Commentary

Transcriptional Corepressors in Cancer

Emerging Targets for Therapeutic Intervention

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The normal cell transcriptional process entails a high degree of combinatorial effects and time-dependent "flexibility" to translate cellular signaling into differential gene expression levels. Transcriptional corepressors can function as histone-modifying enzymes to regulate epigenetic events, modulate chromatin structure, and hence control transcriptional activity. Various corepressor complexes have been described; qualitative and quantitative alterations of corepressors can crucially influence the transcriptional output of both normal and malignant cells. Because these molecules can exert epigenetic control of tumorigenic signaling pathways, they can be considered potential regulators of cancer cell-related phenomena. Alterations of the expression level and/or function of transcriptional corepressors have been reported in a wide range of human cancers; thus, corepressors may present rational therapeutic targets as well as potential biomarkers of response to selective therapeutic interventions. Deeper insights into the context-specific and time-specific physical connections among transcription factors, coregulators, and gene regulatory elements, as well as epigenetic modifications, and their interactions, can enhance the capacity to interfere with small molecules that may restore the normal transcriptome/interactome in a cancer cell. There are several conceivable mechanisms of corepressor targeting in cancer that create enthusiasm. However, design, discovery, and testing of such innovative treatment approaches require extensive elaboration before they can achieve practical implementation in the clinic. ***Cancer*** **2013;119:1120-8.** © *2012 American Cancer Society.*

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INTRODUCTION

Carcinogenesis is a multistep process emanating from the accumulation of genetic and epigenetic alterations in genes that regulate cell proliferation, growth, differentiation, adhesion, migration, angiogenesis, and apoptosis. Modulation of gene expression relies on the dynamic balance and spatiotemporal control of specific transcription factors that interact with the basal transcriptional apparatus as well as with transcriptional coregulators (corepressors and coactivators), resulting in a multicomplex protein network.[1,2] Transcriptional coregulators contribute to the accuracy of this circuitry and can function as (or cross-talk with) histone-modifying enzymes to control epigenetic events; modify chromatin structure; and, thus, regulate gene expression patterns.[3] Specifically, corepressor protein complexes can mediate enzymatic repression of gene transcription through multiple biochemical mechanisms.[3,4]

Diverse corepressor complexes have been identified. These include nuclear receptor corepressor 1 (NCoR1), NCoR2/silencing mediator for retinoid and thyroid hormone receptors (NCoR2/SMRT), C-terminal–binding proteins (CTBP1, CTBP2), RE1-silencing transcription factor (REST)/neural-restrictive silencing factor (REST/NRSF), Rest corepressor (RCOR/CoREST), runt-related transcription factor (RUNX), breast cancer metastasis suppressor 1 (BRMS1), BTG3-associated nuclear protein/scaffold-matrix–associated region 1-binding protein (BANP/SMAR1), zinc-finger and breast cancer type 1 susceptibility protein (BRCA1)-interacting protein with a Kruppel-associated box (KRAB) zinc finger domain 1 (ZBRK1/ZNF350), nuclear receptor-interacting protein 1 (NRIP1/RIP140), nucleosome remodeling and histone deacetylase (NURD), and Swi-independent 3 (SIN3).

Mechanistically, it is suggested that transcriptional corepressors bind to nuclear receptors in the absence of their ligand; whereas the presence of ligand can change the receptor configuration, favoring binding to coactivators, thus stimulating gene transcription.[5] The NCoR1-SMRT complex, the SIN3 complex, the corepressor Alien, and orphan nuclear

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receptors are paradigms of this mechanism, acting as constitutive repressors.[5] Alternatively, a "competitive" dynamic balance between coactivators and corepressors is proposed as another putative mechanism of transcription regulation, with the release of a corepressor and the binding of a coactivator marking a new transcription cycle. A unique category of corepressors, namely, receptor-interacting protein 140 (RIP140) and ligand-dependent corepressor (LCoR), manifests agonist/antagonist bound-dependent corepression.[5,6] Overall, cells can modulate corepressor complexes in a multilevel manner to achieve a high degree of transcriptional fine-tuning. Apart from ligand-binding–induced, conformational changes resulting in the dissociation of corepressors, such modulation also can be achieved through post-translational modifications followed by nuclear export and/or degradation of the corepressor.[7] Therefore, qualitative and quantitative changes of corepressors can play a key role in the transcriptional output of both normal and tumor cells. The functional role of transcriptional corepressors can vary in a temporal and spatial manner ("pleiotropic activity"), participating in normal cell differentiation and tissue homeostasis as well as in cell transformation and tumor progression (Table 1).[4,8-30] In that these intricate protein complexes can affect oncogenic signal-transduction cascades, they constitute potential regulators of self-sustained cell proliferation, resistance to apoptosis, unrestrained migration, angiogenesis, and metastasis.

A volume of data has documented alterations in the structure, expression level, and/or function of transcriptional corepressors in a broad array of human malignancies.[8,31,32] It becomes evident that corepressors may function as rational therapeutic targets and/or potential biomarkers of response to selective chemotherapy regimens. Here, we present selected examples of this relatively novel and challenging therapeutic concept.

Transcriptional Corepressors: Paradigms of Potential Tumor Targeting

Nuclear receptor corepressor 1 and nuclear receptor corepressor 2/silencing mediator for retinoid and thyroid hormone receptors

NCoR1 and NcoR2 are archetype transcriptional corepressors, and their structure and role have been well described.[33-36] Tumorigenic roles have been recognized in acute promyelocytic leukemia (APL), which results in fusion oncoproteins (promyelocytic leukemia [PML]-retinoic acid receptor alpha [RARα] or PML zinc finger [PLZF]-RARα) that sustain NCoR1 interactions, leading to a condensed structure of chromatin and, hence, preventing RARα-mediated cell differentiation.[37] In contrast to PML-RARα, PLZF fusion protein is resistant to pharmacologic doses of retinoic acid; this resistance might be overcome by the addition of histone deacetylase (HDAC) inhibitors. In acute myeloid leukemia (AML), the AML1-821 corepressor (ETO) fusion oncoprotein can recruit NCoR1, hindering transcription regulation.[38] The revelation of a functional role for NCoR1 in leukemia illustrates the importance of transcriptional corepressors in mediating tumorigenic actions and provides a rational for HDAC targeting. For example, HDAC inhibitors resulted in myeloid cell differentiation in vitro, corresponding to increased histone 3 and histone 4 acetylation; however, they did not alter *ncor1* or *ncor2* gene expression.[39]

Expression profiling of solid tumors has revealed alterations in NCoR1/NCoR2 expression and localization.[40-43] These alterations may have prognostic value and/or may predict response to specific therapeutic interventions, such as response to tamoxifen in estrogen receptor (ER)-positive breast cancer. In colorectal cancer, which is not considered hormone-dependent, post-translational modifications of NCoR1 and NCoR2 can affect their cytoplasmic localization, hindering β-catenin binding to lymphoid enhancer factor/T-cell factor (LEF/TCF) target genes and promoting TCF4 transcriptional repression.[44,45] It is noteworthy that corepressor changes can occur within the tumor cells and/or in the surrounding microenvironment, which appears to exert an important role in tumor initiation and progression. There is considerable uncertainty in the timing and degree of corepressor alteration with regard to tumor development; however, thorough understanding of such molecular interplays can provide a platform for testing novel therapeutic regimens with or without additional hormone and/or biologic treatments.

C-terminal–binding proteins 1 and 2

CTBP1 and CTBP2 are 2 similar, highly conserved corepressors that have been linked to cancer progression and can be regarded as putative therapeutic targets.[10,11] CTBPs may promote cell proliferation, epithelial-mesenchymal transition (EMT), and invasiveness, although they may inhibit apoptosis through suppression of *INK4* (cyclin-dependent kinase inhibitor 2A gene) cell-cycle control proapoptotic genes[11]; this appears to be relevant in hepatocellular carcinoma.[12] The activities of CTBP1 and CTBP2 are context-dependent and time-dependent. CTBP1 is associated with ERα *trans*-repression, whereas its deregulation can deform normal transcriptional activity in breast cancer cells.[13,46] In colorectal cancer, CTBP

TABLE 1. Corepressor Complexes: Functions and Roles in Human Cancers

Corepressor Complex	Molecular Interactions	Functions and Roles	Implication in Cancer Type	References
NCoR1, NCoR2/SMRT	NRs, NF-κB, AP-1, MYOD, ETO, CBF, TFIIB, MAD/MXI	HDAC: multiple roles in a context-specific manner	Bladder, breast, prostate, colorectal, endometrial, glioma, leukemia	Perissi 2010,[4] Battaglia 2010,[8] Liu 2007[9]
CTBP1/2	ER, INK4a/b, Bax, CoREST, p21, PERP, PTEN, E-cadherin, Noxa	HDAC, HDEM: promotes cell proliferation, invasiveness/migration, EMT; antagonizes apoptosis	Breast, colorectal, hepatocellular	Battaglia 2010,[8] Straza 2010,[10] Chinnadurai 2009,[11] Chen 2008,[12] Stossi 2009[13]
RUNX1/2/3	CBF, TLE1, NRs, AP-1, HDAC3, MYST4, STUB1, SMAD1/3, SUV39H1	Transcription factors: RUNX1, hematopoietic cell differentiation; RUNX2, bone development; RUNX3, T-cell regulation, neuronal differentiation; role in mitosis	Leukemia, lymphoma, breast, gastric, thyroid, prostate, embryonal carcinoma	Battaglia 2010,[8] Chua 2009,[14] Chuang 2012,[15] Niu 2012[16]
CoREST	Rest, CTBP, SWI/SNF, ZNF217	HDAC, HDEM: multiple roles in a context-specific manner	Breast, prostate, colorectal	Battaglia 2010,[8] Lakowski 2006,[17] Thillainadesan 2008[18]
BRMS1	SIN3a HDAC chromatin remodeling complexes (e.g. ARID4A, RBP-1)	HDAC (mSin3a family): metastasis suppressor; apoptosis inducer; NF-κB/EGFR down-regulation; miRNA regulation	Breast, melanoma	Battaglia 2010,[8] Liu 2006,[19] Edmonds 2009,[20] Metge 2008,[21] Meehan 2004,[22] Hurst & Welch 2011[23]
NURD	ER, AP-1, TWIST, SNAIL, FOG1, IKAROS, Rb, BCL6, BCL11B, PML–RARα, INK4, NAB2, ZIP, LSD1, MYC, BRCA, HIF1a, HER2, PAX, p53	HDAC, ATP-dependent chromatin remodeling: tumor promoter *or* suppressor in a context-specific manner; hematopoietic stem cell differentiation; role in cell cycle, genomic stability, EMT, metastasis	Breast, colorectal, gastric, esophageal, endometrial, pancreatic, ovarian, nonsmall-cell lung, prostate, hepatocellular, diffuse large B-cell lymphoma, leukemia	Lay & Wade 2011,[24] Manavathi 2007,[25] Wang 2009,[26] Ramierz 2010,[27] Kai 2010,[28] Hsia 2020,[29] Fujita 2003[30]

Abbreviations: AP-1, activator protein-1; ARID4A, AT-rich interactive domain-containing protein A4; ATP, adenosine triphosphate; Bax, B-cell lymphoma 2-associated X protein; BCL6, B-cell lymphoma 6 protein; BCL11B, B-cell lymphoma/leukemia 11B protein; BRCA, breast cancer susceptibility protein; BRMS1, breast cancer metastasis suppressor 1; CBF, core binding factor; CoREST, RE1-silencing transcription factor (REST) corepressor; CTBP1/2, C-terminal binding proteins 1 and 2; EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; ER, estrogen receptor; ETO, 821 protein; FOG1, friend of GATA protein 1; HDAC, histone deacetylase; HDEM, histone demethylase; HER2, human epidermal growth factor receptor 2; HIF1a, hypoxia-inducible factor 1, α subunit; IKAROS, Ikaros zinc finger protein (lymphoid cell-specific transcription factor); INK4a/b, tumor suppressor proteins associated with the p16INK4a and p16INK4b genes; LSD1, lysine-specific histone demethylase 1; MAD/MXI, mothers against decapentaplegic/max-interacting protein 1; miRNA, microRNA; mSin3a, Swi-independent 3a (paired amphipathic helix protein) multiprotein complex; MYC, v-myc myelocytomatosis viral oncogene homolog (avian); MYOD, myogenic regulatory factor; MYST4, MUST histone acetyltransferase (monocytic leukemia) 4; NAB2, early growth response-1 binding protein 2; NCoR1, nuclear receptor corepressor 1; NCoR2/SMRT, nuclear receptor corepressor2/silencing mediator for retinoid and thyroid hormone receptors; NF-κB, nuclear factor κB; Noxa, phorbol-12-myrisate-13-acetate-induced protein 1; NRs, nuclear receptors; NURD, nucleosome remodeling and histone deacetylase; p21, cyclin-dependent kinase inhibitor 1; p53, protein 53; PAX, paired box protein; PERP, protein 53 apoptosis effector related to peripheral myelin protein 22; PML-RARα, promyelocytic leukemia-retinoic acid receptor α; PTEN, phosphatase and tensin homolog; Rb, retinoblastoma protein; RBP-1, retinoblastoma binding protein-1; RUNX1/2/3, Runt-related transcription factors 1, 2, and 3; SIN3a, Swi-independent 3a (paired amphipathic helix protein); SMAD1/3, mothers against decapentaplegic (MAD)-related proteins 1 through 3 (transforming growth factor β signaling proteins); SNAIL, snail zinc finger protein; STUB1, STIP1 homology and U box-containing protein 1; SUV39H1, suppressor of variegation 3-9 homolog 1A; SWI/SNF, switch/sucrose nonfermentable; TFIIB, transcription initiation factor IIB; TLE1, trypsin-like enzyme; TWIST, twist-related protein; ZIP, zipper-interacting protein; ZNF217, zinc-finger protein 217.

up-regulation has been recognized as an event downstream of adenomatous polyposis coli (APC) loss and has been correlated with alternative reading frame (ARF) loss.[47] However, low CTBP levels in melanoma allow up-regulation of LEF/TCF-related genes, contributing to invasion.[48] The compound 4 methylthio-2-oxobutyric acid (MTOB) can bind to CTBP, triggering conformational changes that result in CTBP dislocation from target-gene promoters.[10] Alternatively, reduction of nicotinamide adenine dinucleotide (NADH) levels with antioxidants may distort CTBP binding to its interacting proteins.[8,49]

Rest corepressor and runt-related transcription factor

CoREST forms a complex with REST to repress target-gene transcription and acts as a docking platform for the assemblage of HDAC1/HDAC2, BRCA2-associated factor 35 (BRAF35), BRAF35-HDAC complex protein (BHC80), and lysine-specific histone demethylase 1 (LSD1).[17] LSD1 is up-regulated in a gamut of solid tumors and has been associated with a poor prognosis.[50-52] However, abnormal epigenetic silencing of tumor suppressor genes by LSD1 has been demonstrated in colorectal cancer cell lines.[53] LSD1 inhibition, combined with DNA methyltransferase inhibition, may re-establish the expression of repressed genes. CoREST can also form a larger complex with ZNF217, a candidate oncogene in breast cancer; this has been correlated with loss of transforming growth factor-beta (TGF-β) responsiveness and repression of tumor suppressor genes, such as *p15ink4b*.[18] CoREST can also participate in complex formation with switch/sucrose nonfermentable (SWI/SNF) and CTBP, resulting in tumorigenic activity.[8,54]

Transcription factors of the RUNX family were identified in embryonal carcinoma cells after RAR-induced differentiation. The oncogenic capacity of RUNX2 was unveiled by the creation of a transgenic mouse with *runx2* under the control of *cd2* promoter, which resulted in the perturbation of thymocyte development and spontaneous lymphoma.[55] Augmented RUNX2 expression levels have been noted in breast cancer cell lines; and RUNX2 may be implicated in bone metastasis, reflecting its role in bone biology.[56] RUNX2 seems to be downstream of Wnt and forms a network that is pertinent in prostate cancer cell growth, rendering it a potential therapeutic target.[8,14,57]

Breast cancer metastasis suppressor 1 and nucleosome remodeling and histone deacetylase

BRMS1 interacts with several proteins, such as retinoblastoma-binding protein-1 (RBP-1), the mammalian SIN3-HDAC complex, and heat-shock protein 90 (Hsp90). BRMS1 complexes can suppress nuclear factor-kappaB (NF-κB) activity through the inhibition of inhibitor of NF-κB-alpha (IκBα) phosphorylation and subsequent degradation.[58] BRMS1 contributes to direct suppression of the transcription factor p65 (RelA)/p65 subunit of NF-κB through HDAC1-catalyzed deacetylation, whereas *BRMS1* knockdown permits the recruitment of acetylated RelA/p65 to NF-κB–dependent antiapoptotic target genes.[19] In addition, BRMS1 can regulate the levels of microRNAs (miRNAs) that play a role in metastasis.[8,20]

The NURD complex is an ATP-dependent chromatin remodeling complex that can recruit HDAC1, HDAC2, and LSD1. It is involved in the preservation of DNA integrity and can function either as a tumor promoter or a tumor suppressor in a context-specific manner.[24,25] It is suggested to play a role in tumor initiation, progression, and invasion. NURD complexes inhibit p53 through deacetylation interactions with snail zinc finger protein (SNAIL) and twist-related protein (TWIST) during EMT. Metastasis-associated protein 1 (MTA1), a NURD component, can be up-regulated by the oncogene *myc* (v-myc-myelocytomatosis viral oncogene homolog [avian]), correlating with invasion and a poor outcome in a wide spectrum of tumors.[24,25] In breast cancer, human epidermal growth factor receptor 2 (HER2) signaling up-regulates MTA1, whereas MTA1 and MTA2 inhibit estrogen activity. MTA3, another NURD component, competes with MTA1, inhibits EMT, and inhibits TGF-β and mitogen-activated protein kinase (MAPK) tumorigenic signaling.[24-26] In APL, NURD is recruited by the fusion protein PML-RARα, impairing cell differentiation.[27] In addition, resveratrol (3,4′,5-trihydroxystilbene; a natural polyphenolic compound) activates p53 in prostate cancer cells by MTA down-regulation and NURD destabilization, an effect that is enhanced by HDAC inhibition.[28] A low-molecular-mass compound that mimics the function of an MTA1 splice variant, which regulates estrogen nuclear localization, has demonstrated anticancer effects in an in vivo model.[29]

DISCUSSION

Outlook

The main role of transcriptional corepressors is to modify the structure of chromatin and, thus, decelerate gene transcription. In cancer, elevated levels (or enhanced function) of corepressors may be linked to tumor suppression gene silencing. Conversely, reduced levels (or impaired function) of corepressors can result in overexpression of

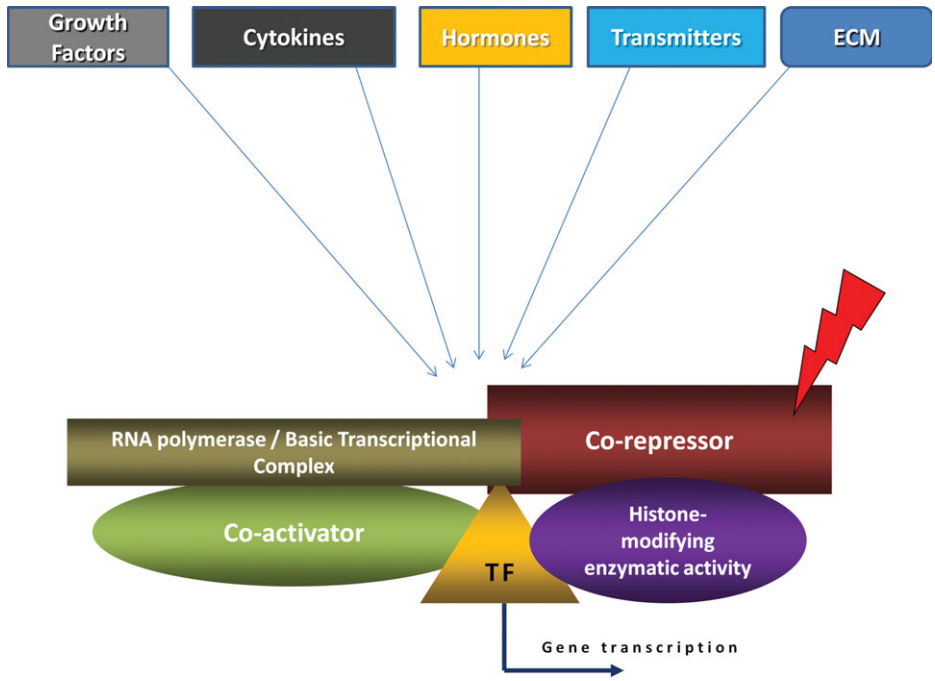

Figure 1. Various extracellular cues can instigate signals that are transduced through the cytoplasm and enter the nucleus to regulate the activity and fine-tune protein-protein interactions of transcriptional regulators in a context-specific and time-specific manner (spatiotemporal control). Targeting selective aspects of corepressor biochemistry can alter chromatin structure/functional dynamics and, thus, influence transcriptional activity of cancer-related target genes. ECM indicates extracellular matrix; TF, transcription factor.

oncogenes. In cancer, the relative "plasticity" that characterizes the normal cell transcriptional process may be disrupted and replaced by a dysfunctional "rigidity" that can stimulate uncontrolled cell proliferation, lack of differentiation, and inability of the cell to undergo apoptosis. Abnormal corepressor expression, localization, structure, and biomolecular interactions in the nuclear microenvironment can translate into the deregulated activity of histone-modifying enzymes, altered histone modification (ie, methylation, acetylation), and either transcription aberration or impairment.[8]

Contrary to fixed gene mutations, epigenetic modifications can be reversible and potentially altered by the administration of small-molecule drugs that can interfere with the structure and functional interactions of transcriptional coregulators, and corepressors in particular (Fig. 1). Many strategies for therapeutic targeting of transcriptional corepressors are plausible (Table 2).[31] Transcriptional coregulators are characterized by modular composition and occasionally by unique presence in cancer cells (ie, fusion oncoproteins). Nevertheless, the pharmacologic manipulation of both transcription factors and transcriptional coregulators remains a difficult task. Traditional drug discovery tools, such as high-throughput screening of compound libraries or de novo drug synthesis by organic chemistry, have failed to generate effective corepressor-targeting compounds that can be used as therapeutic agents. Transcription (co-)factors have convoluted structures with large surfaces; they lack "hot spots" or deep binding sites; and they are located in the nucleus and, thus, are considered "poorer" drug targets. The pharmaceutical industry has indicated little interest in investing in the development of small-molecule drugs against these complicated macromolecules and, instead, has attempted to target upstream ligands, receptors, or kinases that participate in signal transduction. However, this approach lacks specificity and has limited potential, considering the redundancy as well as the highly dynamic, continuous cross-talk among signaling cascades (Fig. 2). Targeting distinct domains/motifs as well as DNA-protein/protein-protein interactions of transcription factors and coregulators (corepressors, in this case) through small molecules can exert more selective effects at the exact site of gene transcription, where the final "transcription decisions" are made by integrating incoming signals (Fig. 2).

Rapid advances in biotechnology, systems biology, translational bioinformatics, and computational chemistry hold the promise of discovering and/or synthesizing sophisticated, targeted agents that can alter the activity of transcriptional corepressors. Structure-based design

TABLE 2. Potential Mechanisms of Corepressor Targeting in Cancer

Translation inhibition (antisense or decoy oligonucleotides, RNAi)
Post-translational modifications
Stability/degradation/recycling/localization/derepression
Protein-protein complex interaction/dissociation
DNA-protein interaction
Direct inhibition of enzymatic or binding domain
"Allosteric" inhibition of enzymatic or binding domain
Artificial corepressors with high specificity (mimics)
Corepressor-related pathways/synthetic lethality

Abbreviations: RNAi, RNA interference.

techniques, such as nuclear magnetic resonance (NMR) spectroscopy, protein-ligand x-ray crystallography, and molecular modeling with robust computational mathematical platforms of ligand docking and conformational analysis, can support the generation of refined compounds that target specific interactions of transcriptional corepressors. The candidate molecules should be further assessed in validated in vitro and in vivo cancer models before they enter clinical investigation in humans.

Novel HDAC inhibitors already have demonstrated clinical benefit and have been officially incorporated into the anticancer armamentarium. Other promising alternatives include artificial transcription (co-)factor mimics, consisting of normal factors conjugated with synthetic compounds targeting DNA-protein or protein-protein interactions. RNA interference (RNAi) approaches and antisense or decoy oligonucleotides also can be used to manipulate transcriptional events. In that regard, the development of novel drug-delivery systems is becoming inevitable, including cell-penetrating peptides (natural or synthetic membrane-crossing molecules), viral delivery vectors (generated from engineered viruses), and nonviral formulations (liposomes, [lipo]polyplexes, nanoparticles). The notion of synthetic lethality offers an additional mechanism for transcriptional corepressor targeting. It is believed that 2 genes (or proteins) are synthetically lethal when concurrent impairment in both results in cell death, whereas either alone can sustain survival. Thus, targeting a gene/protein that is synthetically lethal to a tumor-engaged, abnormal corepressor could selectively harm tumor cells and spare normal cells.[59] Such approaches can increase the therapeutic window and, thus, provide high efficacy with less toxic adverse effects.

There are several examples of transcriptional corepressor targeting. In the clinical arena, vorinostat, a pan-HDAC inhibitor, and romidepsin, a bicyclic pan-HDAC inhibitor, have been approved by the US Food and Drug Administration for the management of relapsed or refractory, cutaneous T-cell lymphoma.[60] Currently, there are

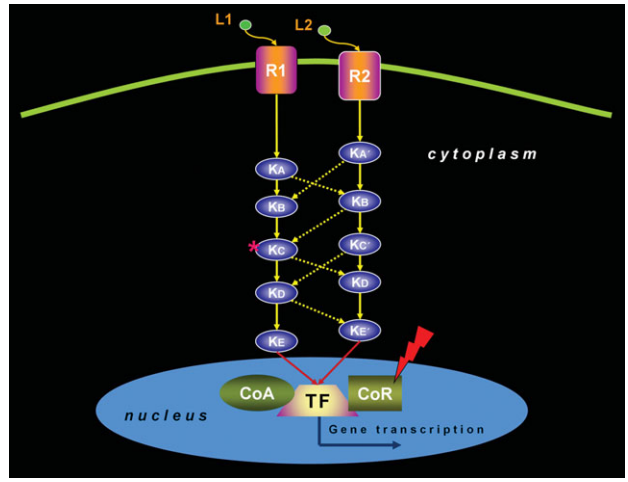

Figure 2. This schematic representation of 2 intracellular signaling pathways illustrates redundancy (ie, kinase B [KB] and KD are used by both) and extensive cross-talk (dotted arrows; a kinase in 1 pathway may use a downstream kinase in the other pathway as substrate). The frequently deregulated in cancer mitogen-activated protein kinase pathway, with its various isoforms, can be viewed as a representative example. Conventional therapeutics target extracellular ligands (L1 and L2; eg growth factors), transmembrane receptors (R1 and R2; eg receptor tyrosine kinases), or kinases (KA-KE, KA′-KE′) that participate in signal transduction rather than transcription factors (TF) (common effector of KE and KE′ activation), coactivators (CoA), or corepressors (CoR). KA through KE and KA′ through KE′ are hierarchical kinase cascades (vertical arrows) in the 2 pathways. The asterisk on KC indicates a putative mutation that may have an impact on its catalytic activity.

210 clinical trials evaluating the role of vorinostat and 50 trials of romidepsin in various hematologic and solid organ malignancies (available at: http://clinicaltrials.gov; accessed October 1, 2012).

In the preclinical setting, it has been demonstrated that NCoR1 expression and activity distort the peroxisome proliferator-activated receptor (PPAR)α/γ gene targets that regulate cell-cycle proteins, such as cyclin-dependent kinase inhibitor 1A (CDKN1A) and TGF-β receptor-associated protein-1 (TRAP1; also TGFBRAP1). Both HDAC inhibition and *ncor1* knockdown augment antiproliferative sensitivity to PPARα/γ ligands in prostate cancer cell lines.[61] In addition, HDAC inhibitors exert antiproliferative and proapoptotic effects on prostate cancer cells, and the implication of HDAC in silencing androgen receptor (AR) signaling has therapeutic potential.[62] These effects appear to be specific for prostate cancer AR-positive cells and correspond to formation of the AR-SMRT complex. Although HDAC inhibitors can be potent anticancer agents, they act against several HDAC family members, potentially resulting in various toxicities.[63] Therefore, it is critical to specify the cancer-related

HDACs in a tumor type and to design selective inhibitors that target specific biochemical interactions.

Considering the expression and role of NCoR in maintaining an undifferentiated neural stem cell state through transcriptional repression, agents that promote NCoR phosphorylation and subsequent translocation to the cytoplasm can result in astroglial differentiation.[64] The treatment of glioma cells with a combination of retinoic acid and low-dose okadaic acid reduced the corepressor effect of NCoR and exerted a significant synergistic effect on growth inhibition, providing the rational for differentiation-based therapeutic strategies in brain tumors.[64] Moreover, a competitive small molecule effectively disrupted NCoR complex function/expression, leading to NCoR cytoplasmic translocation and subsequent tumor cell differentiation and/or death. Therefore, targeting of NCoR function in the cancer stem cell component of gliomas may be beneficial and should be further evaluated.[65] In addition, miRNA-10a/b can affect neural cell differentiation through direct targeting of NCoR2, inducing a major reprogramming of transcriptome, including N-*myc* down-regulation.[66] What is more, genistein, a potent modifier of NCoR protein conformation, induced apoptosis and cell-cycle arrest and promoted cell differentiation in both retinoic acid-sensitive and retinoic acid-resistant APL cells.[67] Genistein up-regulated PML and NCoR expression, resulted in PML-RAR degradation, and reorganized the microspeckled distribution of PML oncogenic domains, underlining the potential value of protein conformation-based therapies in APL. In breast cancer cells, targeting of the adaptor protein Tab2 (TGFβ-activated kinase 1-binding protein 2), which interacts with ERα/NCoR, has been suggested as a novel approach to revert tamoxifen resistance.[68] Another treatment paradigm is to block NCoR2 binding by oncogenic fusion proteins (RUNX1-ETO) and, thus, disrupt the repressor protein complex, restoring cell differentiation in leukemia cells.[69]

The CTBP dehydrogenase substrate MTOB can act as a CTBP inhibitor at high concentrations, causing cytotoxicity and apoptosis through a p53-independent mechanism, as mentioned above.[10] These effects correlate with the derepression of the proapoptotic CTBP repression target Bik (Bcl-2–interacting killer), suggesting that CTBP inhibition may provide a suitable anticancer strategy. In human colon cancer cell peritoneal xenografts, MTOB therapy decreased tumor burden and induced apoptosis. In addition, the potential role of CTBP targeting to reverse breast cancer chemoresistance merits further investigation.[70]

Another example of epigenetic targeting in cancer includes the use of hypomethylating agents (azacitidine, decitabine) in myelodysplastic syndrome and acute myeloid leukemia.[71] Currently, there are 223 clinical trials investigating the role of azacitidine and 127 trials of decitabine in several cancers (available at: http://clinicaltrials.gov; accessed October 1, 2012). However, there is a need to understand the biologic mechanisms that underlie treatment response to these compounds and a need to identify specific epigenetic targets. It is interesting to note that BRMS1 recently was identified as a novel target of epigenetic silencing because of promoter hypermethylation, which supposedly may be susceptible to therapeutic manipulations.[21]

Another way to view the potential clinical usefulness of transcriptional corepressors is their use as prognostic and/or predictive biomarkers. A prognostic biomarker is a surrogate of outcome, such as cancer stage and grade. A predictive biomarker is a surrogate that can yield information about the probability of benefit or toxicity from a specific therapeutic intervention. Examples include the expression of ER concerning response to antiestrogen therapy or HER2 expression regarding response to trastuzumab (anti-HER2 humanized monoclonal antibody) in metastatic breast and gastric cancer. A candidate biomarker can be identified, for instance, through genome-wide association studies that can identify a gene signature, which can distinguish normal tissue from cancer tissue. However, before a corepressor can be considered a suitable biomarker toward better tailored treatment among the available drugs, it needs to display vigorous analytic validity, biologic relevance, and clinical significance through appropriate and rigid testing methods.

To conclude, transcriptional corepressors are considered vital for the regulation of gene transcription, and they often are deregulated in malignant cells, promoting transcriptional "inflexibility." Elucidating the spatiotemporal circuit of DNA/chromatin-transcription factors-coregulators, noncoding RNAs (including miRNAs), epigenetic alterations, and their interactions, in the cancerous state (transcriptome/epigenome mapping of tumor cells from individual patient samples), can open new routes for the development of low-molecular-mass compounds that may re-establish transcriptional "plasticity" and, thus, bridle the anomalous cancer cell behavior. Nonetheless, drug discovery and design tools should undergo major refinement before successful corepressor-targeted approaches demonstrate actual benefits in the clinical setting.

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