# The Clinical Relevance of Steroid Hormone Receptor Corepressors

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**Abstract** Steroid hormone receptors are ligand-dependent transcription factors that control a variety of essential physiologic and developmental processes in humans. The functional activity of a steroid receptor is regulated not only by hormones but also by an array of regulatory proteins such as coactivators, corepressors, and chromatin modifiers. Contrary to an earlier notion that corepressors and coactivators exist in separate complexes, these molecules, which have apparently opposite functions, are increasingly being found in the same complex, which allows for efficient transcriptional control mechanisms. These control mechanisms are in turn regulated by an array of post-translational modifications under the influence of upstream and local signaling networks. Because the outcome of steroidal hormone receptor transcriptional complexes is measured in terms of the expression of target genes, any dysregulation of coregulator complexes perturbs normal homeostasis and could contribute to the development and maintenance of malignant phenotypes. Increasing evidence implicating steroid hormone receptors and their coregulators in various pathophysiologic conditions has elicited interest in their structure and biology. Further advances in this field of study should open up a unique window for novel targeted therapies for diseases such as cancer. Here we briefly review the clinical relevance of corepressors, with a particular focus on their role in the development of cancerous phenotypes.

Steroid hormone receptors (also known as nuclear receptors) are ligand-dependent transcription factors that control a variety of essential physiologic and developmental processes in humans (1–3). Widely studied steroid hormone receptors include estrogen receptors, progesterone receptors, androgen receptors, and glucocorticoid receptors. The nuclear receptors primarily regulate the initiation of transcription by directly binding to specific DNA sequences in the regulatory region of target genes called hormone response elements and recruiting diverse ancillary factors characterized as coregulators along with the basal transcriptional machinery (4). For transcription factors to access DNA in the chromatin, the packaged chromatin structure needs to be partially unwound. The ligand-activated nuclear receptors are thought to mediate their transactivation functions by facilitating this regional loosening of the chromatin in the following manner. Ligand binding results in the dismissal of histone deacetylase–containing corepressor complexes and the concomitant recruitment of coactivator complexes. The coactivator complexes use their histone acetyltransferase activities to remodel the nucleosomal structures, thus opening up the chromatin and helping recruit the basal transcriptional machinery. In general, coregulators do not bind to DNA but are recruited to the target promoters via protein-protein interactions with the sequence-specific and context-dependent transcription factors (5). The relaxation and condensation of chromatin is tightly controlled by post-translational modifications of nuclear receptors, coregulators, and the nucleosomal histones themselves around the target promoter. Excellent reviews detailing the mode of functioning of nuclear receptors and the importance of various post-translational modifications in this event can be found elsewhere (6).

In recent years, it has been increasingly accepted that the diverse functions of nuclear receptors stem from differential recruitment or the interaction of coactivators and corepressors with the nuclear receptors, or both, combined with a variety of modifications such as phosphorylation, methylation, acetylation, and sumoylation under the influence of upstream signaling cascades of these molecules. In this context, it is believed that any perturbation in the balance of coregulators is likely to influence the expression of target genes and thus could participate in the development of disorders in an important manner. Because the transcriptional control of nuclear receptors continues to be an intensely investigated area, and because coactivators have been extensively reviewed elsewhere in the recent past (7, 8), this review concentrates on the clinical relevance of corepressors in the biology of steroid hormone receptors (Table 1). To place the corepressors in a larger context, in this review, we present the role of corepressors in various diseases with emphasis on cancers.

## Modulation of Coactivators by Corepressors

Although the components of coactivator and corepressor complexes have been identified, little is known about the

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processes that lead to the exchange of these complexes by transcription factors. The existing cofactor exchange models suggest that biochemically distinct coactivator and corepressor complexes are present in a preformed state and are recruited to the chromatin by nuclear receptors, depending on the activation state of the nuclear receptor. An interesting hypothesis that argues against this model has been put forward recently. It proposes that coactivators and corepressors are present in the same complex and the ligand-activated nuclear receptor just reorients the complex to elicit rapid transcriptional activation of the genes (9).

**Table 1.** Corepressors and the disorders due to their aberrant expression/function

| Corepressors | Clinical implications | References |
|---|---|---|
| NCoR and SMRT | Resistance to thyroid hormone syndrome | (22) |
| | Huntington's disease | (45) |
| | Acute promyelocytic leukemia | (60) |
| | Acute myeloid leukemia | (73) |
| | Hormone independence of breast cancers | (85) |
| PPARγ | Insulin resistance | (25) |
| SHP | Obesity | (37) |
| | Hormone independence of breast cancers | (87) |
| DAX1 | Adrenal hypoplasia congenita and dosage-sensitive sex reversal | (38, 42) |
| | Breast cancer | (115, 118) |
| | Adrenal and pituitary adenoma | (123) |
| | Prostate cancer | (128) |
| RIP140 | Female infertility | (43) |
| | Hormone independence of breast cancers | (88) |
| CtBP | Female infertility | (44) |
| | Huntington's disease | (48) |
| | Breast cancer | (107) |
| | Melanoma | (121) |
| TBL1 | Sensorineural deafness | (57) |
| BCL-6 | Lymphoma | (79) |
| REA | Estrogen receptor-positive breast cancer | (92) |
| BRCA1 | Breast cancer | (93) |
| | Ovarian cancer | (94) |
| MTA1 | Breast cancer metastasis | (95) |
| | Ovarian cancer, gastrointestinal cancer, prostrate cancer, esophageal and laryngeal squamous cell carcinoma, hepatocellular carcinoma, lung cancer, pancreatic cancer | (96, 119, 120) |
| MTA1s | Hormone independence of breast cancers | (102) |
| Cyclin D1 | Breast cancer | (129) |
| | Bladder cancer | (131) |
| | Prostate adenocarcinoma | (132) |
| EZH2 | Prostate cancer | (130) |
| TEL | Leukemia | (75) |
| SAFB | Breast cancer | (112) |

Several studies have yielded findings that support the coexistence of coactivators and corepressors in a complex. Close links between activation and repression were implicated in various studies. Groucho (a CoR) and p300 (a CoA) were shown to bind to separate domains of NK-4, a transcription factor (10). Likewise, nuclear hormone receptor corepressor (NCoR) and CREB-binding protein, a coactivator, have been shown to bind to the pbx (pre–B cell leukemia transcription factor)-hox (homeobox protein) heterodimer simultaneously, suggesting that all the factors may be in the same complex (11). The most convincing of such interactions is the direct interaction of NCoR with the coactivator, AIB1 (9). In the same study, AIB1 was also found to interact with the corepressor, silencing mediator of retinoid and thyroid hormone receptors (SMRT). Additional evidence for direct interactions of coactivators and corepressors also came with the identification of a variety of coactivators as metastasis-associated antigen 1 (a CoR; MTA1) binding partners. MTA1-interacting coactivator, a novel estrogen receptor coactivator, was identified from a yeast two-hybrid screen for MTA1 binding proteins (12). MTA1 also interacts with proline-, glutamic acid-, leucine-rich protein 1/modulator of non-genomic activity of estrogen receptor (13) and integrin β3 binding protein (14), two recently discovered estrogen receptor coactivators that make breast cancer cells hypersensitive to estrogen. Because these coactivators and corepressors physically interact with each other, both proteins could affect each other's functions. There are also examples wherein corepressor-coactivator cross-talk may involve additional binding factors. For example, the SMRT/histone deacetylase-1-associated repressor protein interacts with the nuclear receptor, histone deacetylase, coactivator steroid receptor RNA activator, and corepressor SMRT hormone receptors (15). Although unlikely, another possible explanation for the interactions of coactivators and corepressors could be transitional interactions between coactivator and corepressor complexes during the exchange of complexes by activated nuclear receptors. However, considering that the functions of many of these factors are also regulated by acetylation/deacetylation, which is brought about by the histone acetyltransferase/histone deacetylase complexes, we are compelled to consider the existence of stable associations between coactivator/corepressor complexes that occur for regulatory purposes. Furthermore, corepressor-coactivator interactions could also be important to enforce a feedback mechanism to prevent excessive activation. In this context, it is expected that any dysregulation of coregulators is likely to influence the expression of target genes and hence the development of pathophysiologic conditions.

## Resistance-to-Thyroid-Hormone Syndrome

Patients who have resistance-to-thyroid-hormone syndrome typically exhibit hyposensitivity to thyroid hormone, associated with increased levels of circulating serum triiodothyronine and thyroxine, and elevated levels of thyroid-stimulating hormone. Unliganded thyroxine receptors bind to thyroxine receptor response elements and repress the

transcription of positively regulated target genes (16). Two major corepressors, NCoR and SMRT, preferentially interact with unliganded thyroxine receptors and retinoic acid receptor (RAR) and repress the basal transcription of target genes in the absence of their cognate ligands (17). NCoR and SMRT contain three transferable repression domains and two COOH-terminal α-helical interaction domains. Both SMRT and NCoR participate in the assembly of multiprotein repressor complexes through the repression domains, which interact with chromatin deacetylating enzymes, the histone deacetylases. Thus, local histone deacetylation plays a crucial role in the basal repression brought about by the unliganded thyroxine receptor-corepressor complex by maintaining local chromatin structure in a state that shuts down basal transcription (17).

A tight linkage between the mutations in the ligand-binding domain of thyroxine receptor-β1 and resistance-to-thyroid-hormone syndrome was independently shown by two groups (18, 19). These mutant thyroxine receptor-βs had decreased triiodothyronine-binding affinity and transcriptional activity. Furthermore, other thyroxine receptor-β mutants that are defective in the ligand-induced release of corepressor have been shown to have strong dominant-negative activity (20–22). Taken together, these results support the notion that dominant-negative activity is mediated by transcriptionally inactive thyroxine receptor complexes that bind to thyroxine receptor response elements. Recently, thyroxine receptor-β mutations in a third "hotspot" have been identified (23). Two of the thyroxine receptor-β mutants (R243Q and R243W) have normal triiodothyronine-binding affinity but transactivate poorly in the presence of triiodothyronine and have dominant-negative activity (24).

## Insulin Resistance

Common disorders, such as obesity, type II diabetes, and polycystic ovary syndrome, are associated with moderate to high insulin resistance. Screening for mutations in these disorders led to the identification of two different heterozygous, missense mutations in the PPARγ ligand-binding domain (25). Functional studies showed that both mutant receptors exhibited reduced ligand binding and coactivator recruitment, resulting in impaired transcriptional activity. Analogous to the thyroxine receptor-β mutations in the resistance-to-thyroid-hormone syndrome, the natural PPARγ mutants inhibited the action of coexpressed wild-type receptors in a dominant-negative manner. In addition, these mutants have been shown to repress basal gene transcription. This property is particularly significant, because wild-type PPARγ (unlike thyroxine receptor-β or the RAR) does not repress basal gene transcription or recruit corepressors when bound to DNA (26). Analysis of an artificial dominant-negative PPARγ mutant indicated that the mutant inhibits basal gene transcription, recruits corepressors, and exhibits delayed ligand-dependent corepressor release (27).

## Obesity

The small heterodimer partner (SHP) is an atypical orphan member of the nuclear receptor superfamily that contains the dimerization and ligand-binding domain found in other members but lacks the conserved DNA-binding domain. SHP interacts with a variety of nuclear receptors, including PPARα (28), estrogen receptor (29), liver receptor homologous protein-1 (30–32), liver X receptor (33), and the hepatocyte nuclear factor-4α (34). In most cases, SHP inhibits the activity of the nuclear receptor with which it interacts (35). In addition, SHP can act as a direct transcriptional repressor (36). Genetic variation in the SHP gene, leading to the loss of SHP activity, is associated with mild obesity (37). Interestingly, SHP mutations also seem to correlate with high birthweight. This phenotype could be related to the effects of SHP on several nuclear receptors associated with metabolic regulation.

## Adrenal Hypoplasia Congenita and Dosage-Sensitive Sex Reversal

Adrenal hypoplasia congenita is an inherited disorder characterized by underdevelopment of the adrenal cortex, which has two manifestations: the miniature adult form and the cytomegalic form. This is an X-linked disorder, and mutations or deletions in the orphan receptor DAX1 (dosage-sensitive sex reversal adrenal hypoplasia critical region on the X chromosome gene 1; *DAX1* gene) were identified in adrenal hypoplasia congenita patients. Adrenal hypoplasia congenita patients with defects in the *DAX1* gene also show hypogonadotropic hypogonadism, thus identifying the mutant *DAX1* as the causative agent of both disorders. Furthermore, duplication of the gene that encodes DAX1 in sex-reversed patients makes the *DAX1* gene a very strong candidate for the dosage-sensitive sex reversal gene (*DSS*; ref. 38). It has been proposed that DAX1 inhibits the expression of steroidogenic acute regulatory protein by binding to DNA hairpin structures in the steroidogenic acute regulatory promoter (39). Most notably, however, DAX1 was shown to act as an inhibitor of steroidogenic factor 1–mediated transcriptional transactivation (40, 41). Steroidogenic factor 1 functions as a transcriptional activator of many genes involved in steroid hormone biosynthesis in the hypothalamic-pituitary-adrenal-gonadal axis, and DAX1 seems to act by complexing with and inhibiting the activator function of steroidogenic factor 1 (42).

## Female Fertility

Ovulatory dysfunction is the most common cause of female infertility. It has been shown that the corepressor receptor interacting protein 140 (RIP140), also called nuclear-receptor-interacting protein 1, is essential for ovulation (43). RIP140 represses steroid receptor transcriptional responses in both a histone deacetylase–dependent and –independent manner. RIP140-mediated repression involves multiple repression domains and the recruitment of COOH-terminal binding protein (CtBP; ref. 44). Mice null for this protein are viable, but female mice are infertile because of the complete failure of mature follicles to release oocytes at ovulation.

## Huntington's Disease

NCoR has also been implicated in nervous system disorders. For example, the COOH terminus of NCoR interacts with the $NH_2$ terminus of the Huntington's disease gene product,

huntingtin, in both yeast two-hybrid screens and pull-down assays (45). Although NCoR is generally thought to exert its action in the nucleus (46), immunohistochemical studies of the brains of patients with Huntington's disease and controls revealed that the localization of NCoR and transcriptional repressor, mSin3 in the diseased cortex and caudate is exclusively cytoplasmic, whereas in the normal brain they are localized in the nucleus and the cytoplasm of the cells in these structures. This suggests that relocalization of corepressor proteins in diseased brains alters transcription and is thus involved in the pathogenesis of this disease. It is interesting but perhaps counterintuitive that inhibitors of histone deacetylase activity can arrest the neurodegeneration associated with Huntington's disease in a *Drosophila* model (47).

Huntingtin has also been shown to interact with another corepressor protein, CtBP (48). The CtBP family of proteins are conserved transcriptional regulators (49). CtBP functions as a corepressor for a wide array of DNA-binding transcriptional factors (50–53). CtBP's interaction with its binding partners usually involves a conserved PXDLS interaction motif (where X is any amino acid) in CtBP interacting proteins (54). CtBP mediates its corepressor function via multiple mechanisms including histone deacetylases, mSin3, and polycomb complexes (55). Because of huntingtin's altered interactions with CtBP and other nuclear proteins, mutant huntingtin may disrupt the formation of protein complexes that regulate transcription and RNA processing. For example, the proteolysis of mutant huntingtin in the nucleus in patients with Huntington's disease may produce $NH_2$-terminal fragments of huntingtin that repress transcription aberrantly and cause neuronal dysfunction (48).

## Sensorineural Deafness

Ocular albinism with late-onset sensorineural deafness is an X-linked recessive disorder characterized by ocular albinism and progressive sensorineural hearing loss in the fourth and fifth decades of life. Transducin (β)-like 1 (TBL1), a non–histone deacetylase protein found in corepressor complexes, has been shown to be involved in this disorder (56, 57). TBL1/TBLR1 (TBLR1, the TBL1-related F box/WD-40-containing factor) binds to histones H2B and H4, and repression by TBL1/TBLR1 correlates with their interaction with histones (58). TBL1 and TBLR1 serve as specific adaptors for the recruitment of the ubiquitin conjugating/19S proteasome complex, with TBLR1 selectively serving to mediate a required exchange of the NCoR and SMRT, for coactivators upon ligand binding (59).

## Corepressors and Cancer

A plethora of corepressors have been shown to be deregulated in cancers of all types. Because a listing of all these is not practical and would, in fact, be counterproductive, we will enumerate and discuss only the well-studied examples in specific cancer types.

***Leukemia.*** Roles for NCoR and SMRT in several types of leukemia are well characterized. Acute promyelocytic leukemia, which is caused by a block in myeloid differentiation, is associated with rearrangements of *RAR-α*, which most commonly results in fusions of the *RAR-α* gene with the promyelocytic leukemia gene (*PML*) or the promyelocytic leukemia zinc finger gene (*PLZF*; ref. 60). Clinical treatment of PML-RAR acute promyelocytic leukemias with retinoic acid induces differentiation of leukemic blasts and disease remission, whereas PLZF-RAR acute promyelocytic leukemias are resistant to retinoic acid (60). Both RAR-α fusion proteins retain the ability to interact with NCoR and SMRT and recruit the nuclear hormone receptor corepressor-histone deacetylase-TBLR1 complex (61). This ability is critical for PML-RAR to be able to block the differentiation of hematopoietic precursor U937 cells. Conversely, a triple-point mutation in the corepressor box, a region required for corepressor recruitment in RAR, abolishes both the NCoR interaction and the ability of PML-RAR to block the differentiation of U937 cells (62).

Interestingly, retinoic acid can dissociate corepressors from both RAR and PML-RAR complexes both in solution and upon DNA binding, but substantially higher concentrations of retinoic acid are required to dissociate corepressors from PML-RAR (62–65). This result provides a biochemical explanation for the requirement of pharmacologic doses of retinoic acid *in vivo* to induce the differentiation of PML-RAR-expressing myeloid blasts. Surprisingly, PLZF-RAR associates with corepressor complexes in a retinoic acid–resistant way (66). Indeed, PLZF is able to interact with many components of the corepressor complex, i.e., NCoR-SMRT, Sin3a, and histone deacetylase directly, offering a retinoic acid–resistant interaction surface in the PLZF-RAR fusion protein (62–65). For example, combined treatment with retinoic acid and histone deacetylase inhibitors transforms PLZF-RAR from an inhibitor into an activator of the retinoic acid signaling pathway (62–64). In addition to pinpointing the link between transcriptional silencing and acute promyelocytic leukemia pathogenesis, these findings might have important clinical implications as well. A combination therapy of retinoic acid along with histone deacetylase inhibitors, trichostatin A and butyric acid could overcome the unresponsiveness of PLZF-RAR acute promyelocytic leukemias to retinoic acid. Indeed, there has been one preliminary report in which one patient with relapsed acute promyelocytic leukemia showed a response to combined retinoic acid-butyrate treatment (67). Although corepressor interactions with PML-RAR-α fusions are less sensitive to the effects of retinoic acid than are interactions with wild-type RAR-α, pharmacologic concentrations of retinoic acid do result in the dismissal of the corepressor complex and in the activation of transcription. In contrast, the PLZF-RAR-α fusion protein interacts with the corepressor even in the presence of retinoic acid. These observations correlate corepressor with the disease state, because the relative retinoic acid sensitivity of the interaction between the RAR-α fusions and NCoR or SMRT correlates with their response to retinoic acid treatment.

Strikingly, the aberrant recruitment of the corepressor complex does not seem to be restricted to acute promyelocytic leukemia. Some (12-15%) acute myeloid leukemias (AML) result from the t(8;21) translocation between *AML1* and *ETO*, which results in the fusion of the hematopoietic transcription factor AML1 to the zinc finger nuclear protein ETO (68). AML1 is a canonical transcriptional activator of several genes involved in hematopoiesis, whereas ETO is isolated as an interactor for NCoR-SMRT in yeast two-hybrid screens (69). The p300-interacting domain of AML1 is lost in the chromosomal translocation and is replaced with ETO, which retains the

corepressor interaction domain. The resulting AML1-ETO fusion protein, therefore, has lost the ability of AML1 to recruit p300 and is endowed with the ability of ETO to recruit histone deacetylase through NCoR-SMRT (69–73).

A third class of leukemia results from chromosomal rearrangements of the E26 transforming specific–related gene *TEL*, which encodes a strong transcriptional repressor that recruits a corepressor complex including SMRT, mSin3A, and histone deacetylase-3 (74, 75). The common theme for the involvement of NCoR and SMRT in the progression of these leukemias thus seems to be the ability of histone deacetylase–associated repression to block differentiation and allow the uncontrolled growth of hematopoietic cells, which ultimately results in the disease state.

***Lymphoma.*** B cell lymphoma-6 (BCL6), a member of a subfamily of BTB proteins that includes PLZF and hypermethylated in cancer-1, encodes a transcription factor that represses genes necessary for the terminal differentiation of lymphocytes within germinal centers, and the misregulated expression of this factor is strongly implicated in several types of B cell lymphomas (76). The homodimeric BTB domain of BCL6 (also known as the POZ domain) is required for the repression activity of the protein and interacts directly with the SMRT and nuclear hormone receptor corepressors (77, 78). Regulatory elements of the *BCL6* gene are frequently mutated in human diffuse large B cell lymphomas (79). This leads to constitutive expression of *BCL6*, which is postulated to favor B cell proliferation, survival, and differentiation blockade in the face of ongoing mutagenesis by the somatic hypermutation machinery (80, 81).

## Cancers of the Female Reproductive System

Progesterone and estrogen are essential regulators of female reproduction. Through their cognate receptors, estrogen and progesterone regulate the normal development of the ovary, the uterus, and the mammary gland and play key roles in the tumorigenesis of these tissues. Repressors for these hormones thus have a critical role in cancer genesis and progression in these organs.

***Corepressors in hormone independence.*** Antiestrogens and selective estrogen receptor modulators are effective in retarding the progression of hormone-dependent breast tumors (82). For example, tamoxifen, a selective estrogen receptor modulator, is a commonly used antagonist in the treatment of hormone-dependent breast cancers. However, a large proportion of these patients with metastatic breast cancer eventually become resistant to hormonal treatment (82). Changes in the relative ratios of coactivator and corepressor in breast cancer cells are implicated in the differential responsiveness of estrogen receptors to an agonist or antagonist (83).

Breast cancer cells resistant to the growth-inhibitory action of tamoxifen sometimes also contain mutations in the estrogen receptors (84). For example, a mutant estrogen receptor (D351Y) has shown a reduced interaction with NCoR and SMRT, suggesting that potential interference with corepressor binding to nuclear receptor might promote tamoxifen resistance (85). In addition, decreased levels of NCoR correlated well with the acquisition of tamoxifen resistance in a mouse model system for human breast cancer, suggesting that NCoR and SMRT-containing complexes act as rate-limiting components in the actions of specific nuclear receptors (86). In addition, SHP could also interact with tamoxifen-bound estrogen receptor and inhibit tamoxifen-stimulated transactivation by blocking estrogen receptor dimerization, suggesting that SHP serves as a novel target for blocking tamoxifen agonist activity in endometrial cells (87). Furthermore, in a study delineating the expression of receptor-interacting proteins in tamoxifen-sensitive and -resistant cells, the expression of RIP140 mRNA was found to be lower in the resistant cell line (88).

Studies have shown that progesterone receptor antagonist action may also involve, in part, the recruitment of corepressors (89). Antiprogestin RU486 is known for its partial agonist activities in a cell type–dependent manner. Because RU486-bound progesterone receptors could bind to both coactivator SRC1 and corepressor SMRT, the ability of RU486 to activate transcription has been linked with the ratio of coactivator to corepressor (90).

***Role in tumorigenesis and metastasis.*** The repressor of estrogen receptor activity is a unique molecule that acts as a corepressor of steroid receptor transactivation functions. The *REA* gene encodes a 37-kDa protein that is an estrogen receptor–selective corepressor and competitively reverses the enhancement of estrogen receptor activity by the steroid receptor RNA activator 1. The repressor of estrogen receptor activity directly interacts with the ligand-activated estrogen receptor and controls the sensitivity to antiestrogens and estrogens in breast cancer cells (91). The expression of repressor of estrogen receptor activity was also found to be up-regulated in estrogen receptor–positive breast tumors and correlated inversely with the tumor grade (92).

*BRCA1* is a breast cancer susceptibility gene, and its inherited mutations are correlated with an increased risk of breast and ovarian cancer (93). *BRCA1* acts as a ligand-independent corepressor for estrogen receptors, androgen receptors, and progesterone receptors (94). If *BRCA1* is mutated, all these pathways will be more or less impaired. The effect of *BRCA1* in cancer development might therefore be multifactorial.

The up-regulation of MTA1 in human cells is associated with the increased invasiveness and metastatic potential of several human cancers, including carcinomas of the breast and ovaries (95, 96). MTA1 was originally identified as an overexpressed gene in rat metastasis breast tumors (97). The MTA family consists of three separately encoded members and six reported forms: MTA1, MTA1s, MTA-ZG29p, MTA2, MTA3, and MTA3L (95, 96). The corepressor function of MTA1 has been linked, in part, with its ability to directly interact with histone deacetylases. Results from experimental models suggest that the dysregulated expression of MTA1 promotes migration, anchorage-independent growth (98–100), the growth of breast cancer cells in nude mice, and the development of mammary carcinomas in transgenic mice (101). Consistent with its role in metastasis, the inhibition of MTA1 protein expression by antisense phosphorothioate oligonucleotides resulted in the inhibition of growth and invasiveness of MDA-MB231 breast cancer cells (95).

MTA1 inhibits estrogen receptor transactivation by recruiting the histone deacetylases to the estrogen receptor target site (99). In contrast, MTA1s (a naturally occurring variant of MTA1) is overexpressed in estrogen receptor–negative tumors. MTA1s inhibits estrogen receptor nuclear signaling by sequestering

estrogen receptor in the cytoplasm via its nuclear receptor–binding motif but exhibits an enhanced estrogen receptor's nongenomic signaling and tumorigenesis (102). Recent studies have also suggested that MTA3 modulates the metastatic potential of breast cancer by influencing the expression of Snail, a master regulator of epithelial to mesenchymal transition (103). Furthermore, MTA3 was shown to be an estrogen receptor–inducible gene via an estrogen receptor response element, which could be repressed by MTA1 and MTA1s, resulting in the up-regulation of Snail and enhanced epithelial to mesenchymal transition (104, 105). These studies suggest a complex role for MTA1 and MTA1s in modulating the hormone-dependence function of the estrogen receptor. MTA1 expression was also found to be increased not only in primary ovarian carcinoma but also in lymph node metastasis (106).

Because CtBP contains two nuclear receptor–interacting motifs, it is possible that CtBP interacts with estrogen receptor. Indeed, it has been recently discovered that CtBP interacts with estrogen receptor-α both *in vitro* and *in vivo* (107). Because CtBP corepressor activity is also inactivated by p21-activated kinase 1 (108–110), these findings raise the possibility that the CtBP-estrogen receptor interaction influences estrogen receptor transcriptional regulation.

Scaffold attachment factor B1 (SAFB1) and B2 (SAFB2) are large, multifunctional proteins implicated in numerous cellular processes including chromatin organization and transcriptional regulation. A significant association between SAFB protein levels and aneuploidy has been reported. More recently, it was shown that low levels of SAFB were associated with worse overall survival of patients with invasive breast tumors (111). SAFB expression is lost in approximately 20% of breast cancers and is suspected to have a tumor-suppressor function. Interestingly, SAFB genes reside near chromosome 19p13, a locus that is frequently lost in clinical breast cancer specimens. Furthermore, SAFB1 mutations have been identified in breast tumors but not in adjacent normal tissue (112). The recruitment of SAFB1 corepressor down-regulates E-cadherin expression in an estrogen receptor–dependent manner (113).

The overexpression of aromatase in adipose tissue has been closely linked with the development and progression of breast cancer. Because corepressor SHP is a potent inhibitor of aromatase transcription in preadipocytes, the modulation of SHP expression or activity, or both, in adipose tissue has been proposed to influence the level of aromatase expression and estrogen production in breast adipose tissue (114).

DAX1, an estrogen receptor corepressor, has been shown to inhibit estrogen receptor activation in mammalian cells. The underlying mechanism involves occupation of the ligand-induced coactivator-binding surface and subsequent recruitment of corepressor. Because DAX1 is widely coexpressed with estrogen receptors in reproductive tissues, Zhang et al. (115) have proposed that the reported DAX1-estrogen receptor interaction could affect the outcome of estrogen receptor signaling in a significant manner. In addition, DAX1 could also function as a global negative regulator of steroid hormone production by repressing the expression of multiple genes involved in the steroidogenic pathway (116). The DAX1 corepressor has been found to be positively correlated with the expression of androgen receptors and estrogen receptors in breast cancer specimens. Agoulnik et al. (117) proposed that the presence of DAX1 in cancer cells might contribute to the failure of endocrine therapies. DAX1 also represses the agonist-dependent activity of progesterone receptor by disrupting receptor-dimer interactions (118).

## Cancers Other than of the Female Reproductive System

The deregulated expression of corepressor has been noted in other cancers besides those of the female reproductive system, leukemia and lymphomas. For example, MTA1 overexpression has been linked to the tumorigenesis and metastasis of gastrointestinal carcinoma, prostrate cancer, esophageal and laryngeal squamous cell carcinomas, hepatocellular carcinoma, lung cancer, and pancreatic cancer (96, 119, 120). Likewise, CtBP expression is lost in malignant melanoma (121). Whereas melanocytes revealed CtBP expression at the mRNA and protein level, CtBP levels were considerably reduced in melanoma cell lines and tissue samples. As CtBP is proven as a transcriptional repressor of pangolin (LEF/TCF), these data suggest that loss of CtBP leads to the expression of LEF/TCF-controlled genes.

Furthermore, loss of wild-type CtBP in melanoma cells promoted the expression of melanoma inhibitory activity (MIA) via transcription factor-4. MIA is a secreted protein that is expressed in melanoma cells but not in melanocytes. It is a key molecule involved in the progression and metastasis of malignant melanomas (122). DAX1 has been proposed to be involved in the development of cancers of a variety of tissues, including adrenal and pituitary adenomas and prostate cancer. High levels of DAX1 expression are associated with a nonfunctional phenotype in adrenal adenomas (123). In addition, low levels of DAX1 are detected in cortisol-producing tumors causing Cushing syndrome, and high levels of DAX1 are detected in deoxycorticosterone-producing adenomas, suggesting that DAX1 is involved in the regulation of steroidogenesis of adrenal tumors (124). DAX1 expression has also been detected in nonfunctioning gonadotropic pituitary adenomas, in some instances along with steroidogenic factor 1 (125, 126), and DAX1 expression is considerably reduced in benign prostate hyperplasia compared with normal prostate tissue, suggesting that a lack of repression of androgen receptor accounts for the elevated androgen receptor activity in these tumors (127, 128). Cyclin D1, a cell cycle protein, can also function as a corepressor for androgen receptors. Cyclin D1 selectively inhibits ligand-dependent androgen receptor function in several cell types, including breast cancer, bladder cancer, and androgen-independent prostate adenocarcinoma cell lines (129–132). The mechanism by which cyclin D1 inhibits liganded-androgen receptor seems to depend in part on histone deacetylases or histone acetyltransferases (129, 132). Both cyclin D1 and the androgen receptors bind to similar domains of p300/CREB-binding protein–associated factor, and cyclin D1 displaces the binding of the androgen receptor to p300/CREB-binding protein–associated factor *in vitro* (129). Together, these studies suggest that cyclin D1 binding to the androgen receptor represses ligand-dependent androgen receptor activity by competing for androgen receptor coactivator or by recruiting androgen receptor corepressors with histone deacetylase activity.

EZH2 (a group protein enhancer of zeste homologue 2) is a master corepressor that is up-regulated during the progression of prostate cancer, a process accompanied by the silencing of a

number of genes. Interestingly, EZH2-mediated repression of cellular genes was attenuated when histone deacetylase activity was inhibited, implying a dependence of EZH2 targets upon chromatin remodeling. The investigators of this study suggested that EZH2 targets tumor suppressor genes, because EZH2 overexpression not only repressed a significant number of genes but also resulted in increased metastasis (130).

## Upstream Regulators of Corepressor Functions

Aberrant growth factor signaling is a common feature of tumor cells. The functions of corepressor and their associated proteins are subject to regulation by signaling kinases and post-translational modifications. Thus, these upstream factors could potentially dysregulate corepressor functions. The function of histone deacetylases was shown to be affected by phosphorylation, and phosphorylation-dependent signaling has been recently proposed as a potential mechanism for relief of deacetylase-catalyzed transcriptional repression (133). p21-activated kinase 1 phosphorylates CtBP on $Ser^{158}$ within a putative regulatory loop, leading to the redistribution of CtBP to the cytoplasm and blocking its corepressor functions in the nucleus (108). p21-activated kinase 1 regulation of CtBP represents a new model of corepressor regulation, whereby cellular signaling cascades may influence gene expression by derepressing the critical target genes in mammalian cells (108). In addition to phosphorylation, the interaction of CtBP and RIP140 could also be regulated by acetylation, because acetylation disrupts the RIP140-CtBP complex and derepresses nuclear hormone receptor-regulated genes (134). Furthermore, the ability of MTA1s to sequester estrogen receptor in the cytoplasm could be potentiated by MTA1s phosphorylation at $Ser^{321}$ by casein kinase 1-γ2 (135). Thus, signaling pathways could also affect the hormone-independent growth of breast cancer cells.

The ability of SMRT to associate with nuclear receptor to exert its corepressor function has been shown to be strongly inhibited by the activation of tyrosine kinase signaling, because epidermal growth factor receptor–triggered mitogen-activated protein kinase cascade affects the affinity of SMRT for nuclear receptors (136). Disruption of corepressor functions by phosphorylation may also constitute another means of controlling gene expression (134).

Regulation of corepressor functions by controlling the expression of corepressor constitutes another means of controlling gene expression. Heregulin, a growth factor, increases MTA1 expression, leading to estrogen receptor-transcriptional repression and hormone independence (99). Deregulation of human epidermal growth factor receptor-2 in breast cancer cells also enhances the expression of MTA1s and sequesters estrogen receptor in the cytoplasm, thus stimulating nongenomic estrogen receptor signaling, which promotes malignant phenotypes (102).

## Future Perspectives

Transcriptional regulation plays an important role in numerous fundamental cellular processes. Many earlier studies in this area were focused on the regulation of steroid hormone receptor functions by coactivator. However, in recent years, investigation of corepressor biology has become an area of active research. This has led to substantial progress in the identification of a number of novel corepressors with critical roles in determining the action of steroid receptors. On the basis of the progress made in the past few years, it is certain that additional corepressor with diverse physiologic functions will continue to be discovered, adding further complexity to the area of steroid receptor signaling. Emerging data re-emphasizes that the regulation of steroid receptors by corepressor is as significant as that by coactivator. Because chromatin is a dynamic structure, it is tempting to speculate that the coexistence of corepressors and coactivators within the same complex(s) allows for a diverse interaction to achieve rapid and controlled changes in the transcription of target genes.

Uncontrolled alterations in the level of corepressor change the fine balance between corepressor and coactivator and hence modulate signaling by steroid receptors bound by antagonists and partial agonists. The dysregulation of corepressor levels or functions, or both, also presents an opportunity to cancer cells, with unopposed activation of steroid receptors. Because many of the corepressor complexes also contain histone deacetylases, chromatin modification represents one of the widely studied areas of corepressor functions. However, the emerging identification of novel corepressors suggests the prevalence of alternative mechanisms to account for transcriptional repression. Such putative mechanisms might include competitive binding to corepressor, disruption of the formation of steroid hormone receptor dimers, recruitment of polycomb proteins, and alter the localization of coregulators. In addition, the contact of corepressor with the basal transcriptional machinery could also play a role in the corepression functions of corepressor. It is likely that an increased understanding of the mechanisms by which corepressors are normally associated with, and dissociated from, wild-type and mutant steroidal receptors should also assist in the development of clinically relevant pharmacologic interventions.

Mutually antagonistic equilibrium interactions of corepressor and coactivator can also modulate the dose-response and partial agonist activity of some nuclear receptors. Therefore, the dysregulation of corepressor functions, or its expression, may indirectly affect the duration of the hormonal response and thus may have significant clinical implications. Because signaling pathways represent upstream regulators of the functions and interactions of a steroid receptor with corepressor, blocking signaling pathways that are uniquely deregulated in tumors, such as the growth factor signaling pathway, along with antiestrogen treatment could be effectively exploited to improve the treatment of hormonally responsive cancers. In this context, ongoing efforts to further define the molecular action of corepressor and its structural basis will eventually feed into the design and development of better selective antagonists and selective estrogen receptor modulators.

The past decade has been one of the most exciting periods in steroid receptor molecular biology. Although much more work clearly remains to be done, we have begun to gain a deeper insight into the transcriptional function of steroidal receptors, the role played by chromatin modifications, and the contribution of corepressors and coactivators in the development of pathologic conditions. As discussed in the preceding sections, many of the investigations have focused on understanding the modifications on a given target of steroid receptor and delineating the precise steps involved. Although this research

has been tremendously rewarding and fruitful, it is now clear that a more complete understanding of key coregulators in physiologically relevant animal models and human tumor specimens should be combined with tissue culture models for further significant gains.

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