

SRC-3: a transcriptional coactivator and therapeutic target in cancer

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Abstract:

Steroid receptor coactivator-3 (SRC-3), also known as amplified in breast cancer 1 (AIB1), a transcription coactivator, is frequently overexpressed in multiple type of human cancers, including breast, ovarian, prostate, pancreatic, lung, and colorectal cancers. SRC-3 not only interacts with multiple nuclear hormone receptors but also broad-spectrum of non-nuclear-receptor transcription factors to enhance their transcriptional activities. Furthermore, dynamic posttranslational modifications of SRC-3 efficiently modulate this protein in different signaling pathways, and regulate its coactivator activity during transcription. In addition, SRC-3 modulates primary tumor initiation and expansion, and also plays important roles during tumor cell motility and invasion, and metastasis. As previous studies has validated SRC-3 as an oncogene, and indicated that SRC-3-dependent cancers are frequently resistant to traditional chemotherapeutics, the development of small molecule inhibitors that target SRC-3 becomes a feasible way to develop novel anti-cancer agents.

Introduction

Steroid receptor coactivator-3 (SRC-3, also known as NCOA3, AIB1[1], TRAM-1[2,3], RAC3[4,5], pCIP[6,7], and ACTR[8]), is a member of the p160 steroid receptor co-activator (SRC) family, which includes SRC-1[9] and SRC-2 (TIF2)[10]. Recently, SRC-3 has gradually been a research hotspot, because growing evidence has indicated that overexpression of SRC-3 could promote cancer initiation and progression. In this review, we will focus on functions of SRC-3 in multiple signal transduction pathways, carcinogenesis, and tumor metastasis; posttranslational modifications of SRC-3; and

discuss its potential as an anticancer therapeutic target.

Structural characteristics of SRC-3

SRC-3 gene localizes on the chromosomal region 20q12. The protein SRC-3 contains three functional domains, including an N-terminal basic helix-loop-helix (bHLH)/Per/Arnt/Sim (bHLH-PAS) domain, a nuclear receptor interaction domain (NRID), and a C-terminal CREB-binding protein (CBP)/p300 interaction domain (CID) (Figure 1). The bHLH-PAS domain can interact with some transcription factors such as myogenin, MEF-2C and transcriptional enhancer factor

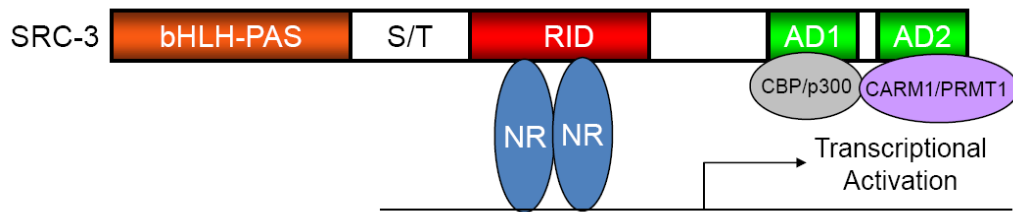


Figure 1. Structure of SRC-3.

(TEF) to regulate their transcription activities[11,12]. The central NRID domain contains three LXXLL motifs, forms amphipathic α -helices, and interacts with multiple nuclear receptors[13-15]. The C-terminal CID domain contains two transcriptional activation domains AD1 and AD2. AD1 binds to p300/CBP, and AD2 interacts with two histone methyltransferases, coactivator-associated arginine methyltransferase 1 (CARM1) and protein arginine methyltransferases (PRMT1)[16-19]. Based on SRC-3 protein structure, it could be an adaptor protein to recruit transcription factors and coactivators, form transcriptional machinery on gene promoter, and regulate downstream target gene transcription.

SRC-3 functions in signal transduction pathways

A number of studies demonstrated that SRC-3 interacts with multiple nuclear hormone receptors to enhance their transcriptional activities, such as estrogen receptor (ER)[20], androgen receptor (AR)[3], progesterone receptor (PR)[21], and thyroid receptor[22]. Estrogen receptor (ER) and progesterone receptor (PR) status are closely related to tumorigenesis in breast, prostate and ovarian cancer[23]. SRC-3 is a steroid receptor coactivator and is critical for ER and PR functions[24-26]. SRC-3 stimulates ER-dependent gene expression[1,27], and also plays an important role in estrogen-mediated breast cancer progression. Cyclin D is an ER

target gene, and SRC-3 upregulates cyclin D expression through interaction with estrogen receptor onto cyclin D1 promoter[28]. On the other hand, downregulation of SRC-3 protein level in ER-positive MCF-7 human breast cancer cells results in reduction of estrogen-stimulated cell proliferation and survival[29] and growth inhibition of MCF-7 xenograft tumors[26]. Based on these findings, SRC-3 is critical for activity of ER and other hormone receptors.

Multiple studies have demonstrated that SRC-3 can also act as coregulators for a broad-spectrum of non-nuclear-receptor transcription factors, including AP-1, NF- κ B, STAT-6, and E2F1 etc.[15]. SRC-3 can interact with and regulate functions of other transcription factors, and play important roles in hormone-independent signal transduction pathways, including IGF-1/Akt signal pathway[30-35], NF- κ B signaling pathway[36,37], EGFR signaling pathway[38], E2F1 signaling pathway[29,39], and MAPK signal pathway[40-43]. Extensive evidence reveal that SRC-3 overexpression correlates with the absence of ER and PR in breast cancer[44], and also involved in many types of tumors that are not targeted by steroid hormones[45-47]. Yan et al.'s results showed that SRC-3 and transcription factor activator protein-1 (AP-1) can coordinately recruit to target gene promoters to regulate the transcription of proteins in the IGF/Akt pathway to promote cancer cell proliferation and survival[32]. It is reported that SRC-3 may

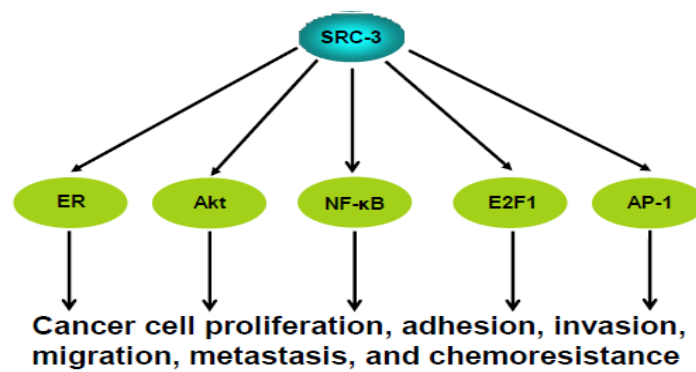


Figure 2. SRC-3 integrates with multiple signal transduction pathways.

not only bind to the active form of NF- κ B as a coactivator, but also interact with the I κ B kinase, induce degradation of I κ B, and then enhance NF- κ B-mediated gene expression[36,48]. Arimura *et al.* demonstrated that SRC-3 interacts with STAT-6 via p300/CBP, and enhance STAT-6 transcriptional activity through formation of a SRC-3, p300/CBP, and STAT-6 transcription complex[49]. Louie *et al.*'s data indicated that SRC-3 directly interacts with E2F1 through its N-terminal domain, and then is recruited to E2F1 target gene promoters as an E2F1 coactivator to promote breast cancer cell proliferation and antiestrogen resistance[29]. Moreover, SRC-3 also functions as an Esrrb coactivator to affect the Oct4-Sox2-Nanog circuitry in embryonic stem cell self-renewal and reprogramming[50]. Taken together, SRC-3 plays important roles in many aspects of physiology and pathology that are both dependent and independent on hormone signaling.

It has been well known that SRC-3 interacts with multiple nuclear hormone receptors and other transcription factors to enhance their transcriptional activities during tumorigenesis (Figure 2). SRC-3 overexpression could promote tumor initiation and progression by regulating various important signal transduction pathways, including hormone-dependent and independent pathways.

Therefore, overexpression of SRC-3 can affect various signal transduction pathways, other than hormone hormone-dependent pathways, and facilitate cancer cell proliferation, survival and metastasis.

Posttranslational modifications of SRC-3

A number of studies have shown that various extracellular stimuli such as hormones, growth factors and cytokines might induce multiple posttranslational modifications of SRC-3, including phosphorylation, ubiquitination, sumoylation, acetylation and methylation. Different posttranslational modifications of SRC-3 determine its protein stability and transcriptional activity. Dynamic posttranslational modifications of SRC-3 selectivity allow this protein to integrate different signal transduction pathways, and affect SRC-3's potency and regulation of different downstream target gene expression.

Multiple kinases can phosphorylate and activate SRC-3, including MAPKs, IKKs, and PKA[37]. Seven Ser/Thr (Thr24, Ser505, Ser543, Ser601, Ser857, Ser860 and Ser867) and one Tyr (Tyr1357) phosphorylation sites of SRC-3 are critical for its function[37,51,52]. Wu *et al.* demonstrated that selective phosphorylations of the SRC-3 involve in multiple signaling pathways. All six phosphorylation sites (Thr24, Ser505, Ser543, Ser857, Ser860 and Ser867) are

necessary for estrogen and androgen receptor coactivation, but not all sites for NF- κ B coactivation[37]. In addition, TNF- α induced IL-6 gene expression is dependent on different combinations of site-specific phosphorylations of SRC-3[37]. Moreover, CK1 δ interacts with and phosphorylates SRC-3 at the site Ser601, modulates ER α -SRC-3 interactions and regulates the transcriptional activity of ER α through SRC-3[51]. SRC-3 is also a target of the c-Abl (v-Abl Abelson murine leukemia viral oncogene homolog 1) tyrosine kinase that can phosphorylate SRC-3 at a C-terminal tyrosine residue Tyr1357 after estrogen and growth factors treatment. Phosphorylation of Tyr1357 by c-Abl modulates the association of SRC-3 with p300 and CARM1, and facilitates SRC-3 coactivation of ER α , PR, NF- κ B, and AP-1-dependent transcription. Additionally, Tyr1357 phosphorylation is increased in HER2/neu breast tumors[52]. Long et al. showed that an atypical MAP kinase ERK3 interacted with and phosphorylated SRC-3 at Ser857, promoted PEA3-mediated upregulation of MMP gene expression and invasion in lung cancer cells[40]. Overall, SRC-3 can function as a molecular switch and mediate the cross talk between hormones, growth factors and kinase signal transduction pathways in cancer.

SRC-3 phosphorylation is not only important in association with other transcription factors and cofactors, but also in regulation of SRC-3 protein stability. Atypical protein kinase C (aPKC) phosphorylates and specifically stabilizes SRC-3 via targeting an acidic region of amino acid residues 1031–1097. In addition, aPKC inhibits SRC-3 degradation through disrupting the interaction between SRC-3 and the C8 subunit of the 20S proteasome[53]. Therefore, SRC-3 can be more stable through phosphorylation. However, dephosphorylation might be also a potential regulatory mechanism for SRC-3. Li et al. reported that the

phosphatases PDXP, PP1, and PP2A were negative regulators of SRC-3. PDXP and PP2A dephosphorylate SRC-3, inhibit its interaction with ER and transcriptional activity. Furthermore, PP1 stabilizes SRC-3 protein through dephosphorylation of two sites (Ser101 and Ser102) and decreases SRC-3 transcriptional activity[54]. Taken together, regulation of SRC-3 phosphorylation plays an important role in its transcription coactivator functions.

Abundant evidences have indicated that stability of SRC-3 can be affected by ubiquitination. Several ubiquitin E3 ligases have been demonstrated to mediate SRC-3 ubiquitination and degradation. E6AP regulates proteasomal degradation of SRC-3[55]. CHIP, a U-box-type ubiquitin ligase, promotes SRC-3 degradation and inhibits anchorage-independent cell growth and metastasis[56]. Li et al. showed that speckle-type POZ protein (SPOP), a cullin 3 (CUL3)-based ubiquitin ligase, induces SRC-3 ubiquitination through directly interacting with SRC-3 in a phosphorylation-dependent manner. Casein kinase I ϵ might phosphorylate the site Ser102 and promotes SPOP-mediated degradation of SRC-3[57]. Moreover, Cullin 3 regulates SRC-3 ubiquitination and degradation after retinoic acid (RA) treatment, and this RA-induced SRC-3 ubiquitination depends on its phosphorylation at Ser860[58]. Although these E3 ligases above can regulate SRC-3 ubiquitination and degradation, interestingly, Wu et al. demonstrated that ubiquitination of SRC-3 regulated by SCF (Fbw7 α) is a phospho-mediated biphasic event, and a transition from multi-(mono)ubiquitination (SRC-3 activation) to long-chain polyubiquitination (SRC-3 degradation) might control both the activation and degradation of SRC-3[59]. Overall, both stability and activity of SRC-3 can be regulated via ubiquitination.

In addition to phosphorylation and ubiquitination,

recent studies have indicated that sumoylation is another important posttranslational modification of SRC-3. The small ubiquitin-related modifier (SUMO) is attached to a lysine residue in a ψ KXE sequence (ψ is a large hydrophobic residue and X represents any amino acid) of a substrate protein through a process similar to ubiquitination dependent on activating (E1), conjugating (E2), and ligating (E3) enzymes[60]. It has been reported that SRC-3 can be sumoylated at sites Lys723, Lys786, and Lys1194, and its transactivation activity might be attenuated by sumoylation[61]. In addition, sumoylation of SRC-3 is negatively correlated with MAPK-mediated phosphorylation. Therefore, phosphorylation and sumoylation coordinately regulate the transcriptional activity of SRC-3. Moreover, although SUMO has similar structure as ubiquitin, sumoylation can antagonize ubiquitination by targeting a common lysine site to prevent protein degradation[15].

Moreover, the function of SRC-3 can be modulated by acetylation and methylation. SRC-3 interacts with nuclear hormone receptors and regulates transcription by directly recruiting other cofactors to gene promoters, such as the acetyltransferase CBP/p300 and the coactivator arginine methyltransferase CARM1. SRC-3 is acetylated by p300/CBP after transcriptional initiation, and the acetylation neutralizes the positive charges of two lysine residues adjacent to the LXXLL motif and results in disassociation of SRC-3 coactivator complexes with estrogen receptors[62]. SRC-3 can be also methylated by CARM1 on Arg1171 in its CARM1 binding region. This methylation attenuates the association between SRC-3 and p300/CBP, disassembles the SRC-3 coactivator complex, decreases ER α -mediated transcription, and increases SRC-3 degradation[63,64]. Collectively, the activity and stability of SRC-3 are modulated by p300/CBP-dependent acetylation and CARM1-dependent methylation.

Postranslational modifications greatly influence both the stability and the coactivator activity of SRC-3, and these modifications are frequently combined and sequential. Initially, SRC-3 is sumoylated at amino acids 723 and 786[59] and hyperphosphorylated[37] as an inactive isoform. When SRC-3 is phosphorylated at Ser505/509 in a GSK3-dependent manner, SRC-3 becomes mono-ubiquitinated at amino acids 723 and 786, and then functions as a transcriptional coactivator[59]. Polyubiquitinated SRC-3 will be degraded by the 26S proteasome[65]. In summary, multiple posttranslational modifications of SRC-3 determine its inactivation, transcriptional activity, and degradation. Dynamic posttranslational modifications of SRC-3 efficiently modulate this protein in different signaling pathways, and regulate transcription of various downstream target genes.

SRC-3 regulates cell motility and invasion

SRC-3 is firstly reported to be involved in cell invasion and migration in *Drosophila* ovary. A *Drosophila* gene Taiman, which encodes a protein related to SRC-3, regulates the migration of follicle cells and border cells in the *Drosophila* ovary[66]. Similar functions of SRC-3 have also been found in mammalian cell motility and invasion. SRC-3 expression was low in normal ovarian epithelium and ovarian tumors that show less stromal invasion. However, SRC-3 was overexpressed in high-grade ovarian tumors. After SRC-3 expression was inhibited, ovarian cancer cells remarkably failed to exhibit cell spreading and migration, which was independent of its ER signaling[67].

In the case of breast cancer, SRC-3(-)/PyMT mice have significantly reduced mammary tumor lung metastasis compared with WT/PyMT mice[68]. Furthermore, SRC-3(-)/PyMT tumor

cells maintain the expression of epithelial markers, show disruption of EMT process and inhibition of cell migration and invasion. Moreover, SRC-3 functions as a coactivator of the ETS transcription factor PEA3, and promotes MMP-2 and MMP-9 expression in mouse breast cancer cells. In addition, SRC-3 expression is positively associated with PEA3, MMP-2, and MMP-9 in human breast tumors[68]. SRC-3 also serves as a coactivator of AP-1 driven MMP-7 and MMP-10 expression[69]. Overall, these data suggested that SRC-3 plays an important role in breast tumor invasion, migration, and metastasis.

SRC-3 also plays an important role in prostate cancer cell invasion and metastasis. SRC-3 is positively associated with human prostate cancer cell migration, invasion, and lymph node metastasis[70]. Furthermore, SRC-3 is necessary for focal adhesion turnover and focal adhesion kinase activation. Moreover, SRC-3 regulates MMP-2 and MMP-13 transcription via AP-1 and PEA3[70]. Similarly, atypical MAPK ERK3 interacted with and phosphorylated SRC-3 at Ser857, mediated association of SRC-3 and PEA3, promotes upregulation of MMP-2 and MMP-10, and induces human lung cancer cell invasion both in vitro and in vivo[40].

Previous extensive studies have demonstrated that full-length SRC-3 modulates cancer cell invasion-metastasis cascade. However, new research findings reveal that a truncated isoform of SRC-3 named SRC-3Δ4 indicates similar effects on cancer cell migration. PAK1-mediated phosphorylation of SRC-3Δ4 promote the localization of SRC-3Δ4 to the plasma membrane, then SRC-3Δ4 serves as a critical adaptor that links EGFR and FAK, and promotes EGF-induced phosphorylations of FAK and c-Src[38]. Furthermore, knockdown of SRC-3Δ4 significantly blocks EGF-induced

c-Src activation and FAK phosphorylation. In addition, SRC-3Δ4 overexpression promotes breast tumor metastasis to lung in a xenograft model[38]. Importantly, clinical data indicated that SRC-3Δ4 expression levels are elevated in human breast cancer specimens[24]. In this regard, SRC-3Δ4 is a predominant regulator for cell motility and invasion during mammary tumor metastasis.

According to these findings, SRC-3 plays important roles in primary tumor initiation and expansion, and functions as a critical coregulator and signal transduction adapter during tumor cell motility and invasion, followed by tumor metastasis[71].

Clinical analysis of SRC-3 overexpression in cancer

SRC-3, localized on a frequently amplified chromosomal region 20q12[1,72,73], is associated with multiple types of cancers, including breast[74], gastric[46], pancreatic[45], ovarian[73], prostate[75], colorectal[76] and esophageal squamous cell carcinomas[77]. Amplification and/or overexpression of SRC-3 have been found in human breast tumors[1,44,78], and correlates with poor disease-free survival[79]. Higher SRC-3 levels are also preferentially present in higher grade invasive tumors[80], and Young et al.'s results also indicate an association between high SRC-3 levels and breast cancer recurrence[81]. Moreover, SRC-3 overexpression is associated with high levels of HER-2/*neu* and tamoxifen resistance in breast cancer patients[44,81-83]. These results suggest that SRC-3 is involved in steroid-targeted tumors. However, SRC-3 overexpression is not only detected in various hormone-sensitive tumors, but also correlated with the absence of ER and PR in breast cancer[44]. Consistently, SRC-3 is also

involved in many types of hormone-independent tumors, such as pancreatic cancer[45,84], colon cancer[76], gastric cancer[46], and liver cancer[47]. These data reveal that SRC-3 can also interact and function through other transcription factors during hormone-independent tumorigenesis. Moreover, SRC-3 is also associated with tumor metastasis and recurrence in gastric and liver cancer[46,47]. Overall, these clinical data reveal that SRC-3 might play an important role in human tumorigenesis in both hormone-dependent and -independent manners.

SRC-3 as a therapeutic target in cancer

SRC-3 overexpression has been demonstrated in a variety types of cancers. SRC-3 is a coactivator for various nuclear hormone receptors and other transcription factors, and simultaneously controls multiple cellular signal transduction pathways. Functional studies indicated that SRC-3 can promote cancer initiation, expansion, and metastasis, suggesting that SRC-3 intervention could be a promising strategy for cancer therapy. A recently discovered SRC-3 small molecule inhibitor gossypol provides a novel and important therapeutic approach for many types of human cancers that overexpress SRC-3 for oncogenesis. Wang et al. showed that gossypol can reduce cellular SRC-3 protein concentration through directly binding to receptor interacting domain of SRC-3[85]. Gossypol degrades SRC-3 protein in multiple types of cancer cells. Gossypol preferentially inhibits cell growth in cancer cells but not in primary hepatocytes. In addition, gossypol can sensitize cancer cells to growth factor receptor inhibitors, such as EGFR inhibitor AG1478 and IGF-IR inhibitor AG1024[85]. In this regard, gossypol and its derivatives[86] could be candidate small molecules targeting SRC-3. Therefore, new

approaches that combine existing targeted therapy with SRC-3-targeting drugs might be more effective against cancers.

Most chemotherapeutic drugs can only block a single signaling pathway, and their monotherapeutic clinical efficacy is frequently limited. Cancer cells frequently become adaptable and resistant to individual anticancer agents by activating alternative pathways. For example, in response to treatment with anti-estrogens, PI3K-Akt or NF- κ B pathways are frequently activated in breast cancers to promote cell growth[87]. However, SRC-3 is a central integrator of multiple steroid hormone and cancer-related growth factor signaling pathways[88]. Therefore, small molecule inhibitors that interrupt its coactivator function should simultaneously prevent the activation of multiple signaling pathways that could promote cancer chemotherapy resistance. According to these findings, SRC-3 might be an attractive target for novel anticancer drug discovery.

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