# Minireview: The Link Between ERα Corepressors and Histone Deacetylases in Tamoxifen Resistance in Breast Cancer

Stéphanie Légaré and Mark Basik

Division of Experimental Medicine, Department of Oncology and Surgery, Segal Cancer Center, Lady Davis Institute for Medical Research, Sir Mortimer B. Davis Jewish General Hospital, McGill University, Montréal, Québec, Canada H3T 1E2



Approximately 70% of breast cancers express the estrogen receptor (ER)α and are treated with the ERα antagonist, tamoxifen. However, resistance to tamoxifen frequently develops in advanced breast cancer, in part due to a down-regulation of ERα corepressors. Nuclear receptor corepressors function by attenuating hormone responses and have been shown to potentiate tamoxifen action in various biological systems. Recent genomic data on breast cancers has revealed that genetic and/or genomic events target ERα corepressors in the majority of breast tumors, suggesting that the loss of nuclear receptor corepressor activity may represent an important mechanism that contributes to intrinsic and acquired tamoxifen resistance. Here, the biological functions of ERα corepressors are critically reviewed to elucidate their role in modifying endocrine sensitivity in breast cancer. We highlight a mechanism of gene repression common to corepressors previously shown to enhance the antitumorigenic effects of tamoxifen, which involves the recruitment of histone deacetylases (HDACs) to DNA. As an indicator of epigenetic disequilibrium, the loss of ERα corepressors may predispose cancer cells to the cytotoxic effects of HDAC inhibitors, a class of drug that has been shown to effectively reverse tamoxifen resistance in numerous studies. HDAC inhibition thus appears as a promising therapeutic approach that deserves to be further explored as an avenue to restore drug sensitivity in corepressor-deficient and tamoxifen-resistant breast cancers. **(*Molecular Endocrinology* 30: 965–976, 2016)**

With a lifetime risk estimated to be 1 in 8 in industrialized countries, breast cancer is the most frequent type of cancer among women worldwide and the second leading cause of cancer deaths in women (1). Breast cancer arises in epithelial cells of the mammary gland and is strongly influenced by hormone-dependent risk factors that include early menarche, late menopause and increasing number of productive hormonal cycles, which involve prolonged exposure to estrogen and progesterone, the 2 most dominant female hormones (2–8). Produced by the ovaries from puberty to menopause, estrogen and progesterone have pleiotropic effects in numerous tissues, including the cardiovascular and central nervous system as well as being essential for the induction and maintenance of most female characteristics (9, 10). However, both also have potent mitogenic actions in breast tissues that may lead to genomic instability, favor the accumulation of genetic alterations, and contribute to the development of hormone-sensitive tumors (11–13).

Dependent on hormones for their growth, proliferation and survival, hormone-sensitive breast cancers account for more than 70% of newly diagnosed breast carcinomas and are commonly characterized by the expression of the estrogen receptor (ER) and/or its transcriptional target, the progesterone receptor (14, 15). The ER is a member of the nuclear hormone receptor family of

ISSN Print 0888-8809 ISSN Online 1944-9917
Printed in USA

Received May 8, 2016. Accepted July 13, 2016.
First Published Online July 20, 2016

Abbreviations: COUP-TFII, chicken ovalbumin upstream promoter transcription factor 2; ER, estrogen receptor; FOXO1, Forkhead box protein O1; HAT, histone acetyltransferase; HDAC, histone deacetylase; HDACi, HDAC inhibitor; HR, hazard ratio; NCOR, nuclear corepressor; NRIP1, nuclear receptor-interacting protein 1; PFN1, profilin 1; PHB2, Prohibitin-2; RIP140, receptor-interacting protein 140; SAFB1, scaffold attachment factor B1; SMRT, silencing mediator for retinoid or thyroid hormone receptor; SPEN, split ends; TAFII68, TATA element-binding protein-associated factor.

ligand-dependent transcription factors that exists in 2 closely related forms, usually referred to as ERα and ERβ. Although close to 40% of ERα binding sites overlap with those of ERβ, it is now clear that the 2 receptors regulate distinct transcriptional programs and have opposing actions at certain gene promoters (16). In fact, recent studies intended to characterize ERβ functions in breast cancer revealed that it has antagonistic activities towards its α homolog, possibly through heterodimeric interactions with ERα, a finding that may explain their cooccurrence on chromatin. Although ERβ is coexpressed with ERα in some tumors and has been identified as an independent prognostic marker in breast cancer, the ERα is the most clinically useful prognostic and predictive biomarker in patients with hormone receptor positive breast cancers (17–19).

The protumorigenic functions of ERα in breast cancer are mediated by genomic and nongenomic mechanisms. Typically initiated upon ligand binding, the genomic actions of the ERα involve its direct association with DNA at estrogen-responsive elements and/or genome-wide tethering to DNA by other transcription factors, such as Activator protein 1 (AP1) and Specificity protein 1 (SP1) (20–27). Chromatin-engaged ERα then recruits the transcriptional machinery, including general transcription factors and RNA polymerase II and induces gene expression, a process tightly regulated by complex cyclical and coordinated interactions with coactivators and corepressors (28, 29). Although coactivators potentiate ERα-dependent transcription, ERα corepressors actively repress hormonal responses, in part by recruiting histone-modifying enzymes, competing with coactivators and interfering with ERα dimerization (30).

The ERα is long considered as the main oncogenic driver in hormone-sensitive breast cancers. However, amplifications affecting the *ERS1* gene are only observed in 3% of ERα-positive breast cancers, whereas mutations are rare (<1%) in primary breast cancers and almost exclusively limited to metastatic lesions (31–37). Interestingly, recent next generation sequencing of breast cancers has revealed that genomic events appear to occur at much higher rates in corepressors of the ERα in primary breast tumors, with each ERα corepressor-encoding gene being lost by hetero- or homozygous deletion in up to 13%–55% of ERα-positive breast tumors (Table 1) (38–40). Indeed, the disruption by mutation and/or deletion of ERα corepressors may be intimately linked to the development of hormone-dependent breast cancers (30, 41–43).

**Table 1.** Frequency of Genetic and Genomic Events Affecting ERα Corepressors in Hormone-Receptor-Positive Breast Cancers

| | | CNV | |
|---|---|---|---|
| | **Mutations (% Tumors)** | **Loss (% Tumors)** | **Gain (% Tumors)** |
| DDX54 (DP97) | 0.3 | 14.0 | 17.8 |
| FOXO1 (FKHR) | 0 | 42.1 | 7.6 |
| LCOR | 0.3 | 23.2 | 8.8 |
| NCOR1 | 5.1 | 56.7 | 5.2 |
| NCOR2 | 2.9 | 14.1 | 19.4 |
| NEDD8 | 0.2 | 15.5 | 18.0 |
| NR0B1 (DAX1) | 0 | 16.3 | 13.6 |
| NR0B2 (SHP) | 0.2 | 40.7 | 3.0 |
| NR2C1 (TR2) | 0.2 | 12.0 | 20.2 |
| NR2F2 (COUP-TFII) | 0 | 21.2 | 13.8 |
| NRIP1 (RIP140) | 0.8 | 19.0 | 14.1 |
| PFN1 | 0 | 56.4 | 5.9 |
| PHB2 (REA) | 0.2 | 13.1 | 19.2 |
| RBFOX2 (RTA) | 0.5 | 48.7 | 7.2 |
| SAFB1 | 0.5 | 21.0 | 13.6 |
| SLIRP | 0.2 | 25.9 | 13.1 |
| SMAD4 | 1.0 | 31.3 | 11.1 |
| SPEN | 4.9 | 39.1 | 3.0 |
| TMEM54 (CAC1) | 0.2 | 33.0 | 6.1 |

Prevalence of mutation and copy number variations (CNV) affecting genes coding for ERα corepressors in a cohort of 594 ERα-positive breast cancers from the TCGA (Cell 2015) dataset. Data for each genes was extracted on December 21, 2015 from the cBioportal platform. Shallow deletions and deep deletions were considered as copy number loss, whereas gains and amplifications were considered as copy number gains. DEAD (Asp-Glu-Ala-Asp); box polypeptide 54 (DDX54); ATP-dependent RNA helicase (DP97); Forkhead Transcription Factor (FKHR); Ligand Dependent Nuclear Receptor Corepressor (LCOR); Neural Precursor Cell Expressed, Developmentally Down-Regulated 8 (NEDD8); Nuclear Receptor Subfamily 0, Group B, Member 1 (NR0B1); Dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1 (DAX1); Nuclear Receptor Subfamily 0, Group B, Member 2 (NR0B2); Small Heterodimer Partner (SHP); Nuclear Receptor Subfamily 2, Group C, Member 1 (NR2C1); Testicular Receptor 2 (TR2); Repressor of ER activity (REA); RNA-Binding Protein, Fox-1 Homolog 2 (RBFOX2); Repressor of tamoxifen action (RTA); SRA stem-loop interacting RNA binding protein (SLIRP); SMAD homolog 4 (SMAD4); Transmembrane Protein 54 (TMEM54); CDK-associated Cullin 1 (CAC1).

The expression of the ERα in breast cancer also represents an important therapeutic target for the treatment of hormone-dependent tumors. Drugs antagonizing the ERα in breast cancer in clinical use include tamoxifen, an antiestrogen with mixed agonist and antagonist activities, fulvestrant and aromatase inhibitors. Tamoxifen functions by competing with estrogen for the ligand-binding domain of ERα and induces a conformational change favoring ERα's interaction with corepressors (44, 45). Aided by transcriptional corepressors, tamoxifen represses ERα-mediated transcription, induces cell cycle arrest and leads to cell death (46–48). Although tamoxifen therapy has shown great efficacy in the prevention and treatment of breast cancer, a large proportion (>45%) of patients with advanced breast cancer on tamoxifen relapse during treatment due to adaptive mechanisms often involving the ERα, in which cancer cells become increasingly sensitive to the agonistic effects of tamoxifen and estradiol, or the activation of alternate growth factor pathways (49–54). Some examples of known mechanisms of tamoxifen resistance include an increase in the nongenomic actions of the ERα through cross talk with membrane-associated receptor tyrosine kinases, de novo production of local estrogen, and down-regulation of transcriptional corepressors (49, 54–57).

Due to compelling evidence for the role of ERα corepressors in modulating responses to antiestrogen therapies in both experimental and clinical settings, it is likely that the genomic aberrations involving the ERα corepressors affect such responses (58–60). In this review, we discuss the biological functions of ERα corepressors in the context of tamoxifen resistance to highlight their promising prognostic and predictive value in the clinical management of ERα-positive breast cancers and provide clues to the mechanism underlying their modulation of tamoxifen response.

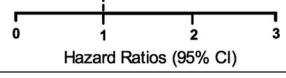
## ERα Corepressors

Over the last 2 decades, a total of 19 transcriptional corepressors of the ERα have been identified and characterized, 12 of which have never been studied in the context of endocrine sensitivity, whereas 7 were shown to affect breast cancer cells' responses to antiestrogens in various biological systems (Table 2) (43, 61–78). Of those, 3 have still poorly defined and ambiguous roles in tamoxifen response (Forkhead box protein O1 [FOXO1], nuclear receptor-interacting protein 1 [NRIP1], and Prohibitin-2 [PHB2]), whereas 4 were shown to potentiate the antitumorigenic effects of tamoxifen in in vitro models (nuclear corepressor [NCOR]1, NCOR2, Nuclear Receptor Subfamily 2, Group F, Member 2 [NR2F2], and split ends



**Table 2.** ERα Corepressors Affect Tamoxifen Sensitivity in Breast Cancer

| | Hazard Ratio RFS (95% CI) *P* Value | Experimentally Observed | Clinically Observed |
|---|---|---|---|
| DDX54 (DP97) | **0.70** (0.45–1.07) *0.095* | | |
| FOXO1 (FKHR) | **0.57** (0.34–0.95) *0.028** | Conflicting results (61, 62) | |
| LCOR | N/A | | |
| NCOR1 | **0.43** (0.26–0.72) *0.00091** | Yes (63, 64) | Yes (65–67) |
| NCOR2 | **0.61** (0.39–0.95) 0.029* | Yes (63, 69) | No (68, 70) |
| NEDD8 | **1.44** (0.95–2.19) *0.088* | | |
| NR0B1 (DAX1) | **1.46** (0.94–2.24) *0.087* | | |
| NR0B2 (SHP) | **0.57** (0.33–0.97) *0.035** | | |
| NR2C1 (TR2) | **1.51** (0.85–2.03) *0.064* | | |
| NR2F2 (COUP-TFII) | **0,60** (0.39–0.93) *0.29* | Yes (71–73) | |
| NRIP1 (RIP140) | **1.34** (0.85–2.12) *0.18* | Conflicting results (68, 74) | No (68) |
| PFN1 | **1.66** (1.08–2.55) *0.019* | | |
| PHB2 (REA) | **1.46** (0.95–2.26) *0.085* | Conflicting results (75–77) | |
| RBFOX2 (RTA) | **0,78** (0.50–1.22) *0.27* | | |
| SAFB1 | **0,70** (0.44–1.11) *0.12* | | No (78) |
| SLIRP | **1.61** (1.05–2.46) *0.028* | | |
| SMAD4 | **0.57** (0.37–0.87) *0.008** | | |
| SPEN | **0.55** (0.36–0.84) *0.005** | Yes (43) | Yes (43) |
| TMEM54 (CAC1) | N/A | | |




Relapse-free survival HRs for each ERα corepressor in a cohort of 424 ERα-positive breast cancer patients (2012 release version) of the luminal A subtype (when identified as such) and treated with tamoxifen alone. The predictive value of each ERα corepressor in tamoxifen-treated breast cancer patients was assessed using an online survival analysis software available at www.kmplot.com. In bold is the hazard ratio, in parenthesis is the 95% confidence interval and in italic is the associated p-value. The average of all probe sets per gene was used for the analysis, and the best cutoff was chosen for each gene. HR marked with an asterisk indicate that high expression of the corresponding ERα corepressors significantly predicts good relapse-free survival ($P < .05$) in tamoxifen-treated ERα-positive breast cancer patients. The last 2 columns indicate whether the expression of the corresponding ERα corepressors has been experimentally or clinically shown to confer tamoxifen sensitivity in breast cancer. N/A indicates that data was not available.

[SPEN]). Providing evidence that ERα corepressors biologically impact the action of tamoxifen in breast cancer, these observations suggest that understanding how the ERα is being modulated by corepressors in tamoxifen-treated cells might help uncover new mechanisms of endocrine resistance and potentially new therapeutic targets in hormone-dependent cancers.

## Nuclear Corepressor 1

The *NCOR1* (NCoR) is one of the first transcriptional corepressor to be identified and also represents the most frequently mutated and genomically altered transcriptional ERα corepressor in breast cancer. With 5% of tumors harboring a somatically acquired mutation in *NCOR1* and more than 55% of hormone-dependent breast carcinomas exhibiting copy number loss at the *NCOR1* locus according to the latest data in cBioPortal, it is expected that most breast cancers display genetic inactivation of *NCOR1* (39, 40). Associated with a generally poor prognosis, low protein levels of NCoR have been shown to confer tamoxifen resistance in in vitro and in vivo experiments and to predict lack of therapeutic response to tamoxifen in a number of clinical studies (66, 67). In vitro, reduced NCoR levels relieve the inhibition of *MYC*, *CCND1*, and *SDF1* gene transcription and result in tamoxifen behaving as a partial agonist for cell cycle progression (63). Furthermore, decreased NCoR protein expression levels correlate with the development of tamoxifen resistance in a xenograft mouse model of breast cancer (64). Clinically, lower *NCOR1* mRNA and protein expression levels are associated with the acquisition of tamoxifen resistance and shorter relapse-free survival in several cohorts of hormone-dependent breast cancer patients treated with tamoxifen (66, 67).

NCoR is a large protein of 270 kDa that interacts with unliganded or tamoxifen-bound ERα. It regulates chromatin accessibility by recruiting and activating histone deacetylase (HDAC)3, which leads to histone deacetylation, chromatin condensation and the loss of RNA polymerase II from DNA-engaged ERα complexes (Figure 1A) (79–81). Cumulative evidence also demonstrates an interaction between NCoR and HDAC4–HDAC7, although only a small fraction of endogenous NCoR appears to be associated with these class II HDACs (82, 83). NCoR also represses gene expression through its inhibition of histone acetyltransferases (HATs), including the coactivating and HAT enzyme, cAMP response element-binding protein, which further favors the establishment of a compacted heterochromatin structure (84).

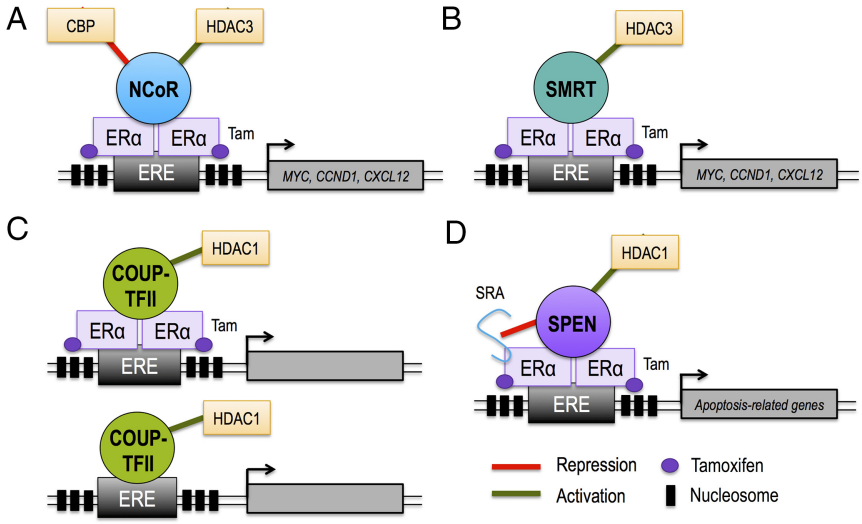



**Figure 1.** Mechanisms of transcriptional repression by ERα corepressors that confer tamoxifen sensitivity. A and B, NCoR (A) and SMRT (B) mediate transcriptional repression of the ERα by recruiting HDAC3 to the ER complex. NCoR (A) also represses hormonal responses by inhibiting cAMP response element-binding protein function. NCoR (A) and SMRT (B) confer tamoxifen sensitivity in part by interacting with tamoxifen-bound ERα and repressing the expression of ERα-target genes, including *MYC*, *CCND1*, and *CXCL12*. C, COUP-TFII attenuates hormone-dependent signaling by interacting with DNA at sites recognized by the ERα and by recruiting HDAC1. D, SPEN interacts with liganded and unliganded ERα and modulates hormonal responses by interacting with HDAC1 and repressing the activity of Steroid Receptor RNA Activator (SRA). SPEN-mediated repression of the ERα also up-regulates the expression of apoptosis-related genes.

## Nuclear Corepressor 2

*NCOR2* also referred to as the silencing mediator for retinoid or thyroid hormone receptors (SMRTs) shows 41% amino acid sequence similarity with NCoR. Despite the high homology and shared mechanisms of actions between NCoR and SMRT, the frequency of genetic events to which each gene is subjected is very different. Indeed, *NCOR2* is much less commonly inactivated by mutation or chromosomal aberrations than *NCOR1*, with mutations and deletions occurring in 3% and 14% of ERα-positive breast cancers, respectively (39, 40). Functionally, SMRT and NCoR both actively repress the ERα through mechanisms involving interaction with the ligand binding domain of the receptor and depending

on HDAC3 histone decacetylase activity (Figure 1B) (79, 85). Like NCoR, SMRT is recruited to tamoxifen-bound ERα and has been shown to play a role in tamoxifen response. Indeed, in vitro assays conducted with SMRT demonstrated that its overexpression confers tamoxifen sensitivity, whereas its silencing stimulates cell cycle progression in tamoxifen-treated MCF-7 cells (63, 69). Although established in vitro, a clinical role for SMRT protein expression in tamoxifen resistance could not be validated clinically in a cohort of 330 breast cancer patients treated with tamoxifen (70). SMRT RNA expression levels did, however, predict relapse-free survival (hazard ratio [HR] = 0.61; $P = .029$) in an independent cohort of 424 ERα-positive and luminal A breast cancer patients treated with tamoxifen alone (86).

## NR2F2

The chicken ovalbumin upstream promoter transcription factor 2 (COUP-TFII) is encoded by the *NR2F2* gene, which is infrequently mutated in breast cancer but displays copy number loss in 21% of ERα-positive breast tumors. COUP-TFII is an orphan member of the nuclear receptor superfamily whose transcriptional repressive functions towards the ERα are still poorly defined. A proposed mechanism of gene repression involves the direct interaction of COUP-TFII with DNA at sites containing 5′-AGGTCA-3′ repeats, which encompass the palindromic DNA sequence recognized by dimerized ERα (71). The binding of COUP-TFII at these genomic sites allows it to act as a platform for the recruitment of other corepressors, such as NCoR and SMRT in addition to preventing ERα engagement with chromatin and the subsequent recruitment of the transcriptional machinery (87). In addition, coimmunoprecipitation studies revealed an interaction between COUP-TFII and HDAC1, suggesting that it may also epigenetically repress gene transcription when recruited to DNA-bound ERα transcriptional complexes (88). Another mechanism by which COUP-TFII has been proposed to mediate transcriptional repression is through a direct interaction with the ERα and interference with the receptor's genomic activities (Figure 1C) (71). Interestingly, COUP-TFII's association with the ERα appears to be enhanced after tamoxifen treatment, whereas abrogation of this interaction by COUP-TFII knockdown has been shown to stimulate proliferation in tamoxifen-treated MCF-7 cells. Reciprocal experiments further consolidated a role for COUP-TFII in endocrine response, whereby COUP-TFII overexpression increased the antiproliferative effects of tamoxifen in MCF-7 cells (73). Of note, treatment with fulvestrant, a drug that induces ERα degradation, mirrored these effects, suggesting that COUP-TFII may confer sensitivity to antiestrogens through mechanisms that may involve direct or indirect interaction with the ERα (73). Importantly, consistent with COUP-TFII influencing antiestrogen sensitivity, lower levels of the protein were measured in tamoxifen-resistant human breast cancer cell lines, although no clinical evidence for its expression having prognostic value has yet been reported (73).

## Split Ends

SPEN, also known as SMRT/HDAC1-associated repressor protein (*SHARP*), is located on the Ch1p36 locus and is inactivated by mutation and/or loss of heterozygosity in 4% and 39% of hormone-sensitive breast tumors, respectively, in the latest cBioPortal (39, 40). It encodes a large protein of 402 kDa able to integrate transcriptional activation and repression. Recently, we have characterized SPEN functions in hormone-receptor positive tumors and identified SPEN as an ERα corepressor owing to its capacity to repress the transcription of ERα target genes, including the expression of the progesterone receptor and apoptosis-related genes (43). It has been suggested that SPEN-mediated transrepression is achieved in part by its Spen paralog and ortholog C-terminal domain, which allows it to scaffold a number of ERα corepressors, such as NCoR and SMRT, as well as histone modifying enzymes, including HDAC1 (Figure 1D) (89, 90). Other structural domains that may be important for SPEN transcriptional repression of the ERα include its 4 N-terminal RNA recognition motifs, which allow it to sequester and inactivate the RNA molecule and ERα coactivator, Steroid Receptor RNA Activator (SRA) (89, 91–94). Interestingly, SPEN's ability to interact with both corepressors through its Spen paralog and ortholog C-terminal domain and coactivators via its RNA recognition motifs may explain its unique capacity to bind and repress both liganded and unliganded ERα (43, 89). This was underlined by coimmunoprecipitation studies, revealing that SPEN associates with the ERα in a ligand-independent manner although this interaction was enhanced in hormone-free or tamoxifen-treated conditions (43). In light of these observations, our group has addressed the effect of SPEN on tamoxifen response and found that its reexpression in T47D cells in which SPEN is inactivated by a nonsense mutation conferred tamoxifen sensitivity by increasing tamoxifen-induced cell death. These results could not be replicated with fulvestrant, a pure ERα antagonist, suggesting that SPEN's interaction with the ERα is necessary to predispose cells to apoptosis after treatment with ta-

moxifen in breast cancer (43). Importantly, both SPEN protein and RNA levels were predictive of relapse-free survival in breast cancer patients treated with tamoxifen alone, providing further evidence that SPEN has important biological roles in tamoxifen sensitivity (43, 86).

Although NCoR, SMRT, COUP-TFII, and SPEN are ERα corepressors that were shown to biologically impact cellular responses to tamoxifen, NRIP1 and SAFB1 are 2 examples of ERα corepressors whose expression levels do not impact tamoxifen sensitivity. Although they may be considered of lesser importance, understanding how they transcriptionally repress the ERα may allow us to better define the molecular mechanisms responsible for endocrine resistance emerging from the loss of NCoR, SMRT, COUP-TFII, and SPEN in breast cancer.

## Nuclear Receptor-Interacting Protein 1

NRIP1, also known as the receptor-interacting protein 140 (RIP140), is encoded by the *NRIP1* gene, which is mutated in close to 1% of breast cancers and exhibits copy number loss in 9% of ERα-positive breast carcinomas (39, 40). Unlike most ERα corepressors, NRIP1 or RIP140 uniquely and exclusively associates with estrogen- or ligand-bound ERα and has mixed coregulatory functions (Figure 2A). Indeed, it was observed that RIP140 reduces ERα-dependent transcription in reporter systems (95–97). However, the work of Rosell et al (74 ) recently provided evidence that RIP140 is required for ERα transcriptional complex formation as well as ERα-dependent gene expression, suggesting that RIP140 may act as a coactivator for some genes and as a corepressor for others (74, 95, 98). It has been proposed that the corepressive functions of RIP140 are achieved in part via an interaction with the C-terminal-binding protein 1, which also functions as a transcriptional corepressor (95). In addition, colocalization and glutathione S-transferase-pulldown experiments demonstrated that RIP140 associates with class I and II HDACs, including HDAC1, HDAC4–HDAC7, and HDAC9–HDAC11, suggesting that it may also repress gene transcription through epigenetic mechanisms (99). However, treatment with trichostatin A, an HDAC inhibitor (HDACi), did not reverse the repressive effects of RIP140 on ERα activities, suggesting that its ability to prevent ERα-dependent gene transcription is not dependent on HDAC enzymatic actions (98). Further studies are therefore required to better define and understand the molecular mechanisms underlying the mixed coregulatory functions of RIP140 with respect to the ERα. RIP140's ability to act both as a coactivator and a corepressor may nevertheless provide an explanation for the conflicting in vitro results obtained with RIP140 and tamoxifen. Indeed, it was reported that *NRIP1* knockdown using small-interfering RNAs does not affect MCF-7 cells' sensitivity to tamoxifen or fulvestrant (74). However, another study demonstrated that RIP140 is significantly less expressed in tamoxifen-resistant compared with parental MCF-7 cells (68). Although RIP140's contribution to endocrine resistance remains ambiguous in vitro due to conflicting results, the assessment of RIP140's clinical significance revealed no change in *NRIP1* RNA levels in tumors from tamoxifen-treated compared with untreated breast cancer patients, suggesting that RIP140 has limited impact on tamoxifen sensitivity (68). Consistent with this data, *NRIP1* RNA expression levels had no predictive value when looking at relapse-free survival in a cohort of 424 breast cancer patients with luminal A tumors and treated with tamoxifen (86).

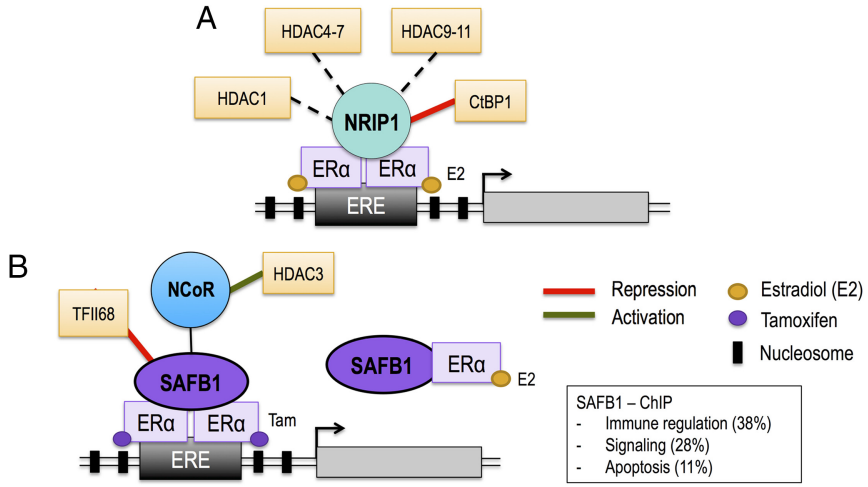



**Figure 2.** Mechanisms of transcriptional repression by ER corepressors that do not affect tamoxifen response. A, NRIP1 mediates transcriptional repression of the ERα by binding liganded-bound ERα. It also represses C-terminal-binding protein 1 (CtBP1) and interacts with numerous HDACs, but it has been shown that its transcriptional repressive actions are not dependent on histone deacetylation (indicated by the dashed line). B, SAFB1 inhibits ERα-dependent gene transcription, including genes involved in immune regulation, signaling and apoptosis by interacting directly with liganded and unliganded receptors. It also inhibits the actions of TF1168 and binds NCoR, which recruits and activates HDAC3. However, histone deacetylation is not required for SAFB1-mediated transcriptional repression of the ERα.

## Scaffold Attachment Factor B1 (SAFB1)

SAFB1 is encoded by the *SAFB1* gene, which is rarely mutated in breast tumors and displays inactivation by loss of heterozygosity in

21% of ERα-positive breast cancers (39, 40). SAFB1 is a large multifunctional protein implicated in numerous processes, including chromatin organization, transcriptional regulation as well as RNA splicing and that has been identified as an ERα corepressor (Figure 2B) (42, 100). Indeed, it was reported that SAFB1 overexpression in cultured cells reduces ERα-mediated transcription, whereas its deletion in a mouse model increases ERα activity in vivo (100, 101). Hammerich-Hill et al expanded on these results, finding that SAFB1 loss causes derepression of estrogen-regulated genes, including known regulators of immunity, signaling and apoptosis (102). Based on structure-function analyses, it has also been shown that SAFB1 mediates transcriptional repression through its C-terminal domain, which is responsible for its association with the TATA element-binding protein-associated factor (TAFII68) (103). TAFII68, also known as RBP56 or TAF15, is a protein that has previously been shown to interact with the general transcription factor IID as well as RNA polymerase II (104, 105). Interestingly, abrogation of SAFB1's association with TAFII68 by deletion of its C-terminal domain resulted in loss of transcriptional repression, suggesting that SAFB1 may function, at least in part, by sequestering TAFII68 and preventing its recruitment of the basal transcription machinery. Other than TAFII68, the C-terminal domain of SAFB1 also mediates interaction with the NCoR/HDAC3 corepressive complex (106). Actually, it was demonstrated that SAFB1 interacts directly with NCoR and coimmunoprecipitates with HDAC3, although no direct association between SAFB1 and HDACs was detected. Of note, treatment with HDACis and the siRNA-mediated knockdown of *NCOR1* or *HDAC3* both partially relieved SAFB1 transcriptional repressive effects, suggesting that SAFB1-mediated attenuation of ERα transcriptional activity is partly dependent on histone deacetylation (106). It was also reported that SAFB1 has the ability to decrease the mobility of liganded ERα and to sequester estrogen-bound receptors to the nuclear matrix, providing an additional mechanism by which it may modulate ERα's genomic actions (107). Although SAFB1 can interact with the ERα in the presence or absence of estrogen, it appears that its interaction with the ERα is significantly enhanced in the presence of antiestrogens, which may be suggestive of a role in tamoxifen resistance (100). However, a possible contribution for SAFB1 in endocrine sensitivity has not been reported in vitro thus far. Further studies are therefore required to address more thoroughly SAFB1's role in tamoxifen resistance even though clinical data from 2 independent cohorts suggest that its expression is not predictive of clinical outcomes in patients treated with tamoxifen-based regimens (78, 86).

Of note, all nuclear receptor corepressors under study are posttranslationally modified and a large body of evidence suggest that these modifications can alter protein functions and localization. However, the literature is very limited when it comes to evaluating the role of posttranslational modifications on the functions of ERα corepressors in drug response. In addition, there is no evidence to support a role for nuclear receptor corepressors in the nongenomic actions of the ERα nor in the recruitment of histone methyltransferases or demethylases to DNA, and thus these fields of investigation remain wide open.

However, from the analysis of the molecular mechanisms underlying the transcriptional repressive functions of NCoR, SMRT, COUP-TFII, SPEN, NRIP1, and SAFB1, which differently impact tamoxifen sensitivity, we can speculate about possible biological processes affecting antiestrogen responses in hormone-receptor positive breast cancers. Very interestingly, it appears that NCoR, SMRT, COUP-TFII, and SPEN all interact with HDAC enzymes and largely mediate transrepression by altering chromatin structure and promoter accessibility. Although it should not be excluded that the aforementioned ERα corepressors may confer tamoxifen sensitivity through totally independent modes of action, the fact that they all rely, at least in part, on HDACs for gene repression is noteworthy and deserves further investigation. Of particular interest is the fact that this epigenetic-based mechanism of repression is not shared with NRIP1 and SAFB1, whose expression levels have not been shown to have predictive value in tamoxifen-treated breast cancer patients. Indeed, although NRIP1 does associate with HDACs, it has become clear from the work of Castet et al (108) that gene repression by the full length NRIP1, which exclusively interacts with estrogen-bound ERα, is not dependent on histone deacetylation. Similarly, although SAFB1-mediated transrepression is partly dependent on HDAC enzyme activity, no direct association between SAFB1 and HDACs exists. Rather, SAFB1 interacts directly with NCoR, which recruits and activates HDAC3. Importantly, this interaction appears to be required for SAFB1-mediated attenuation of ERα genomic activities as *NCOR1* or *HDAC3* knockdown both equally relieved transrepression achieved by SAFB1. Hence, SAFB1's dependency on the NCoR/HDAC3 complex for gene repression may explain why NCoR but not SAFB1 expression predicts clinical outcomes in tamoxifen-treated breast cancer patients.

Decreased histone acetylation has long been recognized to mediate prolonged attenuation of hormonal responses (109). Histone acetylation is a reversible epigenetic modification that governs gene transcription by altering chromatin structure and promoter accessibility.

The acetylation state of histones is influenced by the competing enzymatic activity of HATs and HDACs, which are generally recruited to DNA by transcriptional coactivators and corepressors, respectively. Dysregulation of HATs or HDACs enzymes impairs the homeostatic balance existing between histone acetylation and deacetylation and accelerates tumorigenesis. Since the early 2000s, our understanding that epigenetic changes, such as those arising from the abnormal recruitment of HDAC enzymes to DNA, greatly affect gene expression and contribute to tumorigenesis has provided a rationale for the use of HDACis as anticancer therapies (110).

HDACis are a class of drugs with broad antiproliferative and proapoptotic effects in breast cancer that antagonize the activity of HDACs. After treatment with HDACi, histones become more acetylated and have less affinity for the negatively charged DNA backbone, thereby facilitating the recruitment of transcription factors and other components of the transcriptional machinery at gene promoters (110). Although it is widely accepted that hyperacetylated and hypoacetylated histones are associated with gene transcription and repression, respectively, recent findings indicate that pharmacological inhibition of HDAC does not necessarily translate into increased gene transcription and protein synthesis. In fact, it appears that HDACi also lead to the acetylation of nonhistone proteins, such as DNA-binding proteins, transcription factors and heat shock proteins. Such posttranslational modifications have been shown to greatly alter protein stability and functions and to have important consequences on a multitude of cellular processes. Indeed, with more than 50 nonhistone targets, HDACi were shown to affect a wide range of biological responses, including gene transcription, cell-cycle progression, autophagy as well as migration and to predispose cancer cells to apoptosis (110).

The recruitment of HDACs to DNA is a mechanism of transcriptional repression shared by NCoR, SMRT, COUP-TFII, and SPEN. The loss of any of the latter, due to genetic and/or genomic events, is predicted to impede the recruitment of HDACs to ERα-target genes and alter the epigenetic equilibrium existing between histone acetylation and deacetylation. Although this hypothesis needs to be verified by future studies, we propose that owing to a homeostatic disequilibrium, the loss of NCoR, SMRT, COUP-TFII, or SPEN may predispose cells to the antitumorigenic effects of HDACi. Although the role of transcriptional corepressors in HDACi sensitivity remains to be fully defined, numerous in vitro and in vivo studies have already assessed the efficacy of HDACi in tamoxifen-resistant breast cancer cells and promising results were reported. Indeed, it was showed that suberoylanilide hydroxamic acid, an HDACi, induces $G_2$/M cell cycle arrest in vitro and potently inhibits the growth of tamoxifen-resistant MCF-7 cells in vivo (111). More recently, Raha et al demonstrated that HDACi reverse B-cell lymphoma 2 (Bcl2) overexpression in tamoxifen-resistant MCF-7 and T47D cells and restore drug sensitivity by causing cell cycle arrest and predisposing cells to apoptosis (112). Underlined by many groups, the concept that HDACi are therapeutic agents able to effectively reverse tamoxifen resistance in breast cancer was examined in a phase II study of vorinostat combined with tamoxifen in patients with endocrine-resistant breast cancers (113–116). Although lacking an appropriate control cohort, this study demonstrated that the combination of vorinostat and tamoxifen in patients that had progressed on tamoxifen therapy resulted in interesting clinical responses, with 12% and 20% of patients displaying partial response and stable disease for more than 24 months, respectively (115). In both experimental and clinical settings, HDACi were well tolerated and very limited adverse side effects were reported. Altogether, this data establishes the use of HDACi as a promising therapeutic strategy in breast cancer that should be further explored for the treatment of tamoxifen-resistant breast cancers (Figure 3) and specifically for ERα corepressor-deficient breast cancers. Thus, NCoR, SMRT, COUP-TFII, and SPEN, which are to-

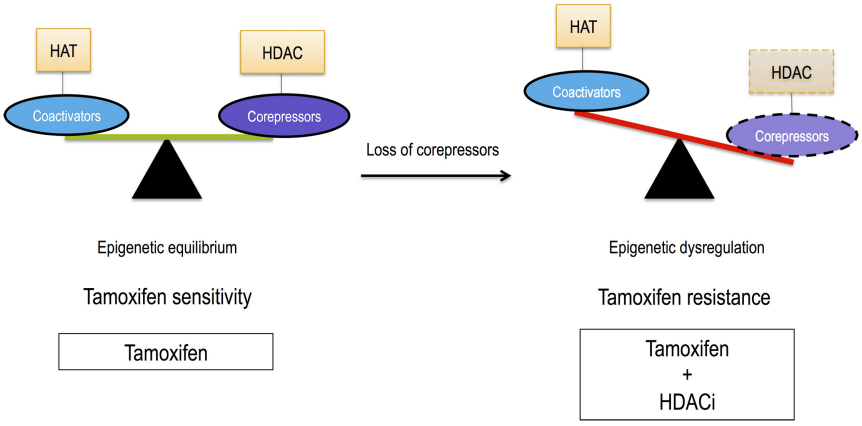



**Figure 3.** Loss of ERα corepressors disrupts the epigenetic equilibrium. The left panel shows that coactivators and corepressors, which recruit HATs and HDACs to DNA, respectively, establish an epigenetic equilibrium between histone acetylation and deacetylation, which is predicted to confer tamoxifen sensitivity. However, the loss of ERα corepressors due to genetic and/or genomic events may disrupt this homeostatic balance (right panel), favoring the acetylation of histones and nonhistone proteins. Thus the epigenetic dysregulation arising from the loss of transcriptional corepressors may predispose cancer cells to the cytotoxic effects of HDACis

gether mutated in 13% of hormone-receptor positive tumors and lost by deletion in more than 70% of ERα-positive breast cancers, may be revealed as candidate biomarkers of HDACi activity in tamoxifen-resistant tumors.

## Conclusion

In the present review, the mechanisms employed by ERα corepressors to attenuate hormonal responses was analyzed to identify biological processes that may confer tamoxifen resistance in breast cancer and expose potentially effective therapeutic strategies to restore drug sensitivity. We noted a mechanism of transcriptional repression common to NCoR, SMRT, COUP-TFII, and SPEN, 4 ERα corepressors that were shown to confer tamoxifen sensitivity, and that involves the recruitment of HDACs to DNA. This mechanism of gene repression is not shared with NRIP1 and SAFB1, whose expression levels do not affect tamoxifen response. It is therefore suggested that histone deacetylation may potentiate tamoxifen actions in breast cancer, whereas the abnormal recruitment of HDACs to tamoxifen-bound ERα, as a consequence to the loss of corepressors, may result in drug resistance. We thus propose that the inactivation of ERα corepressors, as a marker of epigenetic disequilibrium, could predispose cancer cells to the antitumorigenic effects of HDACi and that genomic alterations in ERα corepressors are candidate biomarkers that may predict response to HDACi in tamoxifen-resistant breast cancers. Many groups have already reported reversal of tamoxifen resistance by HDAC inhibition in breast cancer, supporting the concept of evaluating HDACi as a strategy to restore drug sensitivity in ERα corepressor-deficient and tamoxifen-resistant breast cancer cells. Future preclinical and clinical studies evaluating the efficacy of HDACi at reversing tamoxifen resistance in corepressor-deficient breast cancer cells will facilitate our understanding of ERα corepressors' roles in this process. Although the present analysis was restricted to corepressors that had previously been studied in the context of tamoxifen response, more research into the role of ERα coregulators in endocrine response is required to strengthen the link between corepressors, HDACs and tamoxifen resistance. Of particular interest are corepressors whose mRNA expression levels were predictive of relapse-free survival in a cohort of 424 breast cancer patients treated with tamoxifen alone but for which no biological data currently exists (FOXO1, SMAD4, and profilin 1 [PFN1]) (Table 2) (86). In addition to consolidating our current knowledge on ERα corepressors, such studies would also expand our understanding of the mechanisms responsible for endocrine resistance in breast cancer and possibly other hormone-dependent cancers, such as prostate cancer in addition to identifying more clinically relevant ways to stratify patients and ultimately lead to new strategies to treat breast cancer patients with a poor prognosis.

## Acknowledgments

Address all correspondence and requests for reprints to: Mark Basik, MD, Department of Surgical Oncology and Experimental Medicine, McGill University, Lady Davis Institute for Medical Research, 3755 Chemin de la Côte Ste-Catherine, Montréal, Québec, Canada H3T 1E2. E-mail: markbasik@gmail.com.

This work was supported by a McGill Integrated Cancer Research Training Program studentship (FRN53888) and a doctoral award from the Fonds de Recherche du Québec–Santé (to S.L.) and by grants from the Cancer Research Society and the Fonds de recherche du Québec - Santé Réseau de Cancer Axe Cancer du Sein et Ovaire as well as a grant from the Quebec Breast Cancer Foundation (M.B.).

Disclosure Summary: The authors have nothing to disclose.

## References

1. **Bray F, Ren JS, Masuyer E, Ferlay J.** Global estimates of cancer prevalence for 27 sites in the adult population in 2008. *Int J Cancer*. 2013;132(5):1133–1145.
2. **Brisken C.** Progesterone signalling in breast cancer: a neglected hormone coming into the limelight. *Nat Rev Cancer*. 2013;13(6):385–396.
3. **Obr AE, Edwards DP.** The biology of progesterone receptor in the normal mammary gland and in breast cancer. *Mol Cell Endocrinol*. 2012;357(1–2):4–17.
4. **Chlebowski RT, Anderson GL.** Menopausal hormone therapy and cancer: changing clinical observations of target site specificity. *Steroids*. 2014;90:53–59
5. **Lanari C, Lamb CA, Fabris VT, et al.** The MPA mouse breast cancer model: evidence for a role of progesterone receptors in breast cancer. *Endocr Relat Cancer*. 2009;16(2):333–350.
6. **Apter D, Vihko R.** Early menarche, a risk factor for breast cancer, indicates early onset of ovulatory cycles. *J Clin Endocrinol Metab*. 1983;57(1):82–86.
7. **Clavel-Chapelon F, E3N Group.** Cumulative number of menstrual cycles and breast cancer risk: results from the E3N cohort study of French women. *Cancer Causes Control*. 2002;13(9):831–838.
8. **Collaborative Group on Hormonal Factors in Breast C.** Menarche, menopause, and breast cancer risk: individual participant meta-analysis, including 118 964 women with breast cancer from 117 epidemiological studies. *Lancet Oncol*. 2012;13(11):1141–1151.
9. **McEwen BS, Alves SE.** Estrogen actions in the central nervous system. *Endocr Rev*. 1999;20(3):279–307.
10. **Jordan VC.** Estrogen, selective estrogen receptor modulation, and coronary heart disease: something or nothing. *J Natl Cancer Inst*. 2001;93(1):2–4.
11. **Pati D, Haddad BR, Haegele A, et al.** Hormone-induced chromosomal instability in p53-null mammary epithelium. *Cancer Res*. 2004;64(16):5608–5616.
12. **Goepfert TM, McCarthy M, Kittrell FS, et al.** Progesterone facil-

itates chromosome instability (aneuploidy) in p53 null normal mammary epithelial cells. *FASEB J*. 2000;14(14):2221–2229.
13. **Perou CM, Sørlie T, Eisen MB, et al.** Molecular portraits of human breast tumours. *Nature*. 2000;406(6797):747–752.
14. **Osborne CK, Hobbs K, Clark GM.** Effect of estrogens and antiestrogens on growth of human breast cancer cells in athymic nude mice. *Cancer Res*. 1985;45(2):584–590.
15. **McGuire WL.** Hormone receptors: their role in predicting prognosis and response to endocrine therapy. *Semin Oncol*. 1978;5(4):428–433.
16. **Gougelet A, Mueller SO, Korach KS, Renoir JM.** Oestrogen receptors pathways to oestrogen responsive elements: the transactivation function-1 acts as the keystone of oestrogen receptor (ER)β-mediated transcriptional repression of ERα. *J Steroid Biochem Mol Biol*. 2007;104(3–5):110–122.
17. **Maehle BO, Collett K, Tretli S, Akslen LA, Grotmol T.** Estrogen receptor β–an independent prognostic marker in estrogen receptor α and progesterone receptor-positive breast cancer? *APMIS*. 2009;117(9):644–650.
18. **Nakopoulou L, Lazaris AC, Panayotopoulou EG, et al.** The favourable prognostic value of oestrogen receptor β immunohistochemical expression in breast cancer. *J Clin Pathol*. 2004;57(5):523–528.
19. **Speirs V, Parkes AT, Kerin MJ, et al.** Coexpression of estrogen receptor α and β: poor prognostic factors in human breast cancer? *Cancer Res*. 1999;59(3):525–528.
20. **Liao XH, Lu DL, Wang N, et al.** Estrogen receptor α mediates proliferation of breast cancer MCF-7 cells via a p21/PCNA/E2F1-dependent pathway. *FEBS J*. 2014;281(3):927–942.
21. **Doisneau-Sixou SF, Sergio CM, Carroll JS, Hui R, Musgrove EA, Sutherland RL.** Estrogen and antiestrogen regulation of cell cycle progression in breast cancer cells. *Endocr Relat Cancer*. 2003;10(2):179–186.
22. **Kalet BT, Anglin SR, Handschy A, et al.** Transcription factor Ets1 cooperates with estrogen receptor α to stimulate estradiol-dependent growth in breast cancer cells and tumors. *PLoS One*. 2013;8(7):e68815.
23. **Altucci L, Addeo R, Cicatiello L, et al.** 17β-estradiol induces cyclin D1 gene transcription, p36D1–p34cdk4 complex activation and p105Rb phosphorylation during mitogenic stimulation of G(1)-arrested human breast cancer cells. *Oncogene*. 1996;12(11):2315–2324.
24. **Stanculescu A, Bembinster LA, Borgen K, Bergamaschi A, Wiley E, Frasor J.** Estrogen promotes breast cancer cell survival in an inhibitor of apoptosis (IAP)-dependent manner. *Horm Cancer*. 2010;1(3):127–135.
25. **Kim K, Thu N, Saville B, Safe S.** Domains of estrogen receptor α (ERα) required for ERα/Sp1-mediated activation of GC-rich promoters by estrogens and antiestrogens in breast cancer cells. *Mol Endocrinol*. 2003;17(5):804–817.
26. **Scholz A, Truss M, Beato M.** Hormone-induced recruitment of Sp1 mediates estrogen activation of the rabbit uteroglobin gene in endometrial epithelium. *J Biol Chem*. 1998;273(8):4360–4366.
27. **Grober OM, Mutarelli M, Giurato G, et al.** Global analysis of estrogen receptor β binding to breast cancer cell genome reveals an extensive interplay with estrogen receptor α for target gene regulation. *BMC Genomics*. 2011;12:36.
28. **Métivier R, Penot G, Hübner MR, et al.** Estrogen receptor-α directs ordered, cyclical, and combinatorial recruitment of cofactors on a natural target promoter. *Cell*. 2003;115(6):751–763.
29. **Shang Y, Hu X, DiRenzo J, Lazar MA, Brown M.** Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. *Cell*. 2000;103(6):843–852.
30. **Dobrzycka KM, Townson SM, Jiang S, Oesterreich S.** Estrogen receptor corepressors – a role in human breast cancer? *Endocr Relat Cancer*. 2003;10(4):517–536.
31. **Robinson DR, Wu YM, Vats P, et al.** Activating ESR1 mutations in hormone-resistant metastatic breast cancer. *Nat Genet*. 2013;45(12):1446–1451.
32. **Toy W, Shen Y, Won H, et al.** ESR1 ligand-binding domain mutations in hormone-resistant breast cancer. *Nat Genet*. 2013;45(12):1439–1445.
33. **Sefrioui D, Perdrix A, Sarafan-Vasseur N, et al.** Short report: monitoring ESR1 mutations by circulating tumor DNA in aromatase inhibitor resistant metastatic breast cancer. *Int J Cancer*. 2015;137:2513–2519.
34. **Wang T, Liu JH, Zhang J, Wang L, Chen C, Dai PG.** A multiplex allele-specific real-time PCR assay for screening of ESR1 mutations in metastatic breast cancer. *Exp Mol Pathol*. 2015;98(2):152–157.
35. **Chen JR, Hsieh TY, Chen HY, et al.** Absence of estrogen receptor α (ESR1) gene amplification in a series of breast cancers in Taiwan. *Virchows Arch*. 2014;464(6):689–699.
36. **Tremblay GB, Tremblay A, Labrie F, Giguère V.** Ligand-independent activation of the estrogen receptors α and β by mutations of a conserved tyrosine can be abolished by antiestrogens. *Cancer Res*. 1998;58(5):877–881.
37. **Zhang QX, Borg A, Wolf DM, Oesterreich S, Fuqua SA.** An estrogen receptor mutant with strong hormone-independent activity from a metastatic breast cancer. *Cancer Res*. 1997;57(7):1244–1249.
38. **Forbes SA, Bindal N, Bamford S, et al.** COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. *Nucleic Acids Res*. 2011;39(Database issue):D945–D950.
39. **Cerami E, Gao J, Dogrusoz U, et al.** The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov*. 2012;2(5):401–404.
40. **Gao J, Aksoy BA, Dogrusoz U, et al.** Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal*. 2013;6(269):pl1.
41. **Martínez-Iglesias O, Alonso-Merino E, Aranda A.** Tumor suppressive actions of the nuclear receptor corepressor 1. *Pharmacol Res*. 2016;108:75–79.
42. **Oesterreich S.** Scaffold attachment factors SAFB1 and SAFB2: innocent bystanders or critical players in breast tumorigenesis? *J Cell Biochem*. 2003;90(4):653–661.
43. **Légaré S, Cavallone L, Mamo A, et al.** The estrogen receptor cofactor SPEN functions as a tumor suppressor and candidate biomarker of drug responsiveness in hormone-dependent breast cancers. *Cancer Res*. 2015;75(20):4351–4363.
44. **Heldring N, Nilsson M, Buehrer B, Treuter E, Gustafsson JA.** Identification of tamoxifen-induced coregulator interaction surfaces within the ligand-binding domain of estrogen receptors. *Mol Cell Biol*. 2004;24(8):3445–3459.
45. **Shiau AK, Barstad D, Loria PM, et al.** The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. *Cell*. 1998;95(7):927–937.
46. **Ichikawa A, Ando J, Suda K.** G1 arrest and expression of cyclin-dependent kinase inhibitors in tamoxifen-treated MCF-7 human breast cancer cells. *Hum Cell*. 2008;21(2):28–37.
47. **Jones JL, Daley BJ, Enderson BL, Zhou JR, Karlstad MD.** Genistein inhibits tamoxifen effects on cell proliferation and cell cycle arrest in T47D breast cancer cells. *Am Surg*. 2002;68(6):575–577; discussion 577–578.
48. **Varma H, Conrad SE.** Reversal of an antiestrogen-mediated cell cycle arrest of MCF-7 cells by viral tumor antigens requires the retinoblastoma protein-binding domain. *Oncogene*. 2000;19(41):4746–4753.
49. **García-Becerra R, Santos N, Díaz L, Camacho J.** Mechanisms of resistance to endocrine therapy in breast cancer: focus on signaling pathways, miRNAs and genetically based resistance. *Int J Mol Sci*. 2012;14(1):108–145.
50. **Cuzick J, Powles T, Veronesi U, et al.** Overview of the main out-

comes in breast-cancer prevention trials. *Lancet*. 2003;361(9354):296–300.
51. **Jordan VC.** Tamoxifen: a most unlikely pioneering medicine. *Nat Rev Drug Discov*. 2003;2(3):205–213.
52. **Mehta A, Tripathy D.** Co-targeting estrogen receptor and HER2 pathways in breast cancer. *Breast*. 2014;23(1):2–9.
53. **Osborne CK, Shou J, Massarweh S, Schiff R.** Crosstalk between estrogen receptor and growth factor receptor pathways as a cause for endocrine therapy resistance in breast cancer. *Clin Cancer Res*. 2005;11(2 pt 2):865s–870s.
54. **Shou J, Massarweh S, Osborne CK, et al.** Mechanisms of tamoxifen resistance: increased estrogen receptor-HER2/neu cross-talk in ER/HER2-positive breast cancer. *J Natl Cancer Inst*. 2004;96(12):926–935.
55. **Campbell RA, Bhat-Nakshatri P, Patel NM, Constantinidou D, Ali S, Nakshatri H.** Phosphatidylinositol 3-kinase/AKT-mediated activation of estrogen receptor $\alpha$: a new model for anti-estrogen resistance. *J Biol Chem*. 2001;276(13):9817–9824.
56. **Chung YL, Sheu ML, Yang SC, Lin CH, Yen SH.** Resistance to tamoxifen-induced apoptosis is associated with direct interaction between Her2/neu and cell membrane estrogen receptor in breast cancer. *Int J Cancer*. 2002;97(3):306–312.
57. **Ring A, Dowsett M.** Mechanisms of tamoxifen resistance. *Endocr Relat Cancer*. 2004;11(4):643–658.
58. **Feng Q, O'Malley BW.** Nuclear receptor modulation–role of coregulators in selective estrogen receptor modulator (SERM) actions. *Steroids*. 2014;90:39–43.
59. **Girault I, Bièche I, Lidereau R.** Role of estrogen receptor $\alpha$ transcriptional coregulators in tamoxifen resistance in breast cancer. *Maturitas*. 2006;54(4):342–351.
60. **Graham JD, Bain DL, Richer JK, Jackson TA, Tung L, Horwitz KB.** Thoughts on tamoxifen resistant breast cancer. Are coregulators the answer or just a red herring? *J Steroid Biochem Mol Biol*. 2000;74(5):255–259.
61. **Choi HK, Cho KB, Phuong NT, et al.** SIRT1-mediated FoxO1 deacetylation is essential for multidrug resistance-associated protein 2 expression in tamoxifen-resistant breast cancer cells. *Mol Pharm*. 2013;10(7):2517–2527.
62. **Silva J, Cavazos DA, Donzis E, Friedrichs WE, Marciniak R, de-Graffenried LA.** Akt-induced tamoxifen resistance is associated with altered FKHR regulation. *Cancer Invest*. 2007;25(7):569–573.
63. **Keeton EK, Brown M.** Cell cycle progression stimulated by tamoxifen-bound estrogen receptor-$\alpha$ and promoter-specific effects in breast cancer cells deficient in N-CoR and SMRT. *Mol Endocrinol*. 2005;19(6):1543–1554.
64. **Lavinsky RM, Jepsen K, Heinzel T, et al.** Diverse signaling pathways modulate nuclear receptor recruitment of N-CoR and SMRT complexes. *Proc Natl Acad Sci USA*. 1998;95(6):2920–2925.
65. **Tabarestani S, Ghaderian SM, Rezvani H, Mirfakhraie R.** Expression profiling of breast cancer patients treated with tamoxifen: prognostic or predictive significance. *Med Oncol*. 2014;31(4):896.
66. **Girault I, Lerebours F, Amarir S, et al.** Expression analysis of estrogen receptor $\alpha$ coregulators in breast carcinoma: evidence that NCOR1 expression is predictive of the response to tamoxifen. *Clin Cancer Res*. 2003;9(4):1259–1266.
67. **Zhang ZH, Yamashita H, Toyama T, et al.** Nuclear corepressor 1 expression predicts response to first-line endocrine therapy for breast cancer patients on relapse. *Chin Med J (Engl)*. 2009;122(15):1764–1768.
68. **Chan CM, Lykkesfeldt AE, Parker MG, Dowsett M.** Expression of nuclear receptor interacting proteins TIF-1, SUG-1, receptor interacting protein 140, and corepressor SMRT in tamoxifen-resistant breast cancer. *Clin Cancer Res*. 1999;5(11):3460–3467.
69. **Hong W, Chen L, Li J, Yao Z.** Inhibition of MAP kinase promotes the recruitment of corepressor SMRT by tamoxifen-bound estrogen receptor $\alpha$ and potentiates tamoxifen action in MCF-7 cells. *Biochem Biophys Res Commun*. 2010;396(2):299–303.
70. **Smith CL, Migliaccio I, Chaubal V, et al.** Elevated nuclear expression of the SMRT corepressor in breast cancer is associated with earlier tumor recurrence. *Breast Cancer Res Treat*. 2012;136(1):253–265.
71. **Klinge CM, Silver BF, Driscoll MD, Sathya G, Bambara RA, Hilf R.** Chicken ovalbumin upstream promoter-transcription factor interacts with estrogen receptor, binds to estrogen response elements and half-sites, and inhibits estrogen-induced gene expression. *J Biol Chem*. 1997;272(50):31465–31474.
72. **Litchfield LM, Appana SN, Datta S, Klinge CM.** COUP-TFII inhibits NF$\kappa$B activation in endocrine-resistant breast cancer cells. *Mol Cell Endocrinol*. 2014;382(1):358–367.
73. **Riggs KA, Wickramasinghe NS, Cochrum RK, Watts MB, Klinge CM.** Decreased chicken ovalbumin upstream promoter transcription factor II expression in tamoxifen-resistant breast cancer cells. *Cancer Res*. 2006;66(20):10188–10198.
74. **Rosell M, Nevedomskaya E, Stelloo S, et al.** Complex formation and function of estrogen receptor $\alpha$ in transcription requires RIP140. *Cancer Res*. 2014;74(19):5469–5479.
75. **Montano MM, Ekena K, Delage-Mourroux R, Chang W, Martini P, Katzenellenbogen BS.** An estrogen receptor-selective coregulator that potentiates the effectiveness of antiestrogens and represses the activity of estrogens. *Proc Natl Acad Sci USA*. 1999;96(12):6947–6952.
76. **Yoshimaru T, Komatsu M, Matsuo T, et al.** Targeting BIG3-PHB2 interaction to overcome tamoxifen resistance in breast cancer cells. *Nat Commun*. 2013;4:2443.
77. **Yoshimaru T, Komatsu M, Miyoshi Y, Honda J, Sasa M, Katagiri T.** Therapeutic advances in BIG3-PHB2 inhibition targeting the crosstalk between estrogen and growth factors in breast cancer. *Cancer Sci*. 2015;106(5):550–558.
78. **Hammerich-Hille S, Bardout VJ, Hilsenbeck SG, Osborne CK, Oesterreich S.** Low SAFB levels are associated with worse outcome in breast cancer patients. *Breast Cancer Res Treat*. 2010;121(2):503–509.
79. **Li J, Wang J, Wang J, et al.** Both corepressor proteins SMRT and N-CoR exist in large protein complexes containing HDAC3. *EMBO J*. 2000;19(16):4342–4350.
80. **Guenther MG, Barak O, Lazar MA.** The SMRT and N-CoR corepressors are activating cofactors for histone deacetylase 3. *Mol Cell Biol*. 2001;21(18):6091–6101.
81. **Heinzel T, Lavinsky RM, Mullen TM, et al.** A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression. *Nature*. 1997;387(6628):43–48.
82. **Huang EY, Zhang J, Miska EA, Guenther MG, Kouzarides T, Lazar MA.** Nuclear receptor corepressors partner with class II histone deacetylases in a Sin3-independent repression pathway. *Genes Dev*. 2000;14(1):45–54.
83. **Kao HY, Downes M, Ordentlich P, Evans RM.** Isolation of a novel histone deacetylase reveals that class I and class II deacetylases promote SMRT-mediated repression. *Genes Dev*. 2000;14(1):55–66.
84. **Cowger JJ, Torchia J.** Direct association between the CREB-binding protein (CBP) and nuclear receptor corepressor (N-CoR). *Biochemistry*. 2006;45(44):13150–13162.
85. **Yu J, Li Y, Ishizuka T, Guenther MG, Lazar MA.** A SANT motif in the SMRT corepressor interprets the histone code and promotes histone deacetylation. *EMBO J*. 2003;22(13):3403–3410.
86. **Győrffy B, Lanczky A, Eklund AC, et al.** An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. *Breast Cancer Res Treat*. 2010;123(3):725–731.
87. **Shibata H, Nawaz Z, Tsai SY, O'Malley BW, Tsai MJ.** Gene silencing by chicken ovalbumin upstream promoter-transcription factor I (COUP-TFI) is mediated by transcriptional corepressors,

nuclear receptor-corepressor (N-CoR) and silencing mediator for retinoic acid receptor and thyroid hormone receptor (SMRT). *Mol Endocrinol*. 1997;11(6):714–724.
88. **Smirnov DA, Hou S, Ricciardi RP.** Association of histone deacetylase with COUP-TF in tumorigenic Ad12-transformed cells and its potential role in shut-off of MHC class I transcription. *Virology*. 2000;268(2):319–328.
89. **Shi Y, Downes M, Xie W, et al.** Sharp, an inducible cofactor that integrates nuclear receptor repression and activation. *Genes Dev*. 2001;15(9):1140–1151.
90. **Mikami S, Kanaba T, Takizawa N, et al.** Structural insights into the recruitment of SMRT by the corepressor SHARP under phosphorylative regulation. *Structure*. 2014;22(1):35–46.
91. **Ariyoshi M, Schwabe JW.** A conserved structural motif reveals the essential transcriptional repression function of Spen proteins and their role in developmental signaling. *Genes Dev*. 2003;17(15):1909–1920.
92. **Mikami S, Kanaba T, Ito Y, Mishima M.** NMR assignments of SPOC domain of the human transcriptional corepressor SHARP in complex with a C-terminal SMRT peptide. *Biomol NMR Assign*. 2013;7(2):267–270.
93. **Chooniedass-Kothari S, Vincett D, Yan Y, et al.** The protein encoded by the functional steroid receptor RNA activator is a new modulator of ER α transcriptional activity. *FEBS Lett*. 2010;584(6):1174–1180.
94. **Arieti F, Gabus C, Tambalo M, Huet T, Round A, Thore S.** The crystal structure of the Split End protein SHARP adds a new layer of complexity to proteins containing RNA recognition motifs. *Nucleic Acids Res*. 2014;42(10):6742–6752.
95. **Augereau P, Badia E, Balaguer P, et al.** Negative regulation of hormone signaling by RIP140. *J Steroid Biochem Mol Biol*. 2006;102(1–5):51–59.
96. **Cavaillès V, Dauvois S, L'Horset F, et al.** Nuclear factor RIP140 modulates transcriptional activation by the estrogen receptor. *EMBO J*. 1995;14(15):3741–3751.
97. **Carroll JS, Meyer CA, Song J, et al.** Genome-wide analysis of estrogen receptor binding sites. *Nat Genet*. 2006;38(11):1289–1297.
98. **Castet A, Herledan A, Bonnet S, Jalaguier S, Vanacker JM, Cavaillès V.** Receptor-interacting protein 140 differentially regulates estrogen receptor-related receptor transactivation depending on target genes. *Mol Endocrinol*. 2006;20(5):1035–1047.
99. **Wei LN, Hu X, Chandra D, Seto E, Farooqui M.** Receptor-interacting protein 140 directly recruits histone deacetylases for gene silencing. *J Biol Chem*. 2000;275(52):40782–40787.
100. **Ivanova M, Dobrzycka KM, Jiang S, et al.** Scaffold attachment factor B1 functions in development, growth, and reproduction. *Mol Cell Biol*. 2005;25(8):2995–3006.
101. **Townson SM, Dobrzycka KM, Lee AV, et al.** SAFB2, a new scaffold attachment factor homolog and estrogen receptor corepressor. *J Biol Chem*. 2003;278(22):20059–20068.
102. **Hammerich-Hille S, Kaipparettu BA, Tsimelzon A, et al.** SAFB1 mediates repression of immune regulators and apoptotic genes in breast cancer cells. *J Biol Chem*. 2010;285(6):3608–3616.
103. **Townson SM, Kang K, Lee AV, Oesterreich S.** Structure-function analysis of the estrogen receptor α corepressor scaffold attachment factor-B1: identification of a potent transcriptional repression domain. *J Biol Chem*. 2004;279(25):26074–26081.
104. **Bertolotti A, Lutz Y, Heard DJ, Chambon P, Tora L.** hTAF(II)68, a novel RNA/ssDNA-binding protein with homology to the pro-oncoproteins TLS/FUS and EWS is associated with both TFIID and RNA polymerase II. *EMBO J*. 1996;15(18):5022–5031.
105. **Bertolotti A, Melot T, Acker J, Vigneron M, Delattre O, Tora L.** EWS, but not EWS-FLI-1, is associated with both TFIID and RNA polymerase II: interactions between two members of the TET family, EWS and hTAFII68, and subunits of TFIID and RNA polymerase II complexes. *Mol Cell Biol*. 1998;18(3):1489–1497.
106. **Jiang S, Meyer R, Kang K, Osborne CK, Wong J, Oesterreich S.** Scaffold attachment factor SAFB1 suppresses estrogen receptor α-mediated transcription in part via interaction with nuclear receptor corepressor. *Mol Endocrinol*. 2006;20(2):311–320.
107. **Hashimoto T, Matsuda K, Kawata M.** Scaffold attachment factor B (SAFB)1 and SAFB2 cooperatively inhibit the intranuclear mobility and function of ERα. *J Cell Biochem*. 2012;113(9):3039–3050.
108. **Castet A, Boulahtouf A, Versini G, et al.** Multiple domains of the receptor-interacting protein 140 contribute to transcription inhibition. *Nucleic Acids Res*. 2004;32(6):1957–1966.
109. **Chen H, Lin RJ, Xie W, Wilpitz D, Evans RM.** Regulation of hormone-induced histone hyperacetylation and gene activation via acetylation of an acetylase. *Cell*. 1999;98(5):675–686.
110. **Ververis K, Hiong A, Karagiannis TC, Licciardi PV.** Histone deacetylase inhibitors (HDACIs): multitargeted anticancer agents. *Biologics*. 2013;7:47–60.
111. **Lee YJ, Won AJ, Lee J, et al.** Molecular mechanism of SAHA on regulation of autophagic cell death in tamoxifen-resistant MCF-7 breast cancer cells. *Int J Med Sci*. 2012;9(10):881–893.
112. **Raha P, Thomas S, Thurn KT, Park J, Munster PN.** Combined histone deacetylase inhibition and tamoxifen induces apoptosis in tamoxifen-resistant breast cancer models, by reversing Bcl-2 overexpression. *Breast Cancer Res*. 2015;17:26.
113. **Park JH, Ahn MY, Kim TH, et al.** A new synthetic HDAC inhibitor, MHY218, induces apoptosis or autophagy-related cell death in tamoxifen-resistant MCF-7 breast cancer cells. *Invest New Drugs*. 2012;30(5):1887–1898.
114. **Restall C, Doherty J, Liu HB, et al.** A novel histone deacetylase inhibitor augments tamoxifen-mediated attenuation of breast carcinoma growth. *Int J Cancer*. 2009;125(2):483–487.
115. **Munster PN, Thurn KT, Thomas S, et al.** A phase II study of the histone deacetylase inhibitor vorinostat combined with tamoxifen for the treatment of patients with hormone therapy-resistant breast cancer. *Br J Cancer*. 2011;104(12):1828–1835.
116. **Thomas S, Thurn KT, Raha P, Chen S, Munster PN.** Efficacy of histone deacetylase and estrogen receptor inhibition in breast cancer cells due to concerted down regulation of Akt. *PLoS One*. 2013;8(7):e68973.