Chapter

Transcriptional Regulation by ERR and Its Role in NAFLD Pathogenesis

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Abstract

Members of estrogen-related receptors (ERRs) are orphan nuclear receptors (NRs) that play primary roles in mitochondrial biogenesis and bioenergetics. The ERRs regulate a range of cellular functions, including oxidative phosphorylation (OXPHOS) as well as glucose and lipid metabolism. ERRs are considered important targets for the treatment of metabolic diseases, particularly type II diabetes (T2D), insulin resistance (IR) and obesity. In this review, we will overview the transcriptional network regulated by the members of ERR transcriptional factors and elaborate on the regulation of ERR via its binding to PGC-1α, the primary co-activator of ERR as well as post-translational regulation of ERRs by upstream kinase signals. Recent development in ERR's cellular function has identified lipid metabolism/lipogenesis as a process that ERR regulates, and this function significantly impacts metabolic syndrome. Here, we will focus on their roles in lipid metabolic regulation and discuss the *in vivo* functions of ERRs in the development of non-alcoholic fatty liver disease (NAFLD), a comorbid metabolic syndrome concurrent with T2D, IR as well as obesity. Finally, we will explore ERRs as potential therapeutic targets by discussing the ligands that serve as antagonist/agonists for ERRs as well as efforts that target DNA binding of ERR as a transcriptional factor.

Keywords: estrogen receptor, nuclear receptor, energy metabolism, metabolic syndrome, mitochondria, fatty liver

1. Introduction

The ERR family of transcription factors are orphan NRs that are characterized for their functions in the transcriptional regulation of genes involved in mitochondrial bioenergetics and function [1]. Members of the ERR family orphan NRs regulate a range of cellular functions, including OXPHOS as well as glucose and lipid metabolism [2] and play critical roles in the transcriptional regulation of genes involved in mitochondrial bioenergetics, TCA cycle, mitochondrial OXPHOS, and fatty acid β-oxidation [3]. The ERRs in general serve as positive transcriptional regulators of genes regulating mitochondrial respiration and negative regulators for genes

regulating gluconeogenesis [4–6]. These properties make ERRs potential targets for understanding and treating metabolic diseases, particularly T2D, IR, and obesity. In this review, we will summarize the overall function of ERRs and their regulations. We will then focus on their roles in the development of liver steatosis, particularly NAFLD and non-alcoholic steatohepatitis (NASH).

2. Discovery of ERRs

ERR was first identified during the efforts of searching for isoforms of estrogen receptors (ER) using a reduced stringency hybridization protocol to screen recombinant DNA libraries and discover novel receptors [7]. In this screening, the estrogen receptor DNA-binding domain (DBD) was used as the hybridization probe to screen the human testis cDNA library. This screening resulted in the identification of three positive clones of which two encoded for known ERs. The third clone demonstrated partial sequence similarity with ER. This sequence was later identified as a novel receptor and termed the ERRs [7]. Like other NRs, members of the ERR family contain six conserved functional domain structures (A-F) [8, 9]. The most highly conserved region is the C domain, which contains the DBD. This domain is composed of two highly conserved zinc finger motifs, CI and CII, which are DNA-binding motifs that allow ERR to bind to the estrogen related response element (ERRE), which contains the hexanucleotide DNA sequence 5′AGGTCA-3′ that is also recognized by ER [10] (**Figure 1**). The ERRs are able to bind to ERRE as either monomers, homodimers, or heterodimers. The A/B domain located at the N-terminal, is also referred to as the activation-function-1 (AF-1) domain that is ligand-independent. This domain is the least conserved region compared to other NRs, and its activation capacity varies between different NRs [11]. The D domain contains the hinge region that is needed for receptor dimerization and is also involved in the interaction of ERR

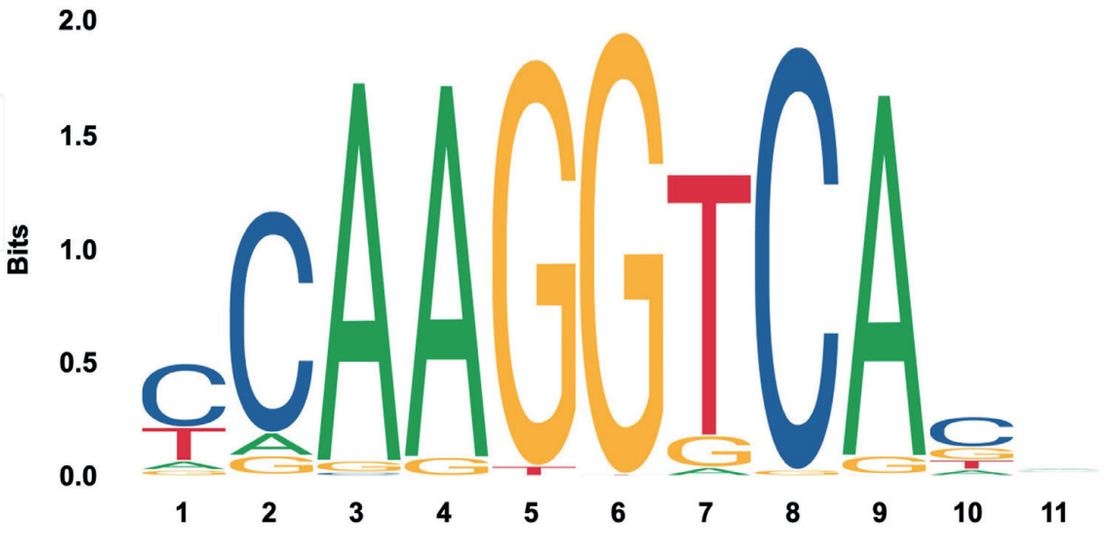

Figure 1.
ERRE binding motif sequence for ERR. JASPAR2022 database was used to generate a logo of the DNA binding motif sequence for ERR. The total height of the letters C, G, T and A at each position was derived from the mean information content available from a collection of transcription factor binding sites in units of bits (y-axis). The height of each letter is representative of the nucleotide frequency in a specific position (x-axis) in the aligned promoter sequence. The three isoforms of human estrogen related receptors have the consensus DNA sequence 5′AGGTCA-3′, which is referred to as the estrogen related receptor response element (ERRE).

with co-regulatory proteins [12]. The E/F domain located at the C-terminal contains the ligand binding domain (LBD). Once a ligand binds to the receptor, ligand-induced conformational changes to the LBD occur, and the ligand becomes trapped in the hydrophobic-binding pocket from the hydrophobic core of the LBD [12–14]. The LBD is also part of the AF-2 domain that is ligand-dependent. The full transcriptional activity for a NR requires the synergistic cooperation between AF-1 and AF-2 [11].

The DBD of ERR shares 54–68% amino acid homology with other known NRs including ER, but with very little target gene similarities [8, 15]. While structurally similar to ER, ERRs preferentially bind to 5′-TCAAGGTCA-3′ rather than the direct repeat sequence of two hexanucleotide sequence of 5′-AGGTCA-3′ that is preferentially recognized by ER [10]. This ERRE sequence represents a 3 bp 5′ extension of the classical hexanucleotide repeat response elements for classical nuclear receptors. Such response elements have been observed for receptors that bind to DNA as monomers, including the rev-Erb and retinoid-related orphan receptors among others. Thus, ERR is expected to be capable of binding to ERRE as a monomer. In addition, ERRs also share high LBD sequence similarity with ERs. However, despite the high sequence and structure similarities of the LBD, studies have found that ERR is unable to bind to estrogen as endogenous ligands. Crystal structures of the coactivator bound LBD domains of ERR show that they adopt an active conformation without the binding of a ligand [16–18]. This conformation is similar in structure as the estrogen bound active ERα. The putative ligand binding pocket formed by the LBD domains of ERR appears to be the smallest observed among nuclear receptors and can only accommodate a structure that is half the size of estrogen. In addition, docking studies show that steric hindrance of D ring on estrogen with L345 and F435, precludes binding of estrogen to ERR in the active conformation.

3. Estrogen related receptor (ERR) family of transcriptional factors and the transcriptional networks they regulate

3.1 Regulation of estrogen-responsive genes by ERRs

Despite their sequence homology (36%) with ERs in the LBD, ERRs do not (or only very weakly) respond to estradiol (E2) and are constitutively active [18–22]. Their LBD interacts with the steroid receptor coactivator 1 (SRC-1) in the absence of any ligand and resumes an active conformation [18]. Since ERRs are identified using DBD of ER and the two receptors share high DBD domain similarities, all three members of the ERR family are able to bind to the half-site hexanucleotide repeat of the classical estrogen response element (ERE) that are recognized by ER [8]. Because of these characteristics of ERRs, earlier studies focused on identifying target genes that are shared by ERR and ER. These studies identified a small handful of genes of which the transcriptions are co-regulated by ERR and ER [23–25]. These genes were associated with clinical outcomes in a COX regression analysis. Among them, pS2, a well-recognized marker for breast cancer was the first common ERR and ER target identified [25]. It was demonstrated that ERRα is a transcriptional activator that interacts with coactivators and binds to EREs in the absence of a ligand in ER+ breast cancers. This ERR induced activity was accredited for the ability of diethylstilbestrol, an ER/ERR antagonist to inhibit pS2 expression in ER- breast cancer cells. Using luciferase reporters in ER+ MCF-7 cells, it was shown that the ERRα competes with ER for binding to ERE and acts as a repressor for the transcription regulation of ER

responsive genes. On the other hand, ERRα acts as a transcription activator in Hela cells when ER and estrogen are not present [13].

3.2 Isoform specific transcriptional regulation by members of the ERR family of transcriptional factors

The ERR subfamily of nuclear receptors comprises three members: ERRα (NR3B1), ERRβ (NR3B2), and ERRγ (NR3B3), with all members having high amino acid sequence homology. ERRβ and ERRγ have high conservations in their LBD domain, where they share less similarities with ERRα. A distinct difference in ERRα is the presence of a phenylalanine at F382 that significantly alters the size and shape of the ligand binding pocket. As a result, ERRα cannot bind to 4-hydroxytamoxifen which acts as antagonist for ERRβ, γ as well as ERs. ERRα, the first orphan nuclear receptor identified from its close homology to Erα [26], is ubiquitously expressed in all cells and tissues, and highly expressed in high oxidative organs. In these tissues/cells, ERRα regulates the expression of genes involved in glycolysis, such as glyceraldehyde dehydrogenase (GAPDH), and binds to the glucose transporter family members [27]. In breast cancer cells, decreased uptake of glucose is observed in the absence of ERRα [28]. Therefore, ERRα is recognized as an important transcriptional activator for cellular glucose metabolism in response to environmental stimuli [29].

In addition, ERRα is identified as the key transcriptional factor for the regulation of OXPHOS [19–22]. Using mouse myocytes to screen for cis-regulatory elements responsible for the regulation of OXPHOS by PCG-1α and β (Peroxisomal proliferation activated receptor γ (PPARγ) coactivator-1), ERRα was identified together with a ETS family of transcription regulators. In this study, 20 common motifs are identified from over 5000 differentially expressed genes induced by the exogenous expression of PGC-1α (1–3 days) [20]. A majority of the 20 motifs, particularly those that displayed changes early in days 1 and 2, are related to ERR regulated promoters. The ERR motifs are found in >50% of the OXPHOS genes coregulated by PGC-1α. Supporting this analysis, a study performed in livers from type 2 diabetes patients identified ERRα and PPARγ as the two nuclear factors correlated with OXPHOS and can be used as predictors for fasting glucose levels [19]. Adenovirus-mediated expression of PGC-1α in ERRα positive and negative mouse embryonic fibroblasts (mEFs) derived showed that genes regulating mitochondrial functions were among the primary transcripts differentially regulated when ERRα is lost [30]. In particular, the inability of PGC-1α to induce citrate synthase activity, a key indicator of mitochondrial activity in the absence of ERRα supports the role of ERRα in the regulation of mitochondrial function.

ERRα regulates the expression of genes that form the mitochondrial respiratory system, including those that encode proteins involved in the TCA mitochondrial oxidative phosphorylation, respiratory chain, and TCA cycle (**Figure 2**). In addition to regulating the expression of genes encoding mitochondrial proteins, inhibition of ERRα also diminishes the ability of PGC-1α to increase mitochondrial DNA content [21]. In SAOS2 cells where PGC-1α induces mitochondrial biogenesis and function, ERRα is needed for sustaining the expression of TFAM, a mitochondrial transcriptional factor that induces mitochondrial DNA replication and transcription, Tim22, a core translocase protein responsible for the integrity of mitochondrial inner membrane proteins, isocitrate dehydrogenase α, which catalyzes the irreversible oxidative decarboxylation of isocitrate to yield α-ketoglutarate (α-KG) and CO2 as part of the TCA cycle, carnitine/acylcarnitine translocase, the rate limiting enzyme for fatty

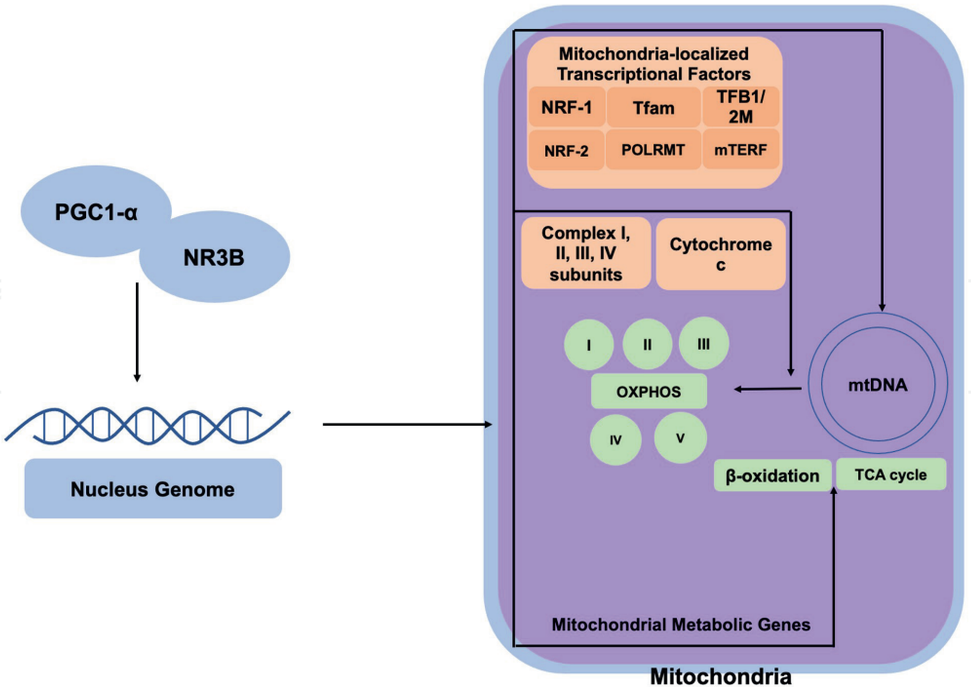

Figure 2.
ERR is a master regulator for mitochondrial functions and biogenesis. ERR regulates the gene expression of metabolic enzymes and mitochondrial respiratory complexes in the nucleus and mitochondria. PGC-1α binds to NR3B as a coactivator, entering subsequent transcriptional process. It further impacts transcriptional factors such as NRF-1/2, TFAM, POLRMT, TFB1/2M, METERF. Mitochondrial DNA (mtDNA) transcriptional activity is then activated. Meanwhile, genes encode proteins involved in mitochondrial oxidative phosphorylation, respiratory chain, and TCA cycle, including cytochrome C (Cytc), NADH dehydrogenase are regulated as well.

acid oxidation, as well as Cytochrome *c* and ATP synβ, both directly involved in the electron transportation during OXPHOS [21].

The role of ERRα as a regulator of mitochondrial function and OXPHOS is validated in neonatal cardiomyocytes, where a significant number of genes induced by ERRα expression are involved in cellular energy metabolic pathways [22]. In addition, a number of genes involved in mitochondrial fatty acid oxidation and lipid uptake are also induced by ERRα overexpression. Notably, medium chain acyl-CoA decarboxylase (MCAD), the rate limiting enzyme involved in fatty acid β-oxidation was confirmed as a direct transcriptional target for ERR by several other studies as well [10, 22].

Despite the differences in LBD, gene-chip analysis shows that ERRα and ERRγ target a common set of promoters of genes related to OXPHOS and fatty acid oxidation [31, 32]. In this context, both ERRs serve as positive transcriptional regulators of genes regulating mitochondrial respiration and fatty acid oxidation as well regulators for genes involved in gluconeogenesis [31, 33, 34]. In addition, ERRγ also positively regulate the promoters of G6Pase and PEPCK, two rate limiting enzymes of gluconeogenesis, while ERRα has been shown to be the transcriptional repressor of PEPCK [34]. ERRγ, the newest member identified in the ERR superfamily, plays a role in controlling metabolic switching in the perinatal heart and acts as a direct transcriptional regulator of GATA4 [35]. Compared to ERRα, which is associated with poor breast cancer outcomes, the overexpression of ERRγ was reported to associate with a better prognosis [36]. ERRγ was also identified as a potential tumor suppressor in gastric cancer by negatively regulating the Wnt signaling pathway [37]. Similar to ERRα, ERRγ plays a role in the regulation of mitochondrial gene expression [38].

In recent years, a role of ERRs in pluripotency has been identified primarily through studies of reprogramming of somatic cells to immortalized pluripotent stem cells (iPS) [39–41]. This was first recognized with ERRβ, which was found to replace Nanog or Klf4 during reprograming of iPS [39, 42]. In particular, ERRβ was found to bind to many target sites co-occupied by OCT4-SOX2-Nanog (OSN) [40, 41, 43], the transcriptional network characterized for their functions in maintaining "stemness". ERRβ participates in the regulation of these factors and their targets and is also a direct transcriptional target of Nanog [39]. Conversely, ERRβ also interacts with Oct4 within the *Nanog* promoter, a component also regulated by the Wnt/Gsk3 pathway [27, 41]. Further studies show that ERRβ is regulated by leukemia inhibitory factor (LIF), Wnt, PROX1, Ncoa3 as well as nucleostemin [44–48], all involved in pluripotency regulation.

In vivo, ERRβ is highly expressed during embryogenesis and is involved in the development and physiologic function of different tissues and organs, including the placenta, inner ear, and retina [49]. Embryos carrying homozygous deletions of ERRβ displayed impaired placental formation and died *in utero*, indicating that ERRβ plays a crucial role during early placental stages [50]. Consistently, knockdown or knockout of ERR allows for differentiation and calcium deposition while suppressing expression of genes associates with progenitor cells [51–53].

It was later reported that all three ERR isoforms are capable of supporting iPS reprogramming [54]. Using ERRα and γ and their cofactors to induce OXPHOS, it was demonstrated that at least an initial burst of the OXPHOS activity is necessary for the reprogramming of iPS [54]. ERRβ also regulates OXPHOS, similar to ERRα and γ [55]. Together, this work established that ERR transcriptional factors and the transcriptional network regulated by ERRs play an important role in the regulation of cell fate.

4. Regulation of ERRα

4.1 Regulation via binding with co-activator PGC-1

Like other NRs, ERRs bind to co-activators for their functions. Although the p160 family of coactivator SRC-1 was used to co-crystalize with LBD of ERRγ, the p160 family of coactivators only weakly bind with ERRs. On the other hand, ERRα is identified as one of the primary partner for PGC-1α in the regulation of mitochondrial biogenesis [56]. PGC-1 was discovered to bind both ERRα and γ via a LXXLL motif that is also necessary for PGC-1 to bind other nuclear receptors [57]. The AF-2 domains on ERRs are needed for this binding to occur. PGC-1 is highly expressed in tissues with high energy demands including heart, kidney, brown fat, and muscle [58], similar to the tissue distribution of ERRα and γ. Studies suggest that PGC-1 can be considered as the protein "ligand" for ERR since there is no endogenous lipophilic ligand identified for ERRs [59]. Binding to PGC-1α turns ERRα from a weak to a strong transcriptional factor. This is achieved by directly increasing of ERRα expression as PGC-1α-ERRα complex binds to the promoters of ERRα itself [60, 61]. In addition, cofactor binding allows ERRs to assume active conformation and increase its activity as a transcriptional factor [16, 62]. Together, the PGC-1α-ERR complex activates genes that encode proteins critical for mitochondrial components or activating transcription factors involved in mitochondrial biogenesis. In addition, PGC-1β, another isoform of PGC-1 coactivators that is often considered regulators of basal mitochondrial biogenesis, has also been reported to bind and regulate the transcriptional activities of ERRs.

PGCs are originally identified as transcriptional coactivators of PPARγ for adaptive thermogenesis in response to cold induction [58]. All three members of the PGC family (PGC-1α, PGC-1β and PRC) of coactivators play roles in mitochondrial biogenesis by regulating the expression of overlapping genes. Unlike the p160 family of coactivators, PGC-1 family of coactivators does not possess histone acetyltransferase activities. Instead, they provide docking sites for histone acetyltransferases including SRC-1 as well as CBP/p300 [63]. PGC-1α responds to different stimuli to induce mitochondrial biogenesis via binding to ERRs as well as others nuclear receptors such as PPARγ and nuclear respiratory factors (NRF). PGC-1 s also have the capacity to bind other transcriptional factor including the forkhead and the yin-yang transcriptional factors among others [6]. Thus, the activity of these transcription factors including ERRs are coordinately coregulated through their competition and coordination in binding to PGC-1. In addition, the binding of PGC-1α to ERRs and other nuclear factors is regulated by Prox1, a homeobox protein that is tethered with ERRs and other nuclear factors to participate in their transcriptional activity [44].

PGC-1 s are subject themselves to post-translational regulation, and these regulations play important roles in their response to different stimuli. In general, PGC-1α responds to the different stimuli and is regulated by cell signaling pathways to control mitochondrial biogenesis and function. Notably, AMP activated kinase (AMPK) phosphorylates PGC-1α on Thr177 and Ser 538 and increases its transcriptional activity [64]. On the other hand, PGC-1α phosphorylation by AKT or S6K integrates nutrient signals to suppress its gluconeogenesis and activity towards fatty acid oxidation [65, 66]. PGC-1α also cross-talks with the sirtuin family of protein deacetylases to regulate metabolism. PGC-1α is activated by SIRT1-mediated deacetylation when cells sense changes of NAD+/NADH ratios [67]. In addition, PGC-1α is also methylated and ubiquitinated to meet different cellular energy demands [68].

4.2 Post-translational modifications and signaling

In MCF-7 cells, treatment of epidermal growth factor (EGF) leads to phosphorylation of ERRα and enhances its transcriptional activity [69]. In this study, PKCd was found capable of phosphorylating ERRα on the DBD, resulting in its enrichment at the ERRE containing promoters [69]. Screening breast cancer samples for expression of ER and ERR isoforms identified ERRα as the potential biomarker for poor prognosis for ER- and ErbB2 high expressing tumors [36]. In MCF-7 cells, overexpression of ErbB2 leads to hyperphosphorylation of ERRα and increased transcriptional activity [70]. This ErbB2 induced phosphorylation is readily inhibited by anti-ErbB2 as well as U0126 and LY294002, inhibitors for MAP kinase and AKT, two major signaling kinases downstream of ErbB2 signaling pathway. *In vitro*, both MAPK and AKT were found to phosphorylate AKT. Multiple phospho-sites are found throughout the protein for MAPK and phospho-sites for AKT are also predicted based on these in vitro kinase studies. However, no phospho-site has been identified thus far for each of the kinases.

Being the downstream signal induced by insulin signal, the PI3K/AKT signal plays major roles in regulating glucose metabolism, including glycolysis, gluconeogenesis as well as the TCA cycle and mitochondrial functions [71] (**Figure 3**). In hepatocytes and livers where PI3K/AKT signal is induced due to loss of negative regulator phosphatase and tensin homolog deleted on chromosome 10 (PTEN) expression, upregulation of ERRα as well as OXPHOS are observed [72]. Activation of PI3K/AKT leads to increased oxygen consumption (OCR) as well as induction of mitochondrial biogenesis [72, 73], whereas inhibiting ERRα activity blocks the induction of

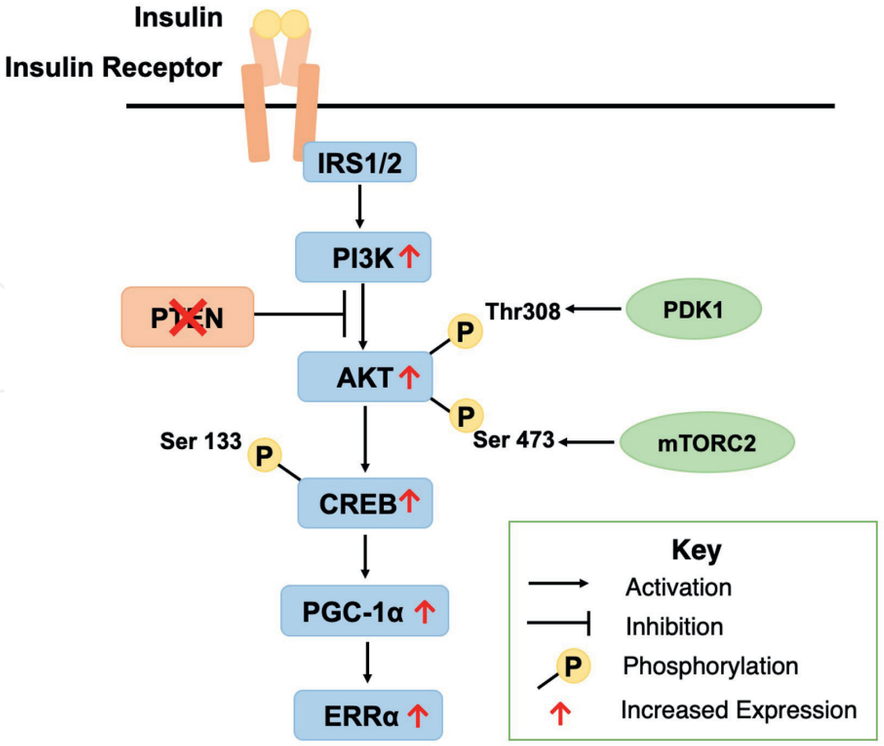

Figure 3.
PI3K/AKT signalling regulates ERRα. Activation and phosphorylation of insulin receptors results in recruitment of PI3K and the subsequent conversion of phosphatidylinositol (3,4)-biphosphate (PIP_2) to phosphatidylinositol (3,4,5)-triphosphate (PIP_3). PTEN, a negative regulator of the PI3K/AKT pathway, converts PIP_3 back to PIP_2. Following binding to PIP_3, the serine/threonine kinase AKT becomes fully activated via phosphorylation at Thr308 and Ser473 by 3-phosphoinositide-dependent kinase 1 (PDK1) and mammalian target of rapamycin complex 2 (mTORC2), respectively. Activated AKT then phosphorylates various downstream substrates, including forkhead box O (FOXO) transcription factors, glycogen synthase kinase-3 (GSK3α/β), and tuberous sclerosis complex-2 (TSC2), a critical negative regulator of mTORC1 signaling. Activated AKT also phosphorylates CREB at Ser133, leading to an increase in PGC-1α and ERRα expression.

mitochondrial function by PI3K/AKT signal [72]. Phosphorylation of CREB by AKT is thought to play a role in the regulation of ERRα by AKT in these cells, though direct phosphorylation of ERRα by AKT cannot be ruled out.

Due to the lack of endogenous ligands identified, ERRα is thought to be regulated primarily via transactivation and by upstream signaling pathways. However, not much has been elucidated for post-translation modification of ERRs beyond the reported phosphorylation of ERRα associated with breast cancer cell growth and survival. One addition modification reported is sumoylation at lysine 14 (Lys14), which suppresses its transcriptional activity with unexplored mechanisms [74]. It was found that this sumoylation of ERRα is dependent on its phosphorylation at serine 19 (Ser19).

The discovery of the roles ERR play in iPS have led to studies exploring how ERR signals crosstalk with those regulating pluripotency. These studies led to the discovery that ERRα physically interacts with β-catenin and lymphoid enhanced-binding factor-1 (LEF-1), with an overlap among genes previously demonstrated to be regulated by either β-catenin or ERRα [75]. A reduction of migratory capacity of breast, prostate, and colon cancer cell lines was observed following silencing of either β-catenin or ERRα with siRNAs, and this effect was further enhanced when the expression of both proteins was reduced simultaneously. The increased migratory capacity of cancer cells was suggested to occur as a result of the ERRα/β-catenin-dependent induction of Wnt 11, an activator of noncanonical Wnt signaling pathway [75]. Furthermore, ERRα

is also reported to regulate osteoblast differentiation via the Wnt/β-catenin signaling pathway. In C3H10T1/2 cells overexpression of ERRα with PGC-1α or overexpression of Wnt3a, a significant overlap in gene expression is observed. These results suggest that the expression of ERRα and PGC-1α causes similar gene changes within the Wnt pathway as activation by Wnt3a alone [51].

5. The role of ERR in lipid metabolism

The functions of ERRs have been defined by their interactions with PGC-1 s as coactivators. Beside inducing OXPHOS and supply the cellular energy demand, ERRs also activate transcription of numerous genes involved in oxidative metabolism that depends on mitochondrial respiration. ERRE is located in the promoter region of the gene that encodes carnitine-acylcarnitine carrier (SLC25A29), which is involved in the net transport of fatty acyl units to the mitochondrial matrix, where they are oxidized through β-oxidation. In addition, ERRE is located in the 5′-flanking region of the gene encoding for MCAD, a key enzyme involved in the initial step of mitochondrial β-oxidation [10]. AMPK-mediated expression of ERRα and PGC-1β and subsequent expression of MCAD/CPT1 is found to play a role in fatty acid β-oxidation in tamoxifen-resistant MCF-7 cells.

Genetic deletion of ERRα or ERRγ in mice confirmed the role of these ERRs in mitochondrial biogenesis and oxidative capacity, particularly in tissues with high energy demand [26, 76]. In cardiac muscles and brown adipose tissue where mitochondrial biogenesis and bioenergetic is needed for the function of the tissue, impaired adaptation to hemodynamic stressors and thermogenesis is observed respectively when ERRα is lost [77, 78]. Loss of ERRγ also resulted in the failure to switch to a oxidative transcriptome [76].

Given these positive regulatory roles of ERRs in mitochondrial respiration and fatty acid oxidation, loss of ERRs is expected to inhibit catabolic metabolism. However, deletion of ERRα, while viable and fertile, exhibited reduced fat mass and resistant to high-fat diet induced obesity [26]. Although mice lacking ERRβ or ERRγ are not viable to adulthood due to placenta and cardiac failures respectively [50, 58, 61, 76], pharmacological inhibition of either ERRγ (the dominant form of ERR in cardiac and skeletal muscles) or ERRα have led to improved insulin response and better tolerance to diet induced metabolic changes [79, 80]. Consistent with the reduced fat mass phenotype, lipogenic genes such as fatty acid synthase (Fasn) and elongase (Elov3) in the adipose tissue are all inhibited when ERRα is absent [26]. In agreement with this putative function of ERRs in lipogenesis, ERRα and PGC1-α expression are concurrently upregulated in response to adipogenic inductions [81]. ERR and PGC1-α together are found to be required for adipogenic differentiation induced by glucocorticoid, cAMP and insulin [81, 82]. Consistent with these observations, ChIP-on-Chip and ChIP-seq analysis indeed show that ERRα can occupy the promoter regions of Fasn and acetyl-CoA carboxylase (ACC), the two rate-limiting enzymes in the lipogenic pathways [83]. Inhibition of ERRα led to reduced triglyceride (TG) content in the liver accompanied by attenuated expression of Fasn and ACC [84].

The storage of lipid into TG starts with esterification of long-chain fatty acids to glycerol 3-phosphate [85] (**Figure 4**). This committed step is catalyzed by GPATs, the rate-limiting enzymes for the process. Acylation at carbon 1 leads to formation of lysophosphatidic acid (LPA) which is converted to phosphatidic acid (PA) via the action of AGPAT. During biosynthesis of triglycerides, PA is converted to

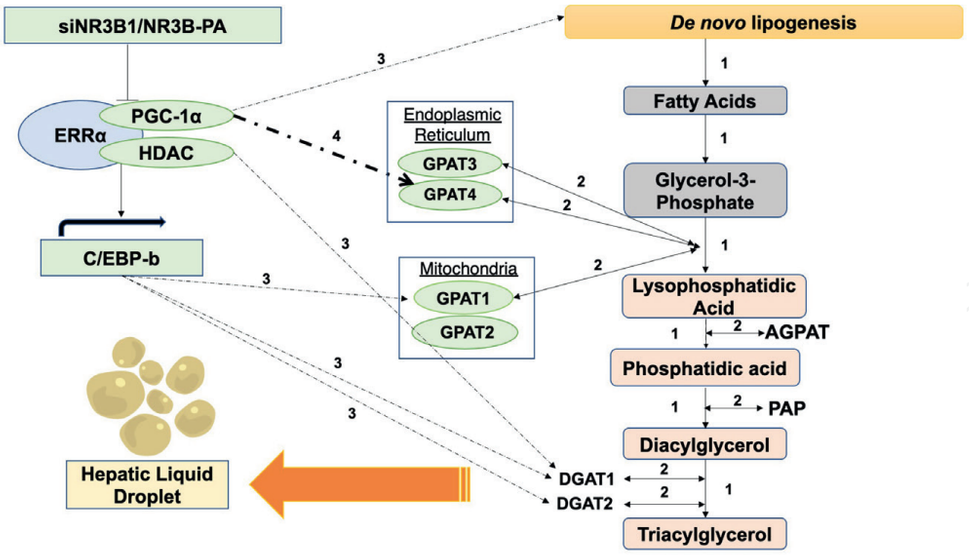

Figure 4.
Glycerolipid biosynthesis pathway. Arrows labeled 1 describe the steps leading to the formation of triacylglycerol. Hepatic de novo lipogenesis results in the synthesis of fatty acids through acylation, which is catalysed by GPAT. G3P and acyl-CoA is converted to lysophosphatidic acid with the help of GPAT. Following this, LPA is converted to phosphatidic acid, which is catalysed by AGPAT. Dephosphorylation of PA by PAP generates diacylglycerol, which further serves as the substrate for DGATs in the synthesis of triglycerides. Arrows labeled 2 indicate a catalysis reaction. Arrows labeled 3 indicate the transcriptional regulation of GPAT enzymes by PGC-1α, and arrow 4 indicates how GPAT4 is transcriptionally regulated by ERRα and co-activator PGC-1α.

diacylglyceride (DAG) via the actions of LIPIN, a group of enzymes recently gained significant attention relating to their functions in lipid particle formation and autophagy. The final step of TG biosynthesis is catalyzed by the actions of DGATs. The preferentially ERRE binding motif, which has a high frequency of 5′GGTCA-3′ was screened for and found in the promoters of *Esrra*, *Dgat1*, *Gpat4*, *Agpat1* and *Agpat3*. Histone marks were also recognized for *Esrra*, *Dgat1*, and *Gpat4*, suggesting that ERRα is being recruited near the transcription initiation sites [84].

Together, these studies thus suggest that ERRα is a broad spectrum of regulator for lipid metabolism including fatty acid b-oxidation, *de novo* lipogenesis as well as glycerolipid biosynthesis. However, how ERRα may play roles in lipid metabolism may be dependent on the metabolic state and physiological stimuli. For example, in liver steatosis induced by rapamycin treatment, lack of ERRα was shown to impair fatty acid oxidation while buildup of citrate due to downregulation of the TCA cycle is redirected towards lipid biosynthesis [83]. Thus, unexplored transcriptional roles of ERRs, at least ERRα in lipid biosynthesis vs. β-oxidation likely play a role in the *in vivo* phenotype observed with ERR function under different metabolic conditions.

6. ERRs in the development and progression of non-alcoholic steatohepatitis

NAFLD and NASH are common chronic liver conditions and comorbid diseases for more severe liver disease [86, 87]. Simple fatty liver or steatosis is readily reversible while NASH can progress to more morbid forms of liver pathologies. In subsets of patients, the disease can progress to fibrosis/cirrhosis and liver cancer.

Lipid metabolic dysfunctions in the liver is a key contributing factor to the development of liver steatosis [88, 89]. Depending on the metabolic state, different cellular

processes contribute to liver steatosis. High fat diet (HFD) induces hyperinsulinemia and hyperglycemia concurrent with glucose intolerance and IR together with NAFLD. During HFD feeding, dietary lipids may directly contribute to steatosis in addition to stimulating *de novo* lipogenesis in the liver. High carbohydrate diet (HCD) feeding, on the other hand, induces liver steatosis via carbohydrate-induced *de novo* lipogenesis. In both diets that induce steatosis, ERRα, the dominant liver isoform of ERRs, is upregulated in the liver [84]. Interestingly, ERRα is also induced in the steatotic livers of EtOH diet fed mice, further indicating a potential role of ERRα in liver steatosis.

In addition to diet induction, steatosis develops in patients with metabolic syndromes, including obesity, IR and Diabetes [86, 87]. As high as 80% of T2D patients exhibit NAFLD/NASH in the liver [87, 90]. Under these pathological conditions, abnormal insulin signals also contribute to liver lipid buildup in addition to the excess lipid and carbohydrate content coming from the diet. In obese individuals and particularly individuals with IR, "selective hepatic insulin resistance" is observed [91], where hyperinsulinemia cannot suppress hepatic gluconeogenesis (resistance) but continues to induce lipogenesis (non-resistance), leading to steatosis in the liver. Previous studies have established that insulin regulates *de novo* lipogenesis by activating phosphoinositide 3-kinases (PI3Ks), lipid kinases involved in the PI3K/AKT/mammalian target of rapamycin (mTOR) signaling [92]. In the livers, loss of PTEN expression, a negative regulator of PI3K/AKT, leads to *de novo* lipogenesis and NAFLD development [93, 94]. Loss of AKT2 resulted in attenuation of lipid and blocked NAFLD development [95–97].

Evidence suggests that the PI3K/AKT signal induces CREB cyclic AMP (cAMP)-response element-binding protein) to activate ERRα and mitochondrial biogenesis and bioenergetics in the hepatocytes [72, 98]. AKT was found to directly phosphorylate CREB at Ser133 as CREB contains a RXX(S/T) sequence that is a potential AKT substrate motif72. CREB is a 43 kDa basic/leucine zipper transcription factor expressed in most tissues. It is suggested to control the expression of over 4000 genes [99], including genes involved in regulation of hepatic glucose and lipid metabolism [100, 101]. Here, PGC-1 was found to be necessary for CREB to induce the gluconeogenesis program [101]. Notably, mice deficient in CREB function are prevented from hepatic lipid accumulation in models of metabolic syndromes such as the Zucker Rat, ob/ob mice, STZ induced T2D, as well as HFD-induced NAFLD [102]. However, loss of CREB function also led to fatty liver development in unchallenged mice [100]. The divergent response of CREB-ERRα regulation in hepatocytes where PI3K/AKT is active vs. inactive may be responsible for this paradoxical observation with CREB deficiency [72]. Consistent with this idea, mice deficient for ERRα (*Esrra*−/− mice) have been reported to be resistant to HFD-induced obesity and steatosis in the liver even though ERRα promotes a catabolic metabolic program [26].

It has been suggested that in addition to promoting TG breakdown during refeeding, ERRα also promotes TG buildup during chronic conditions [84, 103]. In NAFLD induced by rapamycin treatment, however, lack of ERRα was shown to impair fatty acid oxidation, while buildup of citrate due to downregulation of the TCA cycle is redirected towards lipid biosynthesis [83]. Thus, unexplored transcriptional roles of ERRs, at least ERRα in lipid biosynthesis vs. β-oxidation, likely play a role in the *in vivo* phenotype observed with ERR function under these different metabolic conditions. Indeed, in addition to the well characterized target MCAD, lipogenic genes are among the list of lipid metabolic genes that ERR regulates in data collected from a ChIP study, suggesting potential function of ERRs in lipid biosynthesis [44].

7. Therapeutic potential for targeting ERRα in NAFLD treatment

Fatty liver and associated diseases including ASH/NASH are comorbid diseases with diabetes, particularly type 2 diabetes (T2D) which accounts for 95% of all diabetes cases [104]. Fatty liver disease occurs in 80% of T2D patients [87, 90]. While simple fatty liver is readily reversible, NASH can progress to more morbid forms of liver pathologies including fibrosis/cirrhosis and even liver cancer. Currently, there is no therapy besides caloric restriction for the treatment of fatty liver disease [105]. A potential therapy is under development in clinical trials that activates FXR, a bile acid receptor. In a completed clinical trial (NCT01265498), FXR ligand obeticholic acid treatment led to NAFLD resolution in 21% of the subjects vs. 13% in placebo treated subjects after 72 weeks of treatment. While these clinical trials are underway, studies exploring molecules that play roles in liver lipid dysfunction have the potential to discover novel therapies. In mouse models, caloric restriction is capable of curing fatty liver disease [105].

The function of ERRs as master regulators for metabolism made them interesting targets for the treatment of T2D, as activating ERRs has the potential to improve overall mitochondrial respiratory function and suppress hepatic glucose output. Earlier studies, tethered with the elucidation of LBD structures, have focused on developing agonists or antagonists. These studies show that ERRs are constitutively active and identified several estrogen-related hydrophilic molecules that can bind and block the establishment of active conformations. These molecules such as the diethylstilbestrol (DES) and 4-hydroxytamoxifen (4-OHT) have been described as antagonists for ERRs, where DES binds to all three ERRs, and 4-OHT does not bind to ERRα [106, 107].

Due to the lack of endogenous ligand, XCT-790 was identified as a potent inverse agonist for ERRα and is used in many studies as inhibitors of ERR functions [108]. Using the NIH compound library in a couple high throughput screening studies, it was found that several pesticides contain ERRβ genes promoting activities, and these compounds may act as potential "ligands" for ERRs [109, 110]. The activity of ERRα has been reported to be antagonized by the organochlorine pesticides chlordane and toxaphene [111, 112]. Screening approaches also identified small molecules with the ability to alter ERR transcriptional activity. An example is the discovery of compound 11 that potently inhibits ERRα's transcriptional activity by preventing binding of ERRα to PGC-1α and suppressing the proliferation of different cancer cell lines [113].

Troglitazone also interferes with binding of ERRα and γ to PGC-1α. Troglitazone was recently discovered to be an inverse agonist for ERRα and γ [114]. Troglitazone is an FDA approved therapy for T2D. In hepatocytes, troglitazone inhibits oleic acid induced liquid buildup. Thus, activity of troglitazone towards ERRα likely plays a role in this effect. In addition, pharmacological inhibition of either ERRα or ERRγ has led to improved insulin response and better tolerance to diet induced metabolic changes [79, 80].

Beyond identification of ligand-like molecules that can serve as either antagonist or agonist for ERR, effort was put into blocking its binding to DNA. Pyrrole-imidazole (Py-Im) polyamides are a class of synthetic ligands for the sequence-specific recognition in double-helical DNA minor groove [84]. Polyamides targeted at the ERRE (ERR-PA) were designed to block binding of ERR onto the promoters of genes regulated by it. An *in vitro* study showed that there was over a 50% reduction in basal and maximal respiration with 0.2 μM ERR-PA treatment and around 70% reduction with 1 μM, emphasizing the dose dependency of the polyamide molecule [84]. ERR-PA was highly effective at reducing liver steatosis in multiple NAFLD models, including one with deletion of *Pten*, the negative regulator of

insulin signal; a HFD model, which induces lipid transport into the liver; and a HCD model that induces *de novo* lipogenesis. ERR-PA also reversed the NASH phenotypes observed in the mice where NAFLD/NASH is developed due to loss of hepatic *Pten*. This evidence suggest that inhibiting ERR activity can serve as a viable approach to treat NAFLD/NASH development.

TGs are the primary content induced in NAFLD/NASH. TGs are synthesized via the glycerolipid biosynthesis pathway, where Glycerol-3-phosphate (G3P) and acyl-CoA is converted to LPA with the help of GPAT enzyme activity followed by incorporation into DAG and TG. Enzymes catalyzing the steps in this biosynthesis are subjected to regulations by ERR and other nuclear transcriptional factors. *Gpat1*-null mice were found to have lower triacylglycerol and DAG concentrations and were protected from the HFD-induced insulin resistance, which was attributed to lower DAG-mediated PKCϵ activation [115]. Mice lacking GPAT3 expression had increased liver size with dysregulated cholesterol metabolism, implying that *Gpat3* plays a crucial role in regulating energy, glucose, and lipid levels [116]. GPAT4 is the major isoform in the liver and mammary gland [117]. Recent work has identified GPAT4 as a direct transcriptional target for ERRα and could be responsible for ERRα regulated NAFLD development [84].

8. Conclusion

ERRs are a family of orphan NRs that do not have a known endogenous ligand. Of the three isoforms of ERRs (ERRα, ERRβ, and ERRγ), ERRα and ERRγ are involved in the transcriptional regulation of mitochondrial metabolism and integrity, OXPHOS, glucose and lipid metabolism metabolism, while ERRβ plays a role in embryonic development. The transcriptional activity of ERRs requires binding with coactivator PGC-1α. ERRs share overlapping functions with PGC-1α regulated transcriptional networks and are subjected to the factors that regulate PGC-1α. In addition, ERRs are regulated post-translationally by upstream signal that also include the insulin regulated PI3K/AKT signaling pathways. In recent years, a major development in the cellular functions regulated by ERRs is the discovery of lipogenesis and glycerolipid biosynthesis regulation by ERR. These functions of ERRs allow them to play major roles in NAFLD/NASH development. Pharmacologically, significant efforts have been put forth to identify ligands for ERRs and these studies identified several agonists and antagonists for ERRs that can be further developed for future therapeutical efforts. Notably, DBD antagonists are also being developed and shown strong promise at targeting NAFLD. This review provides a brief and comprehensive view for the transcriptional network regulated by ERRs and their functions in NAFLD and potential therapeutical developments targeted at ERRs.

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Conflict of interest

The authors declare no conflict of interest.

Acronyms and abbreviations

ERR	estrogen related receptor
NR	nuclear receptor
OXPHOS	oxidative phosphorylation
TCA	tricarboxylic acid
T2D	type II diabetes
IR	insulin resistance
NAFLD	non-alcoholic liver disease
NASH	non-alcoholic steatohepatitis
ER	estrogen receptor
DBD	DNA-binding domain
ERRE	estrogen related response element
AF-1	activation-function-1
LBD	ligand binding domain
SRC-1	steroid receptor coactivator 1
ERE	estrogen response element
GAPDH	glyceraldehyde dehydrogenase
PPARγ	peroxisomal proliferation activated receptor γ
PGC-1	coactivator-1
mEFs	mouse embryonic fibroblasts
α-KG	α-ketoglutarate
MCAD	medium chain acyl-CoA decarboxylase
iPS	immortalized pluripotent stem cells
OSN	OCT4-SOX2-Nanog
LIF	leukemia inhibitory factor
NRF	nuclear respiratory factor
AMPK	AMP activated kinase
EGF	epidermal growth factor
PTEN	phosphatase and tensin homolog deleted on chromosome 10
OCR	oxygen consumption
LEF-1	lymphoid enhanced-binding factor-1
Fasn	fatty acid synthase
ACC	acetyl-CoA carboxylase
TG	triglyceride
LPA	lysophosphatidic acid
PA	phosphatidic acid
DAG	diacylglycerol
HFD	high fat diet
HCD	high carbohydrate diet
PI3K	phosphoinositide 3-kinase
mTOR	mammalian target of rapamycin
cyclic AMP (cAMP)	response element binding protein (CREB)
DES	diethylstilbestrol
4-OHT	4-hydroxytamoxifen
Py-Im	pyrole-imidazole
G3P	glycerol-3-phosphate

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