LRH-1 Governs Vital Transcriptional Programs in Endocrine-Sensitive and -Resistant Breast Cancer Cells

Stéphanie Bianco¹, Mylène Brunelle¹, Maïka Jangal¹, Luca Magnani², and Nicolas Gévy¹

Abstract

Tumor characteristics are decisive in the determination of treatment strategy for patients with breast cancer. Patients with estrogen receptor α (ERα)–positive breast cancer can benefit from long-term hormonal treatment. Nonetheless, the majority of patients will develop resistance to these therapies. Here, we investigated the role of the nuclear receptor liver homolog-1 (LRH-1, NR5A2) in antiestrogen-sensitive and -resistant breast cancer cells. We identified genome-wide LRH-1–binding sites using ChIP-seq (chromatin immunoprecipitation sequencing), uncovering preferential binding to regions distal to transcriptional start sites. We further characterized these LRH-1–binding sites by integrating overlapping layers of specific chromatin marks, revealing that many LRH-1–binding sites are active and could be involved in long-range enhancer–promoter looping. Combined with transcriptome analysis of LRH-1–depleted cells, these results show that LRH-1 regulates specific subsets of genes involved in cell proliferation in antiestrogen-sensitive and antiestrogen-resistant breast cancer cells. Furthermore, the LRH-1 transcriptional program is highly associated with a signature of poor outcome and high-grade breast cancer tumors in vivo. Herein, we report the genome-wide location and molecular function of LRH-1 in breast cancer cells and reveal its therapeutic potential for the treatment of breast cancers, notably for tumors resistant to treatments currently used in therapies. Cancer Res; 74(7): 2015–25. ©2014 AACR.

Introduction

During the last 40 years, antihormonal therapy targeting estrogen receptor α (ERα) activity in breast cancer has allowed both an increase in relapse-free survival and a 30% decrease in mortality in the 15 years following treatment initiation (1, 2). Long-term adjuvant treatments currently used following tumor surgery consist in blocking estrogen action at the receptor using tamoxifen or fulvestrant/ICI182780 (ICI), or inhibiting estrogen biosynthesis using inhibitors of the cytochrome P450 aromatase (CYP19 aka aromatase). Despite the success of these endocrine therapies, nearly half of breast cancers are, or will become, resistant to these treatments. Multiple mechanisms have been proposed to explain antiestrone resistance, such as loss or change of ERα expression, alteration in coregulator expression and/or activity, and aberrant growth factor signaling (2, 3). Endocrine resistance results in uncontrolled proliferation and deregulation of the ERα transcriptome, notably in genes involved in cell cycle and apoptosis (3, 4). Recent technological advances in genomic studies have enabled us to determine the chromatin structure and epigenetic signatures that are liable for the aberrant transcriptional program in antiestrogen resistance. For instance, it has been shown that the pioneer factors Forkhead box A1 protein (FOXA1) and pre–B-cell leukemia homeobox 1 (PBX1) influence global chromatin structure and guide ERα to bind specific genes associated with poor clinical prognosis in breast cancer (5–7). This suggests that a better understanding of how these tumors acquire resistance at the epigenetic level is an essential step in developing new strategies to bypass endocrine resistance.

Liver receptor homolog-1 (LRH-1), also known as NR5A2, is a member of the nuclear receptor family and binds DNA as a monomer. Originally classified as an orphan nuclear receptor, crystal structure analyses revealed the presence of a ligand in the pocket of its ligand-binding domain. Several teams have shown that natural phospholipid molecules, such as phosphatidylinositol or phosphatidylcholine, are able to bind LRH-1 in mammals, thereby modifying its transcriptional activity (8, 9). The search of novel strategies to therapeutically target LRH-1 in a variety of pathologies have led to the development of ligands with agonist activity (10) and molecules with inverse agonist activity (11, 12).

LRH-1 regulates multiple essential physiologic functions, particularly in embryonic development and differentiation, fatty acid and cholesterol metabolism, bile acid and pancreatic fluid homeostasis and synthesis (13). In reproduction, LRH-1 is a key regulator of ovulation (14) and placental function (15). It has been reported that LRH-1 has an important role in the development and progression of many cancers, such as colon,
gastric, pancreas, liver, and breast cancers (13). For example, it has been shown that the grafting of pancreatic and hepatic cancer cell lines overexpressing LRH-1 in athymic mice promotes cell proliferation, colony formation, and tumor progression (16). These effects seem to be mediated by the targeting of genes involved directly in cell proliferation such as CCNE1, CCND1, and MYC (16, 17). In breast cancer, LRH-1 is highly expressed and localized in the epithelial compartment of both invasive ductal carcinoma and ductal carcinoma in situ (18, 19). Interestingly, depletion of LRH-1 in breast cancer cells decreases cell mobility, invasion and colony formation (20). Moreover, an important crosstalk seems to exist in estrogen-dependent breast cancer cells, in which LRH-1 is itself regulated by ERα (18). The depletion of LRH-1 in these cells inhibits the proliferative effect of estrogen, suggesting that the mitogenic effects of estrogen may be mediated, in part, by LRH-1. Thus, although the molecular effects of LRH-1 in breast cancer are not fully understood, much evidence suggests that its role is closely integrated with the estrogen signaling pathway and cell proliferation.

In the present study, we determined the cistrome and the transcriptome of LRH-1 in human breast cancer cells. Many LRH-1–binding sites correspond to active open chromatin regions discernable by FAIRE signal (formaldehyde assisted isolation of regulatory elements) and by the active transcription enhancer mark H3K27ac. Some LRH-1–binding sites overlap with Med12 and RNA polymerase II (RNAPII), known to be specific marks for enhancers involved in long-range enhancer–promoter looping. Furthermore, we provide evidence that LRH-1 governs a transcriptional program essential for cell proliferation in antiestrogen-sensitive and antiestrogen-resistant breast cancer cells, in particular the regulation of CCND1, through long-range enhancers, found 125 and 116 kb upstream of its transcriptional start site (TSS). Importantly, LRH-1 transcriptional program is associated with poor outcome in breast cancer. Together, these findings strongly suggest an implication of LRH-1 in the development of resistance to antiestrogen treatments and bring to light the potential of targeting LRH-1 for the development of new breast cancer treatments.

Materials and Methods

Cell line, cell culture, and shRNA lentiviral transduction
MCF7 and HMEC cell lines (American Type Culture Collection) were maintained in Dulbecco’s Modified Eagle Medium (DMEM; Wisent) containing 10% FBS and antibiotics or in HuMEC ready medium (Gibco). The MCF7/LCC2 and MCF7/LCC9 cells (a gift from Robert Clarke, Georgetown University Medical Center, Washington, DC) were cultured in DMEM without phenol red containing 5% charcoal–dextran-treated FBS. Cells were routinely tested for LRH-1 and ERα expression by quantitative PCR (qPCR) and Western blot analyses. MCF7/LCC2 and MCF7/LCC9 cells were routinely checked for resistance to antiestrogen by qPCR on ERα ligands (last test on October 18, 2013) and by growth assays (last test on October 26, 2013). Cells routinely tested were found to be mycoplasma-free. The short hairpin RNAs (shRNA) against LRH-1 (sequences included in Supplementary Table S1) were purchased from Open Biosystems in the pLKO.1 vector. MCF7, MCF7/LCC2, and MCF7/LCC9 were transduced with shRNA lentiviruses, obtained as previously described (4), and collected for mRNA and protein extraction after 4 days of transduction.

RNA, microarray, and protein analysis
Four days after LRH-1 knockdown, total RNA and protein were collected from MCF7, LCC2, and LCC9 cells as previously described (4). For qPCR and Western blot analysis, we used primers and an antibody (listed in Supplementary Table S2 and S3) that target all known LRH-1 variants. Microarray analysis using BeadChip Human Genome (Illumina; HT-12) was performed at the Centre d’innovation at Genome Quebec (McGill University, Montreal, QC, Canada). Log2 transformation and quantile normalization of the data were performed and differentially expressed genes were selected using a P value cutoff <0.05 and fold change >±1.5. The open source software Cluster/TreeView was used to generate microarray heatmap (http://www.eisenlab.org/eisen/).

Transcriptome-based outcome analysis
Genes differentially expressed following LRH-1 knockdown (>2-fold) were compared with poor outcome and high-grade overexpressed gene signatures obtained from in vivo data using Oncomine (https://www.oncomine.com). Clinical studies used in the analysis are listed in Supplementary Table S8. Significant association was established at a P value of at least <0.01 and an OR of >2.

Chromatin immunoprecipitation, FAIRE, and sequencing
Chromatin immunoprecipitation (ChIP) experiments were performed as previously described (21). The antibodies and primers used are listed in Supplementary Table S3 and S4. FAIRE experiments were achieved as previously described (6). ChIP-seq (chromatin immunoprecipitation sequencing) and FAIRE-seq libraries were performed according to the manufacturer’s instruction (Illumina) starting with 10 and 50 ng of DNA, respectively. DNA was purified with solid phase reversible immobilization beads (Agencourt AMPure XP; Beckman Coulter) and quantified with Bioanalyzer (Agilent) prior to being sequenced using the Hi-seq (Illumina). Generated reads were aligned to the human reference genome hg18 using Burrows-Wheeler Aligner (22). Only sequence reads that were uniquely mapped to the genome with a mapping quality score >10 were used. Two biologic replicates were performed and merged for subsequent analyses.

ChIP-seq data analysis and visualization
The peaks were called by Model-based Analysis for ChIP-Seq (MACS; ref. 23) with default parameters. Genomic distributions were determined using Cis-regulatory Element Annotation (CEAS; ref. 24) and Genomic Regions Enrichment of Annotation tool (GREAT; ref. 25). Motif discovery was completed using HOMER known and de novo motif (26). Nearby genes were assigned to ChIP-seq peaks using GREAT and compared with differentially expressed genes from microarray analysis. The k-means linear clustering method was used to generate
heatmaps with seqMINER (27). LRH-1 clusters were intersected with our own RNAPII ChIP-seq data and with RNAPII ChIA-PET (chromatin interaction analysis by paired-end tag sequencing) experiment (GSM832458 and GSM832459; ref. 28). Random genomic regions with the same length, size, and genomic distribution of LRH-1 clusters were used as control, and compared with RNAPII ChIP-seq and RNAPII ChIA-PET data. Gene ontology (GO; biologic processes) analyses were performed using GREAT. Aligned tags were converted to WIG files by F-Seq (29) and visualized with an Integrative Genomics Viewer (30).

Cell proliferation assays
On day 1 after lentiviral transduction, cells were seeded (1 × 10^5) in 48-well plates and treated with vehicle (EtOH), tamoxifen 10^{-6} mol/L (Sigma-Aldrich), or ICT182780 10^{-7} mol/L (Tocris Bioscience) every 3 days. At the indicated time, cell counting was performed using TC20 Automated Cell Counter (Bio-Rad). For cells treated with the LRH-1 inverse agonist ML180 (a gift from Patrick Griffin, The Scripps Research Institute, Jupiter, FL), cell counting was performed according to the manufacturer’s protocol [CellTiter 96 AQuous One Solution Cell Proliferation Assay (MTS); Promega]. MCF7, LCC2, and LCC9 cells were seeded (2 × 10^3 or 5 × 10^3) in 96-well plates and treated with ML180 at concentrations of 5 and 10 μmol/L during 6 days. Cell-cycle analysis was performed as previously described (4).

GEO accession number
All genomic data have been submitted to GEO database under accession number GSE54892: ChIP-seq data (GSE47027) and expression profiling (GSE54891).

Results

LRH-1 regulates genes associated with poor outcome in breast cancer
As described previously, LRH-1 has been shown to be involved in breast cancer cell proliferation. However, its specific transcriptional program had not yet been established. To evaluate the LRH-1–dependent transcription program, we investigated global gene expression in the MCF7, MCF7/LCC2 (LCC2), and MCF7/LCC9 (LCC9) cell lines, depleted or not of LRH-1. In earlier studies, resistance to tamoxifen was enriched in parental MCF7 cells treated with tamoxifen, whereas LCC9 cells presented a cross-resistance to fulvestrant/ICI180782 and to tamoxifen (31, 32). First, we determined the level of LRH-1 in these endocrine-resistant cell lines that still expressed ERα (Supplementary Fig. S1). Surprisingly, LRH-1 was overexpressed in antiestrogen-resistant cells at mRNA (Fig. 1A) and protein levels (Fig. 1B) compared with either the antiestrogen-sensitive MCF7 or to a noncancerous breast cell line (HMEC). We then depleted LRH-1 in MCF7, LCC2, and LCC9 cells using a lentiviral-based shRNA approach. The two constructs shLRH-1 #1 and #2 efficiently depleted (~70%–90%) both mRNA (Fig. 1D) and protein levels (Supplementary Fig. S2A), and were used in the following experiments. Duplicate microarray analyses were performed to identify genes differentially expressed upon knockdown in the three different cell lines (Supplementary Tables S5–S7). Notably, the profile of the LRH-1 transcriptional program displayed greater similarity between cell lines LCC2, LCC9, and MCF7 (Fig. 1C). Indeed, 303 genes were similarly downregulated and 310 upregulated in the three cell lines following LRH-1 knockdown. These results were confirmed by qPCR in MCF7, LCC2, and LCC9 cells, in which cellular depletion of LRH-1 was found to significantly affect the expression of important genes involved in cellular proliferation such as BCL2, MYC (Fig. 1D and Supplementary Fig. S2B). In support of these results, MYC downregulation upon LRH-1 knockdown is consistent with previously published data on LRH-1 target genes described in intestine and pancreatic cancer cells (16, 17).

To further evaluate the impact of LRH-1 in breast cancer cells, genes differentially expressed in absence of LRH-1 in these three cell lines were compared with in vivo breast cancer data using Oncomine Concepts Map analysis (Compendia Bioscience). Genes downregulated in LRH-1–depleted MCF7, LCC2, and LCC9 cell lines were significantly associated with the signatures of genes overexpressed in poor outcome and high-grade tumors in vivo (Fig. 1E). However, upregulated genes observed in absence of LRH-1 in MCF7, LCC2, and LCC9 cell lines had no association with overexpressed gene signatures. Taken together, these results suggest that LRH-1 plays a similar role in terms of transcriptional regulation in antiestrogen-sensitive and anti-estrogen-resistant breast cancer cells, positively regulating genes associated with poor prognosis in breast cancer.

Genome-wide identification of LRH-1–binding sites in breast cancer cells
To expand our understanding of the role of LRH-1 in breast cancer, we performed ChIP with LRH-1 antibody followed by next-generation sequencing (ChIP-seq) in asynchronous MCF7 cells. As a result, we detected 7,014 LRH-1–binding sites using MACS (23) in MCF7 ChIP-seq data. Peak distribution analysis showed that the vast majority of the LRH-1–binding sites were located in intragenic and distal intergenic region (Fig. 2A). Indeed, slightly more than 50% of LRH-1–binding sites were localized at distant regulatory elements (>50 kb) from the TSS, whereas almost 40% were localized between 5 and 50 kb and the remainder around the TSS (Fig. 2B). De novo motif analysis of LRH-1–binding sites revealed the enrichment of a canonical nuclear receptor half-site sequence (Fig. 2C). This result is consistent with the known LRH-1 motif previously determined in mouse embryonic stem cells by ChIP-seq analysis (33).

To examine the relationship between LRH-1 binding and global gene expression differences observed in MCF7 LRH-1–depleted cells, the LRH-1–binding regions were individually assigned to a total of 5,636 human UCSC known genes using GREAT (25). Genes that were differentially expressed when LRH-1 was depleted were significantly associated with genes bound by LRH-1 compared with random peaks (P < 1.85 × 10^{-5}; Fig. 2D). We confirmed these results by ChIP–qPCR on selected genes affected by the depletion of LRH-1 in MCF7 cells such as MYC, BCL2, and IL24 (Supplementary Fig. S3). Taken together, our results suggest that LRH-1 regulates a specific transcriptional program primarily through the binding of distal enhancer sites in breast cancer cells.
Chromatin pattern associated with LRH-1–binding events

To further define the chromatin environment of LRH-1–binding sites and to better understand how LRH-1 acts on its target genes across the genome, we performed ChIP-seq experiments identifying specific chromatin and transcriptional marks in MCF7 cells. FAIRE-seq and histone mark H3K27ac ChIP-seq were carried out to identify regulatory elements characterized by open chromatin (34) and separate active enhancers from poised or nonactive enhancers (35). FAIRE and H3K27ac signal were aligned on LRH-1–binding sites and divided into high and low signal intensity (Fig. 3A, left). The aggregate profiles of the high and low clusters are also shown in Fig. 3A, right.

Med12, a component of the large mediator transcriptional adaptor complex, was recently showed to mediate enhancer looping and to interact directly with nuclear receptors (36, 37). The cluster corresponding to active LRH-1–binding sites (FAIRE/\text{high}_H3K27a\text{c/\text{high}}), was subdivided into high, medium, and low signal according to our Med12 signal generated by ChIP-seq in the same cells (Fig. 3B). As expected, the inactive LRH-1 cluster (FAIRE/\text{low}_H3K27a\text{c/\text{low}}) showed an overall lower Med12 enrichment (Supplementary Fig. S4A). These results suggest that active LRH-1–binding sites, which are strongly associated with Med12, are involved in enhancer-promoter looping.

To demonstrate the involvement of LRH-1 in long-range chromatin interactions, we first compared our LRH-1 clusters with RNAPII-binding sites generated by ChIP-seq in the same cells. RNAPII binds distal genomic regions with active enhancer signatures and genomic loci involved in chromatin looping (28, 38, 39). Our results showed that 26% of the active LRH-1–binding sites overlap with RNAPII-binding sites, whereas only 5% of the inactive LRH-1–binding sites are enriched for RNAPII (Fig. 4A). As expected, this proportion increased to 41% of the active LRH-1 subgroup with the highest Med12 enrichment (Fig. 4A), whereas the inactive LRH-1 weakly overlapped with RNAPII (less than 15%; Supplementary Fig. S4B).

ChIA-PET was developed to explore the three-dimensional chromatin interactions involving specific protein factors at a
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Figure 2. Genome-wide distribution of LRH-1–binding events in breast cancer cells. A, genomic distribution of LRH-1–binding sites and random peaks in MCF7 cells. B, graphical representation of distance of LRH-1 binding to known TSS. C, prediction of LRH-1–binding motifs obtained using HOMER de novo and known motif computational tools. D, graphical representation of the proportion (~32%) of differentially expressed genes with LRH-1 depletion (microarray data, n = 1,803 differentially expressed genes in MCF7), which also have at least one LRH-1–binding site within 300 kb from their respective TSS (ChIP-seq data, n = 5,636 genes associated with LRH-1–binding sites). Random peaks were used as control. A P value is shown. NR, nuclear receptor.

LRH-1 controls cell proliferation in antiestrogen-sensitive and antiestrogen-resistant breast cancer cells

To further annotate and assign potential function to LRH-1–bound DNA regions, GREAT, and GO analyses were performed on ChIP-seq dataset. GO analysis showed a strong correlation with cell proliferation processes for the active LRH-1–binding sites when compared with the inactive LRH-1–binding sites (Fig. 5A). Indeed, the active LRH-1–binding sites displayed strong enrichment with GO terms involved in cellular growth, such as the regulation of cell–matrix adhesion, gland morphogenesis, and mammary gland epithelial cell proliferation and development. Given the propensity for the occurrence of GO terms involved in cell growth, we next wanted to test the impact of LRH-1 depletion on proliferation and cell-cycle distribution in MCF7, LCC2, and LCC9 cells. Using the shLRH-1 #1 and shLRH-1 #2 constructs, we observed a strong reduction in cell proliferation over a period of 9 days following lentiviral infection (Fig. 5B). MCF7 cells, which are sensitive to antiestrogen treatment such as tamoxifen or ICI, showed a very slow or arrested growth after LRH-1 knockdown. Moreover, LCC2 and LCC9 cell lines showed a complete growth inhibition when treated with LRH-1 shRNA while they are nonresponsive to tamoxifen and/or ICI treatment. To further characterize the arrested growth, we performed analysis of the cell-cycle distribution by fluorescent-activated cell sorting. MCF7, LCC2, and LCC9 cells showed an approximately 20% increase in G1-phase, increasing from 60% to 65% in shCTL to 80% to 90% in shLRH-1 cells (Supplementary Fig. S7A). To assess the potential use of LRH-1 inhibitors to overcome antiestrogen resistance in breast cancer, we treated cells with the LRH-1 inverse agonist ML180 (11) and measured cell proliferation over a period of 6 days. As observed in LRH-1 knockdown experiments (Fig. 5B), treatment with the ML180 compound significantly reduced the growth of MCF7 cells and, importantly, the LRH-1 inverse agonist had a stronger antiproliferative effect on antiestrogen-resistant cell lines (Supplementary Fig. S7B). We observed a dose-dependent reduction in cell proliferation of up to 30% in LCC2 and 60% in LCC9 cells after 6 days of treatment, whereas a reduction of 15% to 30% in cell proliferation was achieved for MCF7 cells. Taken together, these data show that perturbation of the transcriptional program of LRH-1 by repressing its expression/activity negatively affects cell proliferation, notably in antiestrogen-resistant breast cancer cells, which fail to respond to current breast cancer therapeutic treatments.

LRH-1 regulates CCND1 expression by distal enhancers

The gene encoding cyclin D1, CCND1, is very important for the G1→S transition during cell cycle. Overexpression, amplification, and mutations of this gene are often observed in
breast cancer and may contribute to tumorigenesis and endocrine resistance. As expected, CCND1 expression was down-regulated, as shown in our microarray data of LRH-1–depleted MCF7 cells (Supplementary Table S5). Interestingly, this effect was also observed and confirmed by qPCR in LRH-1–depleted LCC2 and LCC9 antiestrogen-resistant cells for which tamoxifen and/or ICI did not affect the high level of expression of CCND1 in these cells (Fig. 6A; Supplementary Fig. S8; Supplementary Table S6–S7). Remarkably, ChIP-seq analysis revealed distal regulatory elements bound by LRH-1 at −125, −116, and +13 kb from the CCND1 TSS (Fig. 6B). In line with the previous finding, these CCND1 LRH-1–binding sites associated with
specific chromatin marks such as strong FAIRE, H3K27ac, Med12 signal, and lower RNAPII peak. Specifically, the ChIA-PET RNAPII data showed that these distal sites are involved in long-range interaction, notably by looping with CCND1 promoter region (Fig. 6B). The presence of LRH-1 was validated by ChIP–qPCR at these distal enhancers in MCF7, LCC2, and LCC9 breast cancer cells (Fig. 6C). Altogether, these results suggest that LRH-1 regulates CCND1 transcription through distal enhancer in antiestrogen-sensitive and -resistant breast cancer cells.

Discussion

Previous studies have allowed the emergence of the understanding of the physiologic and pathologic functions of LRH-1. In this study, we provide genomic evidence that LRH-1 plays a critical role in breast cancer cell proliferation and demonstrate a new role of this receptor in the acquisition of antiestrogen resistance.

Using ChIP-seq, we mapped LRH-1 binding sites across the genome and showed that LRH-1 preferentially binds at distal regulatory element from TSS of target genes. A similar pattern
of LRH-1 distribution has been observed in mouse pancreas ChIP-seq analysis (40). These results are also consistent with previously published nuclear receptor cistrome analyses showing a preferential binding of LRH-1 to introns and distal intergenic regions (41). Epigenetic modifications, such as histone posttranslational modifications, are essential to define active and inactive chromatin regulatory elements (35, 42, 43). Close to 40% of LRH-1–binding sites are marked by FAIRE and H3K27ac signals, which are specific markers of open and active chromatin regions. These chromatin features strongly delineate genome-wide LRH-1–responsive elements, the LRH-1 cistrome, which clearly corresponds to breast cancer pathophysiology and cellular proliferation as shown by GO analysis. In mouse stem cells, genes bound by LRH-1 are strongly associated with embryonic development and pluripotency maintenance (33), whereas in pancreas and liver, its transcriptional program is linked to glucose and lipid metabolism (40, 44). Our GO results are consistent with previous studies showing a role for LRH-1 in cell proliferation, notably targeting CCND1 and MYC, and support the hypothesis that the genome-wide–binding pattern of LRH-1 is cell specific. Furthermore, many of LRH-1–binding sites overlap with Med12 and RNAPII binding, suggesting that they could be involved in long-range interaction. Indeed, many LRH-1–bound regions overlap with RNAPII–ChIA-PET interactions, showing that distal LRH-1–binding sites are involved in enhancer–promoter looping. Similar observations were made in ERα ChIA-PET experiments revealing interactions.
between intergenic ERα-binding sites and respective target gene promoters (45, 46).

Interestingly, genes regulated by LRH-1 significantly correlate with genes overexpressed in breast cancer tumor and associated with poor clinical prognosis. We have obtained similar observations with the transcriptional program of LRH-1–depleted cell lines LCC2 and LCC9. Remarkably, antiestrogen-resistant LCC2 and LCC9 cell lines derived from parental MCF7 cells overexpressed LRH-1. Depletion of LRH-1 in these cells showed that LRH-1 regulates gene subsets similar to those we reported in MCF7 cells. Important regulators of cell proliferation, CCND1, MYC, and BLC2, are often overexpressed in antiestrogen-resistant breast tumors (3) and were downregulated in our antiestrogen-resistant cell models following LRH-1 depletion. These observations corroborate our results showing a decrease of cell proliferation in antiestrogen-sensitive and antiestrogen-resistant breast cancer cells after LRH-1 knockdown.

LRH-1 plays a direct role in tumor development and progression via a positive feedback loop with ERα. Indeed, LRH-1 expression is regulated by ERα (18) and ERα is also regulated by LRH-1 (47) because LRH-1 directly binds the ERα promoter and vice versa. In addition, LRH-1 was shown to cooperate with ERα to regulate estrogen-responsive genes such as GREG1 and TFF1 (48). We also observed this cooperation because we found an overlap of approximately 50% between the LRH-1 and ERα cistromes in MCF7 cells (unpublished data). This result suggests that for some genes, LRH-1 and ERα may act together to control gene expression. The overlap between LRH-1– and ERα–binding sites provide a reasonable molecular basis for the effects observed when blocking LRH-1 expression in antiestrogen-sensitive and -resistant cell lines. Further work will be required to establish the mechanism responsible for this cooperation. Despite this close link between LRH-1 and ERα, it was also found that LRH-1 is expressed in ERα-negative breast cancer cells in which it controlled cell migration and invasion (20, 49), suggesting that LRH-1 may also have an important role in hormone-independent cancers.

The G protein-coupled receptor 30 (GPR30) was shown to be involved in activation of LRH-1 via stimulation of the phosphoinositide 3-kinase and mitogen-activated protein kinase pathways in endometrial cancers (50). GPR30 signaling contributes to tumor growth and resistance to antiestrogen in breast cancers (51). Furthermore, GPR30 cooperates with EGFR/HER2, a gene known to be overexpressed in endocrine-resistant breast cancer, to mediate signaling pathway activation and cell proliferation (52, 53).

LRH-1 may also act on breast cancer via an indirect mechanism such as the tumor microenvironment. Indeed, LRH-1 induces aromatase expression, the essential enzyme required for the conversion of androgens to estrogens in breast adipose tissues, acting in a paracrine manner on neighboring tumor cells (54).

Together, our findings underpin an important role in the acquisition of antiestrogen resistance in breast cancer with LRH-1 acting as a key factor in central signaling pathways. Furthermore, we demonstrated the therapeutic potential of targeting LRH-1 using an inverse agonist ligand, which significantly decreased cell proliferation in antiestrogen-resistant cells.

In summary, LRH-1 regulates a specific transcriptional program substantially involved in cell proliferation in antiestrogen-sensitive but also and especially in antiestrogen-resistant breast cancer cells. A better understanding of LRH-1 action and the use of small-molecule inhibitors with high specificity and affinity for this receptor may provide an alternate prognostic marker or breast cancer treatment, a particularly attractive prospect for tumors resistant to endocrine therapy.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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