Sterol regulatory element-binding protein 1 is required for ovarian tumor growth

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Received February 20, 2013; Accepted April 22, 2013

DOI: 10.3892/or.2013.2575

Abstract. Re-programming of lipogenic signaling is one of the most significant alterations of tumor cell pathology. Consistent with a large demand for lipids, tumor cells express high levels of lipogenic enzymes, most of which are transcriptional targets of sterol regulatory element-binding protein 1 (SREBP1). However, the expression levels and the function of SREBP1 in ovarian cancer are largely unknown. Our study aimed to assess the oncogenic potential of SREBP1 in ovarian cancer. In this study, we showed that the SREBP1 protein expression was significantly higher in human ovarian cancer compared to benign and borderline ovarian tumors by immunohistochemical staining. Knockdown of SREBP1 by small hairpin RNA (shRNA) in ovarian cancer cells retarded cell growth, migration and invasion and enhanced cell apoptosis without significant effects on cell cycle distribution. In a xenograft SCID mouse model, SREBP1 silencing inhibited tumor growth *in vivo* and reduced the expression of SREBP1 downstream lipogenic genes at both the protein and mRNA levels. Taken together, the results from this study demonstrate a crucial role of SREBP1 in ovarian cancer growth, which establish SREBP1 as a novel therapeutic target for antitumor therapy.

Introduction

Ovarian cancer is the third most common gynecologic malignancy and is the most lethal form of gynecological cancer. Aberrations of metabolic pathways are the characteristic physiological changes occurring in cancer cells. In the 1920s, Warburg (1) discovered that cancer cells reprogram energy production mechanism through high aerobic glycolysis. Furthermore, it was found that cancer cells derive most fatty acids from *de novo* synthesis (2). Cancer is a complex blend of genetic and metabolic perturbations and *de novo* lipogenesis is believed to be active in oncogenesis. Expression of most enzymes required for the synthesis of fatty acids, cholesterol, triacylglycerols and phosphoglycerides is regulated by the SREBPs which have now been established as global lipid synthetic regulators (3,4). The SREBPs have many targets such as fatty acid synthase (FASN), ATP citrate lyase (ACLY), acetyl-CoA carboxylase (ACAC) and stearoyl-CoA desaturase-1 (SCD1) (5-8). The regulatory function of sterol regulatory element-binding protein 1 (SREBP1) indicates a role in sensing and regulating cancer-associated lipogenesis. Altered expression of SREBP1 has been reported in various human cancers, such as hepatocellular carcinoma, colorectal carcinoma, breast and prostate cancers (9-12). However, the functional studies on the role of SREBP1 in human ovarian cancer are limited to date.

In this study, we investigated the relationship between SREBP1 expression levels and ovarian cancer. We studied the association between expression of SREBP1 in ovarian cancer and its effect on ovarian cancer progression and metabolic changes *in vitro* and *in vivo*.

Materials and methods

Ovarian cancer specimen and immunohistochemical (IHC) staining. Human ovarian tumor specimens were obtained from the primary ovarian sites of previously untreated patients. All of the specimens were anonymous and tissues were obtained in compliance with institutional review board regulations. Patient features and tumor characteristics for this study are reported in Tables I and II. On follow-up, 26 patients survived, 59 patients were deceased, and 11 patients were lost, adding up to a follow-up rate of 88.54%. Follow-up records until December 2012 (from 9 to 120 months) are presented, with a mean follow-up time of 60.03 months.

The immunohistochemical staining was carried out by cutting out 4-μm sections from the formaldehyde-fixed and paraffin-embedded tissue specimens. The tissue sections were baked, deparaffinized with xylene, and rehydrated through a

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Key words: sterol regulatory enzyme binding protein 1, ovarian cancer, lipogenesis, cell growth, tumorigenesis

Table I. Correlation between SREBP1 expression and different phenotypes of ovarian tissues.

Characteristics	No. of patients	SREBP1 n+ (%)	SREBP1 n- (%)	P-value
Benign ovarian tumor	15	2	13	<0.001
Borderline ovarian tumor	16	5	11	
Ovarian cancer	96	65	31	

graded alcohol series. Endogenous peroxidase activity was blocked with a 3% hydrogen peroxide solution. After antigen retrieval and blocking the nonspecific binding of the primary antibodies, the slides were then incubated with the anti-SREBP1 rabbit polyclonal antibody (ab93638, Abcam, Cambridge, MA, USA) and the anti-FASN rabbit polyclonal antibody (ab96866 Abcam) overnight. The slides were incubated in biotinylated secondary antibody (horseradish peroxidase-conjugated anti-mouse/rabbit IgG) and the avidin biotin peroxidase complex. 3,3'-Diaminobenzidine (DAB) substrate chromogen solution was applied subsequently for visualization. For each specimen, immunoreactive score (H-score) was analyzed according to the total percentage of positive cells and the intensity of the cytoplasmic staining (1+, 2+, or 3+), where H = (%1+ x1) + (%2+ x2) + (%3+ x3). A minimum of 100 cells were evaluated in calculating the H-score.

Cancer cell lines, plasmids and cell culture. Human ovarian cancer cell lines HO8910PM, A2780, 3AO, SKOV3 were obtained from Shanghai Institute for Biological Sciences, Chinese Academy of Sciences. Three shRNAs targeting human SREBF-1 and empty vector were from Open Biosystems (catalogue no. RHS4533, Lafayette, CO, USA). A2780, SKOV3, 3AO, HO8910PM cell lines were cultured in RPMI-1640 medium. The human embryonic kidney 293T cells (HEK293T) were maintained in DMEM medium. For lipid-free culture condition, the basal medium was supplemented with 10% lipid-depleted FBS purchased from Cocalico Biological (Reamstown, PA, USA) (catalogue no. 55-0116).

Transfection and constructing stable cell lines. Superfect Transfection Reagent (Qiagen, Valencia, CA, USA) was used for transient transfection according to manufacturer's instructions. Lenti-vira were prepared for cell transduction through Trans-Lentiviral shRNA packaging kit (Open Biosystems). The lenti-viral vector expressing shRNA were then introduced into HEK 293T cells by transient co-transfection with helper virus with calcium phosphate precipitation. After the viral production, the medium was filtered through a 0.45-μm filter. The ovarian cancer cells were infected at ~70% confluence in DMEM medium containing 8 μg/ml of polybrene. After a 24-h culture, the medium was replenished with fresh medium. Then the stable cell lines were selected using 2 μg/ml puromycin for two weeks. The knockdown efficiency was determined by both western blotting and qRT-PCR.

Gene expression analysis (RNA isolation, quantitative real-time PCR). Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The cDNAs were synthesized from total RNA using the SuperScript™ II Reverse Transcriptase kit (Invitrogen). PCR was carried out using SYBR® Green Real-time PCR master mix (code no. QPK-201, Toyobo Co., Ltd.). The thermal cycling conditions were maintained at an initial denaturation at 95˚C for 30 sec, followed by 40 cycles of PCR using the following profile: 94˚C, 5 sec; 60˚C, 10 sec; and 72˚C, 15 sec. GAPDH was used as a control of normalization, the primers used for qRT-PCR are listed in Table III.

Western blot analysis. The cells were collected and lysed in lysis buffer, the boiled lysates were resolved by SDS-PAGE and then blotted onto PVDF membrane. Afer incubating with primary antibodies against SREBP1, FASN, ACLY, SCD, ACAC (Abcam) and β-actin (#IE9A3 ZSGB-BIO). The membranes were incubated with appropriate horseradish peroxidase-labeled secondary antibodies and visualized by ECL Plus system (Amersham Life Sciences, Piscataway, NJ, USA). Quantitative data were assigned using a computing densitometer with Image-Pro Plus software.

Cell apoptosis and cell cycle assays. Cell apoptosis was performed by Annexin V-FITC/PI apoptosis detection kit (BD Biosciences Clontech) following manufacturer's instructions. Cell cycle was determined by RNase A and PI. The mixture was immediately subjected to cell apoptosis analysis and cell cycle assays on FACS calibur flow cytometer (Becton-Dickinson, Franklin Lakes, NJ, USA).

Cell proliferation, transwell migration and invasion assay. For cell proliferation assay using the Cell Counting Kit-8 (CCK-8), cells ($2x10^3$ cells per well) were seeded on 96-well plates for 5-day incubation. Then cells were harvested and numbered by enzyme-linked immunosorbent assay (ELISA) reader. The 24-well transwell cell culture chambers were utilized to examine *in vitro* cell migration and invasion of ovarian cancer cells. Briefly, the undersides of the upper chambers were precoated with extracellular matrix (ECM) gel (1:4 dilution, for invasion assay) or not (for migration assay). Cells ($1x10^5$) were seeded inside the precoated upper chambers. After 24 h of incubation, the numbers of migrated or invading cells were measured by the crystal violet staining method.

Mouse xenograft experiments. In order to analyze the tumorigenic potential of SREBP1, Female (4-6 weeks old) SCID mice were purchased from Medical College of Peking University and all animal work was performed in conformity with the National Institutes of Health Guide for Care and Use of Laboratory Animals (publication no. 85-23, revised 1985). The mice were subcutaneously inoculated with highly metastatic HO8910PM cells in the axillary fossa. Comparisons were made among control-shRNA, shRNA-#1 and shRNA-#3

Table II. Correlation between clinicopathological parameters and SREBP1 expression in ovarian cancer.

Characteristics	No. of patients	SREBP1 n+ (%)	SREBP1 n- (%)	P-value
Age (years)				
≤50	35	20 (20.83%)	15 (15.63%)	0.094
>50	61	45 (46.88%)	16 (16.67%)	
FIGO stage				
I-II	25	7 (7.29%)	18 (18.75%)	<0.001
III-IV	71	58 (60.42%)	13 (13.54%)	
Histologic grade				
G1-G2	36	16 (16.67%)	14 (14.58%)	0.042
G3	60	49 (51.04%)	17 (17.71%)	
Ascites/peritoneal washings				
Negative	25	15 (15.63%)	10 (10.42%)	0.335
Positive	71	50 (52.08%)	21 (21.88%)	
Lymph node metastasis				
Negative	70	51 (53.13%)	19 (19.79%)	0.582
Positive	26	14 (14.58%)	12 (12.50%)	
Pathologic type				
Ovarian serous carcinoma	70	48 (50.00%)	22 (22.92%)	0.944
Mucinous ovarian cancer	15	10 (10.42%)	5 (5.21%)	
Endometrioid adenocarcinoma	11	7 (7.29%)	4 (4.17%)	
FASN expression				
Positive	63	60 (62.50%)	3 (3.12%)	<0.001
Negative	32	5 (5.21%)	28 (29.17%)	

Table III. The primers used for real-time PCR.

Gene symbol	Forward primer sequence	Reverse primer sequence	Amplicon size (bp)
SREBP1a	5'-CGGCGCTGCTGACCGACATC	5'-CCCTGCCCCACTCCCAGCAT	104
SREBP1c	5'-GCGCAGATCGCGGAGCCAT	5'-CCCTGCCCCACTCCCAGCAT	116
FASN	5'-CACAGGGACAACCTGGAGTT	5'-ACTCCACAGGTGGGAACAAG	97
ACLY	5'-GCCCATCCCCAACCAGCCAC	5'-TTGCAGGCGCCACCTCATCG	137
ACACa	5'-CGGAAGGGACAGTAGAAATCA	5'-AGTCGCTCAGCCAAGTGGA	94
SCD1	5'-CGACGTGGCTTTTTCTTCTC	5'-CCTTCTCTTTGACAGCTGGG	70
GAPDH	5'-GAGTCAACGGATTTGGTCGT	5'-TTGAGGTCAATGAAGGGGTC	103

groups ($3x10^6$ cells/mouse) with 9 mice in each group. The tumor volume was calculated using the following equation: $V = a \times b^2/2$, where a and b represented the longest and shortest diameter of the tumor, respectively. At the time of sacrifice, tumors were excised, weighed and preserved in order to perform further experiments.

Statistical analysis. For *in vitro* experiments, results are presented as means ± standard deviation (SD). Least significant difference t test (LSD-t) and t-test were performed for group comparison. An exponential regression model was performed for estimate of *in vivo* experiment. Fisher's exact tests for contingency tables were used in Tables I and II. The relative risk of death from ovarian cancer was evaluated through the Cox proportional hazards regression model. Kaplan-Meier survival curves were used and significance was assessed by the Log-rank test. The overall survival was also compared by Log-rank test. For all of the statistical tests, all P-values were 2-sided and $P<0.05$ was defined as statistically significant. In all cases, SPSS 17.0 software (SPSS Inc., Chicago, IL) was used.

Results

Increased SREBP1 protein expression in ovarian cancer. We performed immunohistochemical (IHC) staining on different subtypes of ovarian cancer specimens and observed

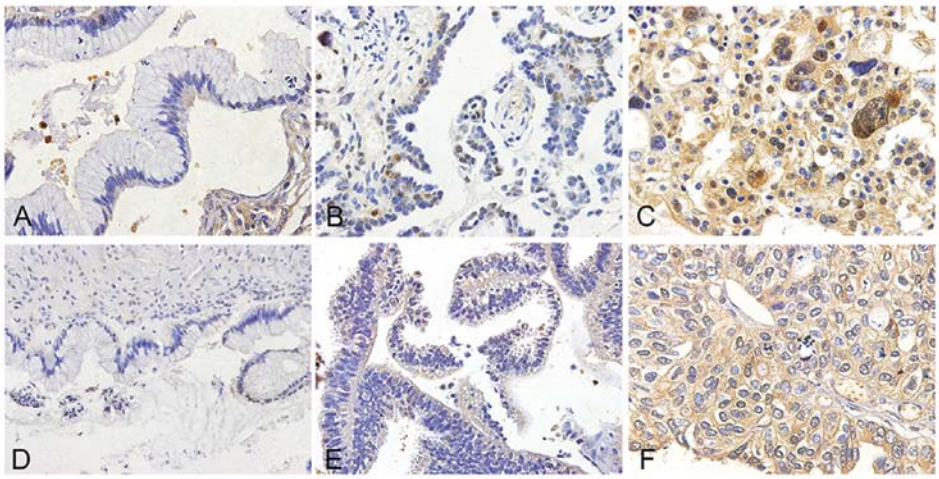

Figure 1. SREBP1 and FASN expression in ovarian tissues determined by IHC. Ovarian benign tumor (A) and borderline ovarian tumor (B) showed a lower staining of SREBP1, but ovarian cancer (C) showed higher density staining (immunohistochemical staining, x400). Ovarian benign tumor (D) and borderline ovarian tumor (E) also showed a lower staining of FASN, but ovarian cancer (F) showed higher density staining (immunohistochemical staining, x400).

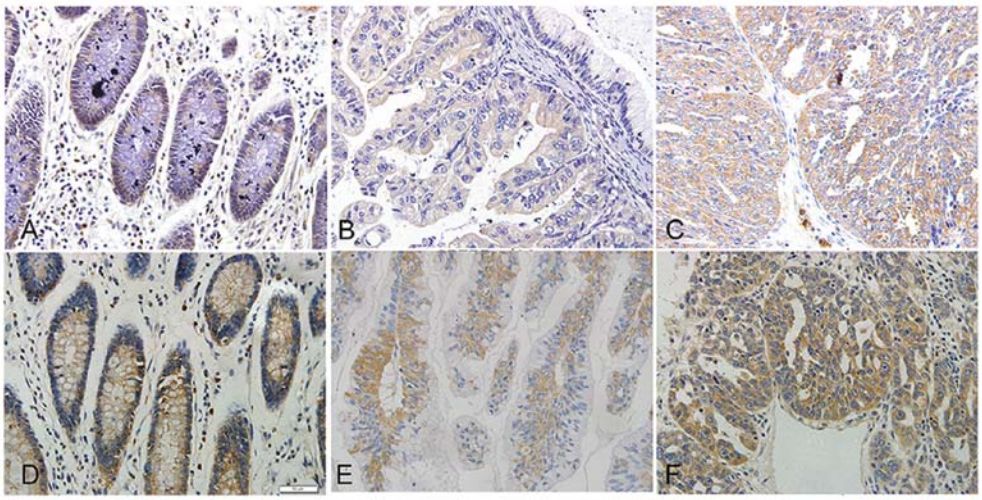

Figure 2. SREBP1 and FASN expression in ovarian cancer of histologic grade determined by IHC. G1 (A) and G2 (B) showed a lower staining of SREBP1, but G3 (C) showed higher density staining (immunohistochemical staining, x400). G1 (D) and G2 (E) showed a lower staining of FASN, but G3 (F) showed higher density staining (immunohistochemical staining, x400).

that SREBP1 expression was detected in epithelial cells but not in stromal cells. Cytoplasmic distribution of SREBP1 was enhanced in ovarian cancer when compared to benign and borderline ovarian tumor (P<0.001), (Figs. 1A-C and 3A; Table I). The detection implies SREBP1 maybe active in ovarian cancer genesis.

Correlation between SREBP1 expression and clinicopathological parameters. SREBP1 expression levels were significantly correlated with FIGO surgical stage, histological grade and FASN expression of ovarian cancer (Table II, Figs. 1 and 2). Expression of SREBP1 protein increased with higher FIGO surgical stage and histological grade of disease [from 16.05% (stage I-II) to 73.95% (stage III-IV) (P<0.001); from 37.50% (G1-G2) to 62.50% (G3) (P=0.042); Table I]. The more aggressive phenotypes of ovarian cancer have more intensive staining of SREBP1 (Fig. 2A-C). However, SREBP1 expression did not show any significant correlation with pathological type, patient age, lymph node metastasis or ascitic cytology (Table II). Elevated expression of nuclear SREBP1 in ovarian cancer was observed, however it did not show any significant correlation with the clinicopathological parameters, which may be due to the size of the patient cohort. These results suggest that SREBP1 is overexpressed in ovarian cancer, which may contribute to cancer progression.

Prognostic factor analysis for ovarian cancer and predictive value of SREBP1 in patient survival. We examined the relationship between SREBP1 expression and survival in patients with ovarian cancer (n=96). The mean survival for patients, tested positive for SREBP1, was significantly shorter than the mean survival for those tested negative (46.06 months; 95%CI, 37.44-54.68 months vs. 75.90 months; 95%CI, 59.67-92.12 months; P=0.017, Fig. 3B). Univariate survival analysis showed that FIGO stage and SREBP1 expression were significantly associated with survival (P<0.05). Furthermore, Cox proportional hazards regression model indicated the FIGO stages to be the definitive prognostic factor (P<0.001). Histological

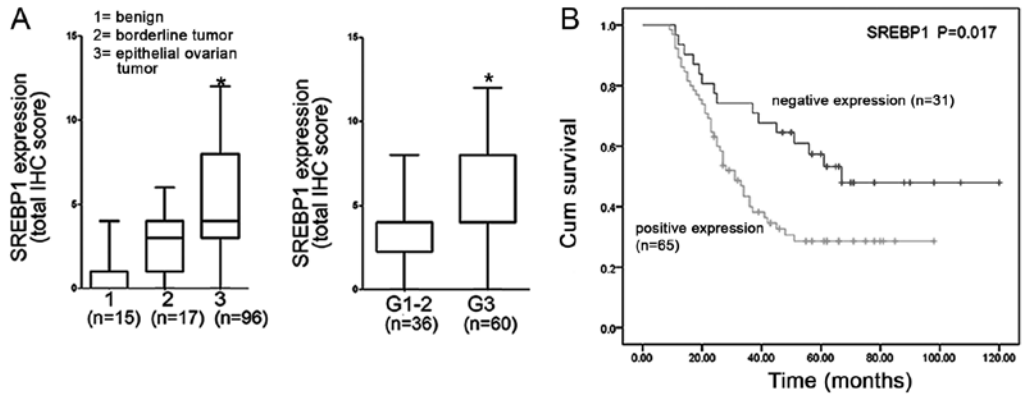

Figure 3. Quantification of SREBP1 expression in various phenotypes of ovarian tissues and predictive value of SREBP1 in patient survival. (A) Box plot of IHC staining score for SREBP1 in cytoplasm in various phenotypes of ovarian tissues and histological grades of ovarian cancer, *P<0.05. (B) Cumulative survival of ovarian cancer patients with reference to SREBP1 expression. Differences between the two groups were evaluated with the Kaplan-Meier curves.

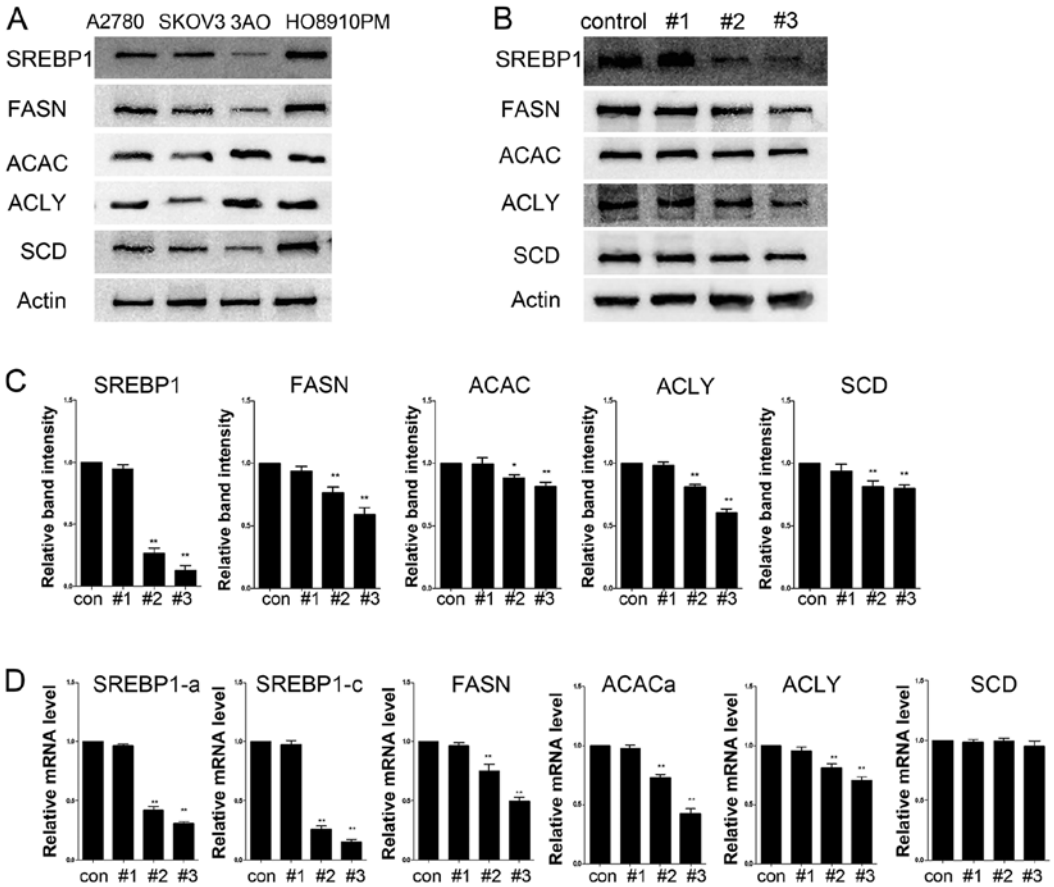

Figure 4. SREBP1 is required for lipogenic genes in ovarian cancer cells. (A) Western blot analysis of lipogenic gene expression in commonly used ovarian cancer cell lines. (B) HO8910PM cells were transduced with a set of lentiviral vector expressing shRNA targeting SREBF1 (#1, #3 and control-vector). Western blot analysis of SREBP1, FASN, ACACα, ACLY and SCD1 were performed. (C) Western blot analysis showed a successful knockdown of SREBP1 and reduced expression of FASN, ACLY, ACAC, SCD as transcriptional targets of SREBP1. *P<0.05, **P <0.001, compared with the control-shRNA group. (D) Quantitative RT-PCR analysis of mRNA abundance of SREBP1 and their targets in transduced cells. *P<0.05, **P<0.001, compared with the SREBP1 group.

classification, pathologic stage, ascitic cytology, lymphatic metastasis, age, FASN and SREBP1 expression appeared to be independent factor in predicting the overall survival in ovarian cancer patients.

SREBP1 is responsible for lipogenic gene expression in ovarian cancer cells. We performed the initial screening for SREBP1 expression in four aforementioned ovarian cancer cell lines. SREBP1 expression was almost undetectable in the 3AO cells, moderately expressed in SKOV3 cells and highly expressed in A2780 and HO8910PM cells (Fig. 4A). SREBP1 downstream target genes FASN, ACLY, ACAC and SCD1 were also highly expressed in HO8910PM. HO8910PM cell line was selected for most of the experiments in this study (13). To verify whether the

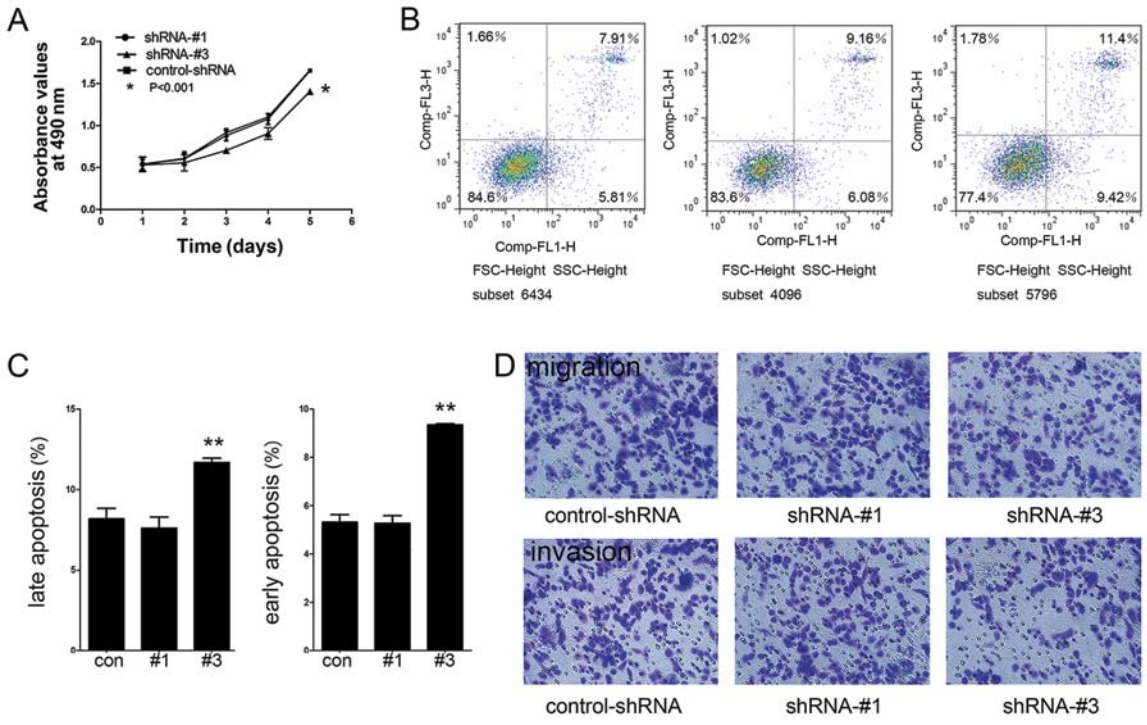

Figure 5. Effect of SREBP1-shRNA transfection on the cell growth, apoptosis, migration and invasion of HO8910PM cells. (A) Curves of cell growth after transfection at different time points by CCK8 assay. HO8910PM cells were divided into three groups (shRNA-#1, shRNA-#3 and control-shRNA group). Data are presented as means ± SD from triplicates. *P<0.001, compared with the control-shRNA group. (B) shRNA-#1, shRNA-#3 and control-shRNA group were subjected to cell apoptosis analysis by flow cytometry. (C) Early and late apoptotic cell death is presented as percentage to the total cells counted. Results are given as means ± SD. The experiment was conducted thrice, **P<0.05, compared with the control-shRNA group. (D) HO8910PM cells crossed the filter and the ECM gel-coated filter (crystal violet staining, original magnification x400) for migration and invasion. A significantly reduced number of migrated and invaded cells in shRNA-#3 group compared to control-shRNA group, P<0.05.

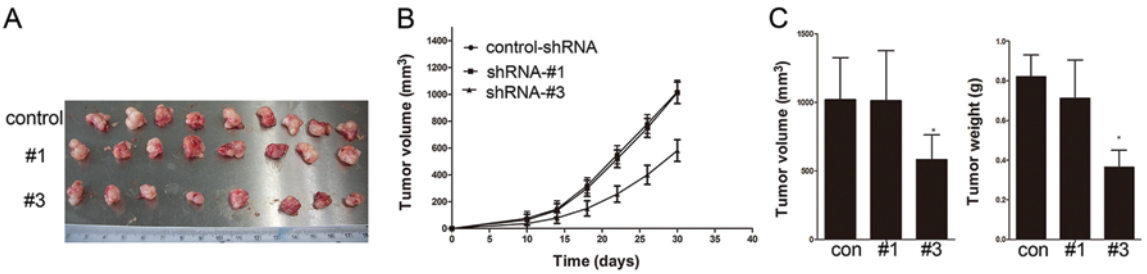

Figure 6. Xenograft tumor growth of ovarian cancer cells is retarded by SREBP1-shRNA. (A and B) HO8910PM cells (shRNA-#1, shRNA-#3 and control-shRNA group) were implanted into BALB/C mice subcutaneously. Tumor growth was measured every four days and tumor volume was calculated. (C) tumor weight and volume were compared between shRNA-#1, shRNA-#3 and control-shRNA group.

endogenous SREBP1 is required for lipogenic gene expression, we constructed four shRNA expression vectors (#1-3 and control-vector) targeting SREBP1, and transfected them into HO8910M cells. A high knockdown efficiency of SREBP1 expression was observed by Western blot and real-time PCR in vectors #2 and #3 (Fig. 4B-D). Simultaneous reduction in the expression of SREBP1 target genes indicated an SREBP1-dependent expression of these genes (Fig. 4B-D).

SREBP1 regulation of cellular proliferation, migration, invasion and apoptosis. In order to compare cell proliferation rate among cells with knockdown of SREBP1, lipid-free culture medium was used to culture shRNA-#1, shRNA-#3 and control-shRNA cells. Statistical analysis indicated a significant reduction of cell proliferation in shRNA-#3 group compared to control-shRNA (P<0.05) (Fig. 5A). We also found that knockdown of SREBP1, though attenuating cell growth, had no significant influence on a specific phase of the cell cycle. These observations suggested that the depletion of SREBP1 may increase the overall cell cycle duration.

As shown in Fig. 5B, the rate of late apoptosis in the shRNA-#3 group (11.68±0.27%) was significantly higher than that of the control-shRNA group (8.17±0.66%) (P<0.001) (Fig. 5C). Cells in early apoptosis were low in all three cell lines, which showed an increase from 5.27% in control-shRNA group to 9.34% in shRNA-#3 group (P<0.01) (Fig. 5C). Next, we analyzed the migratory and invasive properties of SREBP1 knockdown cells. We found a significantly reduced number of

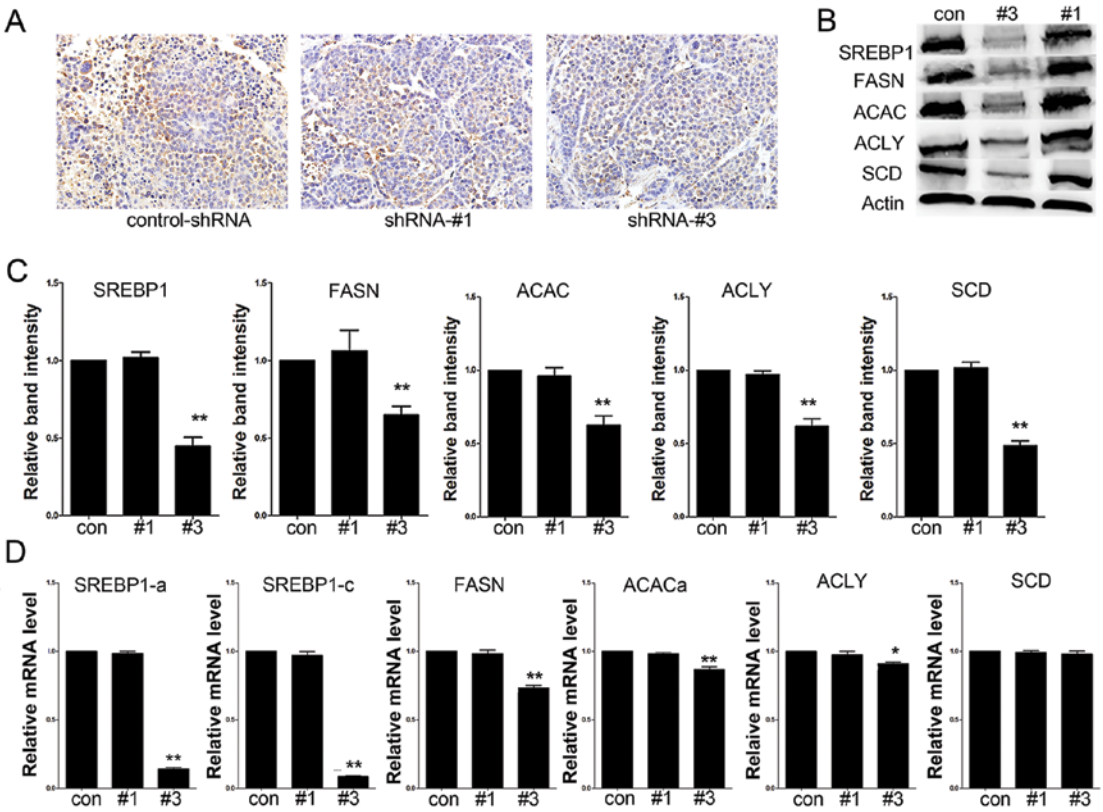

Figure 7. Knockdown of SREBP1 expression represses the downstream genes in xenografted ovarian tumor tissues. (A) Immunohistochemical staining of SREBP1 protein in xenografted ovarian tumor tissues. Attenuated immunoreactivity for SREBP1 was detected in the shRNA-#3 group of mice. (B and C) Western blotting was performed to show a successful knockdown of SREBP1 and significantly reduced expression of its targets in xenografted ovarian tumor tissues. (D) Quantitative RT-PCR were performed to show a successful knockdown of SREBP1 and significantly reduced expression of its targets in xenografted ovarian tumor tissues.

migrating cells in shRNA-#3 group (128.34±6.72), compared with control-shRNA group (170.45±5.64) and invading cells in shRNA-#3 group (91.32±5.26), compared with control-shRNA group (137.36±8.72) (Fig. 5D). These data revealed that SREBP1 functions in regulating cell migration and invasion of ovarian cancer cells.

Knockdown of SREBP1 impairs ovarian tumor growth in vivo. The xenograft female SCID mouse model exhibited a markedly slowed growth pattern at 10 days after tumor cell inoculation of shRNA-#3 group (Fig. 6A and B). Control-shRNA and shRNA-#1 groups were consistent with comparatively larger tumors (Fig. 6C). The shRNA-#3 group showed a significant inhibition of tumor growth ($P<0.001$). These results demonstrate that SREBP1 plays an important role in tumor growth *in vivo*. Immunohistochemical analysis of tumor sections showed a decrease of the SREBP1 expression in tumors from shRNA-#3 groups compared to control-shRNA group (Fig. 7A). As shown in Fig. 7B-D, the expression levels of SREBP1 and its downstream target genes were decreased in the tumors in #3 group.

Discussion

In cancer cells, *de novo* lipogenesis is closely related to increased synthesis of membranes, energy production and activation of intracellular signaling pathways during cell proliferation and division as well as cancer development and progression (14-16). SREBPs are major transcriptional regulators of genes involved in *de novo* lipid synthesis and are critical for maintaining lipid homeostasis. SREBP1 overexpression or activation has been reported in hepatocellular carcinoma, colorectal carcinoma, breast, and prostate tumors (9-12). Despite this elaborate research, this is the first report on SREBP1 expression in ovarian cancer. In our study, we found that SREBP1 promoted ovarian cancer growth. SREBP1 expression levels were associated with clinicopathological parameters and with FASN expression. A previous study showed that the lipogenic gene, FASN, was overexpressed in ovarian cancers (17). We anticipated that SREBP1 overexpression in ovarian cancer may contribute to enhanced FASN expression. The survival of patients who expressed high levels of SREBP1 was significantly reduced compared to the patients negative for SREBP1 expression. The cell culture and *in vivo* experimental data collectively suggest that SREBP1 expression plays an important role in regulation of ovarian tumor growth. The observation of SREBP1 overexpression in ovarian cancer further supports the notion that *de novo* lipid synthesis is required in tumor growth.

To further investigate potential function of SREBP1 in ovarian cancer, we conducted several biophysiological experiments using the shRNA knockdown approach targeting SREBP1. Our results confirmed that SREBP1 silencing in ovarian cancer cells inhibited cell growth, migration and invasion. Enhanced cell apoptosis further implies the mechanisms by which SREBP1 is required for tumor growth. The observation of cell proliferation was only made in cell culture

in the presence of fat-depleted serum; where the cultured cells completely rely on the *de novo* lipogenesis to satisfy the demand for cholesterol and phospholipids of rapidly proliferating cells. Re-activation of *de novo* fatty acid synthesis emerges in many cancers, suggesting that lipogenesis may be a rate-limiting stage in rapidly growing tissues (18). Induction of *de novo* lipid synthesis in response to Akt activation requires the activation of SREBP (19). Furthermore, Akt acts as an anti-apoptotic signaling molecule and PI3K/Akt/TOR pathway regulates lipid and protein biosynthesis. Both of these processes are critically required for cell growth (20). Overactive Akt (often found in cancer) activates SREBP and increases lipid synthesis, promoting Akt signaling (21). Thus SREBP1 expression by stimulating lipogenic genes, promotes cancer cell proliferation. This is the first report that systematically investigated the function of SREBP1 in ovarian cancer cell proliferation and tumor growth and may ultimately provides a novel therapeutic strategy in the therapeutic aspect of this disease.

Several proteins including FASN, ACAC, ACLY and SCD inlipogenic pathway have been reported to play important roles in tumor progression and potentially targeted in cancer therapy. Inhibition of the expression and activity of these genes with either interfering RNA or small molecule inhibitors restrain tumor cell proliferation in cell culture *in vitro* and tumor growth *in vivo* (22-29). Furthermore, chemical inhibitors of FASN have been shown to be effective in repressing tumor growth (30). However, the efficacy of these genes in cancer therapy requires further investigation. Out results showed that silencing SREBP1 blocked the expression of SREBP1 target genes FASN, ACAC and ACLY at both protein and mRNA levels in cell culture. The effect of shRNA targeting SREBP1 was more significant in repressing the expression of FASN, ACAC and ACLY in mouse xenograft model *in vivo*. Our results demonstrated that as a master regulator of lipogenic gene transcription, SREBP1 regulates ovarian cancer cell growth and survival through accommodating downstream *de novo* lipogenic genes. The expression and activation of FASN, ACAC, ACLY and SCD could be regulated not only by transcription factor SREBP1 but also by other signal regulating pathways such as PI3K/Akt and MAPK pathway in human (31). Our study verified the significance of the lipogenic enzymes in ovarian cancer pathogenesis, however, the underlying mechanisms, through which SREBP1 regulates the downstream lipogenic genes in ovarian cancer cells, are still poorly understood.

In summary, we have confirmed that SREBP1 plays important roles in regulating lipid biosynthesis and homeostasis, promoting ovarian tumor growth. Although further investigations are needed to elucidate the molecular mechanisms underlying the overexpression of SREBP1 and its ancillary regulatory signaling pathways in cancers, our study suggests that SREBP1 may serve as a therapeutic target for antitumor therapy.

Acknowledgements

This study was funded by the National Natural Science Foundation of China [81173614 (J.J.)] and [81173614 (Q.T.L)] and also funded by Science and Technology Development planning of Shandong [2011GSF12122 (X.Z.)] and [2012G0021823 (J.J)].

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