Estrogen-Related Receptor Gamma Promotes Mesenchymal-to-Epithelial Transition and Suppresses Breast Tumor Growth

Claire Tiraby, Bethany C. Hazen, Marin L. Gantner, and Anastasia Kralli

Abstract

Estrogen-related receptors (ERR), ERR alpha (ERRα) and ERR gamma (ERRγ), are orphan nuclear receptors implicated in breast cancer that function similarly in the regulation of oxidative metabolism genes. Paradoxically, in clinical studies, high levels of ERRα are associated with poor outcomes whereas high levels of ERRγ are associated with a favorable course. Recent studies suggest that ERRα may indeed promote breast tumor growth. The roles of ERRγ in breast cancer progression and how ERRα and ERRγ may differentially affect cancer growth are unclear. In mammary carcinoma cells that do not express endogenous ERRγ, we found that ectopic expression of ERRγ enhanced oxidative metabolism in vitro and inhibited the growth of tumor xenografts in vivo. In contrast, ectopic expression of the ERRα coactivator PGC-1α enhanced oxidative metabolism but did not affect tumor growth. Notably, ERRγ activated expression of a genetic program characteristic of mesenchymal-to-epithelial transition (MET). This program was apparent by changes in cellular morphology, upregulation of epithelial cell markers, downregulation of mesenchymal markers, and decreased cellular invasiveness. We determined that this program was also associated with upregulation of E-cadherin, which is activated directly by ERRγ. In contrast, PGC-1α activated only a subset of genes characteristic of the MET program and, unlike ERRγ, did not upregulate E-cadherin. In conclusion, these results show that ERRγ induces E-cadherin, promotes MET, and suppresses breast cancer growth. Our findings suggest that ERRγ agonists may have applications in the treatment of breast cancer. Cancer Res; 71(7); 2518–28. ©2011 AACR.

Introduction

Breast cancer is the most common cancer in American women, with an incidence of approximately 1 in 10 women. Estrogens play a major role in breast cancer by promoting the initiation and growth of tumors. About 60% to 70% of breast cancers are positive for estrogen receptor (ER), and a significant proportion of the affected patients benefit from endocrine therapies that inhibit estrogen signaling. This leaves approximately 30% to 40% of breast cancers that are negative for ER and do not respond to endocrine regiments, illustrating the need to identify other breast cancer biomarkers for development of therapies. Recent studies suggest that 2 estrogen-related receptors (ERR), ERR alpha (ERRα) and ERR gamma (ERRγ), have prognostic marker value for clinical outcome in breast carcinoma (1, 2). The roles and mechanisms of action of these 2 receptors in breast cancer progression are still poorly understood.

The 3 ERRs, (ERRα, ERRβ, and ERRγ), comprise a subfamily of orphan nuclear receptors that share sequence similarity with the ERs (3, 4). Despite this similarity, ERRs are not activated by estrogen-like molecules and remain to date orphans with no known endogenous natural ligands. Nevertheless, ERRs have a hydrophobic pocket that is accessible to synthetic ligands. There exist synthetic inverse agonists for all ERRs, such as diethylstilbestrol, or selective inverse agonists, such as 4-hydroxytamoxifen (4OHT) for ERRβ and ERRγ and XCT790 for ERRα (3, 4). Synthetic ERR agonists have so far been identified only for ERRβ and ERRγ (3, 4). In lieu of regulation by small molecule ligands, the activity of ERRs is determined by interactions with coregulators and posttranslational modifications (5–7). ERRα transcriptional activity in particular depends on interactions with members of the PGC-1 coactivator family (8). In contrast, ERRβ and ERRγ are transcriptionally active in cells lacking PGC-1 proteins, likely due to efficient interactions with other coactivators (9). The 3 ERRs recognize a common DNA sequence, TNAAGGTCA, termed ERR response element (ERRE), and act as transcription factors for genes bearing functional ERREs (4, 10). Although they can activate some known ER-responsive genes, such as TFF1 (Trefoil factor 1; refs. 9, 11, 12), ERRs are major regulators of energy metabolism pathways, a function that is not shared by the ERs (4, 8). ERRα cooperates with the
coactivators PGC-1α and PGC-1β to induce the expression of genes important for mitochondrial biogenesis, lipid transport, and oxidative capacity (13–17), and ERRα-deficient mice display tissue-specific defects in oxidative metabolism (18–20). ERRγ recognizes and induces many ERRα targets with roles in energy metabolism, contributing to oxidative capacity in the heart and playing a role in hormone homeostasis (17, 21, 22). ERRα and ERRγ also target and regulate the expression of genes with roles outside metabolism, suggesting that their actions are not confined to metabolic regulation (11, 12, 17).

In contrast to the overlapping metabolic functions of ERRα and ERRγ, breast cancer association studies suggest that ERRα and ERRγ play opposite roles in cancer progression (1, 2). High levels of ERRα are associated with the expression of ErbB2, increased risk of recurrence, and adverse clinical outcome (1, 2). Conversely, ERRγ expression is associated with markers predictive of responsiveness to endocrine treatment and is higher in diploid (less aggressive) than in aneuploid (more aggressive) tumors (1). These associations suggest that ERRα and ERRγ might promote and suppress aggressive breast cancer progression, respectively. In support for ERRα promoting tumor growth, suppression of ERRα expression leads to reduced growth of MDA-MB-231 breast cancer tumors in mice (11). Furthermore, inverse agonists of ERRα inhibit proliferation, induce cell death, and reduce tumorigenicity of cancer cell lines (23–25). To date, the extent to which ERRγ can affect breast tumor progression in vivo has not been determined.

In this study, we use a xenograft model to address the role of ERRγ in breast tumor growth. We find that ectopic ERRγ expression suppresses the growth of MDA-MB-231 tumors in mice. In contrast, ectopic expression of the coactivator PGC-1α, which activates endogenous ERRα, does not affect xenograft growth. Both ERRγ and PGC-1α enhance oxidative metabolism, suggesting that the increased oxidative capacity cannot by itself explain the reduced growth of ERRγ-expressing tumors. Instead, ERRγ activates the program of mesenchymal-to-epithelial transition (MET), upregulating the expression of the cell adhesion molecule E-cadherin and decreasing invasiveness. Our findings show for the first time that ERRγ can play an active role in breast cancer growth. They also provide evidence for ERRα- and ERRγ-selective targets, which may explain the distinct action of the 2 related receptors in breast cancer.

Materials and Methods

DNA vectors

For retroviral packaging, 293T cells were cotransfected with pMSCV-puro (expressing Flag-ERRγ, Flag-PGC-1α, or EGFP), pVPack-GP, and pVPack-VSV-G plasmids, and supernatants were concentrated by ultracentrifugation. For production of lentiviral particles, 293T cells were cotransfected with pLVTHM [expressing short hairpin RNAs (shRNA) for CDH1, ERRγ, or controls (see Supplement)], psPAX2, and pMD2.G plasmids (Addgene), and supernatants were collected. For the E-cadherin ERRE luciferase constructs, oligonucleotides containing the ERREs plus 14 to 16 flanking nucleotides were cloned as trimers upstream of the minimal promoter of pΔ-ΔLuc (26).

Cell lines

MDA-MB-231, PC-3, and BT474 cells were obtained from American Type Culture Collection (ATCC) in 2005, 2009, and 2010, respectively, and cryopreserved. ATCC characterizes cell lines by short-tandem repeat profiling, cell morphology, and karyotyping. All experiments were conducted with cells at less than 20 passages after receipt. Cells were cultured in Dulbecco’s modified Eagle’s medium (MDA-MB-231) or RPMI 1640 (PC-3, BT474) supplemented with 10% FBS and 100 units/mL penicillin/streptomycin. ERRγ-, PGC-1α-, and EGFP-expressing cells were derived by transducing MDA-MB-231 cells with retroviruses and selecting populations in puromycin (InvivoGen; 1 μg/mL). Populations were independently derived twice, to confirm reproducibility of findings. Transgene expression was confirmed by quantitative reverse transcriptase PCR (RT-qPCR), Western blot, and immunofluorescence. E-cadherin and ERRγ expression was suppressed by infection with lentiviruses expressing shRNAs. Infection efficiency was monitored by microscopy on the basis of gfp expression by the vectors.

Western blots and immunohistochemistry

For Western blots, cells or tumors were lysed in radioimmunoprecipitation assay buffer. For immunohistochemistry, cells were fixed with ice-cold methanol; tumor sections were deparaffinized, rehydrated in descending ethanol series, and treated with 0.01 mol/L citrate buffer (pH 6.0). Antibodies used were anti-E-cadherin (clone 36; BD Biosciences), Flag M2 antibody (Sigma), anti-GAPDH (gyceraldehyde 3-phosphate dehydrogenase; Chemicon International), and anti-vimentin (clone V9; Dako). Image acquisition was with an Olympus IX70 microscope and Microfire software.

Gene expression

RNA isolation, cDNA synthesis, and gene expression determination were as published elsewhere (18), using quantitative PCR, gene-specific primers (Supplementary Table S1), and normalization to 36B4 as a reference gene.

Oxygen consumption

Oxygen consumption was monitored with a Clark-type oxygen electrode (Hansatech Instruments), using 2 × 10⁶ cells and at 30°C.

Glucose oxidation assay

Glucose oxidation rates were determined as published elsewhere (27). Briefly, cells were incubated with serum-free medium supplemented with 0.5 μCi/ml [U-14C]glucose (200.6 μCi/μmol) for 2 hours at 37°C. 14CO2 released from the medium by acidification with 60% perchloric acid was trapped on filter paper saturated with benzenethionium hydroxide, and counted in a β-counter. The results were normalized to total protein.

Cell growth assay

Cells were plated at 4,000 cells well in a 96-well plate and assayed using the MTT assay (Sigma) or at 5 × 10⁴ cells well in 12-well plates and counted after trypsinization with a hemocytometer.
Breast cancer xenografts

Protocols were approved by the TSRI Institutional Animal Care and Use Committee and carried out under veterinary supervision. Cells (1 × 10^6 cells in 50 µL of serum-free media) were injected orthotopically into the mammary fat pad of 6- to 8-week-old female C.B17 severe combined immunodeficient (SCID) mice (Taconic Farms). Tumor growth was monitored weekly; volume was calculated as (width^2 × length)/2 (mm^3). At 2 months postinjection, mice were sacrificed and tumors were excised, weighed, and processed for RNA, protein, or sections. Representative data were obtained from 8 to 9 mice per experimental group, and the entire experiment was repeated once with independently derived cell populations.

Matrigel Transwell invasion assays

Cells were shed using EDTA-free trypsin (Sigma), supplemented with 0.5 mmol/L CaCl_2, preincubated in media containing 0.5% FBS, and transferred into Matrigel-coated Transwells (BD BioCoat Matrigel Invasion Chamber; Becton Dickinson) at 2 × 10^4 cells per well. The gel including non-migrating cells was removed after 22 hours, and cells invading the membrane were fixed, stained with DAPI, and counted.

Luciferase assays

Cells were transfected with 40 ng of luciferase reporter, 5 ng of pcDNA3/ERRγ, and 5 ng of pcDNA3/PGC-1α, using FuGENE 6 (Roche). Luciferase was read 24 hours later, using BriteLite Reagent (PerkinElmer) and the PHERAstar Spectrophotometer (BMG Labtech).

Chromatin immunoprecipitation

The CDH1 locus was scanned for putative ERR binding sites using the MAPPER search engine (28). Chromatin immunoprecipitations (ChIP) were conducted as published (18), using the anti-Flag antibody (Sigma) and gene-specific primers (Supplementary Table S1).

Results

Increasing ERRγ or PGC-1α levels leads to enhanced oxidative capacity in breast cancer MDA-MB-231 cells

The human mammary adenocarcinoma line MDA-MB-231 is an established model for in vitro and in vivo xenograft studies of breast cancer. MDA-MB-231 cells are ER negative and express high levels of ERRα and low to undetectable levels of ERRγ, PGC-1α, and PGC-1β (Supplementary Fig. S1). To elucidate the functional significance of ERRγ in breast cancer progression, we established MDA-MB-231 populations that express stably human ERRα and PGC-1α. ESRRA, IDH3A, MAOB, and TFF1. Levels of mRNA are expressed relative to levels of each gene in MDA-control cells and are the mean ± SEM of 4 experiments conducted in duplicate. Data are the mean change ± SD in oxygen consumption from 2 experiments conducted in duplicate (left) and mean change ± SD in [U-13C]glucose oxidation rate from 4 experiments (right), relative to MDA-control cells. D, in vitro growth rates of the indicated populations, measured with a colorimetric MTT assay. Data are the mean ± SD of 8 replicates. *, P < 0.05; **, P < 0.01; †**, P < 0.001.

![Figure 1. Generation and characterization of MDA-MB-231 cells expressing ERRγ and PGC-1α.](image-url)
transcriptionally active in MDA-MB-231 cells, as determined by the induction of known ERR targets (Fig. 1B). PGC-1α induced the expression of endogenous ERRα and of the ERR targets isocitrate dehydrogenase 3A (IDH3A), monoamine oxidase B (MAOB), and, to a lesser extent, TFF1 (9, 13, 29, 30). The PGC-1α–driven induction of these genes was mediated by endogenous ERRα ( Supplementary Fig. S2). ERRγ strongly induced MAOB and TFF1, had a modest effect on ERRα, and had no significant effect on IDH3A. Both PGC-1α and ERRγ significantly increased cellular respiration and glucose oxidation (Fig. 1C), consistent with the 2 proteins enhancing oxidative metabolism. This increase was due to changes in mitochondrial function and not due to mitochondrial biogenesis, as mitochondrial DNA copy number remained unchanged (Supplementary Fig. S3). Finally, neither PGC-1α nor ERRγ had a profound effect on cell growth in vitro, though ERRγ cell growth slowed down modestly after 5 days, on reaching confluency (Fig. 1D and Supplementary Fig. S4).

ERRγ, but not PGC-1α, inhibits tumor growth in a breast cancer xenograft mouse model
To test the effect of ERRγ and PGC-1α on the growth of MDA-MB-231 cells in vivo, MDA-EGFP (control), MDA-PGC-1α, and MDA-ERRγ cells were injected orthotopically into the mammary fat pad of female SCID mice. MDA-ERRγ cells gave rise to significantly slower growing and smaller tumors than the ones seen with control cells (Fig. 2A and B). No significant difference was observed in PGC-1α–expressing tumors (Fig. 2A and B). A second cohort of mice with independently derived cell populations showed similar results (Supplementary Fig. S5), providing strong evidence that ERRγ suppresses tumor growth. To assess transgene expression and activity in vivo, tumors were excised at 63 or 70 days and analyzed for protein and mRNA levels. ERRγ and PGC-1α expression was detected in ERRγ and PGC-1α tumors, respectively (Fig. 2C and Supplementary Fig. S6). We also observed sustained increased expression of ERR-regulated genes (ERRα, IDH3A, MAOB, and TFF1; Fig. 2D), suggesting that ERRγ and PGC-1α...
remained active *in vivo*. Notably, the largest ERRγ tumor (outlier in Fig. 2B) had the lowest expression of ERR targets, consistent with ERRγ activity being important for suppressed tumor growth.

**ERRγ promotes MET**

Because both ERRγ and PGC-1α enhanced oxidative metabolism but only ERRγ suppressed tumor growth, we reasoned that the increased oxidative capacity was unlikely to explain by itself the decreased *in vivo* growth of MDA-ERRγ tumors. We thus focused our study on features unique to the ERRγ-expressing cells. Strikingly, ERRγ expression led to changes in the morphology of MDA-MB-231 cells. MDA-ERRγ cells formed epithelium-like clusters that were flatter and less spindle shaped than control and MDA-PGC-1α cells, which exhibit the typical mesenchymal phenotype of MDA-MB-231 cells (Fig. 3A). These changes were suggestive of MET. Both MET and the reverse process EMT (epithelial-to-mesenchymal transition) are important factors in cancer progression, with the latter being associated with epithelial tumor dedifferentiation and enhanced invasion of surrounding tissues and migration (31).

We next assessed whether changes in cell morphology were accompanied by changes in epithelial and mesenchymal marker expression. MDA-ERRγ cells showed increased expression of multiple epithelial markers Fig. 3B), including E-cadherin (*CDH1*), epithelial cell adhesion molecule EpCAM, sciellin (*SCEL*), epiplakin 1 (*EPPK1*), occludin (*OCLN*), mucin 1 (*MUC1*), and the cell polarity gene crumbs 3 (*CRB3*), and decreased expression of the mesenchymal marker N-cadherin (*CDH2*) and vimentin (*VIM*). Increased expression of E-cadherin, a major characteristic of MET, was confirmed at the protein level (Fig. 3C and D). PGC-1α–expressing cells showed a selective and weaker induction of just a subset of epithelial markers (sciellin, mucin 1, and crumbs 3), and repression of the mesenchymal marker N-cadherin; notably, E-cadherin expression was not induced. Importantly, the inability of PGC-1α to induce E-cadherin and other epithelial

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**Figure 3.** ERRγ promotes MET. A, cellular morphology of MDA-control, MDA-PGC-1α, and MDA-ERRγ cells, examined by phase-contrast microscopy. B, levels of mRNA for the indicated epithelial [E-cadherin (*CDH1*), EpCAM, sciellin (*SCEL*), crumbs 3 (*CRB3*), epiplakin 1 (*EPPK1*), occludin (*OCLN*), mucin 1 (*MUC1*)] and mesenchymal [N-cadherin (*CDH2*) and vimentin (*VIM*)] markers in MDA-control, MDA-PGC-1α, and MDA-ERRγ cells. Data are expressed as in Figure 1 and are the mean ± SEM of 4 experiments conducted in duplicate. C and D, increased E-cadherin protein levels in MDA-ERRγ cells, determined by Western blot and immunofluorescence analyses. GAPDH protein levels in (C) are shown as control for protein loading. *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001.
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Figure 4. Knockdown of ERRγ decreases TFF1 and E-cadherin expression. A and C, levels of ERRγ, TFF1, and E-cadherin (CDH1) mRNA in PC-3 (A) or BT474 (C) cells expressing 2 different shRNAs for ERRγ are expressed relative to levels of each gene in cells expressing control shRNA (shControl; set equal to 1) and are the mean ± SEM of 3 experiments conducted in duplicate or triplicate. **, P < 0.01; ***, P < 0.001. Ct, cycle threshold value of RT-qPCR. B, E-cadherin protein levels in PC-3 cells expressing control shRNA, shERRγ-1, or shERRγ-2, determined by Western blotting. Histone H3 protein levels are shown as a control for protein loading.

markers was not due to a weaker transcriptional activity, as PGC-1α was stronger than ERRγ or as good as ERRγ in inducing other ERR targets (e.g., ERRα, IDH3A, MAOB; Fig. 1B).

To confirm that ERRγ in MDA-ERRγ cells was driving the induction of E-cadherin, we treated cells with 4OHT, which inhibits ERRγ activity (32, 33). 4OHT led to a dose-dependent inhibition of known ERRγ targets, such as MAOB and TFF1, and E-cadherin expression in MDA-ERRγ cells but not in control cells, consistent with E-cadherin being induced by ERRγ (Supplementary Fig. S7).

Next, we asked whether ERRγ could regulate the expression of epithelial and mesenchymal markers when expressed acutely in the absence of selection of stable cell populations. ERRγ, PGC-1α, and as an additional control, ERRα, were expressed in MDA-MB-231 cells via adenoviral vectors. Twenty-four hours post-infection, ERRγ but not PGC-1α nor ERRα strongly induced E-cadherin. ERRγ and PGC-1α also induced IDH3A, MAOB, and TFF1, with a similar preference as that in the stable populations, and downregulated N-cadherin and vimentin expression (Supplementary Fig. S8). ERRα by itself did not activate ERR targets, consistent with the lack of ERRα activity in cells that have low endogenous PGC-1 (Supplementary Fig. S8).

Finally, we tested whether endogenous ERRγ regulates E-cadherin, using prostate cancer PC-3 and breast cancer BT474 cells, in which ERRγ is readily detectable; other breast cancer lines we have tested have low to undetectable ERRγ expression. In PC-3 cells, suppression of ERRγ via 2 different shRNAs led to significant decreases in the known ERR target TFF1 and E-cadherin (CDH1) at the mRNA and protein level (Fig. 4). In BT474 cells, suppression of ERRγ led to smaller decreases that reached significance only for TFF1. The smaller effect was possibly due to the higher expression levels of ERRγ, TFF1, and CDH1, and the less efficient suppression of ERRγ in BT474 than PC-3 cells (Fig. 4).

MDA-ERRγ tumors display markers of MET

We next determined the expression of epithelial and mesenchymal markers in the MDA-MB-231 tumor xenografts. E-cadherin mRNA and protein levels were significantly increased in ERRγ tumors compared with PGC-1α and control tumors (Fig. 5A and B). The epithelial markers mucin 1 (MUC1) and keratin 8 (KRT8) were also upregulated in ERRγ tumors, whereas vimentin expression was decreased (Fig. 5A). In addition, we observed positive immunostaining for E-cadherin in multiple ERRγ tumors but not in control and PGC-1α tumors (Fig. 5C). In contrast, vimentin was not detected in 2 of 3 ERRγ tumors analyzed whereas all 3 control and PGC-1α tumors showed positive vimentin staining (Fig. 5D). In conclusion, the transition of mesenchymal-to-epithelial markers is maintained in the ERRγ tumors in vivo.

ERRγ suppresses invasiveness in vitro in an E-cadherin–dependent manner

Mesenchymal cells display a higher invasive ability than epithelial ones. Consistent with MET, MDA-ERRγ cells showed reduced invasiveness compared with control cells (Fig. 6A). Interestingly, PGC-1α also decreased the invasive properties of MDA-MB-231 cells, though to a lesser extent than ERRγ, suggesting that either the partial induction of the MET program seen at the molecular level (Fig. 3B) or another PGC-1α–dependent program affected cell behavior (Fig. 6A). The impaired invasiveness of MDA-ERRγ cells was at least partially dependent on E-cadherin, as silencing of E-cadherin diminished the suppressive effect of ERRγ on invasion (Fig. 6B).

ERRγ is a direct regulator of E-cadherin expression

Given the central role of E-cadherin in MET and tumor growth, we next asked whether ERRγ regulated E-cadherin
expression directly. Putative ERREs in the CDH1 gene were screened for their association with ERRγ, using ChIP assays. Binding of ERRγ was detected at 2 sites in the second intron (Fig. 7A and B), which is important for expression in vivo (34). As controls, ERRγ was also detected at the ERRE of TFF1 but not at a region upstream of the CDH1 promoter that does not harbor ERREs (Fig. 7B).

To determine the ability of the E-cadherin (CDH1) ERREs to respond to ERRγ, we subcloned these ERREs upstream of a basal luciferase reporter construct. ERRγ activated CDH1-ERRE-1- and CDH1-ERRE-2-containing reporters and the positive control ESRRA-ERRE reporter but had no effect on the ERRE less basal promoter (Fig. 7C). Point mutations in the CDH1 ERREs abolished or diminished responsiveness to ERRγ, consistent with these sequences mediating the effect of ERRγ, PGC-1α also activated reporter activity in a CDH1-ERRE- and ERRα-dependent manner (Fig. 7C and Supplementary Fig. S9). In conclusion, ERRE-1 and ERRE-2 of the E-cadherin gene are bound by ERRγ in MDA-ERRγ cells and mediate ERRγ-dependent transcription in reporter assays, suggesting that ERRγ is a direct positive regulator of E-cadherin expression.

Discussion

ERRγ has been suggested as a marker of favorable clinical course in human breast cancer (1). In support of such a role, a survey of the Oncomine cancer database shows significant correlations between decreased ERRγ levels and higher breast cancer grade, metastasis, recurrence, and unfavorable outcome (Supplementary Table S2). The extent to which these correlations reflect an active role of ERRγ in breast cancer progression had not been tested so far. Using MDA-MB-231 breast cancer cells expressing human ERRγ, we show here that ERRγ suppresses cell invasiveness in vitro and breast cancer xenograft growth in mice. Moreover, we provide evidence that ERRγ acts by inducing the expression of epithelial genes, thereby promoting MET.

In contrast to ERRγ, high levels of ERRα in breast cancer have been associated with increased risk of recurrence and
adverse clinical outcome (1, 2). Recent studies support a role of ERRγ in suppressing tumor growth (11, 23–25). In our study, enhancing ERRα levels and activity, via PGC-1α expression, did not enhance tumor growth. It is possible that the tumor-promoting role of ERRα involves PGC-1α–independent activities and is thus not affected by PGC-1α. Alternatively, a tumor-promoting role of ERRα may be antagonized by ERRα-indepen dent tumor suppressive effects of PGC-1α. Notably, low levels of PGC-1α have been associated with poor clinical outcome in breast cancer (35, 36). Additional studies will be needed to test a potential role of PGC-1α in breast cancer and to understand the cellular context in which ERRα promotes tumor growth.

EMT plays an important role in tumorigenesis (31). The ability of epithelial cancer cells to invade surrounding tissues and escape from the primary tumor is associated with loss of intercellular adhesion, loss of contact inhibition, loss of apical-basal polarity, and enhanced migratory potential. During this process, cells transit from a polarized epithelial phenotype to a highly motile mesenchymal one. Loss of E-cadherin expression is often considered a common indicator of EMT, whereas the reverse process MET is usually associated with E-cadherin reexpression. ERRγ promoted multiple aspects of MET, including increased levels of several epithelial markers, decreased levels of mesenchymal markers, morphologic changes, and decreased invasiveness. Interestingly, ectopic expression of ERRα (Supplementary Fig. S8) or activation of endogenous ERRα (via expression of PGC-1α) led to either no effect or weaker and gene-selective induction of epithelial markers, suggesting that ERRγ is a more efficient activator than ERRα with respect to the epithelial program. Notably, PGC-1α/ERRα was as efficient as ERRγ or more efficient than ERRγ in inducing other ERR targets, such as MAOB and IDH3A. These findings suggest that ERRα and ERRγ, which bind common target genes (17), can act differentially at select targets. The underlying mechanisms for such differential action are not yet clear but could reflect differential recruitment of coregulators.

From the genes we have tested, E-cadherin is one of the most differentially affected by ERRγ and PGC-1α. In breast cancer, dysfunction of E-cadherin correlates with loss of epithelial characteristics, acquisition of invasiveness, increased tumor grade, metastatic behavior, and poor prognosis (37, 38). Overexpression of E-cadherin in MDA-MB-231 cells decreases invasiveness (39) and growth in soft agar (40). Additional cellular and animal models support E-cadherin as a suppressor of tumor growth and invasion (41, 42). The mechanism by which ERRγ induces E-cadherin is likely to be direct, via ERREs in the second intron, which is important for expression (34). Interestingly, when taken out of their genomic context, these ERREs also mediate PGC-1α/ERRα-activated transcription, suggesting that epigenetic regulation at the CDH1 locus may keep the endogenous gene refractory to PGC-1α. Consistent with this notion, the CDH1 locus in MDA-MB-231 cells is methylated (38). Although we cannot exclude yet additional mechanisms by which ERRγ induces
E-cadherin, we have not seen significant differences in the levels of other known E-cadherin regulators (43) between ERRγ- and PGC-1α-expressing MDA-MB-231 cells (Supplementary Table S3).

A role of ERRγ for epithelial function may not be restricted to breast cancer. ERRγ is highly expressed in epithelia of several tissues, including intestine, stomach, kidney, and salivary gland, where it may contribute to developmental processes, normal epithelial function, and/or pathophysiologic processes in which EMT/MET transitions have been implicated (e.g., organ fibrosis; ref. 44). Moreover, ERRγ expression has been correlated with a better prognosis in prostate, endometrial, and ovarian carcinomas (45–47) and overexpression of ERRγ suppresses prostate cancer cell proliferation and tumor growth (48). In conclusion, ERRγ may be involved in the pathology of different tumors and development of ERRγ agonists may have general applications in cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Figure 7. ERRγ is a direct regulator of E-cadherin. A, diagram of the human CDH1 (E-cadherin) locus showing ERRE-1 and ERRE-2 (boxed sequences) in intron 2. Numbered bars in diagram represent exons. Small arrows indicate the regions amplified in the ChIP experiments of B. B, ERRγ and PGC-1α recruitment to ERREs, determined by ChIP. Immunoprecipitated products were amplified by qPCR, using primers indicated in (A) or flanking the ERRE of the TFF1 gene. Data are expressed relative to the amount of DNA immunoprecipitated in control cells and are the mean ± SEM of 3 experiments conducted in quadruplicate. The amounts of CDH1-ERRE-1, CDH1-ERRE-2, and TFF1-ERRE-containing DNA in the ERRγ IP were 0.13%, 0.10%, and 0.16% of input DNA, respectively. C, ERRE-1 and ERRE-2 of CDH1 mediate ERRγ-dependent activation of transcription. Luciferase reporter constructs bearing just the basal promoter (pΔ-Luc) or having trimers of ERRE-1 or ERRE-2 of CDH1 (wild-type (WT) or bearing mutations (MUT) in the nucleotides marked with an asterisk (*) in A), were cotransfected in MDA-MB-231 with control plasmid (pcDNA3), PGC-1α, or ERRγ expression constructs. The ERRE-containing regulatory sequence of the ESRRA gene was used as a positive control. Data are the mean ± SEM luciferase activity of 3 independent experiments conducted in quadruplicate. *, P < 0.05; **, P < 0.01; ***, P < 0.001.
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References


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