Molecular Pathways: Targeting Steroid Receptor Coactivators in Cancer

David M. Lonard and Bert W. O’Malley

Abstract

Coactivators represent a large class of proteins that partner with nuclear receptors and other transcription factors to regulate gene expression. Given their pleiotropic roles in the control of transcription, coactivators have been implicated in a broad range of human disease states, including cancer. This is best typified by the three members of the steroid receptor coactivator (SRC) family, each of which integrates steroid hormone signaling and growth factor pathways to drive oncogenic gene expression programs in breast, endometrial, ovarian, prostate, and other cancers. Because of this, coactivators represent emerging targets for cancer therapeutics, and efforts are now being made to develop SRC-targeting agents, such as the SI-2 inhibitor and the novel SRC stimulator, MCB-613, that are able to block cancer growth in cell culture and animal model systems. Here, we will discuss the mechanisms through which coactivators drive cancer progression and how targeting coactivators represent a novel conceptual approach to combat tumor growth that is distinct from the use of other targeted therapeutic agents. We also will describe efforts to develop next-generation SRC inhibitors and stimulators that can be taken into the clinic for the treatment of recurrent, drug-resistant cancers. Clin Cancer Res; 22(22); 5403-5415. ©2016 AACR.

Background

The nuclear receptors (NR) gene superfamily consists of ligand-activated transcription factors that respond to steroid and other lipophilic hormones, or that in some cases are “orphan” receptors for which no ligand exists or has been identified to date (1). NRs regulate a diverse array of physiologic processes and, as a consequence, are often coopted by cancer cells to drive tumor growth. However, a large body of research has reinforced the notion that NRs cannot drive transcription by themselves and rely on coactivators to act as key intermediaries needed to modify and interpret the epigenome and signaling functions as essential enablers for NR-mediated transcription.

At this time, more than 400 coactivators have been reported in the literature (2). The first identified coactivator, steroid receptor coactivator 1 (NCOA1/SRC-1), was characterized as a protein that can stimulate the transcriptional activities of the progesterone receptor, the estrogen receptor α (ESR1/ERα), and other NRs (3). Two other members of the SRC family, SRC-2 (NCOA2/TIF2/GRIP1) and SRC-3 (NCOA3/ABI1/RAC3/ACTR/pCIP), were subsequently identified (4), and SRCs have been substantiated as the primary platform coactivators that serve as scaffolds for building a multi-protein coactivator protein complex containing co-activator chromatin-modifying enzymes, including CREB-binding protein (CREBBP/CRBP), p300 (EP300), coactivator-associated arginine methyltransferase 1 (CARM1), and other proteins (5). In many cases, these co-activators posttranslationally modify SRGs in addition to histones, altering SRC activity and protein stability (6). In addition to SRCs, these SRC-associated co-activators provide an entry for therapeutic interventions with small-molecule inhibitors (SMI), such as the p300 HAT inhibitor C646 (7).

SRCs in breast cancer

Because of their strong connection with NRs, SRC proteins have been recognized as key oncoproteins in hormone-dependent cancers. For instance, elevated SRC expression and activity is a driving force in the initiation and progression of ERα-positive breast cancers. The SRC-3 gene is amplified in 5% to 10% of breast cancers (8, 9), and the SRC-3 mRNA or protein has been found to be overexpressed by approximately 60% in different breast cancer patient cohorts (8, 10, 11). SRC-3 overexpression was found to be associated with more aggressive breast cancers (9, 12) and poor survival rates (13). Consistent with this, clinical data from breast cancer patients indicate that high SRC-3 expression is associated with decreased disease-free survival (14). In genetically engineered MMTV-v-ras and MMTV-ErbB2 mouse models, loss of SRC-3 reduces breast tumor incidence and delays tumor growth and development (15, 16). In both models, insulin-like growth factor 1 (IGF1) and ERBB2 signaling pathways are impaired in the absence of SRC-3, implicating that kinase signaling cascades independent of ERx are also involved in coactivator-dependent breast cancer tumor progression. Expanding on this idea that SRCs can drive multiple cancer growth signaling systems, SRC-3 has been shown to coactivate other transcription factors, such as the Ets transcription factor polyoma enhancer activator 3 (PEA3), leading to increased expression of matrix metalloproteinase 2 (MMP2) and MMP9, leading to metastasis (17).

The multifunctional role for SRC-3 as a broad integrator of additional cancer cell growth programs is further reinforced by the finding that a splice variant of SRC-3 (SRC-3Δ4) functions as a...
Role of SRCs in endocrine therapy–resistant breast cancers
SRC-3 has been implicated in clinical resistance to tamoxifen and aromatase inhibitors, and this has been traditionally attributed to the role that SRC-3 has in stimulating growth factor signaling pathways, circumventing the inhibition of ERα in breast cancer cells (19, 20), consistent with the pleiotropic actions that we have described for SRC-3 above. Recent studies that have sought to uncover genomic alterations that underlie endocrine therapy resistance have provided additional layer of detail pointing to a role for SRC-3 in recurrent breast cancer. Sequencing of tumors from breast cancer patients that have acquired resistance to aromatase inhibitors has revealed the presence of recurrent mutations in the ligand-binding domain of the ERα gene, resulting in activation of the receptor in the absence of estrogens (21). These mutant receptors also are refractory to the pure antiestrogen fulvestrant, and considerable effort has been directed toward the development of next-generation, orally available selective estrogen degraders (SERD) to target these mutant ERα proteins (22). Importantly, these mutant ERα proteins interact with SRCs in the absence of estrogens, pointing to a vital role for SRCs in driving hormone therapy–refractory breast cancers that harbor ESRR mutations (23). We speculate that in addition to the development of newer and more potent SERDs, SRC SMIs may prove to be powerful agents when used in combination with SERDs to better target the transcriptional function of these gain-of-function mutant ERα proteins (Fig. 1).

SRCs in prostate, endometrial, and ovarian cancer
A comprehensive sequencing analysis of human prostate tumors found that 8% of primary and 37% of metastatic tumors either have amplifications of the SRC-2 gene or overexpress its mRNA (24). Primary prostate tumors with SRC-2 gain of function have elevated androgen receptor (AR) signaling, consistent with the known role of SRC-2 as an AR coactivator. These findings are in agreement with earlier studies that showed a correlation between SRC-2 expression and high tumor grade and poor survival (25–27). Interestingly, SRC-2 has been shown to be a key regulator of energy homeostasis (28–31), and this fact has been linked to its role as a regulator of prostate cancer metabolic programming. Specifically, SRC-2 drives glucose-dependent and sterol-regulatory element–binding protein 1 (SREBP1)–mediated de novo lipogenesis that enhances prostate cancer cell survival and metastasis (32). SRC-3 and SRC-1 have also been reported to be overexpressed in prostate cancers, and their expression levels have been positively correlated with tumor grade and disease recurrence (33).

As coactivators for ERα, SRCs have been implicated in other estrogen-related cancers, such as endometrial and ovarian cancer. mRNA levels of all three SRCs are significantly increased in endometrial carcinoma (34), and SRC-3 expression is correlated with clinical stage, depth of myometrial invasion, differentiation, and poor prognosis (35, 36). Earlier on, SRC-3 was found to be bridging adaptor between the EGFR and focal adhesion kinase (FAK), potentiating cancer cell migration and invasion in a non-genomic manner (18).
amplified in ovarian cancer (6, 9), and elevated SRC-3 expression has been found in 64% of high-grade ovarian cancers, and its levels are correlated with tumor progression (37). SRCs have also been implicated in a wide variety of cancers, including pancreatic (38, 39), colon (40), lung (41), and other cancers (2).

**Clinical–Translational Advances**

As coactivators for NRs, SRCs are essential components of steroid hormone signaling, providing a strong impetus to develop SRC-targeting agents for the treatment of endocrine-related cancers. Moreover, as described above, SRCs also function as coactivators for other transcription factors, including NF-κB (NFKB1; ref. 42), SREBP1 (32), PEA3 (43, 44), and AP-1 (JUN; refs. 45, 46), making the potential for SRC-targeting drugs pertinent to a wide range of cancers where steroid hormone signaling is not directly implicated.

An important concept related to the oncogenic role for SRCs in cancer revolves around their roles as integrators of growth factor kinase signaling cascades and growth-promoting transcriptional programs utilized by cancer cells. For instance, SRC-3 sits at a nexus for MAPK, FAK, ABL, and GSK3β (GSK3B) kinase signaling cascades that phosphorylate distinct residues on the SRC-3 protein that forms a phosphorylation code that determines its ability to interact with different classes of transcription factors (47). An important corollary of this fact is in contrast to most targeted therapeutic agents, inhibition of SRC-3 function can simultaneously interfere with multiple growth-related signaling pathways and provides promise to deny the cancer’s ability to develop acquired resistance through the activation of alternative escape pathways (Fig. 1).

**Development of SRC-targeting agents**

Because SRCs lack a high-affinity ligand-binding domain and primarily function as a scaffold for the assembly of co-activators through protein–protein interactions, SRCs have been considered difficult drug targets. However, given their key roles as prominent oncoproteins, this challenge is outweighed by the potential therapeutic benefit that SRC-targeting drugs may bring. Earlier on, several groups performed compound screening campaigns to develop agents to disrupt the binding of SRCs to NRs, such as ERα, ERβ (ESR2), and peroxisome proliferator–activated receptor γ (PPARγ; refs. 48–50); however, these screens specifically focused on targeting the NR-coactivator protein–protein interface. Some additional efforts have been made to identify and develop additional disruptors of SRC-NR binding, and some structure–activity relationship information has been obtained for these molecules (51). However, additional work is needed to identify compounds with greater potency, and none of these are currently ready to take into the clinic.

In contrast, our laboratory has focused on targeting SRC protein directly, and we identified gossypol as a proof-of-concept SMI for SRC-1 and SRC-3, for the first time establishing that SRC proteins can be directly targeted by small-molecule compounds (52). In a subsequent high-throughput screen of a large chemical library containing compounds from the large NIH-Molecular Libraries Probe Production Centers Network, improved SRC SMIs were identified, including bufalin and verrucarin A (53, 54). Bufalin promotes the degradation of SRC-3 and SRC-1 in a proteasome-dependent manner and efficiently blocks cancer cell growth in vitro and in vivo. A water-soluble analogue, 3-phospho bufalin, was developed to overcome the solubility problem of bufalin, and it was proved to be equally efficient in inhibiting tumor growth in an orthotopic breast cancer model (55). Importantly, additional investigations of compounds identified from this high-throughput screen led to the identification and medicinal chemistry development of additional compounds with stronger drug-like properties, such as SI-2 (56). SI-2 is able to selectively kill cancer cells at low nanomolar ranges (3–20 nmol/L) and can inhibit primary tumor growth in a triple-negative breast cancer xenograft tumor model. Pharmacologic parameters of SI-2 and structurally related compounds have been determined, revealing that SI-2 is rapidly cleared from the bloodstream in mice. Ongoing effort is being made to identify SI-2 derivatives with improved stability in vivo.

**SRC small-molecule stimulators: Oncogene hyperstimulation as a novel paradigm for cancer therapy**

Interestingly, the quest for SRC small molecule–targeting agents has led to an unexpected turn and a novel concept for cancer therapy. High-throughput screening led to the discovery of MCB-613, a SRC small-molecule stimulator (SMS; ref. 57). MCB-613 hyperstimulates SRC oncoproteins and can selectively kill cancer cells and inhibit tumor growth in a breast cancer xenograft mouse model. MCB-613 increases SRCs’ interaction with other coactivators, such as CBP and CARM1, and by strongly hyperstimulating SRC-related growth programs, it induces proteostasis and reactive oxygen species stress that is well beyond that which cancer cells can cope. Considering the oncogenic roles of SRCs, it appears to be counterintuitive to employ SRC SMSs in treating cancer. However, in comparison with normal cells, cancer cells are already under high levels of stress from increased protein synthesis/folding and metabolism due to their highly proliferative nature. It is of the utmost importance for them to fully engage their stress response pathways to maintain homeostasis, making them more vulnerable than normal cells to stressors, such as MCB-613. By acutely overstimulating SRC family proteins, MCB-613 overloads the stress response system of cancer cells and selectively kills them. Thus, targeting the SRC coactivators, either by inhibition or stimulation, represents novel and promising approaches for cancer therapeutic development. The identification of MCB-613 not only points to additional ways to target SRCs but suggests that strategies that seek to stimulate other oncogenes represent an unexploited space for future drug development that might bear fruit as well.

**Conclusions**

Coactivators are necessary for NRs and other transcription factors to drive target gene expression and as such, are frequently exploited by cancer cells to drive proliferation, invasion, and other oncogenic programs. By integrating and promoting growth signaling pathways used by cancer cells, SRCs represent emerging targets for cancer therapeutics, and SRC-targeting agents have been identified that show promise as potential new cancer therapies. Because SRCs have been found to be overexpressed in such a wide breadth of cancer types, SRC SMIs and SMSs may ultimately find broad use in the clinic (5). SRCs represent a novel group of oncoprotein drug targets not currently represented in the armamentarium of clinically available drugs, but ongoing drug development efforts promise to
address this unmet clinical need. In a conceptual sense, SRCs’ key roles as growth factor and steroid hormone signaling integrators also make them unique targets for next-generation SRC SMs that can disrupt communication across these steroid and kinase growth-simulating pathways as well as for SRC SMs that can kill cancer through coactivator hyperstimulation-induced tumor cell stress.

**Disclosure of Potential Conflicts of Interest**

D.M. Lonard reports receiving commercial research support from Coregon, Inc. B.W. O’Malley reports receiving commercial research grants from and has ownership interest (including patents) in Coregon, Inc. No other potential conflicts of interest were disclosed.

**References**


**Authors’ Contributions**

**Conception and design:** D.M. Lonard, B.W. O’Malley

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Coactivator Involvement in Cancer

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