Journal of Steroid Biochemistry & Molecular Biology 157 (2016) 13–19

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

There and back again: The journey of the estrogen-related receptors in the cancer realm

Ingrid S. Tam[a], Vincent Giguère[a,b,*]

[a] Goodman Cancer Research Centre, McGill University, 1160 Pine Avenue West, Montréal, QC H3A 1A3, Canada
[b] Departments of Biochemistry, Medicine and Oncology, McGill University, Montréal, PQ H3G 1Y6, Canada

ARTICLE INFO

Article history:
Received 3 April 2015
Received in revised form 9 June 2015
Accepted 16 June 2015
Available online 4 July 2015

Keywords:
Breast cancer
Metabolism
Mitochondria
Nuclear receptor
Warburg effect

ABSTRACT

The identification of two genes encoding polypeptides with structural features common with the estrogen receptor more than a quarter century ago, referred to as the estrogen-related receptors (ERRs), subsequently led to the discovery of several previously unrecognized hormone responsive systems through the application of reverse endocrinology. Paradoxically, the natural ligand(s) associated with members of the ERR subfamily remains to be identified. While initial studies on the mode of action and physiological functions of the ERRs focused on interaction with estrogen signalling in breast cancer, subsequent work showed that the ERRs are ubiquitous master regulators of cellular energy metabolism. This review aims to demonstrate that the ERRs occupy a central node at the interface of cancer and metabolism, and that modulation of their activity may represent a worthwhile strategy to induce metabolic vulnerability in tumors of various origins and thus achieve a more comprehensive response to current therapies.

1. Introduction

The identification of the estrogen-related receptors (ERRs) while probing the human genome for established but yet to be cloned steroid receptors was a pivotal discovery that led to the rapid expansion of nuclear receptor family from the known entity of classic hormone and vitamin receptors to the new frontier of orphan receptors and their unexpected partner ligands [1–3]. Perhaps fitting with their proud status of "first" orphan receptors to be identified, the three members of the ERR family, referred to as ERRα (NR3B1), ERRβ (NR3B2) and ERRγ (NR3B3), are still in search of their natural ligand(s). However, while the application of reverse endocrinology to the study of the ERRs has yet to identify their cognate ligand(s), much has been learned about their roles in development, physiology and disease. Two seemingly distinct themes emerged from the early work focused on finding a biological role for the ERRs: the ERRs interfering with the estrogen pathway [4], especially in the context of breast cancer, and the ERRs as master regulators of energy metabolism [5]. Herein we will review the scientific journey of the ERRs from minor players as putative vestigial estrogen receptors (ERs) to dominant effectors of cellular metabolism and back again as a major force in determining the reprograming of cancer cell metabolism to satisfy the bioenergetic needs of tumors during the progression of the disease.

2. Kinship with the estrogen receptor: initial link to breast cancer

In the early years of the ERRs, studies focused on potential crosstalk with the classic ERs in breast cancer, primarily due to the known structural homology shared between ERRα and ERα in combination with the long-recognized role of ERα in breast cancer. Inhibition of ERα transcriptional activity was already a widely used therapeutic strategy [6], so it was of particular interest to discover that ERRα binds DNA segments harboring the estrogen-response element (ERE) *in vitro* [7–9]. This observation lead to the speculation that the two classes of receptors may have overlapping target genes. Indeed, ERRα was shown to bind to and modulate the expression of several estrogen-inducible genes including lactoferrin and osteopontin, as well as the breast cancer prognostic marker *TFF1* [8,10,11]. In addition, ERRα was demonstrated to be putatively involved in estrogen biosynthesis *via* the transcriptional

Abbreviations: ChIP, chromatin immunoprecipitation; EMT, epithelial-to-mesenchymal transition; eNOS, endothelial nitric oxide synthase; ER, estrogen receptor; ERE, estrogen response element; ERR, estrogen-related receptor; ERRE, estrogen-related response element; HIF-1, hypoxia inducible factor-1; MET, mesenchymal-to-epithelial transition; PGC-1, peroxisome-proliferator activated receptor γ coactivator 1; ROS, reactive oxygen species; TCA, tricarboxylic acid; VEGF, vascular endothelial growth factor.

* Corresponding author at: Goodman Cancer Research Centre, McIntyre Bldg., Suite 710A, 1160 Pine Avenue West, Montréal, Québec, H3A 1A3, Canada.
E-mail address: vincent.giguere@mcgill.ca (V. Giguère).

http://dx.doi.org/10.1016/j.jsbmb.2015.06.009
0960-0760/

regulation of aromatase [12], further associating ERRα to ERα and estrogen physiology. However, stricter binding site specificity experiments revealed that the ERRs preferentially recognize DNA elements containing a single consensus half-site TnAAGGTCA in physiological contexts [13]. The ERR binding site is now referred to as the estrogen-related receptor response element (ERRE), and is clearly distinct from the traditional ERE that harbors two inverted half-sites. Indeed, gene promoters that are common targets of ERRα and ERα such as *TFF1* were often found to be bound by ERRα and ERα through distinct DNA recognition elements located in close proximity [11]. ERRα signaling was eventually mostly dissociated from ERα signaling following the genome-wide identification of ERRα target genes by methods based on chromatin immunoprecipitation (ChIP) [14]. Nonetheless, due to the presence of a common half-site, a small fraction of genes contain overlapping ERE/ERRE elements in domains regulating their expression. These hybrid response elements then allow for competitive dual control by ERRα and ERα, which can be exemplified by their regulation of the oncogene *ERBB2*, at least in the context of breast cancer cells [14,15].

3. ERRs as cancer biomarkers

In addition to their functional kinship with the classic ERs in carcinoma of the breast, all three ERR isoforms have also been explored as potential biomarkers in various cancers [16]. Elevated ERRα expression has been observed in tumor samples from ovarian, cervical, colorectal, and prostate cancer patients when compared to normal tissue [17–20]. High ERRα expression is also associated with tumor aggressiveness, as ERRα expression increases with clinical stage in ovarian, endometrial, and colorectal cancers [17,21,22]. Importantly, ERRα expression is associated with unfavorable outcomes in breast cancer [23]. Further, marked increases of ERRα mRNA levels have been observed in a mouse tumor model of brain metastasis [24]. In contrast, expression of ERRγ correlates with more favorable clinical outcomes. ERRγ expression is linked to progression-free survival in both ovarian and breast cancer [18,23], and is downregulated in prostate cancer cell lines and in medulloblastoma [25,26], indicating that it may play a tumor-suppressing function in these cancers. Similarly, expression of ERRβ is decreased in prostate cancer [27].

4. ERRs as master regulators of cellular metabolism

While the ERRs were being explored in the context of cancer, data began to emerge linking the ERRs to metabolic control. Along with the association of ERRα and ERRγ expression to highly metabolic tissues, three key pieces of evidence further implicated the ERRs in the control of energy metabolism. First, ERRα was shown to activate the *ACADM* gene promoter, thereby regulating a key step in mitochondrial fatty acid β-oxidation [13,28]. Second, phenotypic analysis of the ERRα-null mice demonstrated the null animal to be lean and resistant to high-fat diet-induced obesity [29]. Finally, the ERRs were shown to be expressed with, induced and activated by peroxisome proliferator-activated receptor γ coactivator-1 (PGC-1) α and β [30–33], coactivators known to play important roles in mitochondrial oxidative metabolism as well as in the maintenance of glucose, lipid, and energy homeostasis [34]. These observations placed the ERRs in the purview of cellular energetics, leading to a body of work that solidified the role of the ERRs in energy metabolism. Physiological studies validated the importance of the ERRs in regulating energy balance *in vivo*: experiments in ERRα-null mice demonstrated that ERRα is required to generate energy required to respond to physiological and pathological stresses in various tissues, but is not essential for basal cellular energy needs [35–40]. Conversely, ERRγ is necessary to direct and maintain the metabolic switch in the fetal heart at birth from an energy dependence on primarily carbohydrates during development to fatty acid oxidation in the postnatal heart [41,42]. Meanwhile, the development of functional genomics technologies were pivotal in understanding the consequences of ERR transcriptional activity. Importantly, delineation of ERR transcriptional networks *via* ChIP-based technologies revealed an underlying metabolic function for ERR target genes at a genome-wide level. The ERRs occupy the promoter regions of genes encoding over 700 mitochondrial proteins; moreover, ERRα binds to genes encoding nearly all enzymes involved in the glycolytic pathway, pyruvate metabolism, and the TCA cycle (reviewed in [43,44]). Concurrent with the establishment of the ERRs as master regulators of metabolism was the recognition that altered cellular bioenergetics plays an important role in maintaining the cancer phenotype [45,46], thus generating renewed interest in investigating an estrogen signaling-independent role for the ERRs in cancer.

5. ERRs at the interface of cancer and metabolism

The observation that tumor cells exhibit drastic changes in metabolism when compared to normal cells is an old concept [47]; however, the importance of metabolic reprogramming has only recently been officially recognized as a hallmark of cancer [48]. Metabolic alterations of cancer cells serve to support all aspects of tumor progression: rapid growth, proliferation, environment stresses, migration, metastasis and drug resistance. These metabolic adaptations include but are not exclusive to shifts in rates of aerobic glycolysis and oxidative phosphorylation, elevated glutamine uptake and glutaminolysis, modifications in levels of fatty acid oxidation and lipid biosynthesis, and increased flux through the pentose phosphate pathway [49]. The ERRs have been implicated in the normal functioning of virtually all these processes through the transcriptional regulation of their target genes [44]. In recent years, a range of studies has solidified the pivotal role of the ERRs and associated cofactors, particularly the PGC-1s, in contributing to the distinct metabolic profiles of cancer cells [50].

While a metabolic switch from oxidative to glycolytic metabolism referred to as the Warburg effect is often observed in cancer, it is now recognized that not all tumor types are predominantly reliant upon aerobic glycolysis; rather, cancer cells uniquely balance glycolysis and oxidative phosphorylation to fulfill their bioenergetic needs [51,52]. The reconfiguration of metabolic pathways in tumors is driven by both genetic and epigenetic changes which converge on regulators of cell metabolism. For instance, in breast cancer, expression of miR-378* diminishes ERRγ levels, thereby inhibiting ERRγ-mediated transcription of tricarboxylic acid (TCA) cycle genes and re-directing glucose metabolism through aerobic glycolysis rather than oxidative phosphorylation [53]. In the same manner, ectopic expression of ERRγ increases oxidative phosphorylation in breast cancer cells [54]. The shift to oxidative metabolism *via* ERRγ inhibits breast cancer cell proliferation *in vitro* and tumor growth *in vivo* [53,54]. Conversely, ERRα activates the gene promoters of glycolytic enzymes in breast cancer to maintain aerobic glycolysis and support proliferation by meeting the energy demands of dividing cells [53,55,56]. In contrast to these observations, negative regulation of lactate dehydrogenase activity by ERRα modulation of *LDHB* expression contributes to elevated oxidative metabolism in thyroid tumors [57]. Similarly, ERRα and PGC-1α collaborate to govern metabolic programs of melanoma tumors that exhibit high levels of mitochondrial energy metabolism instead of glycolysis [58]. Taken together, these findings highlight the cell- and context-dependent activities of the ERRs in controlling the coordination between