin, c-erb A1 and aromatase (60, 61). Antisense-mediated disruption of ERRα synthesis inhibits key genes involved in bone formation (runt-related transcription factor, bone sialoprotein and osteocalcin) in primary rat calvaria cells and negatively affects bone nodule formation, and transient overexpression of ERRα in bone cells increases osteoblast differentiation and bone formation (62).

ERRα ligands

A small-molecule natural ligand for ERRα has not yet been identified, and there remains the possibility that ERRα functional activity is not mediated by an endogenous ligand. Structural investigations have helped to assess the potential for developing small-molecule ERRα ligands. The x-ray crystal structure of ERRα ligand binding domain (LBD) has been solved at 2.5 Å resolution (28). Activation function 2 (AF-2) of the unliganded LBD resides in a conformation disposed towards binding of coactivators, consistent with the observed constitutive activity of ERRα. As suggested by earlier homology modeling studies, there is little available volume to accommodate ligand binding (27). In comparison to ERRα and ERRβ, the smaller ligand binding pocket (LBP) volume of ERRα is attributed to the presence of F232 (corresponding to alanine residues in the other two ERR subtypes). Furthermore, mutagenesis studies have demonstrated the importance of this residue to the constitutive activity of ERRα.

The active conformation of unliganded ERRα and the small volume available for ligand binding suggests that such an event would likely require receptor reorganization, for example by rotation of F232. Consequently, it is more likely that a ligand would deactivate rather than further activate the receptor; not surprisingly, most small-molecule ERRα ligands are reported as inverse agonists. The organochlorine pesticides toxaphene and chlordane were profiled as low-affinity (IC_{50} > 10 μM) ERRα inverse agonists in a reporter gene assay (63). The estrogen receptor agonist diethylstilbestrol (DES) was reported to decrease the interaction between ERRα and coactivator peptide SRC-1.2 with an IC_{50} of 10 μM in a cell-free assay, but no effect on ERRα transcriptional activity was observed with DES at similar concentrations (64). In contrast to these earlier reports detailing low-affinity ERRα inverse agonists, a recent communication from X-Ceptor

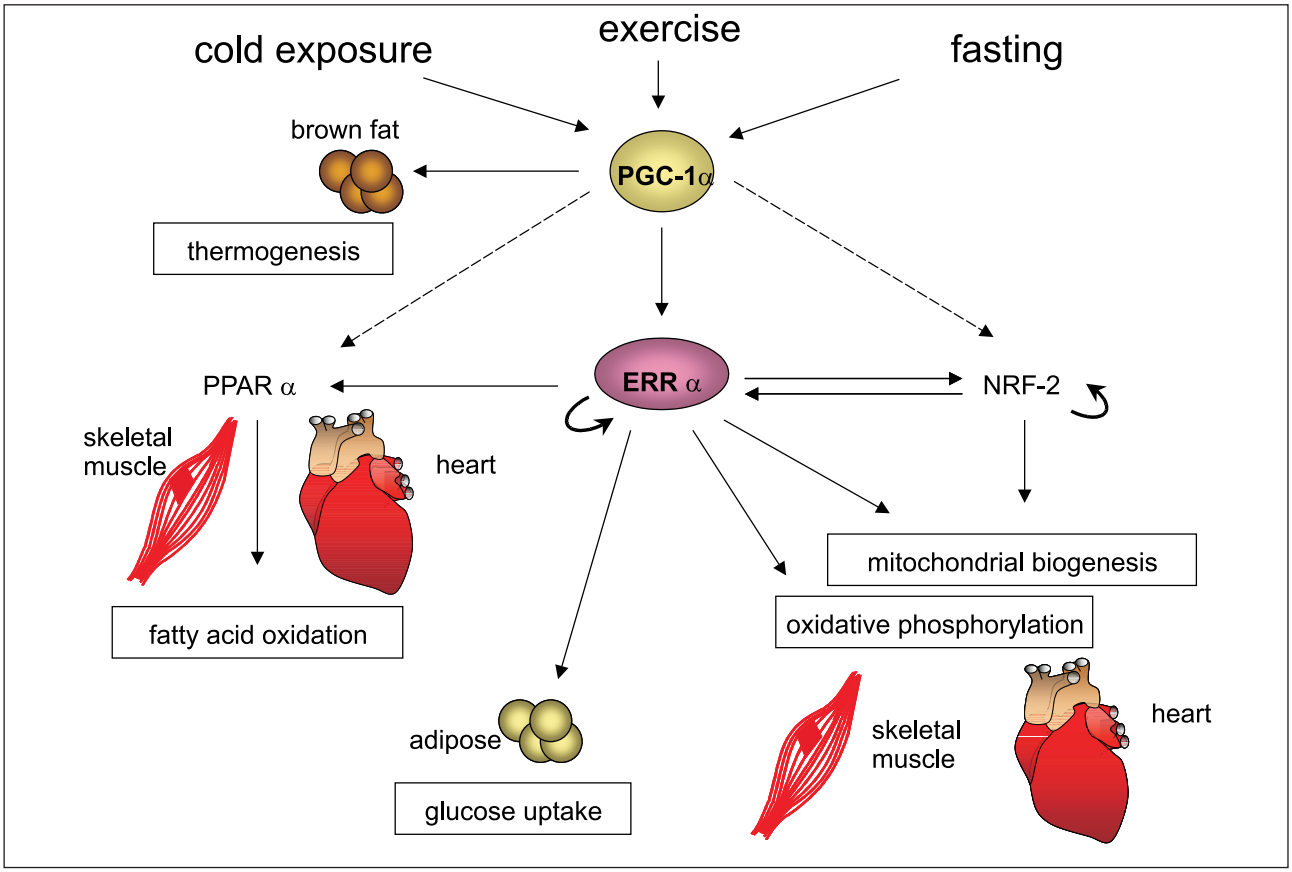

Fig. 3. ERRα is a metabolic regulator of energy homeostasis. A variety of environmental and hormonal stimuli can activate a PGC-1α-mediated response to changing cellular energy demands. Through a feed-forward signaling cascade, PGC-1α, ERRα and NRF-2 can activate downstream target genes directly or indirectly via PPARα and NRFs to regulate fatty acid oxidation, mitochondrial biogenesis, glucose uptake and oxidative phosphorylation in a variety of tissues.

Therapeutics (now Exelixis) describes the discovery, by high-throughput diversity screening, of a series of ERRα inverse agonists and subsequent optimization to XCT-790 (Fig. 4, **1**), which has submicromolar activity (IC_{50} = 0.37 μM in a GAL4-ERRα transfection assay) (65). Other reports of submicromolar ERRα inverse agonists include indole **2** from GlaxoSmithKline (66), pyrazole **3** from Phenex-Lion (67) and thiazolidinedione **4** from Johnson and Johnson (68).

In contrast to the numerous reports of ERRα inverse agonists, there has been less progress in the development of small-molecule ERRα agonists. Suetsugi and coworkers described flavone and isoflavone phytoestrogens as ERRα agonists in mammalian transfection assays (69). These phytoestrogens are weakly potent (IC_{50} > 10 μM) and are cross-reactive with the classical ERs. Moreover, direct binding to ERRα LBD has not been demonstrated with these phytoestrogens. The current understanding of ERRα LBD structure suggests that it may not be possible to develop small-molecule ERRα agonists because of the requisite protein rearrangements for accommodation of the ligand.

The challenge of finding ERRα agonist ligands has prompted the search for alternative avenues for activation of the ERRα program. One potential strategy is increased expression of ERRα by activation of an upstream transcription factor. The classical estrogen receptors as well as ERRα have been reported to regulate ERRα expression (70, 71). Given the availability of chemical tools for the ERs and ERRγ, this pathway activation strategy merits further elucidation as a viable method for ERRα agonism in tissues where ERRα and the targeted upstream regulator are co-expressed to a significant extent.

Summary and conclusions

Investigations into the physiological roles of ERRα suggest it is an attractive therapeutic target with a range of applications. ERRα agonists may be useful in the treatment of metabolic or osteopenic disorders. However, activation of ERRα would likely present the liability of proliferation in tissues including breast. Conversely, ERRα inverse agonists may have utility in the treatment of breast and ovarian cancer but would likely have undesired effects on liver and bone metabolism. Because a therapeutically useful ERRα ligand would require efficacy with minimal liability, one potential approach involves development of a tissue-specific or pathway-selective

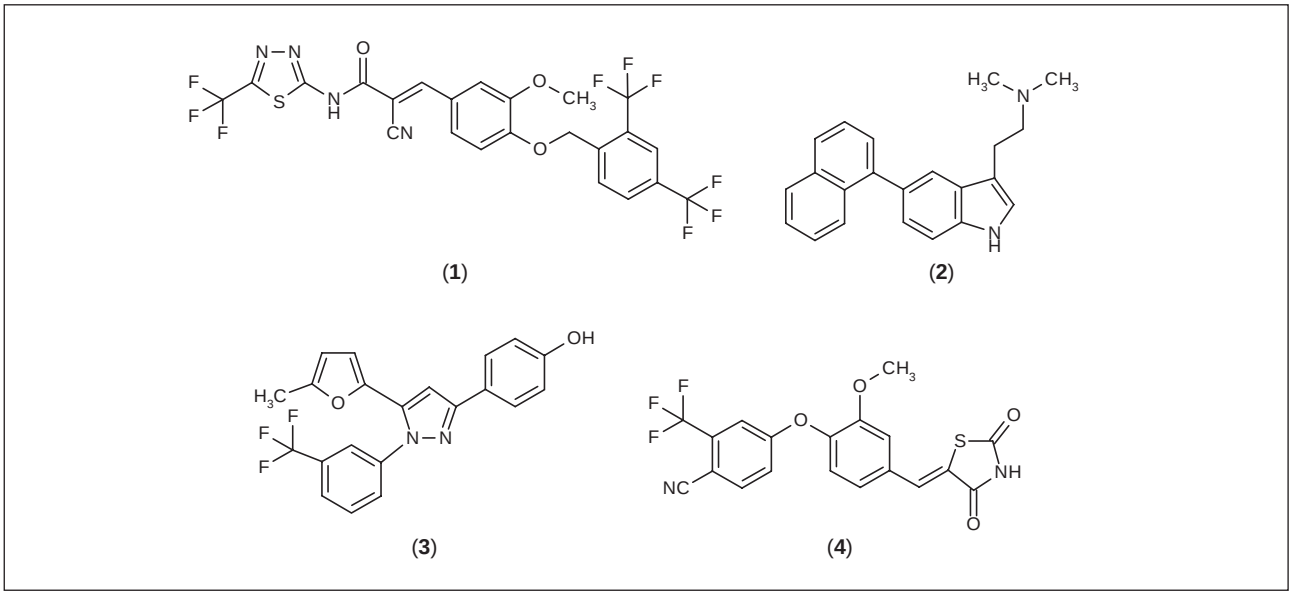

Fig. 4. Reported ERRα inverse agonists.

ERRα ligand. Tissue- or pathway-selective ligands have precedence (*e.g.*, selective estrogen receptor modulators) and a variety of strategies are currently being pursued to develop such compounds to modulate the activity of a growing number of nuclear receptors. Several ERRα inverse agonist ligands have been reported and may serve as useful starting points for ERRα-modulating compounds. However, structural evidence suggests that development of agonist ligands for ERRα will be severely hampered by the paucity of available volume within the ERRα LBP.

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