Imbalanced expression pattern of steroid receptor coactivator-1 and -3 in liver cancer compared with normal liver: An immunohistochemical study with tissue microarray

SHAN LI[1,2*], HUIYAN ZHANG[3*], YANLAN YU[1,2], MENGYING LIU[1], DEYU GUO[4], XUQING ZHANG[3] and JIQIANG ZHANG[1]

[1]Department of Neurobiology, Chongqing Key Laboratory of Neurobiology; [2]Cadet Brigade, Third Military Medical University; [3]Department of Infectious Diseases; [4]Institute of Pathology, Southwest Hospital, Third Military Medical University, Chongqing 400038, P.R. China

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Abstract. Steroids affect normal and pathological functions of the liver through receptors, which require coactivators for their transcriptional activation. Steroid receptor coactivator (SRC)-1 and SRC-3 have been demonstrated to be regulated in numerous cancers; however, their expression profiles in liver cancer including hepatocellular carcinoma (HCC) and cholangiocellular carcinoma (CCC) remain unclear. Using tissue microarray immunohistochemistry, normal liver tissue and HCC tissue exhibited immunoreactivity of SRC-1, which were predominantly localized within extranuclear components; in CCC, they were detected within the cell nuclei; SRC-3 was also detected in the cell nuclei. Furthermore, no altered expression of SRC-1 and SRC-3 was observed in liver cancer compared with normal liver tissue; however, in CCC, the expression of SRC-3 was significantly increased compared with that detected in HCC. Importantly, although expression of SRC-1 and SRC-3 did not reveal any significant differences (30 vs. 40%) in normal liver tissue, HCC and CCC expression of SRC-1 was significantly decreased compared with that of SRC-3 (9.3 vs. 36%, and 6.7 vs. 67.7% for HCC and CCC, respectively). Further comparative analysis revealed that this discrepancy was detected in males with liver cancer, across all ages of HCC cases, younger CCC cases and all stages of liver cancer. The results suggested the presence of an imbalanced expression pattern of SRC-1 and SRC-3 from normal liver tissue to liver cancer (decreased SRC-1 and increased SRC-3), which may affect hepatic function and therefore promote liver carcinogenesis.

Correspondence to: Dr Jiqiang Zhang, Department of Neurobiology, Chongqing Key Laboratory of Neurobiology, Third Military Medical University, 30 Gaotanyan Street, Shapingba, Chongqing 400038, P.R. China
E-mail: zhangjqtmmu@yahoo.com

Dr Xuqing Zhang, Department of Infectious Diseases, Southwest Hospital, Third Military Medical University, 30 Gaotanyan Street, Shapingba, Chongqing 400038, P.R. China
E-mail: xuqing651005@tom.com

*Contributed equally

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Introduction

Steroids, including androgens and estrogens (E_2), have been demonstrated to exert multiple effects not only on reproductive function, but also on numerous other organ systems, including the liver, in men and women (1). For example, previous human studies have demonstrated that female menopause has been associated with the increase in non-alcoholic fatty liver disease, hepatocellular carcinoma (HCC) and the progression of fibrosis (2). Furthermore, animal studies also revealed that bilateral ovariectomy increased the risk of HCC (3). There appears to be a sex difference in the survival of patients with HCC: HCC is a male-dominated cancer, with men 4-8-fold more likely to develop HCC than women; however, testosterone may act to protect against hepatic steatosis (4-6). Additionally, high levels of aromatase, an enzyme catalyzing the conversion of testosterone into E_2, has been detected within the human liver, and aromatase overexpression has also been identified in hepatitis and HCC (7,8). Furthermore, aromatase gene-knockout mice exhibited hepatic glucose intolerance, which was able to be reversed by E_2 administration (9). Recent studies have identified that high circulating E_2 and low testosterone ratio may be associated with adverse clinical outcomes in men with advanced liver disease and patients with primary liver cancer (10,11).

The action of steroids is known to be mediated by their receptors. Androgen receptor (AR) has been detected in normal and cancerous liver tissue and cell lines; estrogen receptor (ER) α and decreased expression of ERβ have also been identified in HCC (5,12,13). These receptors have been demonstrated to regulate lipid and glucose metabolism in the liver. For example, ERs function to decrease lipogenesis, gluconeogenesis and fatty acid uptake, but enhance lipolysis, cholesterol secretion and glucose catabolism; AR functions to

increase insulin receptor expression and glycogen synthesis, decrease glucose uptake and lipogenesis, and promote cholesterol storage (14). Additionally, studies have revealed that expression of ERα was associated with invasion and metastasis in HCC, and AR and ERα, but not ERβ, gene expression contributed to the prevalence of HCC in male rats (15,16).

Cholangiocellular carcinoma (CCC) is difficult to treat due to its chemo-resistance and its inability to be detected at an early stage of disease (17). It has been reported that, compared with males, females are more susceptible to several biliary tract diseases such as primary biliary cirrhosis, debilitating/symptomatic adult polycystic liver disease and autoimmune hepatitis (18). The significant increase of estrogen levels in the serum of patients with CCC has been reported (19), and has also been demonstrated to enhance the proliferation and invasiveness of CCC cells *in vitro*. Furthermore, the survival time of patients with CCC is associated with estrogen levels (20). Additionally, different levels of ERα and ERβ have been detected in CCC cells (18,21), ERα has been demonstrated to mediate estrogenic stimulation of interleukin-6 production and thus influence the pathology of CCC (18), the overexpression of ERβ has also been demonstrated to exhibit protective abilities against CCC (21).

Previous studies have demonstrated that steroid receptor coactivators (SRCs) are required for the transcriptional activation of target genes by a steroid receptor (22,23). Among SRCs, the p160 family members SRC-1 and SRC-3 have been investigated in a number of types of cancer including tissue and cell lines (24). For example, overexpression of SRC-1 and SRC-3 has been detected in breast cancer, non-small cell lung cancer, bone cancer and chondrosarcoma (25-31). Decreased expression of SRC-3 has been reported in astrocytic tumors and decreased expression of SRC-1 and SRC-3 has been identified in meningothelial tumors and neuroepithelial tumors (32,33). In liver tissue, an early study demonstrated that SRCs serve a role in the regulation of hepatic energy homeostasis (34), and further studies highlighted that SRC-1 was a critical mediator of glucose homeostasis as it functioned as the integrator of glucose and oxidized/reduced nicotinamide-adenine dinucleotide homeostasis (35,36). Thus, hepatic SRC-1 activity may have potential relevance for human metabolic pathogenesis (37). However, the expression profiles of SRC-1 and SRC-3 in HCC and CCC have not yet been reported. To address this question, the present study investigated the expression and significance of these two coactivators in HCC and CCC using tissue microarray (TMA) immunohistochemistry.

Materials and methods

Tissue microarray. The two types of hepatic carcinoma and normal TMA used were purchased from US Biomax, Inc. (cat. no. BC03118; Rockville, MD, USA). The TMA contained 75 cases of malignant HCC (65 males and 10 females; mean age, 50.8 years), 15 cases of malignant CCC (8 males and 7 females; mean age, 48.5 years) and 10 cases of normal hepatic tissue (6 males and 4 females; mean age, 26.8 years). In total, 200 tissue cores featured on a single slide as 2 cores were punched from each case.

Immunohistochemistry. TMAs were deparaffinized in xylene, rehydrated with a gradient alcohol series and heat-mediated antigen retrieval (0.01 M sodium citrate buffer, pH 6.0) was performed according to our previous protocol (32,33). The sections (5-μm thick) were washed with PBS (0.01 mol/l, pH 7.4) prior to being blocked with 3% H_2O_2 for 15 min. TMAs were incubated for 30 min with normal goat serum (2%, v/v) to inhibit non-specific binding and then incubated with rabbit polyclonal antibody against SRC-1 (1:200; cat. no. sc-8995; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) or SRC-3 (1:200; cat. no. sc-25742; Santa Cruz Biotechnology, Inc.) at 4˚C for 48 h. Following washing with PBS, sections were incubated with biotinylated goat-anti-rabbit secondary antibody (1:200; cat. no. ZB2010; OriGene Technologies, Inc., Beijing, China) for 1 h at room temperature. The sections were then washed with PBS, incubated with horseradish peroxidase-labeled streptavidin (1:200; cat. no. ZB2404; OriGene Technologies, Inc.) for 1 h at room temperature. Finally, sections were incubated with a 3,3'-diaminobenzidine-peroxidase substrate kit (cat. no. ZLI-9018; OriGene Technologies, Inc.) for 5 min at room temperature. Blank controls were carried out using the same procedure; however, primary antiserum was replaced with Antibody Diluent (ZLI-9028, OriGene Technologies, Inc.) according to that manufacturer's protocol.

Image acquisition and data analysis. Images of immunohistochemical staining were captured using a digital camera (DP70; Leica, Germany) equipped with an Olympus microscope (BX60; Olympus Corporation, Tokyo, Japan; under x20 or x40 magnification). The strength of staining was scored in accordance with a four-point system (0-3) described in a previous study (32) by a pathologist double-blindly. A score of 3 indicated visible dark staining of >50% of cells; a score of 2 indicated either dark focal staining of <50% of cells or moderate staining of >50%; a score of 1 indicated either moderate focal staining of <50% of cells or pale staining in any proportion of cells not easily seen at low power; a score of 0 indicated no positive staining. A high level of expression was defined as a score of 2 or 3, and a low level of expression was defined as a score of 0 or 1. Pathologically, early-stage liver cancer was defined as stage I and II; advanced stage was defined as stage III and IIIb.

Statistical analysis. All data are expressed as n (%) and compared using a χ^2 test or Fisher's exact test with SPSS software (version 18.0; SPSS, Inc., Chicago, IL, USA). All P-values were two-tailed and $P<0.05$ was considered to indicate a statistically significant difference.

Results

Subcellular localization of SRCs in normal and cancerous liver tissue. Results presented in Fig. 1 demonstrate the localization patterns of SRC-1- and SRC-3-immunoreactive materials. In normal liver and HCC tissue, SRC-1-immunoreactive materials were predominantly detected within the extra-nuclear component. However, in CCC, SRC-1 materials were predominantly detected within the cell nuclei. SRC-3-immunoreactive materials were primarily detected within the cell nuclei.

Expression profiles of SRCs in normal and cancerous liver tissue. High levels of SRC-1 expression was detected in 30%

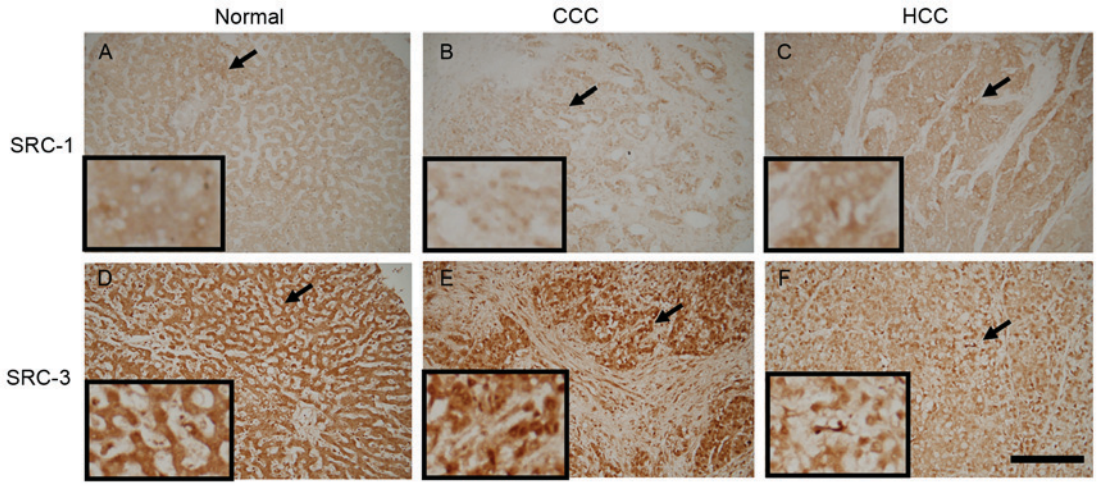

Figure 1. Immunohistochemical staining of SRC-1 and SRC-3 in normal and cancerous liver tissue (HCC and CCC). (A) SRC-1 in normal liver tissue (male; 3-year-old). (B) SRC-1 in cholangiocellular carcinoma (male; 46-year-old). (C) SRC-1 in hepatocellular carcinoma (male; 51-year-old). (D) SRC-3 in normal liver tissue (female; 40-year-old). (E) SRC-3 in cholangiocellular carcinoma (female; 36-year-old). (F) SRC-3 in hepatocellular carcinoma (male; 53-year-old). In normal and hepatocellular carcinoma, SRC-1-immunopositive materials were predominantly localized in extranuclear components; in cholangiocellular carcinoma, SRC-1-immunopositive materials were predominantly localized in the nuclei. For SRC-3-immunopositive materials, they were predominantly detected within the nuclei. The insets present the magnification (x40) of selected areas (indicated by black arrow), which demonstrated the subcellular localization of individual immunopositive materials. SRC, steroid receptor coactivator; CCC, cholangiocellular carcinoma; HCC, hepatocellular carcinoma. Scale bar, 200 μm.

(3/10) of the normal liver tissue, 9.3% (7/75) of HCC tissue and 6.7% (1/15) of CCC tissue. The χ^2 test indicated no statistically significant differences in association with the expression levels of SRC-1 between normal and HCC, normal and CCC, or HCC and CCC samples (Table I and Fig. 2A-C). High levels of SRC-3 expression was detected in 40% (4/10) of the normal liver tissue, 36% (27/75) of HCC tissue and 67.7% (10/15) of CCC tissue. The χ^2 test indicated no statistical significance in the expression levels of SRC-3 between normal and HCC or normal and CCC samples (Table I, Fig. 2D and E). However, expression of SRC-3 in CCC was significantly increased compared with HCC (67.7 vs. 6.7%; P=0.028) as indicated in Table I and Fig. 2F.

Comparison of the expression profile of SRCs in normal and cancerous liver tissue. Comparisons of SRC-1 and SRC-3 expression profiles in normal and liver cancer tissue were performed in order to determine any statistical significance. Normal liver tissue results identified that 30% (3/10) exhibited high levels of SRC-1 expression and 40% (4/10) exhibited high levels of SRC-3 expression. The difference in expression profiles was not statistically significant (P>0.05). In HCC, 9.3% (7/75) exhibited high levels of SRC-1 expression and 36% (27/75) exhibited high levels of SRC-3 expression. The HCC results indicated that SRC-3 expression was significantly increased compared with SRC-1 expression (P<0.01). In CCC results, 6.7% (1/15) exhibited high levels of SRC-1 expression, whereas SCR-3 expression was significantly increased in comparison (P=0.02) at 67.7% (10/15). These results are presented in Table II and Fig. 2G-I.

Sex-specific analysis of SRCs in normal and cancerous liver tissue.

Male-specific analysis. The normal tissue group revealed that of the 6 cases, none exhibited high levels of SRC-1 and only 1 exhibited high levels of SRC-3; these results were not statistically significant (P>0.05). The HCC tissue group revealed that of 65 cases, 7.7% (5/65) exhibited high levels of SRC-1, whereas SCR-3 was significantly increased (P<0.01) at 38.5% (25/65). In CCC, of 8 cases none exhibited high levels of SRC-1, whereas 75% (6/8) exhibited high levels of SRC-3. SRC-3 expression was significantly increased compared with that of SRC-1 (P<0.01). These results are presented in Table III and Fig. 3.

Female-specific analysis. The normal tissue group revealed that of the 4 cases, 75% (3/4) exhibited high levels of SRC-1 and 75% (3/4) exhibited high levels of SRC-3; these results were not statistically significant (P>0.05). The HCC tissue group demonstrated that of 10 cases, 20% (2/10) exhibited high levels of SRC-1 and 20% (2/10) exhibited high levels of SRC-3; these results were not statistically significant (P>0.05). The CCC tissue group demonstrated that of 7 cases of CCC, 14.3% exhibited high levels of SRC-1 (1/7) and 57.1% (4/7) exhibited high levels of SRC-3; these results were not statistically significant (P>0.05). These results are presented in Table III and Fig. 3.

Age-specific analysis of SRCs in normal and cancerous liver tissue. The mean age of normal cases was 26.8 years (n=10). A total of 4 normal cases were ≥26.8 years, none of which exhibited high levels of SRC-1 and 50% (2/4) exhibited high levels of SRC-3; these results were not statistically significant (P>0.05). A total of 6 normal cases were <26.8 years, 50% (3/6) exhibited high levels of SRC-1 and 33.3% (2/6) exhibited high levels of SRC-3; these results were not statistically significant (P>0.05). The mean age of HCC cases was 50.8 years (n=75). A total of 38 HCC cases were ≥50.8 years, of which 5.3% (2/38) exhibited high levels of SRC-1 and 36.8% (14/38) exhibited high levels of SRC-3. This was significantly

Table I. Expression of SRC-1 and SRC-3 in normal, HCC and CCC.

	SRC-1				SRC-3			
Tissue	High	Low	χ^2	P-value	High	Low	χ^2	P-value
Normal	3	7	1.913	>0.05	4	6	0.000	>0.05
HCC	7	68			27	48		
Normal	3	7	1.004	>0.05	4	6	0.818	>0.05
CCC	1	14			10	5		
HCC	7	68	0.000	>0.05	27	48	4.856	0.028
CCC	1	14			10	5		

SRC, steroid receptor coactivator; HCC, hepatocellular carcinoma; CCC, cholangiocellular carcinoma.

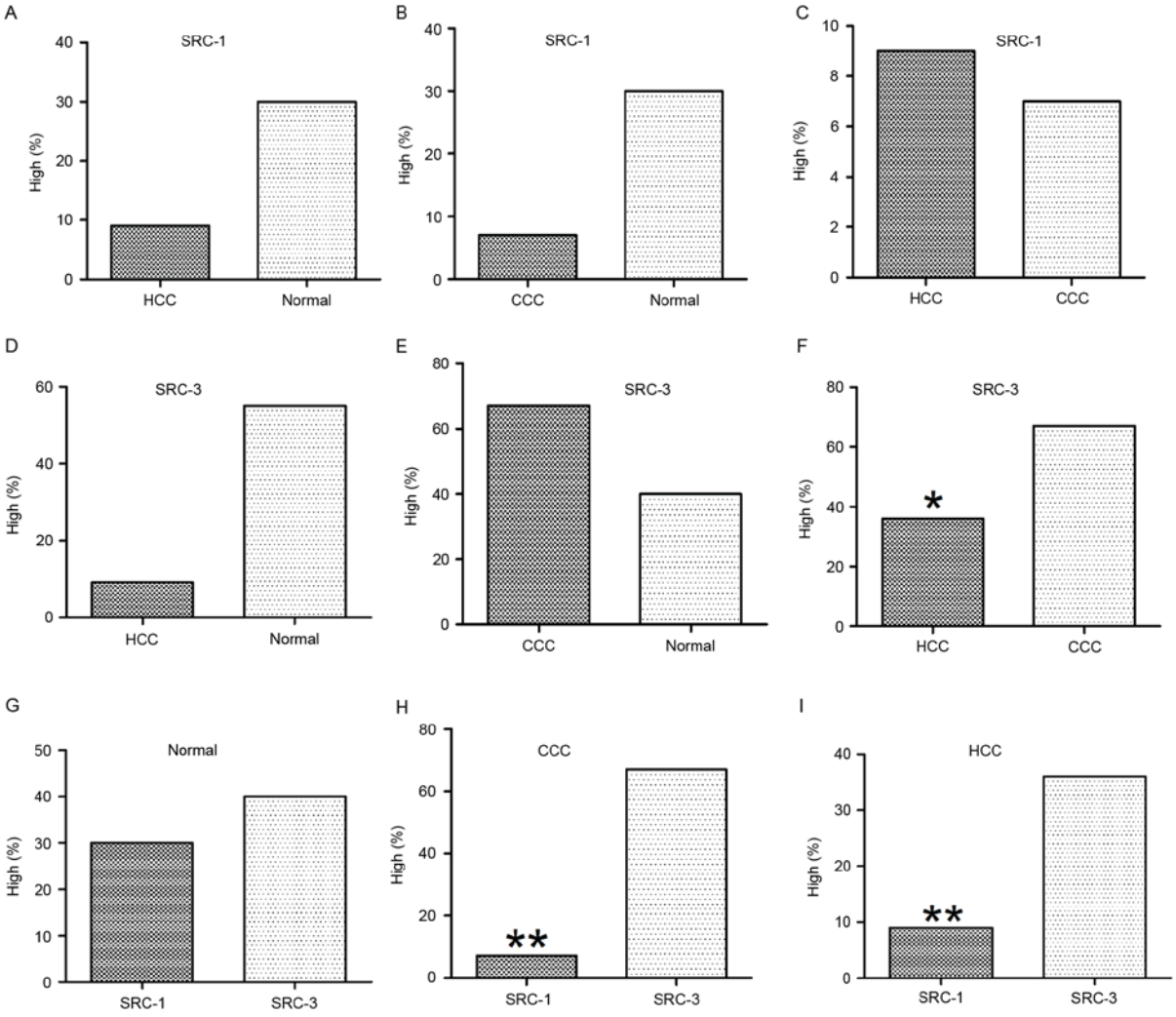

Figure 2. Statistical analysis of SRC-1 and SRC-3 immunoreactivities in normal and cancerous liver tissue (HCC and CCC). The expression of SRC-1 did not reveal significant differences between (A) HCC and normal, (B) CCC and normal, or (C) HCC and CCC. Expression of SRC-3 did not reveal significant differences between (D) HCC and normal, or (E) CCC and normal. (F) However, SRC-3 was significantly increased in CCC when compared with that of HCC. Comparison of SRC-1 and SRC-3 in (G) normal, (H) CCC and (I) HCC tissue. In normal liver tissue, expression of SRC-1 and SRC-3 did not reveal a significant difference. However, in CCC and HCC, expression of SRC-1 was significantly decreased when compared with that of SRC-3. *P<0.05; **P<0.01. SRC, steroid receptor coactivator; CCC, cholangiocellular carcinoma; HCC, hepatocellular carcinoma.

increased compared with that of SRC-1 (P=0.02). The remaining 37 cases were <50.8 years, 13.5% (5/37) exhibited high levels of SRC-1 and 35.1% (13/37) exhibited high levels of SRC-3; this was also significantly increased compared with that of SRC-1 (P=0.030). The mean age of CCC cases was 48.5 years (n=15). A total of 7 CCC cases were ≥48.5 years,

Table II. Comparison of the expression of SRC-1 and SRC-3 in normal, HCC and CCC.

	Normal				HCC				CCC			
SRC	High	Low	χ^2	P-value	High	Low	χ^2	P-value	High	Low	χ^2	P-value
1	3	7	0.000	>0.05	7	68	15.213	<0.01	1	14	9.187	0.002
3	4	6			27	48			10	5		

SRC, steroid receptor coactivator; HCC, hepatocellular carcinoma; CCC, cholangiocellular carcinoma.

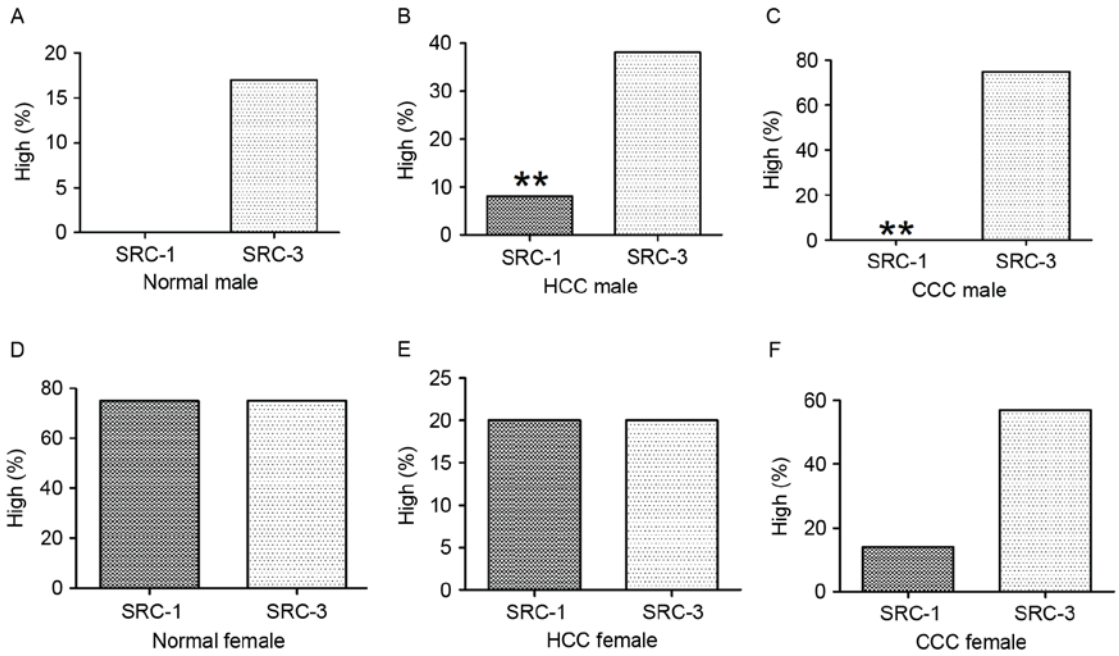

Figure 3. Sex-specific comparison of SRC-1 and SRC-3 in normal, HCC and CCC. In normal liver tissue, no statistical differences between the sexes were detected between the levels of SRC-1 and SRC-3. However, in HCC and CCC, males tended to have higher SRC-1 than that of SRC-1. (A) Expression of SRC-1 and SRC-3 in normal male liver tissue. (B) Expression of SRC-1 and SRC-3 in male HCC. (C) Expression of SRC-1 and SRC-3 in male CCC. (D) Expression of SRC-1 and SRC-3 in normal female liver tissue. (E) Expression of SRC-1 and SRC-3 in female HCC. (F) Expression of SRC-1 and SRC-3 in female CCC. **P<0.01 vs. CRC-3. SRC, steroid receptor coactivator; CCC, cholangiocellular carcinoma; HCC, hepatocellular carcinoma.

14.3% (1/7) exhibited high levels of SRC-1 and 71.4% (5/7) exhibited high levels of SRC-3; these results were not statistically significant (P>0.05). The remaining 8 CCC cases were <48.5 years, none exhibited high levels of SRC-1 and 62.5% (5/8) exhibited high levels of SRC-3, which was significantly increased compared with that of SRC-1 (P=0.031). All results are presented in Table IV and Fig. 4.

Stage-specific analysis of SRCs in HCC and CCC. In the early stage of HCC, 7.9% (3/38) exhibited high levels of SRC-1 and 36.8% (14/38) exhibited high levels of SRC-3, this was significantly increased compared with that of SRC-1 (P=0.006). In the advanced stage of HCC, 10.8% (4/37) exhibited high levels of SRC-1 and 35.1% (13/37) exhibited high levels of SRC-3; this was significantly increased compared with that of SRC-1 (P=0.027). These results are presented in Fig. 5A and B.

In the early stage of CCC, no case exhibited high levels of SRC-1 and 40% (2/5) exhibited high levels of SRC-3; these results were not statistically significant (P>0.05). In the advanced stage of CCC, 10% (1/10) exhibited high levels of SRC-1 and 80% (8/10) indicated high levels of SRC-3; this was significantly increased compared with that of SRC-1 (P=0.007). These results are presented in Fig. 5C and D.

Discussion

It is well known that liver disease is a major health concern worldwide (38). Statistics published in 2014 estimated that the number of new liver cancer cases in the USA totaled 33,190 (24,600 male cases), and the estimated mortality rate was 23,000 (15,870 male cases). Therefore, these statistics indicate that liver cancer is the fifth leading cause of male (15,870) and the ninth leading cause of female (7,130) cancer mortality in the USA in 2014 (39). Similar results were also reported in China, where liver cancer was ranked within the top five causes of cancer-associated mortality with clear male predominance (310,600 vs. 111,500) (40). However, the molecular mechanisms underlying the occurrence and disease progression, as well as sex differences observed in liver cancers are poorly understood. Therefore, in the

Table III. Sex-specific comparison of SRC-1 and SRC-3 in normal, HCC and CCC.

		Male				Female			
Tissue	SRC	High	Low	χ^2	P-value	High	Low	χ^2	P-value
Normal	1	0	6	0.000	>0.05	3	1	0.000	>0.05
	3	1	5			3	1		
HCC	1	5	60	17.333	<0.01	2	8	0.000	>0.05
	3	1	5			3	1		
CCC	1	0	6	6.667	0.010	3	1	1.244	>0.05
	3	1	5			3	1		

SRC, steroid receptor coactivator; HCC, hepatocellular carcinoma; CCC, cholangiocellular carcinoma.

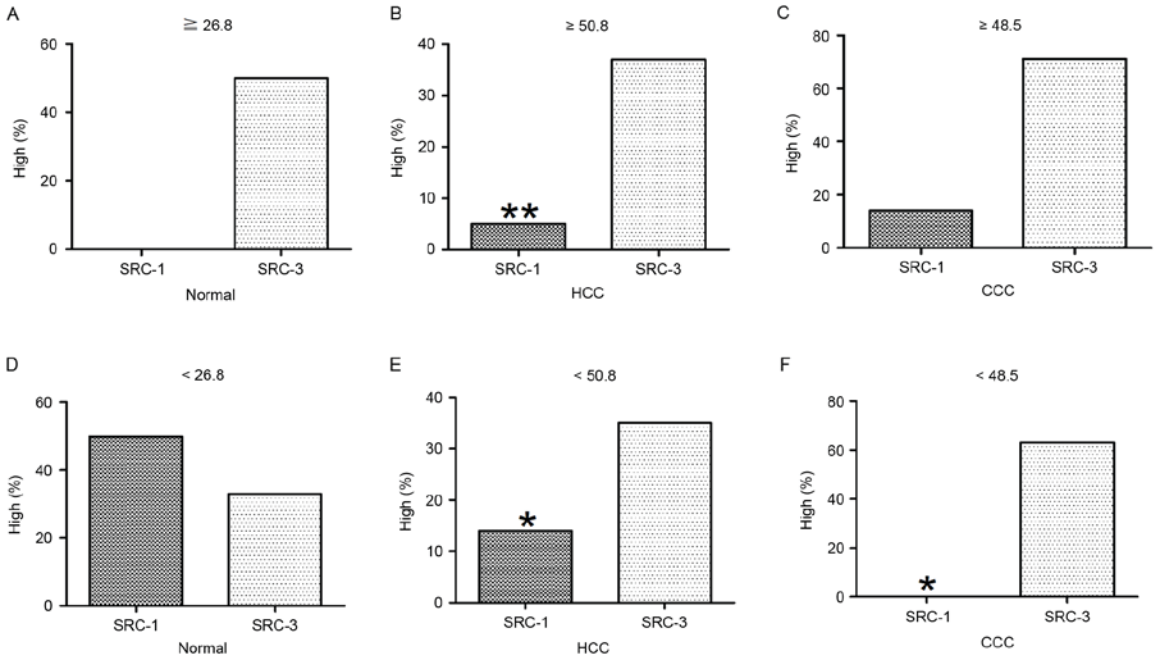

Figure 4. Age-specific comparison of SRC-1 and SRC-3 in normal, HCC and CCC liver tissue. In the older or equal to mean age cases, significantly decreased expression of SRC-1 was detected in the HCC compared to SRC-3, whereas in the normal and CCC liver tissue, no significant difference was detected. Additionally, in the cases, significantly decreased expression of SRC-1 was detected in HCC and CCC compared with SRC-3. (A) Expression of SRC-1 and SRC-3 in normal liver tissue aged ≥26.8 years. (B) Expression of SRC-1 and SRC-3 in HCC tissue aged ≥50.8 years. (C) Expression of SRC-1 and SRC-3 in CCC liver tissue aged ≥48.5 years. (D) Expression of SRC-1 and SRC-3 in normal liver tissue aged <26.8 years. (E) Expression of SRC-1 and SRC-3 in HCC tissue aged <50.8 years. (F) Expression of SRC-1 and SRC-3 in CCC liver tissue aged <48.5 years. *P<0.05 vs. SRC-3; **P<0.01 vs. SRC-3. SRC, steroid receptor coactivator; CCC, cholangiocellular carcinoma; HCC, hepatocellular carcinoma.

present study tissue microarray immunohistochemistry was used in order to compare the expression profiles of SRC-1 and SRC-3 in normal, HCC and CCC. It was observed that SRC-1-immunopositive materials were predominantly detected in the extranuclear component in normal and HCC liver tissue. However, in CCC, SRC-1 was localized in the cell nuclei, indicating that plasma-nucleus translocations may contribute to the pathology of CCC. For SRC-3, the immunoreactive materials were mainly detected within the cell nuclei in all tissue types examined. The diversity of subcellular localization of SRC-immunoreactive materials was in general agreement with previous studies demonstrating that SRC-1 and SRC-3 would be able to be detected in cell nuclei and cytoplasm (29,41).

Furthermore, results indicated that expression of SRC-1 did not demonstrate any significant differences among normal, HCC and CCC liver tissue; similar phenomena for SRC-3 were also detected; however, significantly increased levels of SRC-3 were detected in CCC tissue when compared with those in HCC tissue. These tissue results indicated that there was an unchanged SRC-1 but an evident overexpression of SRC-3 in HCC and CCC when compared with that detected in the normal liver tissue. Some previous studies have also reported different changes of SRC-1 or SRC-3 in specific cancers compared to normal tissue. For example, overexpression of SRC-1 is present in breast and ovarian cancer cell lines as well as primary breast cancer, prostate cancer and endometrial carcinoma (42-44) when compared with normal tissue. Additionally, overexpression of SRC-3 was

Table IV. Age-specific comparison of SRC-1 and SRC-3 in normal, HCC and CCC.

Tissue	High	Low	χ^2	P-value	High	Low	χ^2	P-value
Normal		≥26.8 years				<26.8 years		
SRC-1	0	4	0.667	>0.05	3	3	0.000	>0.05
SRC-3	2	2			2	4		
HCC		≥50.8 years				<50.8 years		
SRC-1	2	36	9.579	0.002	5	32	4.655	0.031
SRC-3	14	24			13	24		
CCC		≥48.5 years				<48.5 years		
SRC-1	1	6	2.625	>0.05	0	8	4.655	0.031
SRC-3	5	2			5	3		

SRC, steroid receptor coactivator; HCC, hepatocellular carcinoma; CCC, cholangiocellular carcinoma.

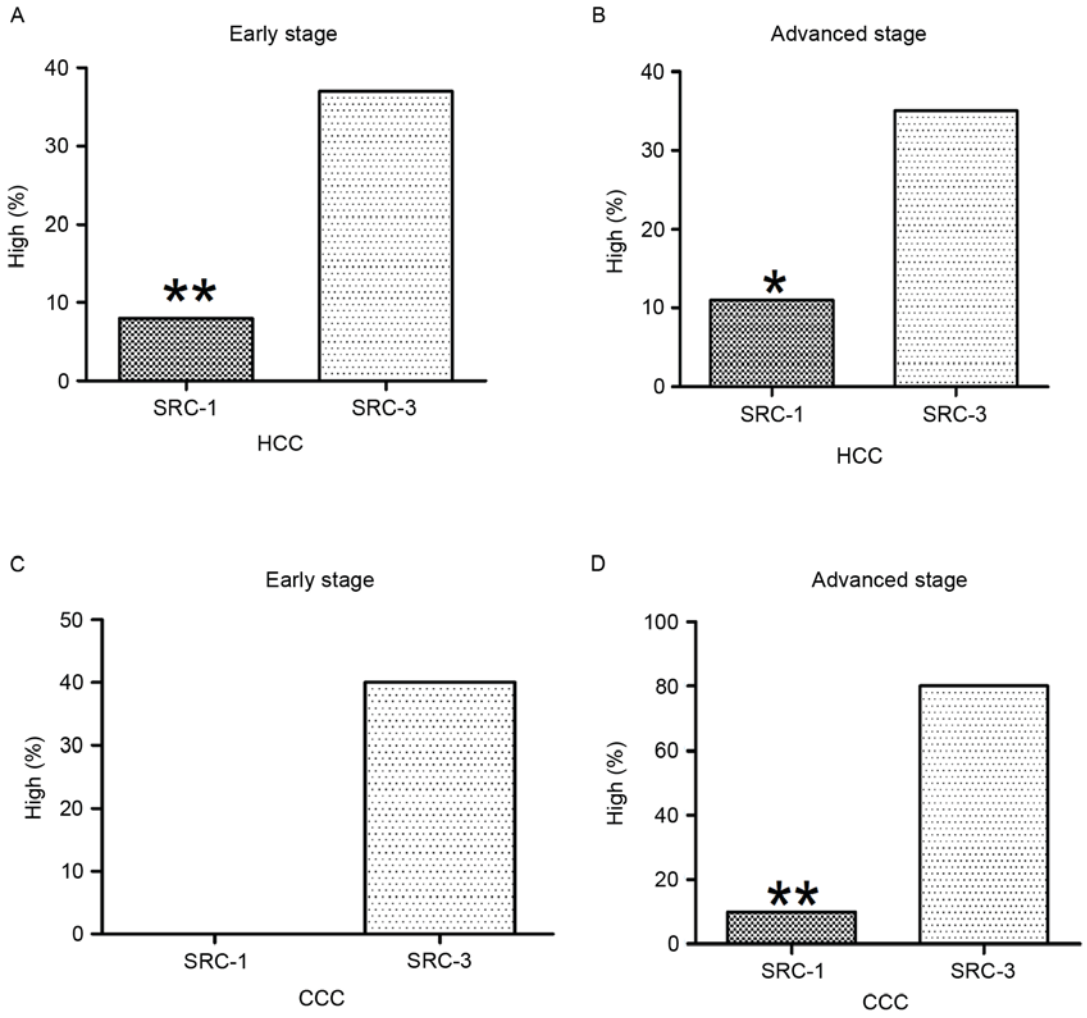

Figure 5. Stage-specific comparison of SRC-1 and SRC-3 in HCC and CCC. In HCC and CCC, levels of SRC-3 were significantly higher than that of SRC-1. (A) At the early stages of HCC, expression of SRC-3 was significantly higher compared with SRC-1. (B) At an advanced stage of HCC, expression of SRC-3 was significantly higher compared with SRC-1. (C) At an early stage of CCC, expression of SRC-3 was significantly higher compared with SRC-1. (D) At an advanced stage of CCC, expression of SRC-3 was significantly higher compared with SRC-1. *P<0.05; **P<0.01.

identified in CCC, which was in agreement with previous studies reporting overexpression of SRC-3 in lung cancer, colorectal carcinoma, endometrial carcinoma, esophageal squamous cell carcinoma and gastric cancer, ovarian cancer and pancreatic cancer when compared with normal tissue (29,45-50). However, previous studies also demonstrated a decrease in SRC-1 protein in endometrial carcinoma, a decrease in SRC-3 but unchanged SRC-1 in the high-grade astrocytic tissue and a decrease in

Table V. Stage-specific comparison of SRC-1 and SRC-3 in HCC and CCC.

		Early stage				Advanced stage			
Tissue	SRC	High	Low	χ^2	P-value	High	Low	χ^2	P-value
HCC	1	3	35	7.577	0.006	4	33	4.888	0.027
	3	14	24			13	24		
CCC	1	0	5	0.625	>0.05	1	9	7.273	0.007
	3	2	3			8	2		

SRC, steroid receptor coactivator; HCC, hepatocellular carcinoma; CCC, cholangiocellular carcinoma.

SRC-1 and SRC-3 in meningothelial tumor and neuroepithelial tumor when compared with normal tissue (32,33,51). With regard to HCC, Martínez-Jiménez *et al* (52) reported decreased SRC-1 levels in human hepatomas; by contrast, Tong *et al* (53) reported SRC-1 overexpression in 62.5% of HCC tissues using western blot analysis. In the present study, levels of SRC-1 and SRC-3 remain unchanged in liver cancer including HCC and CCC when compared with that in normal tissue. The reasons for these differences are unclear; however, the relatively small normal sample size (10 cases) in the present study may be a factor, and thus further examinations are required to confirm these results.

Owing to a lack of significant differences regarding the levels of SRC-1 and SRC-3 between normal and cancerous liver tissue, focus was placed on the increased expression profiles of SRC-3, and comparisons were made between the levels of SCR-1 and SRC-3 in normal and cancerous liver tissue. A key point of interest was the absence of statistical significance regarding the different levels of SRC-1 and SRC-3; however, in liver cancer, including HCC and CCC, significantly decreased expression of SRC-1 was detected when compared with that of SRC-3. Further sex-, age- and stage-specific analysis revealed that significantly decreased expression of SRC-1 was detected in the following groups: HCC cases (male), CCC cases (male), HCC (all ages), CCC cases (below mean age) and liver cancer stages (all stages). However, levels of SRC-1 and SRC-3 did not exhibit any significant differences in the following categories: Normal cases (male), all cases (female), normal cases (all ages) and CCC cases (above or equal to mean age). The decreased SRC-1/SRC-3 ratio in liver cancer but not normal liver tissue may be due to the slight decrease in SRC-1 expression and increase in SRC-3 expression observed. This indicates an imbalanced expression between these two coactivators, and may contribute to the occurrence and progression of liver cancer. Additionally, the loss-of-balance expression pattern of SRC-1/SRC-3, detected in males caused by their distinct expression profiles (decreased SRC-1; increased SRC-3), was positively associated with previous reports demonstrating a high occurrence and mortality in males with liver cancer (39,40). A similar imbalanced expression profile was also detected in other tumors including high-grade astrocytoma, as a decrease in SRC-3 but unchanged SRC-1 was reported (32).

In summary, although no significant changes in SRC-1 and SRC-3 were identified in liver cancer tissue when compared with that detected in the normal liver tissue, it was noted that there was a significantly decreased SRC-1/SRC-3 ratio in liver cancer, compared with that detected in normal liver tissue. The significance of this decreased ratio is currently unclear. Louet *et al* (35) reported that SRC-1 is a key coordinator of the hepatic gluconeogenic program and a critical mediator of liver glucose homeostasis; Ma *et al* (54) reported that SRC-3 serves a crucial role in regulating hepatic lipid metabolism (35,54). Thus, the imbalanced expression of SRC-1 and SRC-3 in the liver tissue may induce abnormal hepatic metabolism and finally induce tumorigenesis. However, further studies are urgently required to explore the precise roles of these two coactivators in both the normal liver and liver disease.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author's contributions

SL, HZ and YY conducted the experiments. ML photographed the pictures. DG conducted the pathological scoring. SL and HZ prepared the draft of this manuscript. JZ and XZ conceived this study and finalized this manuscript.

Ethics approval and consent to participate

Not applicable since the tissue microarray used in the present study is commercial available.

Pateint consent for publication

Not applicable since the tissue microarray used in the present study is commercial available.

Competing interests

The authors declare that they have no conflicts of interest.

References

1. Simpson E and Santen RJ: Celebrating 75 years of oestradiol. J Mol Endocrinol 55: T1-T20, 2015.
2. Brady CW: Liver disease in menopause. World J Gastroenterol 21: 7613-7620, 2015.
3. McGlynn KA, Sahasrabuddhe VV, Campbell PT, Graubard BI, Chen J, Schwartz LM, Petrick JL, Alavanja MC, Andreotti G, Boggs DA, *et al*: Reproductive factors, exogenous hormone use and risk of hepatocellular carcinoma among US women: Results from the liver cancer pooling project. Br J Cancer 112: 1266-1272, 2015.
4. Yang D, Hanna DL, Usher J, LoCoco J, Chaudhari P, Lenz HJ, Setiawan VW and El-Khoueiry A: Impact of sex on the survival of patients with hepatocellular carcinoma: A surveillance, epidemiology, and end results analysis. Cancer 120: 3707-3716, 2014.
5. Kanda T, Jiang X and Yokosuka O: Androgen receptor signaling in hepatocellular carcinoma and pancreatic cancers. World J Gastroenterol 20: 9229-9236, 2014.
6. Kelly DM, Nettleship JE, Akhtar S, Muraleedharan V, Sellers DJ, Brooke JC, McLaren DS, Channer KS and Jones TH: Testosterone suppresses the expression of regulatory enzymes of fatty acid synthesis and protects against hepatic steatosis in cholesterol-fed androgen deficient mice. Life Sci 109: 95-103, 2014.
7. Biegon A, Alexoff DL, Kim SW, Logan J, Pareto D, Schlyer D, Wang GJ and Fowler JS: Aromatase imaging with [N-methyl-11C] vorozole PET in healthy men and women. J Nucl Med 56: 580-585, 2015.
8. Hata S, Miki Y, Saito R, Ishida K, Watanabe M and Sasano H: Aromatase in human liver and its diseases. Cancer Med 2: 305-315, 2013.
9. Van Sinderen ML, Steinberg GR, Jørgensen SB, To SQ, Knower KC, Clyne CD, Honeyman J, Chow JD, Herridge KA, Jones ME, *et al*: Hepatic glucose intolerance precedes hepatic steatosis in the male aromatase knockout (ArKO) mouse. PloS One 9: e87230, 2014.
10. Sinclair M, Gow PJ, Angus PW, Hoermann R, Handelsman DJ, Wittert G, Martin S and Grossmann M: High circulating estrone and low testosterone correlate with adverse clinical outcomes in men with advanced liver disease. Liver Int 36: 1619-1627, 2016.
11. Qian X, Zhan Q, Lv L, Zhang H, Hong Z, Li Y, Xu H, Chai Y, Zhao L and Zhang G: Steroid hormone profiles plus α-fetoprotein for diagnosing primary liver cancer by liquid chromatography tandem mass spectrometry. Clin Chim Acta 457: 92-98, 2016.
12. Wei Q, Guo P, Mu K, Zhang Y, Zhao W, Huai W, Qiu Y, Li T, Ma X, Liu Y, *et al*: Estrogen suppresses hepatocellular carcinoma cells through ERβ-mediated upregulation of the NLRP3 inflammasome. Lab Invest 95: 804-816, 2015.
13. Baldissera VD, Alves AF, Almeida S, Porawski M and Giovenardi M: Hepatocellular carcinoma and estrogen receptors: Polymorphisms and isoforms relations and implications. Med Hypotheses 86: 67-70, 2016.
14. Shen M and Shi H: Sex hormones and their receptors regulate liver energy homeostasis. Int J Endocrinol 2015: 294278, 2015.
15. Sheng ML, Xu GL, Zhang CH, Jia WD, Ren WH, Liu WB, Zhou T, Wang YC, Lu ZL, Liu WF, *et al*: Aberrant estrogen receptor alpha expression correlates with hepatocellular carcinoma metastasis and its mechanisms. Hepatogastroenterology 61: 146-150, 2014.
16. Ahmed HH, Shousha WG, Shalby AB, El-Mezayen HA, Ismaiel NN and Mahmoud NS: Implications of sex hormone receptor gene expression in the predominance of hepatocellular carcinoma in males: Role of natural products. Asian Pac J Cancer Prev 16: 4949-4954, 2015.
17. Wang B, Chen L and Chang HT: Potential diagnostic and prognostic biomarkers for cholangiocarcinoma in serum and bile. Biomark Med 10: 613-619, 2016.
18. Isse K, Specht SM, Lunz JG III, Kang LI, Mizuguchi Y and Demetris AJ: Estrogen stimulates female biliary epithelial cell interleukin-6 expression in mice and humans. Hepatology 51: 869-880, 2010.
19. Hunsawong T, Singsuksawat E, In-chon N, Chawengrattanachot W, Thuwajit C, Sripa B, Paupairoj A, Chau-in S and Thuwajit P: Estrogen is increased in male cholangiocarcinoma patients' serum and stimulates invasion in cholangiocarcinoma cell lines in vitro. J Cancer Res Clin Oncol 138: 1311-1320, 2012.
20. Singsuksawat E, Thuwajit C, Charngkaew K and Thuwajit P: Increased ETV4 expression correlates with estrogen-enhanced proliferation and invasiveness of cholangiocarcinoma cells. Cancer Cell Int 18: 25, 2018.
21. Marzioni M, Torrice A, Saccomanno S, Rychlicki C, Agostinelli L, Pierantonelli I, Rhönnstad P, Trozzi L, Apelqvist T, Gentile R, *et al*: An oestrogen receptor β-selective agonist exerts anti-neoplastic effects in experimental intrahepatic cholangiocarcinoma. Dig Liver Dis 44: 134-142, 2012.
22. Xu J and Li Q: Review of the in vivo functions of the p160 steroid receptor coactivator family. Mol Endocrinol 17: 1681-1692, 2003.
23. York B and O'Malley BW: Steroid receptor coactivator (SRC) family: Masters of systems biology. J Biol Chem 285: 38743-38750, 2010.
24. Xu J, Wu RC and O'Malley BW: Normal and cancer-related functions of the p160 steroid receptor co-activator (SRC) family. Nat Rev Cancer 9: 615-630, 2009.
25. Qin L, Liu Z, Chen H and Xu J: The steroid receptor coactivator-1 regulates twist expression and promotes breast cancer metastasis. Cancer Res 69: 3819-3827, 2009.
26. Qin L, Chen X, Wu Y, Feng Z, He T, Wang L, Liao L and Xu J: Steroid receptor coactivator-1 upregulates integrin α_5 expression to promote breast cancer cell adhesion and migration. Cancer Res 71: 1742-1751, 2011.
27. Zhang Y, Duan C, Bian C, Xiong Y and Zhang J: Steroid receptor coactivator-1: A versatile regulator and promising therapeutic target for breast cancer. J Steroid Biochem Mol Biol 138: 17-23, 2013.
28. Song X, Zhang C, Zhao M, Chen H, Liu X, Chen J, Lonard DM, Qin L, Xu J, Wang X, *et al*: Steroid receptor coactivator-3 (SRC-3/AIB1) as a novel therapeutic target in triple negative breast cancer and its inhibition with a phospho-bufalin prodrug. PLoS One 10: e0140011, 2015.
29. Wang H, Zhang D, Wu W, Zhang J, Guo D, Wang Q, Jing T, Xu C, Bian X and Yang K: Overexpression and gender-specific differences of SRC-3 (SRC-3/AIB1) immunoreactivity in human non-small cell lung cancer: An in vivo study. J Histochem Cytochem 58: 1121-1127, 2010.
30. Luo F, Li W, Zhang J, Huang K, Fu J and Xie Z: Overexpression of steroid receptor coactivator-3 in bone cancers: An in vivo immunohistochemical study with tissue microarray. Pathol Res Pract 209: 790-796, 2013.
31. Li W, Fu J, Bian C, Zhang J and Xie Z: Immunohistochemical localization of steroid receptor coactivators in chondrosarcoma: An in vivo tissue microarray study. Pathol Res Pract 210: 1005-1010, 2014.
32. Liu C, Zhang Y, Zhang K, Bian C, Zhao Y and Zhang J: Expression of estrogen receptors, androgen receptor and steroid receptor coactivator-3 is negatively correlated to the differentiation of astrocytic tumors. Cancer Epidemiol 38: 291-297, 2014.
33. Liu M, Zhang K, Zhao Y, Guo Q, Guo D and Zhang J: Evidence for involvement of steroid receptors and coactivators in neuroepithelial and meningothelial tumors. Tumour Biol 36: 3251-3261, 2015.
34. Jeong JW, Kwak I, Lee KY, White LD, Wang XP, Brunicardi FC, O'Malley BW and DeMayo FJ: The genomic analysis of the impact of steroid receptor coactivators ablation on hepatic metabolism. Mol Endocrinol 20: 1138-1152, 2006.
35. Louet JF, Chopra AR, Sagen JV, An J, York B, Tannour-Louet M, Saha PK, Stevens RD, Wenner BR, Ilkayeva OR, *et al*: The coactivator SRC-1 is an essential coordinator of hepatic glucose production. Cell Metab 12: 606-618, 2010.
36. Motamed M, Rajapakshe KI, Hartig SM, Coarfa C, Moses RE, Lonard DM and O'Malley BW: Steroid receptor coactivator 1 is an integrator of glucose and NAD+/NADH homeostasis. Mol Endocrinol 28: 395-405, 2014.
37. Tannour-Louet M, York B, Tang K, Stashi E, Bouguerra H, Zhou S, Yu H, Wong LJ, Stevens RD, Xu J, *et al*: Hepatic SRC-1 activity orchestrates transcriptional circuitries of amino acid pathways with potential relevance for human metabolic pathogenesis. Mol Endocrinol 28: 1707-1718, 2014.
38. Hansel MC, Davila JC, Vosough M, Gramignoli R, Skvorak KJ, Dorko K, Marongiu F, Blake W and Strom SC: The use of induced pluripotent stem cells for the study and treatment of liver diseases. Curr Protoc Toxicol 67: 14 13 11-14 13 27, 2016.

39. Siegel R, Ma J, Zou Z and Jemal A: Cancer statistics, 2014. CA Cancer J Clin 64: 9-29, 2014.
40. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ and He J: Cancer statistics in China, 2015. CA Cancer J Clin 66: 115-132, 2016.
41. Zhang D, Guo Q, Bian C, Zhang J, Lin S and Su B: Alterations of steroid receptor coactivator-1 (SRC-1) immunoreactivities in specific brain regions of young and middle-aged female Sprague-Dawley rats. Brain Res 1382: 88-97, 2011.
42. Anzick SL, Kononen J, Walker RL, Azorsa DO, Tanner MM, Guan XY, Sauter G, Kallioniemi OP, Trent JM and Meltzer PS: AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. Science 277: 965-968, 1997.
43. Culig Z, Klocker H, Bartsch G and Hobisch A: Androgen receptors in prostate cancer. Endocr Relat Cancer 9: 155-170, 2002.
44. Kershah SM, Desouki MM, Koterba KL and Rowan BG: Expression of estrogen receptor coregulators in normal and malignant human endometrium. Gynecol Oncol 92: 304-313, 2004.
45. Xie D, Sham JS, Zeng WF, Lin HL, Bi J, Che LH, Hu L, Zeng YX and Guan XY: Correlation of AIB1 overexpression with advanced clinical stage of human colorectal carcinoma. Hum Pathol 36: 777-783, 2005.
46. Glaeser M, Floetotto T, Hanstein B, Beckmann MW and Niederacher D: Gene amplification and expression of the steroid receptor coactivator SRC3 (AIB1) in sporadic breast and endometrial carcinomas. Horm Metab Res 33: 121-126, 2001.
47. Xu FP, Xie D, Wen JM, Wu HX, Liu YD, Bi J, Lv ZL, Zeng YX and Guan XY: SRC-3/AIB1 protein and gene amplification levels in human esophageal squamous cell carcinomas. Cancer Lett 245: 69-74, 2007.
48. Sakakura C, Hagiwara A, Yasuoka R, Fujita Y, Nakanishi M, Masuda K, Kimura A, Nakamura Y, Inazawa J, Abe T and Yamagishi H: Amplification and over-expression of the AIB1 nuclear receptor co-activator gene in primary gastric cancers. Int J Cancer 89: 217-223, 2000.
49. Yoshida H, Liu J, Samuel S, Cheng W, Rosen D and Naora H: Steroid receptor coactivator-3, a homolog of Taiman that controls cell migration in the Drosophila ovary, regulates migration of human ovarian cancer cells. Mol Cell Endocrinol 245: 77-85, 2005.
50. Henke RT, Haddad BR, Kim SE, Rone JD, Mani A, Jessup JM, Wellstein A, Maitra A and Riegel AT: Overexpression of the nuclear receptor coactivator AIB1 (SRC-3) during progression of pancreatic adenocarcinoma. Clin Cancer Res 10: 6134-6142, 2004.
51. Uchikawa J, Shiozawa T, Shih HC, Miyamoto T, Feng YZ, Kashima H, Oka K and Konishi I: Expression of steroid receptor coactivators and corepressors in human endometrial hyperplasia and carcinoma with relevance to steroid receptors and Ki-67 expression. Cancer 98: 2207-2213, 2003.
52. Martínez-Jiménez CP, Gómez-Lechón MJ, Castell JV and Jover R: Underexpressed coactivators PGC1alpha and SRC1 impair hepatocyte nuclear factor 4 alpha function and promote dedifferentiation in human hepatoma cells. J Biol Chem 281: 29840-29849, 2006.
53. Tong Z, Li M, Wang W, Mo P, Yu L, Liu K, Ren W, Li W, Zhang H, Xu J and Yu C: Steroid receptor coactivator 1 promotes human hepatocellular carcinoma progression by enhancing Wnt/β-catenin signaling. J Biol Chem 290: 18596-18608, 2015.
54. Ma X, Xu L, Wang S, Cui B, Li X, Xu J and Ning G: Deletion of steroid receptor coactivator-3 gene ameliorates hepatic steatosis. J Hepatol 55: 445-452, 2011.