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REVIEW

The pERK of being a target: Kinase regulation of the orphan nuclear receptor ERRγ

Rebecca B. Riggins

Department of Oncology, Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, DC 20057, USA

Correspondence: Rebecca B. Riggins
E-mail: rbr7@georgetown.edu
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Estrogen-related receptors (ERRs) are orphan members of the nuclear receptor superfamily that are important regulators of mitochondrial metabolism with emerging roles in cancer. In the absence of an endogenous ligand, ERRs are reliant upon other regulatory mechanisms that include protein/protein interactions and post-translational modification, though the cellular and clinical significance of this latter mechanism is unclear. We recently published a study in which we establish estrogen-related receptor gamma (ERRγ) as a target for extracellular signal-regulated kinase (ERK), and show that regulation of ERRγ by ERK has important consequences for the function of this receptor in cellular models of estrogen receptor-positive (ER+) breast cancer. In this Research Highlight, we discuss the implications of these findings from a molecular and clinical perspective.

Keywords: ESRRG, ERRgamma; ERK/MAPK; orphan nuclear receptor; transcription; tamoxifen

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The human genome contains 48 genes encoding members of the nuclear receptor superfamily, thought to have evolved from an ancient ancestor first observed in the early metazoan (marine sponge) *Amphimedon queenslandica* [1]. These specialized, multi-domain sensory proteins respond to environmental cues by binding DNA and initiating gene transcription that drive many important physiological processes. While many of the most commonly studied nuclear receptors are specifically regulated by endogenous ligands such as hormones (*e.g.* the estrogen receptor, or ER), more than half lack endogenous ligands and are thus classified as orphan nuclear receptors. The structural similarity of these orphans to ligand-regulated receptors - *i.e.* the presence of DNA-(DBDs) and ligand-binding domains (LBDs, **Figure 1**) - has led to a concerted effort to identify or modify synthetic ligands and natural products which modulate their constitutive transcriptional activity [2, 3].

Estrogen-related receptors (ERRs) alpha and beta were the first orphan nuclear receptors to be identified, and all three members of the ERR family are now known to be important regulators of mitochondrial metabolism with emerging roles in cancer [4-6]. In the absence of ligand, the major mode of ERR regulation appears to be protein/protein interactions with coregulatory proteins,

most commonly members of the peroxisome proliferator-activated receptor gamma coactivator 1 (PGC-1) gene family[7]. A second mechanism of ERR regulation is post-translational modification (PTM), with phosphorylation, acetylation, and SUMOylation being the two modifications identified thus far [8-12]. However, the impact of these PTMs on the clinically relevant functions of ERR family members is unknown.

In our recent publication[13], we provide the first evidence that ERRγ is a target for pERK, and propose that phosphorylation is integral to this receptor's ability to promote endocrine resistance in ERα positive (ER+) breast cancer. We show that inhibition of ERK, but not the closely related JNK and p38 MAPK family members, reduces receptor protein expression. In contrast, several different means of activating the ERK signal transduction cascade – EGF stimulation, exogenous expression of wild type ERK2 or constitutively active MEK – markedly increase ERRγ expression. Guided by the minimal MAPK phosphorylation site consensus sequence (Serine/Threonine-Proline), we identified Serine residues 57, 81, and/or 219 (**Figure 1**) as important targets for kinase-mediated ERRγ stabilization, since their simultaneous mutation to Alanine reduces basal receptor levels and blunts the effect of ERK inhibition or activation on ERRγ expression.

Serines 57 and 81 are located within the amino-terminal activation function 1 (AF1) domain of ERRγ. AF1 domains of nuclear receptors, liganded and orphaned alike, are intrinsically disordered [14] regions that can recruit coregulatory proteins, a function that has recently become a new avenue for drug development as we seek to identify alternative receptor modulators for clinical use (reviewed in [15, 16]). Serine 219 is located in the hinge region, which commonly includes a nuclear localization signal. Phosphorylation by another member of the MAPK family (p38) at Threonine 311 within the hinge region of classical ERα regulates nuclear localization and transcriptional activity[17].Steroidogenic factor 1 (SF-1) phosphorylation by ERK2 at Serine 203 enhances coactivator recruitment and receptor stability [18,19], while ERK-dependent phosphorylation of the closely related orphan liver receptor homolog 1 (LRH-1) at Serines 238 and 243 has similar effects [20].The molecular functions and relative dominance of ERRγ Serines 57, 81, and 219 in ERK-mediated receptor regulation are important areas of future investigation.

Having previously established that ERRγ plays a critical role in resistance to the growth inhibitory effects of Tamoxifen (TAM) in estrogen receptor-positive (ER+) breast cancer cell lines [21], we determined whether phospho-deficient ERRγ was less able to induce resistance

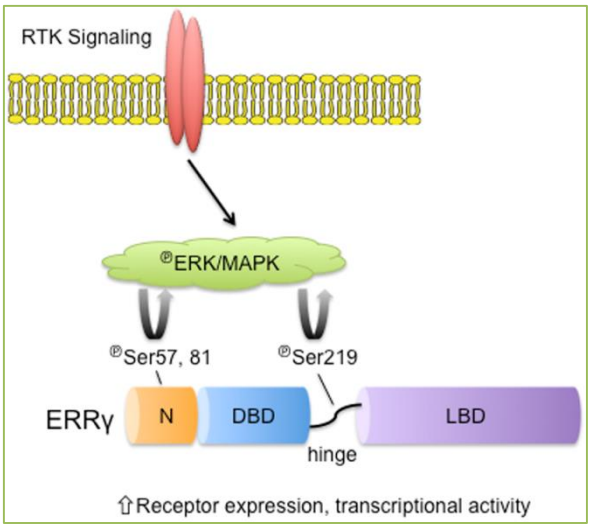

Figure 1. Model for ERK-mediated regulation of ERRγ. Hyper activationof ERK/MAPK, through receptor tyrosine kinase (RTK) engagement or other means, leads to increased ERRγ expression and transcriptional activity in a Serine 57, 81, and/or 219-dependent manner. N - amino-terminal activation function 1 (AF1) region; DBD - DNA binding domain; LBD - ligand binding domain.

than the wild type receptor. Indeed while exogenous expression of wild type ERRγ prevents any reduction in MCF7 breast cancer cell proliferation by TAM, exogenous expression of ERRγ in which Serines 57, 81, and 219 have been mutated to Alanine is unable to do so. The reduced pro-proliferative activity of phospho-deficient ERRγ is also consistent with changes in the expression and/or phosphorylation of the cell cycle regulatory proteins p21, p27, and Rb.

The precise mechanism(s) by which ERRγ promotes TAM resistance is/are unresolved. As a transcription factor, it stands to reason that a specific set of target genes is engaged by ERRγ, and some of our current studies are focusing on the *in silico* identification of such targets in breast cancer clinical datasets. However, the challenge in predicting relevant ERRγ target genes in any context is the relatively broad DNA-binding specificity of this and other members of the ERR family [22-24]. In addition to the estrogen-related response element (ERRE, consensus sequence TCAAGGTCA), ERRγ can act through the inverted repeats of the estrogen response element (ERE) and, as we show in [13], the ERRE/ERE hybrid element[25] as well. The activity of phospho-deficient ERRγ upon luciferase promoter-reporter constructs bearing each of these enhancer elements is inhibited vs. wild type ERRγ. This is most likely the consequence of reduced receptor expression, although we cannot rule out the possibility that positive regulation of ERRγ by ERK can also impact DNA binding.It should also be noted that ERRγ and other members of this subfamily can regulate gene transcription

indirectly by association with AP1[26] and Sp1[27] transcription factor complexes, which may also be impacted by ERK activation status.

Our recent findings have important implications for the management of endocrine resistant breast cancer, which remains a clinically significant problem [28]. Amplified ERK/MAPK signaling has been linked to TAM resistance [29, 30], and recent neoadjuvant studies with the aromatase inhibitor anastrozole show that intrinsic resistance is predicted by high baseline expression of an IGF-1/MAPK gene expression signature [31]. It is therefore plausible that development of an immunohistochemical (IHC) assay to measure of ERRγ protein levels in ER+ breast tumor specimens may be useful as a marker of functionally elevated ERK/MAPK signaling that, if present, would indicate the need to combine inhibition of this pathway with ER-targeted agents. To this end, we have begun optimizing commercially available ERRγ antibodies for IHC in a small series of breast tumors (n=9), and have thus far determined that its expression is positively correlated with that of pERK (Spearman's rank correlation coefficient = 0.5, p = 0.07).

There are also potentially important consequences for ERK-mediated regulation of ERRγ that extend beyond breast cancer. The first of these contexts is proper function of the placenta, in which ERRγ plays an essential role in the induction of the aromatase gene CYP19A1 [32] (leading to increased estrogen production), voltage-gated potassium channel genes, and kallikrein 1 [33] in response to oxygen. Most recently, Luo *et al* [34] have demonstrated that ERRγ is overexpressed in placentas from women affected by preeclampsia, and in an elegant series of *in vivo* studies using pregnant female $ERR\gamma^{+/-}$ mice show that this receptor regulates maternal blood pressure and levels of circulating antiangiogenic peptides which are known to contribute to preeclampsia. Interestingly, aberrant MAPK signaling characterizes subgroup 2 of preeclampsia, which does not exhibit the more well established molecular markers of this condition [35].

Second, aberrant regulation of ERRγ expression and/or function by ERK in hepatocytescould have profound effects on two disparate pathologies: Type 2 diabetes mellitus (T2DM) and response to infection by *Salmonella typhimurium* (*S. typhimurium*). T2DM is characterized by deregulation of glucose response, including the inappropriate production of glucose (gluconeogenesis) by the liver. Using two different mouse models for T2DM, Kim *et al*[36]have shown that hepatic deletion of ERRγ or treatment with its inverse agonist GSK5182 inhibits a pro-gluconeogenic gene expression profile, lowers blood glucose levels, and is as at least as effective as metformin in normalizing overall body weight and hepatic lipid accumulation. This same group has demonstrated that in hepatocytes, ERRγ-mediated production of the peptide hormone hepcidin occurs in response to an interleukin 6/signal transducer and activation of transcription 3 (IL6/STAT3) signaling cascade[37]. In turn, circulating hepcidin promotes the degradation of ferroportin 1 in macrophages, resulting in higher intra-macrophage iron levels that enhance *S. typhimurium* replication. As in their T2DM models, systemic treatment with GSK5182 improves mouse survival by reversing these events. Increased ERK activation in hepatocytes has been implicated in other mouse models of diabetes[38], but its relevance to the production of hepcidin by these cells is much less clear. Further studies will be necessary to determine if ERRγ's role in these pathologies is modified by ERK/MAPK signaling.

Conflicting interests

The authors have declared that no competing interests exist.

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