RESEARCH ARTICLE

Steroid Receptor Coactivator-3 Promotes Bladder Cancer Through Upregulation of CXCR4

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Abstract

  The three homologous members of the p160 SRC family (SRC-1, SRC-2 and SRC-3) mediate the transcriptional functions of nuclear receptors and other transcription factors, and are the most studied of all the transcriptional co-activators. Recent work has indicated that the SRC-3 gene is subject to amplification and overexpression in various human cancers. Some of the molecular mechanisms responsible for SRC overexpression, along with the mechanisms by which SRC-3 promotes breast and prostate cancer cell proliferation and survival, have been identified. However, the function of SRC-3 in bladder cancer remains poorly understood. In the present study, our results indicate that overexpression of SRC-3 promotes bladder cancer cell proliferation whereas knockdown of SRC-3 results in inhibition. At the molecular level, we further established that CXCR4 is a transcriptional target of SRC-3. Therefore, our study first identified that SRC-3 plays a critical role in the bladder cancer, which may be a target beneficial for its prevention and treatment.

Keywords: SRC-3 - bladder cancer - bladder cancer - CXCR4

Introduction

  The worldwide estimate for new cases of bladder cancer is 261,000 annually, while the causes of bladder cancer remain unknown in most cases (Parkin et al., 2008). Nuclear receptors (NR) belong to a large superfamily of ligand-regulated (and orphan) transcription factors that transduce steroid, retinoid, thyroid hormone, and lipophilic endocrine signaling into distinct physiological responses (Lonard et al., 2012). Agonists binding to NR lead to the recruitment of coactivator proteins that are essential for their transcriptional activity. Steroid receptor coactivator (SRC)-1 was first identified to interact with NR in a ligand-dependent manner to enhance their transcriptional function (Xu et al., 2009). Soon after this, two other proteins, transcriptional intermediary factor-2/SRC-2 and amplified in breast cancer-1/SRC-3 (SRC-3) were cloned as NR coactivators that comprise the SRC coactivator family (Xu et al., 2009; Gojis et al., 2010). Besides, the SRC family functions as coactivators not only for NR but also for multiple other transcriptional factors (TF), such as nuclear factor kappa B, E2F1 and IGF-1-dependent TFs (York et al., 2010; Ma et al., 2011; Walsh et al., 2012). By coactivating NR and these NFs, all members of the SRC family can modulate diverse genes expression programs and play important roles in growth, metabolism, reproduction and turmorigenesis (Wu et al., 2007; Coste et al., 2008; Yi et al., 2008; Cai et al., 2010).

  In breast cancers, SRC-3 gene amplification has been found in 9.5% of cancer tissues, and its mRNA was found to be overexpressed as high as 64% of the time (Lydon et al., 2011). Forced overexpression of SRC-3 in mammary epithelial cells has been shown to be sufficient to promote mammary tumor formation, directly indicating it in breast cancer initiation (Lanz et al., 2010). Consistently, mice with SRC-3 deletion had suppressed oncogene- and carcinogen-induced breast cancer initiation, progression, and metastasis (Kuang et al., 2004; Yi et al., 2013). Moreover, overexpression of SRC-3 has been frequently observed in a variety of other cancer types including lung, ovarian, liver, colorectal, pancreatic, and prostate cancers (Long et al., 2012; Palmieri et al., 2013; Geng et al., 2013). However, the biological function of SRC-3 in bladder cancer remains unexplored until now.

Materials and Methods

Tissue samples
  Primary bladder cancer tissues, adjacent normal tissues were collected from routine therapeutic surgery at our department. All samples were obtained with informed consent and approved by Shanghai Changning Center Hospital

Cell culture
  Bladder cancer cells (EJ cells and T42 cells) used

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were purchased from The Cell Bank of Type Culture Collection of Chinese Academy of Sciences (CAS, Shanghai), and cultured in RPMI 1640 medium (Gibco, USA) supplemented with 10% fetal calf serum (Beyotime, Shanghai, China), 100 IU/ml penicillin and 100 mg/ml streptomycin (Gibco, USA).

Adenoviruses construction and transfections

We used Ad5 (full name: tumor-specific replication-defective adenovirus type 5) as the vector. Ad5-EV and Ad5-SRC-3 were constructed by standard molecular cloning. For transfection, cells were cultured in 6-well plates and exposed to viral supernatant in the absence of cytokines and serum with different multiplicities of infection (MOIs): the number of plaque-forming units (pfu) per cell. The efficiency of transfection was estimated by determining the percentage of enhanced green fluorescence protein (EGFP)-positive cells in cells infected with Ad5-EGFP. After transfection for 3 days, half of the virus-containing medium was replaced for the first time, and then plates were further incubated and all the medium was changed every 2 days.

siRNA, RNA extraction and real-time analysis

Cells were seeded on to 6-well plates then transfected with 20nM siGENOME non-targeting siRNA, human SRC-3 (Dharmacon, USA). Total RNAs were isolated from tissues or cells by TRIzol reagent, and reverse transcriptions were performed by Takara RNA PCR kit (Takara, China) following the manufacturer's instructions. In order to quantify the transcripts of the interest genes, real-time PCR was performed using a SYBR Green Premix Ex Taq (Takara, Japan) on an ABI 7300 machine (ABI, Life science).

Western blot

Cells were harvested by trypsinization, lysed in 23 Laemmli buffer (100 mM Tris-HCl at pH 7.0, 200 mM DTT, 4% SDS [w/v], 20% glycerol, 0.05% bromophenol blue), denatured for 10 min at 80°C, sheared with an insulin syringe, and resolved on SDS/PAGE gels. After immunoblotting, the membranes were blocked in PBS/0.1% Tween-20 with 10% nonfat dry milk, and primary antibodies were incubated in PBS/0.1% Tween-20 with 10% nonfat dry milk. Antibodies directed against SRC-3, CXCR4 and GAPDH were purchased from Santa Cruz Biotechnology (Santa Cruz, USA).

Statistical Analysis

All data are presented as mean ± SEM. Statistical differences were determined by a two-tailed t test. Statistical significance is displayed as *($P < 0.05$), **($P < 0.01$) or ***($P < 0.001$).

Results

Up-regulation of SRC-3 is a frequent event in human bladder cancer tissues

First, we analyzed SRC-3 expression in 28 paired bladder cancer tissues and adjacent non-tumor liver tissues by way of real-time PCR. We found that SRC-3 was
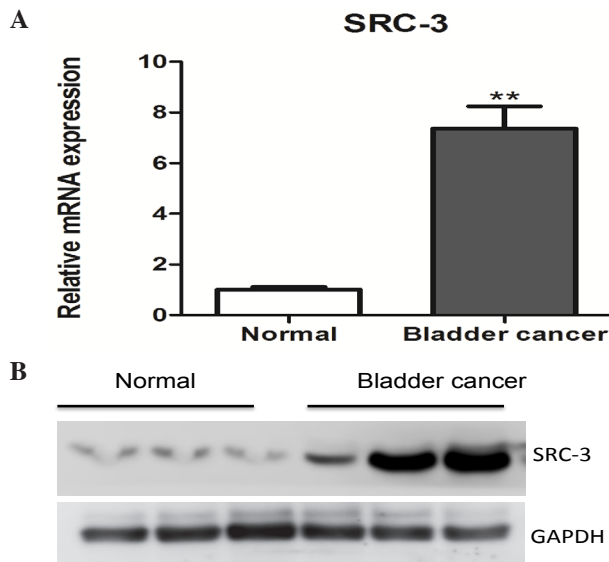
Figure 1. Up-regulation of SRC-3 in Bladder Cancer Tissues. (A-B) mRNA and protein levels of SRC-3 were analyzed by real-time PCR (A) and Western Blot (B) in bladder cancer tissues and normal tissues
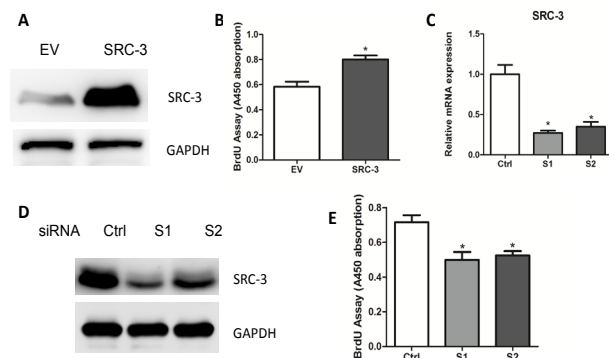

Figure 2. Roles of SRC-3 on EJ Cell Proliferation. (A) SRC-3 expression was determined by western blot in EJ cells transfected with empty vector (EV) or SRC-3. (B) The cell proliferative potential (BrdU) was determined in EJ cells transfected with empty vector (EV) or SRC-3. (C-D) SRC-3 expression was determined by real-time PCR (C) and western blot. (D) in EJ cells transfected with siRNA oligos targeting SRC-3 (S-1, S-2) or scramble siRNA (Ctrl). (E) The cell proliferative potential (BrdU) was determined in EJ cells transfected with SRC-3 or scramble siRNA oligos

significantly up-regulated in cancer tissues (Figure 1A). We then employed Western blot with anti-SRC-3 antibody to detect the protein expression level of SRC-3 in clinic samples. The results showed the increased expression of SRC-3 in bladder cancer samples (Figure 1B).

Effect of SRC-3 on bladder cancer cell proliferation

To further elucidate the functional role of SRC-3 in bladder cancer, we transduced EJ cells with adenoviruses containing SRC-3 (Figure 2A). As a result, cell proliferation was enhanced by SRC-3 overexpression as measured bybromodeoxyuridine (BrdU) analysis (Figure 2B). Moreover, EJ cells were transfected with small interfering RNA (siRNA) targeting SRC-3. Two independent siRNA oligos showed efficient SRC-3 knockdown in EJ cells, compared with scramble siRNA-transduced cells (Figure 2C and 2D). As a result, down-
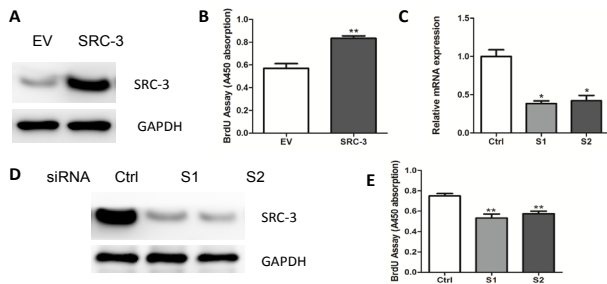
Figure 3. Roles of SRC-3 on T42 Cell Proliferation.
(A) SRC-3 expression was determined by western blot in
T42 cells transfected with empty vector (EV) or SRC-3. (B)
The cell proliferative potential (BrdU) was determined in T42
cells transfected with empty vector (EV) or SRC-3. (C-D)
SRC-3 expression was determined by real-time PCR (C) and
western blot (D) in T42 cells transfected with siRNA oligos
targeting SRC-3 (S-1, S-2) or scramble siRNA (Ctrl). (E) The
cell proliferative potential (BrdU) was determined in T42 cells
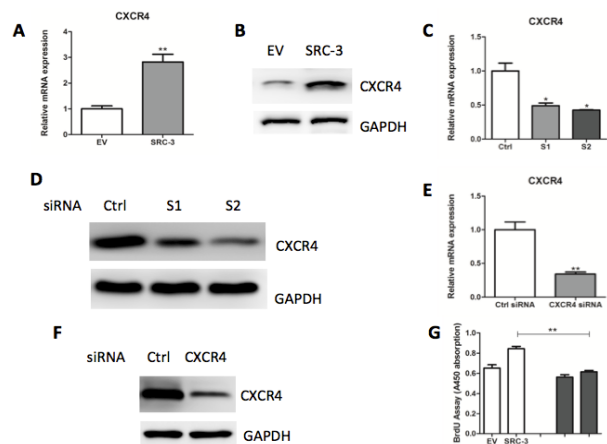transfected with SRC-3 siRNA oligos

**Figure 4. SRC-3 Positively Regulates CXCR4
Expression.** (A-B) mRNA and protein levels of CXCR4 in EJ
cells overexpressing SRC-3 or empty vector (EV). (C-D) mRNA
and protein levels of CXCR4 in EJ cells with SRC-3 knockdown.
(E-F) mRNA and protein levels of CXCR4 in EJ cells transfected
with CXCR4 siRNA oligos. (G) The cell proliferative potential
(BrdU) was determined in EJ cells overexpressing SRC-3
or empty vector (EV), in the absence or presence of CXCR4
siRNA oligos

regulation of SRC-3 led to a marked decrease in cell
proliferation in these cells (Figure 2E). In addition, we
also observed similar results in T42 cell (Figure 3A-E).
Taken together, our results suggest that SRC-3 might be
an important positive regulation of bladder cancer cell
proliferation.

SRC-3 regulation of CXCR4 in bladder cancer cells

Previous studies have shown that the CXCR4
plays an essential role in cell growth in bladder cancer
(Kucukgergin et al., 2012; Yates et al., 2013). We asked
whether overexpression or knockdown of SRC-3 may
affect CXCR4 expression. As shown in Figure 4A and 4B,
overexpression of SRC-3 increased CXCR4 mRNA and
protein levels. In agreement, SRC-3 depletion dramatically
led to a reduction of CXCR4 expression (Figure 4C and
4D), suggesting that SRC-3 is an upstream regulator of
CXCR4. Next, to determine if induction of CXCR4 is
required for the proliferative effect of SRC-3, we carried

out experiments with CXCR4 knockdown using siRNA
oligos (Figure 4E-F). As a result, the siRNA rescued cells
from the effect of SRC-3 on cell proliferation in EJ cells
(Figure 4G), suggesting that the role of SRC-3 to promote
cell proliferation is, at least in part, dependent on the
upregulation of CXCR4.

Discussion

In the present study, our results found that
overexpression of SRC-3 promoted bladder cancer cells
proliferation whereas knockdown of SRC-3 inhibits its
proliferation. At the molecular level, we further identify
that CXCR4 is a transcriptional target of SRC-3. Indeed,
CXCR4 deficiency reversed the proliferative roles of
SRC-3 in bladder cancer cells. Therefore, our study firstly
identify that SRC-3 plays a critical role in the bladder
cancer, which may be beneficial for its prevention and
treatment.

Other studies have explored additional molecular
mechanisms underlying the role of SRC coactivators
in enhancing cancer cell proliferation. For instance,
SRC-1 overexpression has been shown to increase
ERBB2, colony-stimulating factor-1, and Twist gene
expression (Qin et al., 2009; Moi et al., 2012). SRC-3
overexpression has been shown to stimulate IGF and
E2F1-mediated pathways, also pointing to its broad ability
to activate multiple cancer growth pathways (Mussi et
al., 2006). In addition, aberrant expression of SRC-3 in
patients has been associated with resistance to endocrine
therapies and the development of tumor recurrence (Ma
et al., 2011; McBryan et al., 2012). For example, high
expression of both SRC-3 and ERBB2 also was shown
to significantly increase the agonist activities of 4HT,
resulting in resistance to 4HT treatment (McBryan et al.,
2012). In ERBB2-overexpressing breast cancer cells,
overexpression of SRC-3 contributes to resistance against
ERBB2 targeting treatment with trastuzumab (Herceptin)
through activation of IGF signaling pathways (McBryan
et al., 2012).

Previous studies have shown that CXCR4 was the
only chemokine receptor whose mRNA expression level
was upregulated in bladder cancer cell lines as well as
in invasive and locally advanced bladder cancer tissue
samples (Nishizawa et al., 2010; Wang et al., 2011).
Exposure of CXCR4-positive bladder cancer cells to
its ligand (CXCL12) provoked a significant increase in
proliferation as well as invasion across a Matrigel barrier
in a Boyden chamber type assay (Eisenhardt et al., 2005;
Retz et al., 2005). Consistently, enhanced migration and
invasion were inhibited by a CXCR4-specific blocking
antibody (Eisenhardt et al., 2005; Retz et al., 2005).
Moreover, a recent study demonstrated that higher CXCR4
expression was associated with Stat3 signaling activation
in bladder cancer [30]. CXCR4 knockdown in bladder
cancer T24 cells impaired CXCL12-induced cell invasion
and Stat3 activation (Shen et al., 2013). Therefore, it will
be interesting to investigate the regulatory mechanisms
of SRC-3 on CXCR4 expression in the future. Further
studies are also needed to explore the potential cross-talk
between SRC-3 and Stat3 in bladder cancers.

In conclusion, our results found the SRC-3 gene amplification in bladder cancer tissues and indicate a functional role of SRC-3 to promote bladder cancer cell proliferation. Our study identify SRC-3/CXCR4 regulatory axis as a potential target for the attenuation of bladder cancer progression.

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