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Review

Functional and physiological genomics of estrogen-related receptors (ERRs) in health and disease ☆

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ABSTRACT

Orphan nuclear receptors, in a manner comparable to classic steroid hormone receptors, regulate key developmental and physiological processes. However, the lack of appropriate pharmacological tools has often hindered the identification and study of their biological functions. In this review, we demonstrate that functional and physiological genomics are effective alternatives to discover biological functions associated with orphan nuclear receptors. Indeed, we document that these approaches have allowed for the unambiguous identification of the estrogen-related receptors (ERRs) α, β, and γ (NR3B1, 2, and 3) as global regulators of cellular energy metabolism. We further show that although the three ERR isoforms control analogous gene networks, each isoform performs unique biological functions in a tissue-specific manner in response to a variety of physiological stressors. Finally, we discuss how the activity of the three ERR isoforms contributes to the development and progression of metabolic diseases as well as to the adaptation of cancer cells to their unique bioenergetic requirement. This article is part of a Special Issue entitled: Translating nuclear receptors from health to disease.

1. Introduction

As transcription factors, nuclear receptors mediate their biological activities through the transcriptional regulation of specific genes. Therefore, the identification of gene regulatory networks controlled by nuclear receptors is critical to understand the roles of these proteins in development, normal physiology, and diverse pathologies, including metabolic diseases and hormone-dependent cancers. During the last decade, the sequencing of mammalian genomes and the subsequent introduction of high-throughput approaches aimed at the analysis of transcription factor/genome interactions and modulation of transcriptomes have led to the development of the field of functional genomics. In particular, the application of chromatin immunoprecipitation (ChIP)-based technologies along with gene expression analyses has uncovered novel mechanistic paradigms of nuclear receptor action and identified vast gene networks governed by individual nuclear receptors [1]. When functional genomics studies are integrated with comprehensive experimental validation in cell-based and animal models subjected to genetic, pharmacological, and physiological perturbations, it becomes possible to uncover novel nuclear receptor-dependent biological functions (Fig. 1). This amalgamation of approaches, which we referred to herein as physiological genomics, can directly link biological phenotypes to well-defined molecular mechanisms. Although this investigative method can be applied to all nuclear receptors, it is perfectly suited for the study of orphan nuclear receptors. In this review, we highlight the successful use of this method that led to the identification of biological functions influenced by the three members of the estrogen-related receptor (ERR, NR3B) subfamily of orphan nuclear receptors in both normal physiology and in disease state.

2. A brief initiation to the ERRs

Since several recent reviews have described in details the origin, biological, and functional properties of the ERRs [2–5], only salient features of these receptors related to the subject of this review will be presented herein.

The ERR subfamily includes three members referred to as ERRα (NR3B1), ERRβ (NR3B2), and ERRγ (NR3B3). ERRα was originally cloned during a search for new members of the superfamily of nuclear receptors using a complementary cDNA encoding the estrogen receptor α (ERα, NR3A1) [6]. However, ligand binding studies and transient transfection experiments with reporter genes failed to link natural estrogens to, or to identify another class of natural signalling molecules for this new receptor. ERRα was therefore classified as the

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first orphan nuclear receptor [6] but was certainly not the last [7]. Indeed, this attribute was reserved for ERRγ whose existence was uncovered only a decade later [8]. In contrast, ERRβ was identified shortly after ERRα using its cDNA as a probe [6]. But due in part to its low level of expression in most tissues and to the early lethality of the *Esrrb* knock-out mouse [9], ERRβ was not as intensively investigated as its nearest relative, ERRα. In view of the close structural relationship between ERRα and ERα, initial studies on ERRα focused on possible functional interactions between ERRα and ERα in estrogen-responsive tissues and cancer cells, including the sharing of coregulatory proteins and DNA-binding elements [2,10,11]. Although several synthetic drugs, including many estrogenic compounds such as diethylstilbestrol and 4-hydroxytamoxifen, have been shown to influence the transcriptional activity of the ERRs [12–20], all three ERR isoforms are still considered *bona fide* orphan nuclear receptors.

Like other members of the nuclear receptor superfamily, the ERRs regulate gene expression through binding to a specific sequence in the regulatory regions of target genes. The binding site for the ERRs, referred to as an ERRE, was defined as TNAAGGTCA using an unbiased binding site selection approach [21]. The transcriptional activity of the ERRs is constitutive and independent of exogenously added ligands. However, the activity of the ERRs is highly dependent on the presence of coactivator proteins, most notably that of peroxisome proliferator-activated receptor γ (PPARγ)-coactivator 1 α (PGC-1α) and PGC-1β [22–29]. The ERRs can also interact with and be stimulated by members of the steroid receptor coactivator (SRC) family [30–33]. On the other hand, the transcriptional activity of the ERRs can be potently suppressed by the orphan nuclear receptor small heterodimer partner (SHP, NR0B2) [34], the nuclear receptor interacting protein 140 (RIP140, NRIP1) [32,35–37] and the homeodomain-containing protein PROX1 [38]. Therefore, the relative levels of specific coactivator and corepressor proteins present in a given cell type likely dictate whether the ERRs act as activators or repressors of gene expression in a given tissue or cell line.

One or more ERR isoforms are expressed in each cell or tissue investigated to date [39]. In particular, the three ERR isoforms are expressed at high levels in the heart and kidneys, two tissues with elevated energy demand. The expression of ERRα is ubiquitous but can be observed at higher levels in the intestine, brown fat, skeletal muscle, and cytokine-activated macrophages [6,21,40]. The distribution of ERRβ is more limited, but substantial levels of expression have been reported in the eye, inner ear, and the extra-embryonic ectoderm of the developing placenta, as well as in mouse but not human embryonic

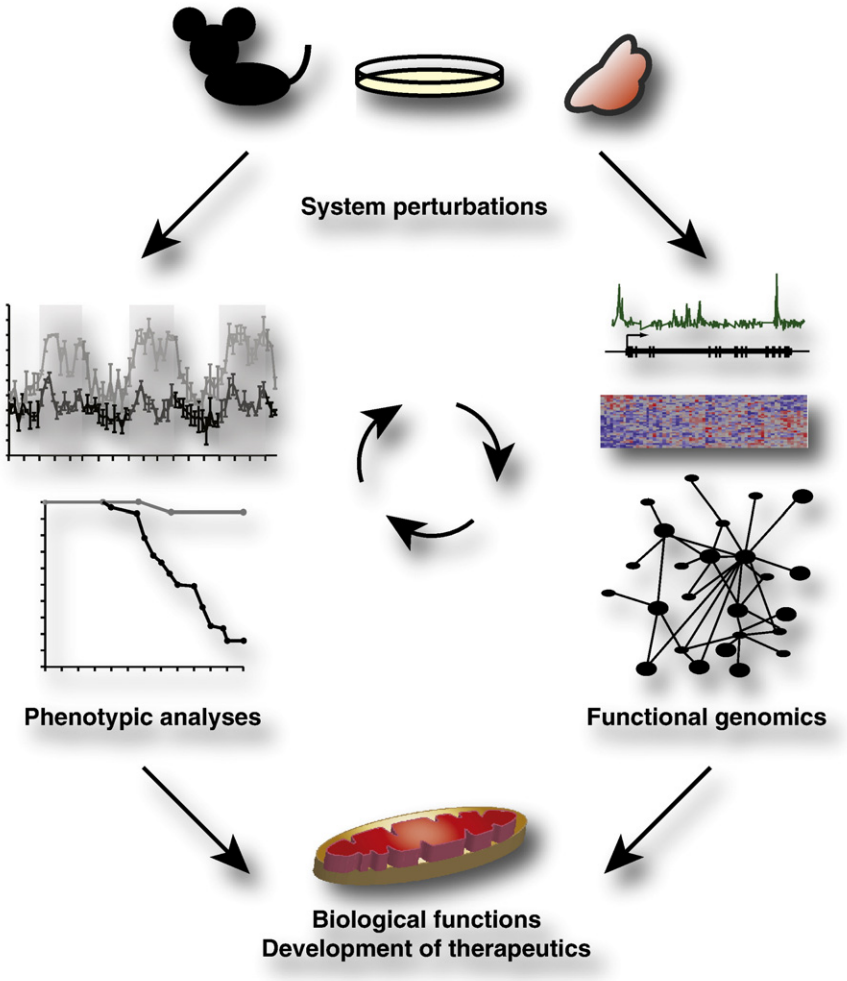

Fig. 1. Physiological genomics of nuclear receptors. Schematic representation of the components of a physiological genomics analysis. A biological system (cells in culture, a specific organ, or the whole organism, top) is perturbed using methods such as gene knock-out or knock-down, overexpression, and/or pharmacological or physiological challenges. The system is then analyzed for alterations in gene expression (DNA microarrays) and occupancy (ChIP-chip or ChIP-seq) profiles and to build regulatory networks (right), as well as for phenotypic changes in the system (left). Information obtained via these two parallel investigative paths are then integrated and used to design more specific perturbations in the system. After one or more reiteration of this cycle, novel biological functions associated with a particular nuclear receptor can be uncovered (e.g., mitochondrial biogenesis for ERRα), a process that can also lead to the development of novel therapeutic applications (bottom).

stem cells [5,6,9,39,41–44]. ERRγ expression is also prominent in the developing heart, the brain stem, and the spinal cord [39,45]. Of note, the expression of the three ERR isoforms has been shown to display diurnal rhythmicity in several tissues that include the liver, skeletal muscle, white fat, kidneys, bones, and uterus [46–48].

3. Functional genomics of nuclear receptors at a glance

3.1. Gene expression analysis

The most common approach to assess the contributions of a nuclear receptor to the gene expression profile in a system is achieved using perturbation assays followed by measurement of variations in mRNA levels of the target genes [49]. Gene expression profiles can subsequently be submitted to various functional and bioinformatic analyses to cluster the transcripts according to their expression pattern and to extract informations about gene ontology and various known biological pathways that are affected by the perturbation.

A convenient approach to perturb the activity of nuclear receptors in the system prior to measurement of RNA transcripts of target genes consists in using specific ligands to modulate the activity of the receptor. For example, several studies have treated estrogen-responsive cells with estradiol and selective estrogen receptor modulators in order to identify target genes affected by various ERα ligands in different contexts [50–52]. There are at least two well-known caveats to these pharmacological studies. First, even with the best-characterized compounds, there is always a possibility that the drug affects, directly or indirectly, other factors and signalling pathways within the cell. Second, this approach cannot easily differentiate between primary and secondary targets of the receptor. Given the rarity of ERR-specific ligands, this approach has not been very helpful to study ERR function to date. However, a recent study using a newly identified inverse agonist for ERRα (Compound A) has shown that the expression of ERR validated metabolic target genes can be modulated by pharmacological agents [53]. Therefore, such an approach should contribute in the near future to elucidate ERR-dependent pathways in cell- and mouse-based models.

Since a ligand-based approach has not yet materialized to analyze ERR-dependent changes in transcriptomes, alternative methods to stimulate the activity of these orphan receptors have been developed. For instance, overexpression of the coactivator PGC-1α or of a variant of the coactivator that specifically interacts with ERRα has been used to modulate the activity of the receptor [29,54–56]. Overexpression of ERRα itself in cells expressing PGC-1α and PGC-1β has also been used to identify potential ERRα target genes [57]. Conversely, methods that consist in deleting (gene knock-out) or partially depleting the receptor (gene knock-down) in the system have been employed to identify biological pathways modulated by the ERRs [45,48,56,58–60].

3.2. Integrative genomics

As mentioned above, while useful to gain insights on the action of specific ligands or perturbation in different cell contexts, the identification of gene expression profiles does not necessarily distinguish between primary and secondary targets of the nuclear receptor being modulated. The initial attempts in identifying nuclear receptor direct target genes were based on reverse functional genomics consisting in inspection of promoters of responsive genes to detect nuclear receptors binding sites, a low-throughput and inefficient process. In the case of ERRα, this was achieved initially through the identification of the ERRα-binding site by unbiased binding site selection, manual inspection of the promoters of ERRα-responsive genes, and/or functional analyses of promoter regions [21,56,58,61–64]. One of the first global approach that identified a significant number of potential ERR direct target genes consisted in the development of large-scale integrative genomics where the promoters of differentially expressed genes modulated upon over-expression of PGC-1α in mouse myoblast cells were analyzed for *de novo* motif discovery [29]. This approach indeed showed an enrichment for ERRE-like sequences in the promoters of these genes.

3.3. Location analysis

Over the last few years, improvement of the ChIP technique [65,66] and development of large-scale genomic technologies such as ChIP-on-chip [67] and ChIP-Seq [68] have allowed global scale location analyses of transcription factors, co-factors, and RNA-polymerase II, as well as histone modification marks in various cellular contexts. Integration of various location analyses in a specific system further puts in relation the binding of nuclear receptors throughout the genome with active or repressed promoters and enhancers delineated by specific epigenomic marks and RNA-PolII recruitment [69]. The development of more advanced techniques like ChIA-PET further allows linkage of distinct genomic regions that together cooperate in gene regulation [70]. Therefore, the identification of functional nuclear receptor binding sites throughout the genome has the potential to provide a more comprehensive and mechanistic view of the transcriptional networks that it directly regulates.

Numerous studies using location analyses approaches have extensively contributed to the discovery of functional nuclear receptor binding sites and networks in various contexts, especially for liganded receptors like ERα [1]. An important observation emerging from these studies is that some nuclear receptors like ERα are preferentially recruited to distal regions from gene promoters [71]. However, chromosome- and genome-wide ERRα [72] and ERRβ [73] location analyses have shown that the proportion of ERR-binding sites located within promoter regions of target genes is significantly higher than for ERα. Detection of transcription factor-binding sites at the genomic scale also allows identification of DNA response elements using bioinformatics tools. The study of the DNA sequences bound by ERRα obtained by ChIP-on-chip in human breast cancer cells has univocally dissociated ERRα recruitment from that of ERα at the genomic level [74]. While both classes of receptors can recognize ERα-binding sites (EREs) and compete for occupancy to these sites *in vitro* [33,75,76], *de novo* binding site discovery on ERRα ChIP-on-chip target sequences revealed that in an *in vivo* context, ERRα specifically binds to ERREs and only competes with ERα on EREs that also contain an embedded ERRE. Future functional genomics studies are thus likely to reveal additional mechanisms that will distinguish between the molecular mode of action of the ERRs from other nuclear receptors.

3.4. Building transcriptional networks

Examination of DNA response elements present in the vicinity of the nuclear receptor binding sites can also reveal functional association between different types of transcription factors. For example, analyses of ERα-bound sequences in MCF-7 cells coupled with extensive experimental validation, identified FOXA1 as a licensing factor for ERα-regulated transcriptional activity in breast cancer cells [77,78]. The initial location analyses studies for ERRα in various tissues also revealed enrichment of binding sites for other transcription factors like STAT3, CREBP, and NRF1 in the vicinity of ERRα bound segments, indicating that these factors might cooperate with ERRα in the transcriptional regulation of a large subset of genes [38,60,72]. Examination of the DNA sequences surrounding nuclear receptor binding sites can also be used to define functional transcription regulatory modules that are informative on the mode of action of the receptor [79]. In addition, chromosome-wide and extended promoter regions location analyses of the ERRα coactivator PGC-1β have shown a strong overlap with ERRα-bound regions in breast cancer cells [72]. Bioinformatic studies have identified the

enrichment of ERRE in PGC-1β-bound regions, reinforcing the concept that PGC-1β is the preferential coactivator for ERRα in these cells.

4. A physiological genomic view of ERR-driven biological functions

Functional genomic techniques are now being used to uncover large-scale interconnections between gene expression profiles, nuclear receptor genomic recruitment, chromatin landscape, and various signalling pathways that contribute and work in relation with one another in establishing the genetic program of the cell. As discussed in the next sections, integration of gene expression profiles, location analyses data, and phenotypic analyses of ERR-perturbed biological systems have been used to uncover several functions of the ERRs in normal physiology and in the disease state. A summary of these findings is presented in Table 1.

4.1. ERRs as global regulators of mitochondrial biogenesis and energy metabolism

The initial characterization of the gene encoding medium-chain acyl coenzyme A dehydrogenase (MCAD or ACADM), a protein that mediates the initial step in mitochondrial β-oxidation of fatty acids, as the first *bona fide* ERRα target led to the suggestion that ERRα might play an important role in regulating cellular energy balance [21,62]. This finding was later corroborated by the discovery that ERRα and ERRγ interact with and are activated by PGC-1α and PGC-1β, two coactivators known for their crucial role in regulating energy metabolism [80]. Indeed, overexpression of PGC-1α or of functionally adapted variants of the protein, as well as of ERRα itself demonstrated an unambiguous link between ERRα, PGC-1α and changes in the expression of genes specifically involved in the control of cellular energy metabolism [29,54,56,57]. Additional studies using various strategies described above also established that one or more ERR isoforms control the expression of genes involved in lipid transport and uptake [58], mitochondrial biogenesis and function [81–83], as well as in oxidative phosphorylation and in the tricarboxylic acid (TCA) cycle [25,45,60,84]. However, the definitive validation that the ERRs directly regulate these metabolic gene networks was provided by a series of genome-wide ChIP-on-chip and ChIP-seq studies [25,38,45,48,60,73,74]. Through this work, significant enrichment of one or more ERR isoforms was detected at regulatory regions of most metabolic genes identified previously via gene expression studies, and bioinformatic analyses confirmed that the ERRs interact with genomic DNA almost exclusively through the well-defined ERRE. Remarkably, ERRα was found to occupy the promoter regions of practically all genes encoding enzymes participating at every step in the glycolytic pathway, pyruvate metabolism, and TCA cycle [38] in liver tissue. Collectively, the ERRs have been shown to occupy the promoter region of more than 700 genes encoding mitochondrial proteins (Table 1). In addition, these studies have also highlighted a global role for the ERRs in regulating gene networks implicated in growth factor/insulin signalling, energy sensing, translation, and the glucosamine pathway (Fig. 2).

Does a correlation exist between the ERR-dependent metabolic gene regulatory networks uncovered by functional genomics and the roles played by the three receptors in the whole organism? At first glance, the initial phenotypic observation that the ERRα-null mice are lean and resistant to high-fat diet-induced obesity does not appear to harmonize very well with a predominant function for ERRα and ERRγ in up-regulating genes involved in mitochondrial oxidative metabolism [85]. However, more in-depth investigations of the ERRα- and ERRγ-null mice revealed a much better than anticipated coordination between the functional genomics data and the phenotypic responses of these mice to diverse physiological challenges.

First, ablation of the ERRα gene in mice accelerates the bioenergetic and functional signatures of heart failure in the context of pressure overload [86]. Indeed, ERRα-null mice subjected to left ventricular pressure overload displayed signatures of heart failure including chamber dilatation, abnormal phosphocreatine depletion, and reduced maximal ATP synthesis rates in the heart. In addition, ERRα target genes involved in energy substrate oxidation, ATP

Table 1
Physiological genomics analyses of ERR biological function.

Methods	Biological system	Biological function	Related disease	Ref
Expression profiling/integrative genomics	C2C12 cells expressing PGC-1α	ERRα partners with PGC-1α	Diabetes	Mootha et al. [29]
Expression profiling	MCF-7 and BT-474 cells treated with an ERRα antagonist	Energy metabolism and OXPHOS	Breast cancer	Chisamore et al. [53]
Expression profiling	HepG2 cells expressing an ERRα-specific PGC-1α mutant	Energy metabolism and OXPHOS, lipid metabolism and transport	N/A	Gaillard et al. [54]
Expression profiling	MCF-7 cells expressing cells an ERRα-specific PGC-1α mutant	Aerobic metabolism and tumor biology	Breast cancer	Stein et al. [55]
Expression profiling	SAOS2 cells expressing PGC-1α	Mitochondrial biogenesis	Diabetes	Schreiber et al. [56]
Expression profiling	Primary neonatal cardiac myocytes expressing ERRα	Mitochondrial energy producing pathways	Diabetes and heart failure	Huss et al. [57]
Expression profiling	Intestine of ERRα-null mice	OXPHOS, dietary lipid digestion and absorption	Intestinal function	Carrier et al. [58]
Expression profiling	Renal, gastric, and cardiac tissues in perinatal ERRγ-null mice	Ion transport, hypertension	Renal, gastric and cardiac dysfunctions	Alaynick et al. [59]
Expression profiling/ChIP-on-chip	ERRα-null mouse macrophages/ macrophages, ERRα Abs	ROS production	Infectious diseases	Sonoda et al. [25]
ChIP-on-chip	Mouse liver, ERRα Abs	Energy metabolism and OXPHOS	Diabetes	Charest-Marcotte et al. [38]
Expression profiling/ChIP-on-chip	ERRγ-null mice hearts/fetal hearts, ERRγ Abs	Energy generation, sensing and utilization	Cardiac hypertrophy	Alaynick et al. [45]
Expression profiling/ChIP-on-chip	ERRα-null mice kidneys/kidney, ERRα Abs	Renal Na+/K+ handling, blood pressure, renin-angiotensin pathway	Bartter syndrome and hypertension	Tremblay et al. [48]
Expression profiling/ChIP-on-chip	ERRα-null mice hearts/adults hearts, ERRα and γ Abs	Energy generation, sensing and utilization	Cardiomyopathies	Dufour et al. [60]
ChIP-on-chip	SKBr3 cells	Gene expression in the ERBB2 amplicon	Breast cancer	Deblois et al. [72]
ChIP-seq	Mouse ES cells, ERR pan-Abs	Self-renewal, pluripotency, reprogramming in ES cells	Stem cell-based therapy	Chen et al. [73]
ChIP-on-chip	MCF-7 and SKBr3 cells, ERRα Abs	Cell growth, signalling and cellular energy producing pathways	Breast cancer	Deblois et al. [74]

Abs: antibodies; ES, embryonic stem; N/A, not applicable; OXPHOS, oxidative phosphorylation; Ref, reference.

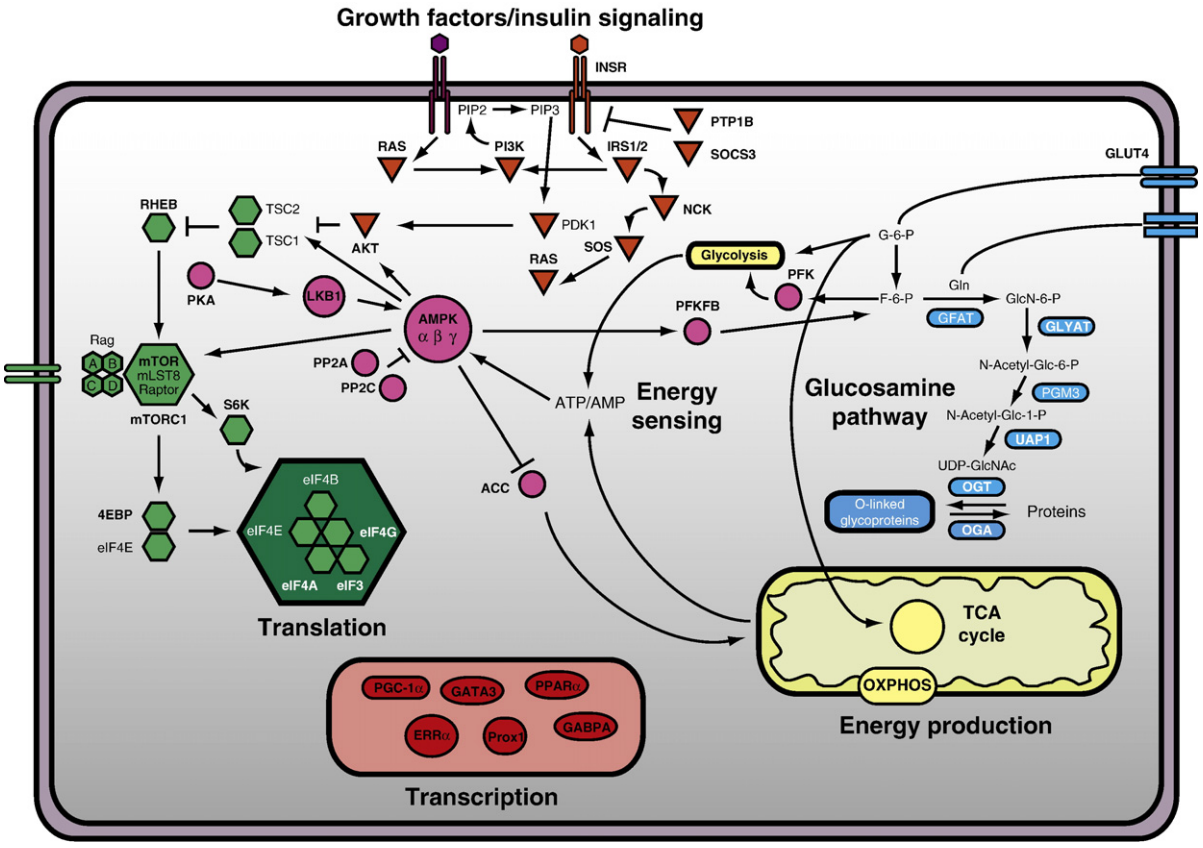

Fig. 2. Physiological targets of ERR involved in energy homeostasis. ERRα regulates target genes involved in growth factors/insulin signalling (orange), energy sensing (magenta), translation (green), glucosamine pathway (blue), transcription (red), and energy generating apparatus (TCA cycle, OXPHOS, and glycolysis, yellow).

synthesis, and phosphate transfer were downregulated in ERRα-null hearts at baseline or with pressure overload. Collectively, the gene expression results were consistent with the metabolic assessment of ERRα-null hearts as altered expression of ERRα target genes involved in fatty acid oxidation, TCA cycle, oxidative phosphorylation, and the synthesis and translocation of ATP complex were observed in the hypertrophied hearts. In addition, the ERRα-null hearts exhibited a reduction in the expression of two AMP kinase subunits, *Prkab1* and *Prkab2*, which is accompanied by reduced AMP kinase and acetyl-CoA carboxylase α (ACC1) phosphorylation [60], as well as by abnormal expression of genes involved in high-energy phosphate transfer such as *Ckmt1* and *Ant1*. Thus, this work not only validated the functional genomic analyses of ERRα as a transcription factor but also identified ERRα as a crucial determinant for the functional adaptation of the heart to hemodynamic stress forced by pressure overload.

Second, disruption of the gene encoding ERRγ was shown to block the metabolic switch that occurs at birth in the heart from a prevalent dependence on carbohydrates during fetal life to a greater reliance on postnatal oxidative metabolism in the newborn heart [45]. Interestingly, these studies showed that while ERRα and ERRγ target the same metabolic gene networks, the two receptors exert distinct functions in the control of energy metabolism in the same tissue in the life-long context of the whole organism.

Third, ERRα-deficient mice exposed to a moderately cold environment are unable to maintain their core body temperature due to a defect in adaptive thermogenesis [84]. While ERRα does not appear to be required for the induction of thermogenic genes in brown adipose tissues, the absence of ERRα correlates once again with a reduction in mitochondrial biogenesis and oxidative capacity required to provide the energy necessary to generate heat.

Fourth, ERRα and its coactivator PGC-1β were shown to regulate mitochondrial output in macrophages activated by interferon γ (IFN-γ) [25]. The deficiency in mitochondrial function in these mice led to a decrease in intracellular reactive oxygen species (ROS) level and clearance of *Listeria monocytogenes* in IFN-γ-activated macrophages. This phenotype correlated with the findings that ERRα and PGC-1β are both required for the induction of ERRα target genes encoding components of the mitochondrial respiratory chain machinery.

Taken together, these studies demonstrated that while ERRα appears to be dispensable for basal cellular energy needs, its presence is definitively required to provide the levels of energy necessary to respond to physiological and pathological insults in diverse tissues. In contrast, ERRγ is essential to establish and maintain the basal oxidative metabolic gene program in the heart. Similarly, disruption of ERRγ activity in human breast cancer and mouse mammary tumor cells via the expression of a micro RNA (miR-378*) also promotes a metabolic shift, known as the Warburg effect, characterized by a reduction in TCA cycle gene expression and oxygen consumption, as well as an increase in lactate production [87].

4.2. ERRα and ERRγ as regulators of ions homeostasis

The kidneys regulate blood pressure via the production of renin, the rate-limiting step of the renin–angiotensin pathway, and the maintenance of electrolyte homeostasis. Using a physiological genomics approach directed at the kidneys, we recently demonstrated that ERRα is located at the promoter and controls the expression of genes encoding channels involved in Na^+ and K^+ handling, the renin–angiotensin pathway, and several genes encoding systemic regulators of blood pressure [48]. Physiological analysis of ERRα-null mice corroborated these findings as the knock-out mice, relative to their

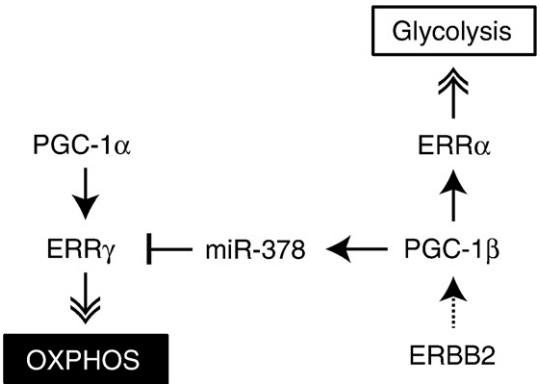

Fig. 3. The ERRs modulate breast cancer cells bioenergetics. ERBB2 signalling modulates the expression of PGC-1β and the activity of ERRα in ERBB2-positive breast cancer cells. PGC-1β contributes to the activity of ERRα in regulating the expression of genes involved in cell growth and proliferation as well as in glycolysis in breast cancer cells (right panel). ERRγ promotes the expression of OXPHOS genes in breast cancer cells (left panel). The PGC-1β gene also encodes for miR-378, a micro-RNA that targets ERRγ and contributes to mediate the metabolic shift towards a glycolytic phenotype that prevails in breast cancer cells.

wild-type siblings, are hypotensive. More specifically, the ERRα-null mice display a deficiency in Na^+ and K^+ handling with a mechanism supporting Na^+ retention. ERRα is required to sustain elevated blood pressure during the period of nocturnal activity of the mice.

In keeping with the theme that ERRα and ERRγ target the same metabolic gene networks but perform distinct physiological functions, ERRγ has been shown to be a regulator of genes central to ion homeostasis in renal, gastric, and cardiac tissues [59]. While ERRα-null mice display a relatively mild renal phenotype, ERRγ-null mice die shortly after birth with elevated serum K^+, a reduction of markers of gastric acid production and cardiac arrhythmia.

4.3. ERRβ is a reprogramming factor in stem cell biology

Pluripotent stem cells have the ability to give rise to cell types of all three germ layers as well as the germ line. Embryonic stem cells can also proliferate in an unlimited manner while maintaining their pluripotent state. Recently, genetic reprogramming has been used to induce pluripotency and self-renewal in mature cell types such as fibroblasts [88]. Several factors and extracellular signalling molecules, working in various combinations, have been shown to be able to reprogram the cellular phenotype of differentiated cells [89]. One of these factor is ERRβ [90]. ERRβ can reprogram mouse fibroblasts when introduced in combination with POU5F1 and SOX2 and can substitute for KLF4, one of the four original reprogramming factors. Interestingly, genome-wide location analysis has shown that sites enriched for ERRβ binding also contain binding sites for KLF family members [73]. In addition, ~60% of ERRβ-binding sites within multiple transcription factor-binding loci (MTL) were found in NANOG/OCT4/SOX2 MTL. However, phenotypic analysis of ERRβ-null mice has shown that knock-out embryos die at mid-gestation with placental

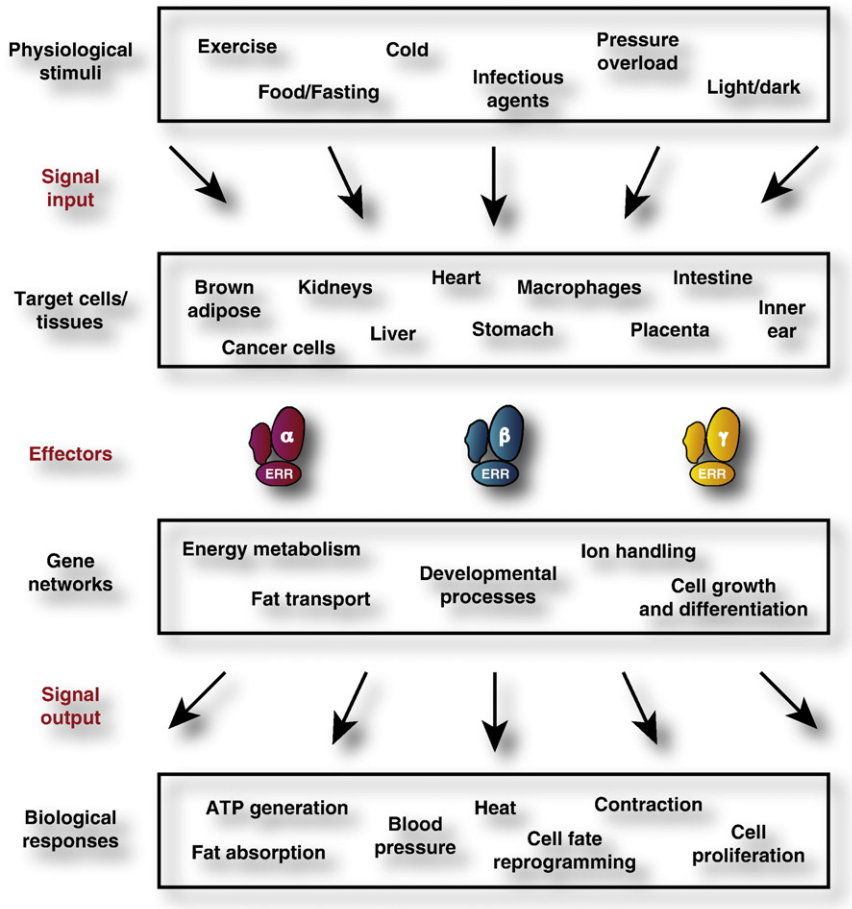

Fig. 4. A physiological response pathway with ERRα, β and γ as its focal point. External stimuli such as exercise and feeding/fasting serve as input signals that elicit a response from a target tissue. Interpretation of the signal leads to the induction of the transcriptional activity of one or more members of the ERR subfamily of nuclear receptors, which then results in the modulation in the expression of vast gene networks. The resulting signal output dictates a fitting cell-specific biological response.

abnormalities [9]. Since ERRγ can substitute for ERRβ to promote reprogramming of mouse fibroblasts, it is likely that genetic redundancy, as previously observed for the three ERRs in other tissues [48,59,60], prevents the observation of a strict requirement for ERRβ in the embryonic lineage. It should be noted that the antibody used to detect ERRβ binding sites in the mouse embryonic stem cells study also recognizes ERRγ [73].

4.4. ERRα as a determinant of breast cancer etiology

Since ERRα shares both structural and functional features with ERα, it was originally suggested that it could contribute to the biology of breast cancer. Initial studies have shown that the expression of ERRα in breast tumors associates with negative prognosis and positively and inversely correlates with ERBB2 and ERα status, respectively [91,92]. Moreover, the transcriptional activity of ERRα is modulated by the EGF/ERBB2 signalling pathways in breast cancer cells [93,94].

Depletion of ERRα in the ERα-negative cells MDA-MB-231 leads to a reduction of the migratory potential of the cells and decreases the tumor growth rate of xenografts [55]. Gene expression profile of MCF-7 cells stably expressing the ERRα-specific variant of PGC-1α revealed that many affected genes play a role in metabolism. ERRα antagonists inhibit tumor growth of both ERα-positive MCF-7 and ERα-negative T47D cells in mouse xenograft [53]. Location analyses in breast cancer cells revealed that the overlap between ERRα and ERα recruitment on extended promoter regions of target genes was much lower than initially expected, suggesting that ERRα also has an ERα-independent function in breast cancer [74]. Both receptors display strict binding site specificity and maintain independent mechanisms of transcriptional activation. Nonetheless, ERRα and ERα co-regulate a small subset of common target genes, many of which have been implicated in the etiology of breast cancer. By intersecting ERRα target genes identified using ChIP-on-chip in breast cancer cells with the gene expression profiles of several cohorts of human breast tumors, it was shown that ERRα signalling is involved in all the established breast tumor subtypes and therefore contributes to the heterogeneity of the disease [74].

ERRα and PGC-1β were shown to be recruited to the 17q12 chromosomal region of breast cancer cells and regulate the expression of ERBB2 and of several co-amplified genes [72]. ERRα and ERα display antagonist activity on the regulation of the ERBB2 gene. The presence of ERRα is required for full tumor development in a mouse model of Neu-initiated mammary tumorigenesis, and tumors lacking ERRα express lower levels of several transcripts of the amplicon.

In contrast, ERRγ is inversely correlated to ERRα expression in breast tumors and is a good prognosis factor [91]. As mentioned above, it was shown that the mir-378*, which is encoded in the PGC-1β gene, is modulated through ERBB2 signalling and targets ERRγ expression in breast cancer cells, contributing to the metabolic shift called the Warburg effect [87]. A schematic representation of the roles played by each ERR and PGC-1 isoform in the regulation of cellular energy metabolism in ErbB2-positive breast cancer cells is shown in Fig. 3.

5. Conclusions and perspectives

Despite their relatively high cost and the lack of consensus analysis methods, the advent of high throughput functional genomic techniques for gene expression profiling and location analyses has transformed the field of nuclear receptor studies. These state-of-the-art techniques are now being used to uncover large-scale interconnections between gene expression profiles, recruitment of nuclear receptors to specific sites in genomes, chromatin landscape, and various signalling pathways that work in relation with one another to establish the genetic programs that contribute to the normal function of the cell or underlying various pathologies. Herein, we have shown that physiological genomics analyses led to the identification of an extensive physiological response pathway that has the ERRs as its focal (Fig. 4). Increasingly, functional/physiological genomics studies will direct future research towards the discovery of novel nuclear receptor-driven biological functions, and hopefully, the development of new therapeutic avenues targeting nuclear receptors for the prevention and treatment of metabolic diseases and hormone-dependent cancers.

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