

# RETRACTED ARTICLE: Down-Regulation of SREBP via PI3K/AKT/mTOR Pathway Inhibits the Proliferation and Invasion of Non-Small-Cell Lung Cancer Cells

This article was published in the following Dove Press journal:  
OncoTargets and Therapy

Bo Zhang<sup>1,\*</sup>  
Jianchun Wu<sup>1,\*</sup>  
Peng Guo<sup>1</sup>  
Yi Wang<sup>2</sup>  
Zhihong Fang<sup>1</sup>  
Jianhui Tian<sup>3</sup>  
Yongchun Yu<sup>4</sup>  
Wenjing Teng<sup>1</sup>  
Yingbin Luo<sup>1</sup>  
Yan Li<sup>1</sup>

<sup>1</sup>Department of Oncology, Shanghai Municipal Hospital of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 200071, People's Republic of China;

<sup>2</sup>Department of Pathology, Cancer Hospital Affiliated Zhengzhou University, Henan, Zhengzhou 450008, People's Republic of China; <sup>3</sup>Department of Oncology, Longhua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai 200030, People's Republic of China; <sup>4</sup>Institute for Thoracic Oncology, Shanghai Chest Hospital, Shanghai Jiao Tong University, Shanghai 200030, People's Republic of China

\*These authors contributed equally to this work

Correspondence: Yan Li; Yingbin Luo  
Department of Oncology, Shanghai Municipal Hospital of Traditional Chinese Medicine, 274 Middle Zhijiang Road, Shanghai, People's Republic of China  
Email: yan.xiaotian@shutcm.edu.cn; dr. luo@qq.com

**Background:** Lung cancer is one of the most common causes of cancer-related deaths worldwide, metabolic disorders are also a problem that puzzles mankind. SREBP is over-expressed in non-small-cell lung cancer (NSCLC) and is also a key regulator of lipid synthesis. However, the mechanisms by which SREBP regulates the proliferation, migration and invasion in NSCLC remain unclear.

**Materials and Methods:** CCK-8, colony formation assay, soft agar assay, scratch wound healing assay and transwell assays were performed to detect proliferation, and invasion in NSCLC cells, respectively. In addition, Western blotting assay, qPCR and immunofluorescence were applied to detect the expression of SREBP1, SREBP2, ki-67, PCNA, Bax, bcl-2, E-cadherin, N-cadherin, Vimentin, PI3K, p-PI3k, AKT, p-AKT, mTOR, p-mTOR in NSCLC cells.

**Results:** In this study, down-regulation of SREBP significantly inhibited the proliferation, migration and invasion of A549 and H1299 cells. Moreover, the method of piecewise inhibition was adopted to prove that SREBP is a downstream molecule of the PI3K/Akt/mTOR signaling pathway.

**Conclusion:** Our study indicated that downregulation of SREBP inhibited the growth in NSCLC cells via PI3K/AKT/mTOR signaling pathway. Thus, we suggested SREBP may serve as a potential target for the treatment of patients with NSCLC.

**Keywords:** non-small-cell lung cancer, SREBP, proliferation, invasion, PI3K/AKT/mTOR

## Introduction

Metabolic reprogramming is one of the important features of tumor cells.<sup>1</sup> In order to satisfy the material and energy needed for rapid proliferation, tumor cells reprogram their metabolic patterns to promote tumor growth. As one of the three major nutrients in human body, lipids can supply and store energy, which is an important substance in cell life activities and closely related to cell proliferation. As one of the most representative features of tumor disease, abnormal lipid metabolism has become an important research direction in the treatment of tumor in recent years.<sup>2</sup> Sterol Regulatory Element-binding Proteins (SREBP) are a key regulator of lipid synthesis,<sup>3</sup> the research and development of new drugs targeting SREBP has attracted much attention.

SREBP is a transcription factor that regulates the synthesis of fatty acids, triglycerides and cholesterol. In mammals, SREBP is divided into three subtypes, named SREBP1a, SREBP1c and SREBP2. Although SREBP1a and SREBP1c are produced

by different promoter codes, their coding genes are the same, and they are collectively called SREBP1, which regulates the metabolism of fatty acids and triglycerides, while SREBP2 regulates the metabolism of cholesterol.<sup>4</sup> Previous studies have focused on its regulatory role in metabolism. Recent studies have found that in addition to its role in regulating metabolism, SREBP also plays a certain role in the occurrence and development of tumors, especially in the proliferation, invasion and migration of tumor cells. Experimental studies have also verified this view. SREBP is highly expressed in prostate cancer.<sup>5</sup> In breast cancer, the expression of SREBP1 is related to the metastasis of tumor, and the activation of SREBP can promote the proliferation of breast cancer cells.<sup>6</sup> Inhibition of SREBP can promote the apoptosis of endometrial cancer cells.<sup>7</sup>

The incidence rate and mortality rate of lung cancer are the first in the world,<sup>8</sup> metabolic disorders are also a problem that puzzles mankind. We made a reasonable guess as to whether the inhibition of SREBP gene, which regulates metabolism, can inhibit the proliferation, invasion and migration of lung cancer cells and other malignant behaviors.

To test this hypothesis, we knocked down SREBP1 and SREBP2 genes of lung cancer cells A549 and H1299 by lentivirus infection, and then observed the proliferation, apoptosis, invasion and migration of lung cancer cells. Our aim was to determine whether SREBP plays a role in promoting the development of lung cancer.

## Materials and Methods

### Cell Culture and Tissue Source

The human NSCLC cell lines A549 and H1299 were from the Shanghai cell bank of the Chinese Academy of Sciences (Shanghai, China). DMEM high-sugar medium containing 10% FBS ((Thermo Fisher Scientific, Waltham, MA, USA)) and 1% penicillin streptomycin mixture was used for culture. The condition of CO<sub>2</sub> incubator were 37 °C, 5% CO<sub>2</sub> and 95% air. Experiments were performed when the cells were in the logarithmic growth phase. At the Cancer Hospital affiliated Zhengzhou University, 4 fresh cases of human non-small cell lung cancer were obtained and paired with normal tissue. All samples were collected with the patient's informed consent.

### Cell Count

The cells were digested and resuspended and diluted to a certain concentration to ensure that the cell density was

not less than 10<sup>4</sup> cells/mL. Draw 10 µl cell suspension and add the cell suspension along the side of the cover slide. Under a microscope, the number of cells in four large squares at the edge of the cell count plate is calculated. A cluster of cells is counted as a cell. After counting, the number of cells per mL of suspension was calculated. Cell suspension number/mL = (total number of 4 large cells n/4)×10<sup>4</sup>× dilution factor; Take the average after three repetitions.

### Lentivirus and Plasmid Transfection

Short hairpin RNA (shRNAs) targeting human LINRIS or non-specific oligonucleotides attached to LV-3 vectors can down-regulate the expression of SREBP1 and SREBP2. The A549 and H1299 cells were transfected with lentivirus according to the manufacturer's instructions. Stable transfected cell lines were obtained after treating with puromycin for 2 weeks (2–3 g/mL). After the knockdown efficiency was verified by quantitative PCR (qPCR) and Western blotting, the cells were used for subsequent experiments. shRNA targeting sequences for SREBP1 are as the following: 5'-GCCATCGACTACATTCGCTTT-3' (a) and 5'-CCAGAAACTCAAGCAGGAGAA-3' (b); for SREBP2: 5'-CTCAGATCATCAAGACAGAT-3' (a) and 5'-TCTGAAGATCGAGGACTT3' (b).

### CCK-8 Assay

After the cell suspension and cells counted, they were inoculated into a 96-well plate. Each well contained 200µL media supplemented with 10% FBS and 1×10<sup>4</sup> cells. Each group was given 3 double holes and another blank hole with media supplemented with 10% FBS. The 96-well plate was placed in an incubator at 37°C for 24h. Replace the media and add 10µL CCK8 reagent (CCK-8, Dojindo, Tokyo, Japan) to 100µL media. After 2 hours of continuous culture, absorbance at 450nm was determined with a micrometer (Thermo Scientific, USA). Cell activity inhibition rate (%) = [(average OD value of the control group – average OD value of the experimental group)/(average OD value of the control group – average OD value of the blank group)]×100%.

### RNA Purification and q-PCR Assay

Total RNA was extracted from A549 and H1299 cells using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc). The first strand cDNA was synthesized using the PrimeScript 1st Strand cDNA Synthesis kit (Takara Bio, Inc.) following the manufacturer's protocol.

PCRs were performed using the rTaq DNA Polymerase (Takara Bio, Inc.) in a total volume of 20  $\mu$ L, and amplification protocol consisted of an initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation for 45 sec at 94°C, annealing for 45 sec at 60°C and extension for 1 min at 72°C, followed by a final extension at 72°C for 10 min.

## Western Blot Assay

The cells were homogenized in a cool tissue lysis buffer, which was centrifuged at 4°C at 12,000 r/min for 15 minutes. The supernatant reflects the extraction of total cellular proteins, the protein concentrations of which were determined using BCA protein assay (Beyotime, China). The samples were boiled in Laemmli buffer at 100°C for 8 minutes. The samples were examined by Western blot. The primary antibody was incubated overnight at 4°C. Antibodies were diluted to 1:1000 for SREBP1 (Thermo Fisher Scientific, USA), SREBP2 (Thermo), Ki-67 (Cell Signalling Technology, USA), p53 (CST), PCNA (CST), bax (CST), bcl-2 (SCST), E-cadherin (CST), N-cadherin (CST), Vimentin (CST), PI3K (CST), p-PI3K (CST), AKT (CST), p-AKT (CST), mTOR (CST), p-mTOR (CST), GAPDH (CST),  $\beta$ -actin (CST). After washing with a secondary antibody; Leave at room temperature for 1 hour. The ECL color rendering solution was prepared, mixed and dropped onto the film surface, exposed and photographed.

## Immunofluorescence

The A549 and H1299 cells were 4% PFA fixed (15min) and then incubated in 1%BSA/10% normal goat serum/0.3M glycine in 0.1%PBS for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with antibody overnight at 4°C. The secondary antibody used was Alexa Fluor 555 goat anti-rabbit IgG used at a 1/1000 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43U/ml.

## Colony Formation Assay

After the cells were digested and resuspended, the cells were counted. According to the density of 500 cells per well, the cells were inoculated into 6-well plates, and 3 double holes were set up in each group. Replenish the medium to 2mL per well; The six-well plate was cultured in an incubator of 5%CO<sub>2</sub> and 37°C for 7 days. When visible clones appeared in petri dishes, the culture medium

was discarded and washed twice with PBS. Fixed with 4% paraformaldehyde for 15min and then dried; Dye with 0.1% crystal violet for 15min and rinse with tap water for 3 times. Take photos after air drying. Clone formation rate = number of clones/number of inoculated cells  $\times$  100%.

## Soft Agar Assay

For soft agar assay,  $5 \times 10^3$  cells were fully mixed with DMEM with 10% agarose and 0.3% agarose, and they were quickly placed on the DMEM curing layer with 0.75% agarose and 10% agarose. DMEM was preheated at 37°C in advance. 0.5mL DMEM containing 10% FBS was added weekly to each well tested Cells. After 3 weeks, colonies were scored in 5 random fields which were larger than 50  $\mu$ m.

## Scratch Wound Healing Assay

In the scratch wound healing assay, cells were plated in a 6-well plate. When the cells were fused to about 90%, a 200 $\mu$ L pipette tip was used to scrape the monolayers of cells in a straight line to create a "scratch". After the defoliated cells and cell fragments were washed, fresh medium containing 1% fetal bovine serum was added and the cells were photographed with a microscope at a predetermined time. Use ImageJ software to evaluate migration distances. The relative mobility of each treatment was calculated by cell relative migration area.

## Transwell Migration Assay

The Transwell migration assay was carried out by 8 $\mu$ m Transwell Chambers (Corning Costar). The upper chambers were filled with  $5 \times 10^3$  cells in 200 $\mu$ L media supplemented without FBS. Five hundred microliter DMEM supplemented with 10%FBS were plated in the lower chambers. After 24 hours of culture at 37°C, residual cells on the upper surface of the membrane were removed. The cells on the submembrane surface were fixed with 4% paraformaldehyde, and stained with crystal violet, and then take pictures under a microscope.

## Xenograft Experiments

Male nude mice aged 6 weeks were injected subcutaneously with  $10^5$  A549, Sh-Control, Sh-SREBP1, Sh-SREBP2 cells. Tumor growth was followed for 28 days. Tumor volume was determined using the ellipsoidal volume formula. The expression of related proteins was detected by immunohistochemistry (IHC).

## Statistical Analysis

Data were presented as Mean $\pm$ SEM and analyzed using GraphPad Prism 8.0. Each experiment was independently repeated three times.  $P < 0.05$  was considered statistically significant.

## Results

### SREBP1 and SREBP2 are Highly Expressed in Lung Cancer Cells and Tissues

In order to clarify the expression of SREBP protein in lung cancer cells, A549 and H1299 cell lines were selected, and human bronchial epithelial cells (BEAS-2B) were used as the control. Western Blot assay was used to detect the expression levels of SREBP1 and SREBP2 proteins. Compared with HBE, SREBP1 and SREBP2 were both highly expressed (Figure 1A). Then, we detected the expression of SREBP1 and SREBP2 proteins in the matched para-carcinoma tissues of 4 lung cancer specimens, and the results showed that SREBP was highly expressed in cancer tissues. (Figure 1B).

### Lentivirus Infection Resulted in the Stable Transformation of Lung Cancer Cells with Reduced SREBP1 and SREBP2

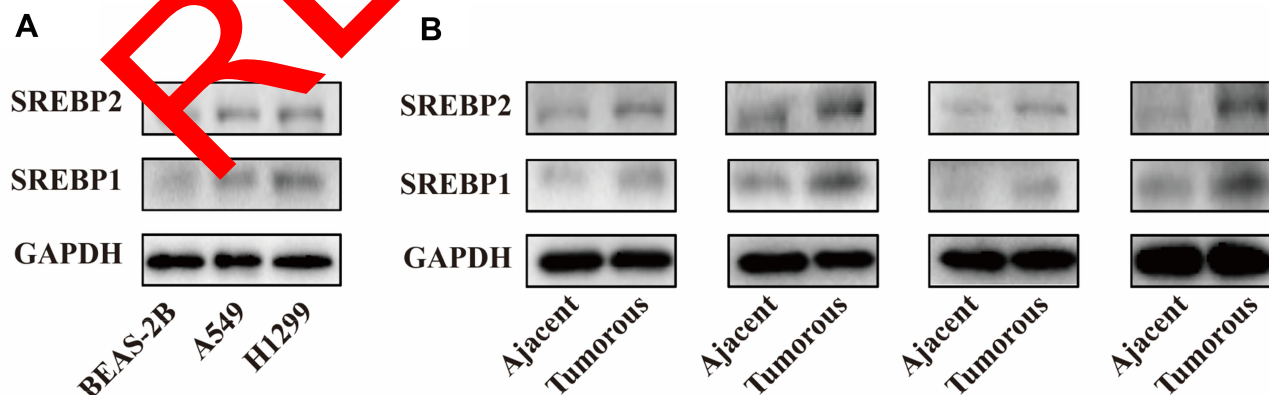
Using lentivirus infection technique, gene knockout, screening and expanding culture were carried out for lung cancer cell lines A549 and H1299, respectively. Western Blot, qPCR and Immunofluorescence (Figure 2A–C) were used to verify the success of gene knockout, and the next experiment could be carried out after successful knockout. In this experiment, the cell infection rate was more than 90%.

### Down-Regulation of SREBP Gene Inhibits the Proliferation of Lung Cancer Cells and Promotes Apoptosis

In the lung cancer cell lines knocked down by SREBP, the expressions of proliferation-related proteins ki-67 and PCNA were reduced (Figure 3A), and the expressions of apoptosis-related proteins P53 and Bax/bcl-2 were increased. The immunofluorescence results were consistent with Western Blot and qPCR (Figure 3B). In terms of functional experiment, CCK8 detected the proliferation ability of cells, and the results showed that the proliferation ability of the knockout cells was significantly decreased (Figure 3C). The results of the cloning experiments showed that the number and size of the cloned cells were lower than that of the normal lung cancer cell lines (Figure 3D–E). The above results showed that SREBP gene could affect the proliferation ability of lung cancer cells A549 and H1299, and inhibit this gene, which could inhibit the proliferation of lung cancer cells.

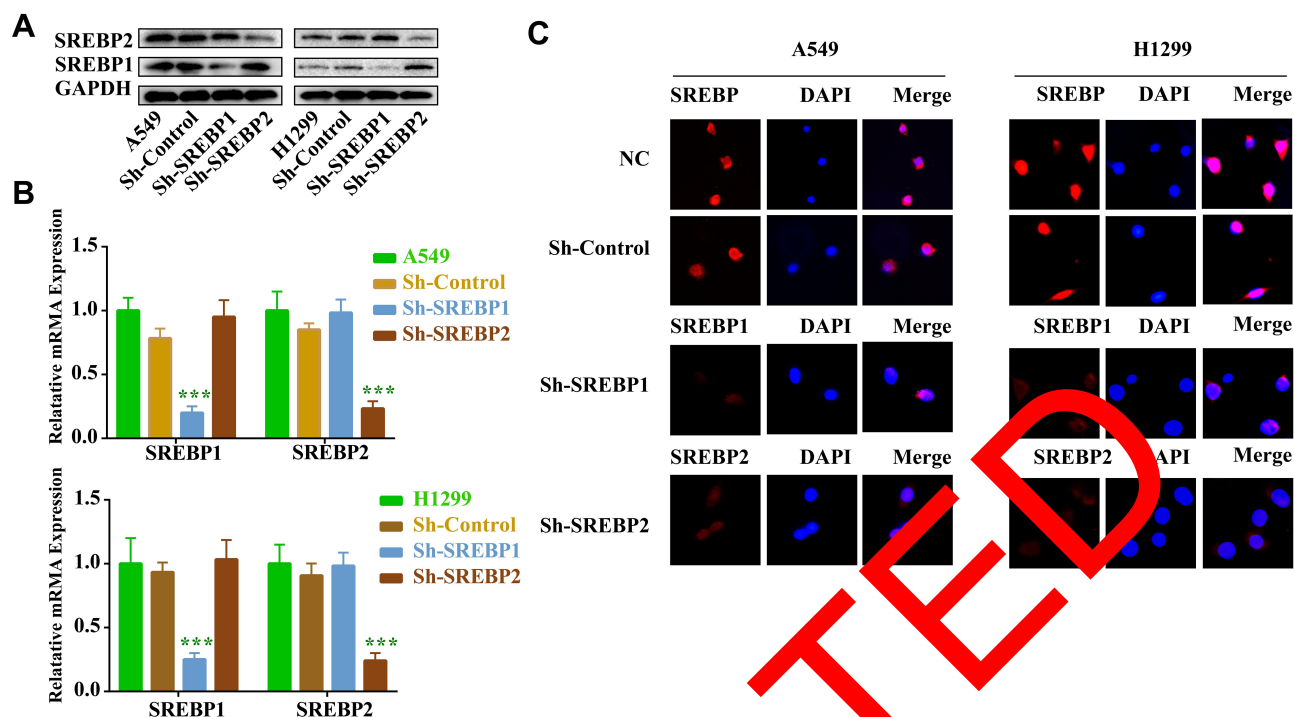
### Down-Regulation of SREBP Gene Can Inhibit the Migration and Invasion of Lung Cancer Cells

In the lung cancer cell lines knocked down by SREBP, the expression of epithelial marker protein E-cadherin was increased, and the expression of mesenchymal marker proteins N-cadherin and Vimentin were decreased (Figure 4A). This result was verified by immunofluorescence results (Figure 4B). Epithelial-mesenchymal transition (EMT) enables tumors to acquire the ability to move and migrate, and invasion and metastasis, which is a key step for the migration and invasion of tumor cells.<sup>9</sup> Therefore, we used scratch test and transwell test to



**Figure 1** SREBP is overexpressed and in non-small cell lung cancer cells and tissues. (A) expression levels of SREBP1 and SREBP2 proteins were both highly expressed in A549 and H1299 cell lines compared with BEAS-2B. (B) SREBP1 and SREBP2 were highly expressed in lung cancer tissues.





**Figure 2** SREBP shRNA2 significantly downregulated the expression of SREBP in NSCLC cells. A549 and H1299 cells were infected with SREBP1 and SREBP2 shRNA1 for 72 h. (A) The level of SREBP in A549 and H1299 cells was detected with Western blotting. (B) Expression level of SREBP in A549 and H1299 cells was analyzed by qPCR. (C) Expression level of SREBP in A549 and H1299 cells was analyzed by immunofluorescence. \*\*\* $P < 0.001$  compared with NC group.

explore the influence of SREBP gene on the migration and invasion of lung cancer cells. Twenty-four hours after the scratch culture, the normal lung cancer cells healed nearly 50%, while the knockdown cells healed less than 20%, which was significantly inhibited (Figure 3C). In the transwell transfer experiment, the number of cells of A549 and H1299 cells crossing the compartment PET membrane was significantly reduced. Matrigel was added to the upper compartment to verify the invasion ability of cells, and the same result was obtained (Figure 4D).

### SREBP Can Be Considered as a Downstream Factor of PI3K/AKT/mTOR

The PI3K/AKT/mTOR signaling pathway is overactivated in a variety of cancers, plays an important role in cell growth and survival, and can maintain the malignant behavior of tumor cells.<sup>10</sup> In order to investigate whether SREBP gene is a downstream factor of this pathway, we adopted the method of block inhibition, respectively using PI3K inhibitor PDK-1 (Thermo), AKT inhibitor MK-2206 (Thermo) and mTOR inhibitor Rapamycin (Thermo) to inhibit this pathway, and obtained the IC<sub>50</sub> concentration

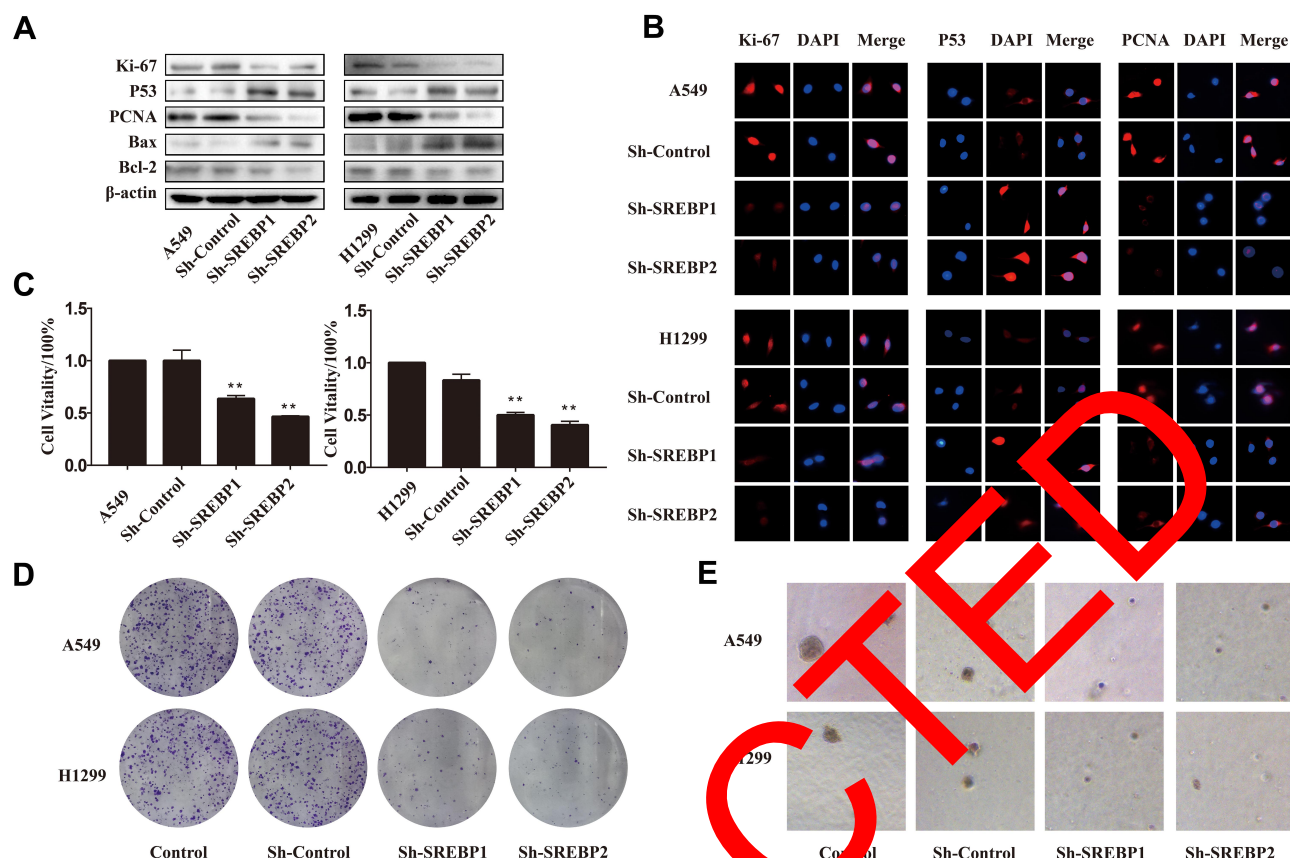
of each inhibitor by CCK8 assay. The inhibitor was added and cultured for 24 hours, and the protein was extracted. The levels of PI3K, p-PI3k, AKT, p-AKT, mTOR, p-mTOR and SREBP1 and SREBP2 were detected by Western Blot. The results showed that with the decrease of the phosphorylation level of the pathway, the expression of SREBP1 and SREBP2 also decreased (Figure 5).

### In vivo Experiments of Sh-SREBP A549 Cells

Normal cells, Sh-Control cells, Sh-SREBP1 and Sh-SREBP2 A549 cells were subcutaneously injected into BALB/C nude mice (6 weeks) and tumor growth was monitored within 28 days. We found that Sh-SREBP1 and Sh-SREBP2 tumor growth rate and average tumor weight (Figure 6A–C). Immunohistochemistry was performed on subcutaneous transplanted tumor of mice, and the expressions of ki-67, PCNA, E-cadherin, N-cadherin were detected. The results were consistent with in vitro (Figure 6D).

### Discussion

Sterol Regulatory Element Binding Protein (SREBP) has the structure of basic helix-loop-helix-leucine zipper

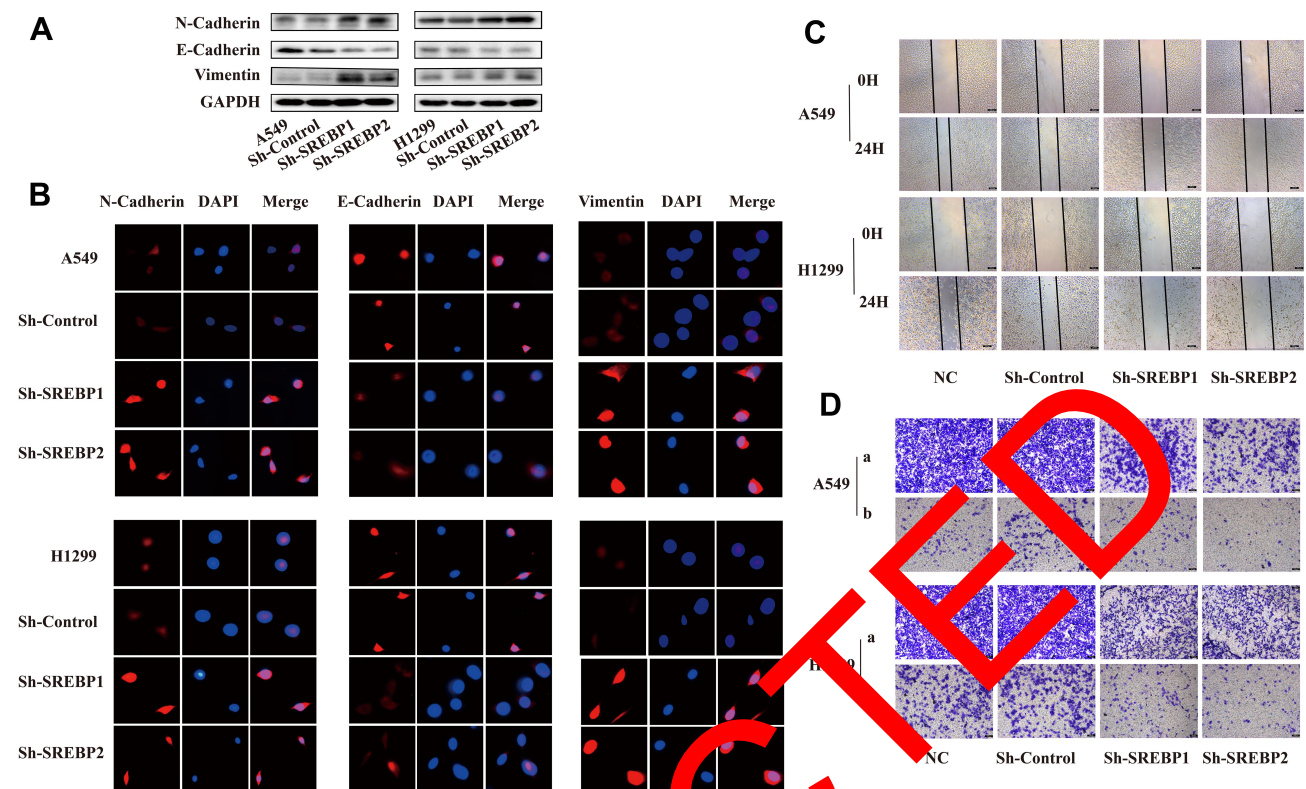


**Figure 3** Downregulation of SREBP inhibited the proliferation and induced apoptosis in HCLC cells. **(A and B)** Expressions of proliferation-related proteins ki-67 and PCNA were reduced, and the expressions of apoptosis-related proteins P53 and bax/bcl-2 were increased. This result was verified by Western Blot and immunofluorescence. **(C)** CCK-8 assay was used to detect the cell viability. **(D)** Colony formation assay. After 10 days, H1299 and A549 cells were fixed and stained with crystal violet. Representative well were photographed and shown. **(E)** Soft agar assay. After 2 weeks, colonies were photographed and representative images were shown. \*\*P < 0.01 compared with NC group.

(Bhlh-ZIP), which plays an important role in the regulation of cholesterol metabolism and fatty acid metabolism in mammals.<sup>11</sup> New synthesis precursor SREBP is inactive, which attached to the endoplasmic reticulum membrane, when cells with lipid needs, endoplasmic reticulum of SREBP precursor proteins are transferred to the golgi apparatus, through two pyrophosphatase activation and release properties (nSREBPs), and then be transferred to the nucleus, together with the corresponding target gene promoter, promotes its downstream proteins ACC, ACLY, FASN, SCD1, LDLR, this enzyme gene transcription, which regulates cholesterol and fatty acid synthesis.<sup>12</sup> SREBP1 mature body is mainly involved in fatty acid and triglyceride metabolism, while SREBP2 mature body is the main regulator of cholesterol metabolism.<sup>13</sup>

Under normal physiological conditions, lipid dynamic equilibrium is strictly maintained. However, under certain pathological conditions, the expression level of SREBP is often maladjusted. Lipid metabolism plays an important

role in regulating cell growth, proliferation, differentiation, survival, apoptosis, and membrane homeostasis.<sup>14,15</sup> Studies have shown that abnormal lipid metabolism is involved in the occurrence and development of various tumor diseases, such as prostate cancer, breast cancer, and colorectal cancer.<sup>16</sup> Studies have also found that SREBP is overexpressed in many cancers, and it can regulate the expression of genes needed to maintain the lipid homeostasis of cells,<sup>14</sup> which has a great effect on the proliferation and metastasis of tumors. For example, SREBP1 and its downstream target genes ACC and FASN were highly expressed in human glioblastoma tissues.<sup>17</sup> The expression level of SREBP1 is related to tumor size, histological grade and invasion, and metastasis.<sup>18</sup> The functionally deficient SREBP1 inhibits cell proliferation, invasion and metastasis and induces apoptosis, which has been found in liver cancer, ovarian cancer and endometrial cancer.<sup>19,20</sup> Inhibition of SREBP1 expression at the gene level or through drugs will cause

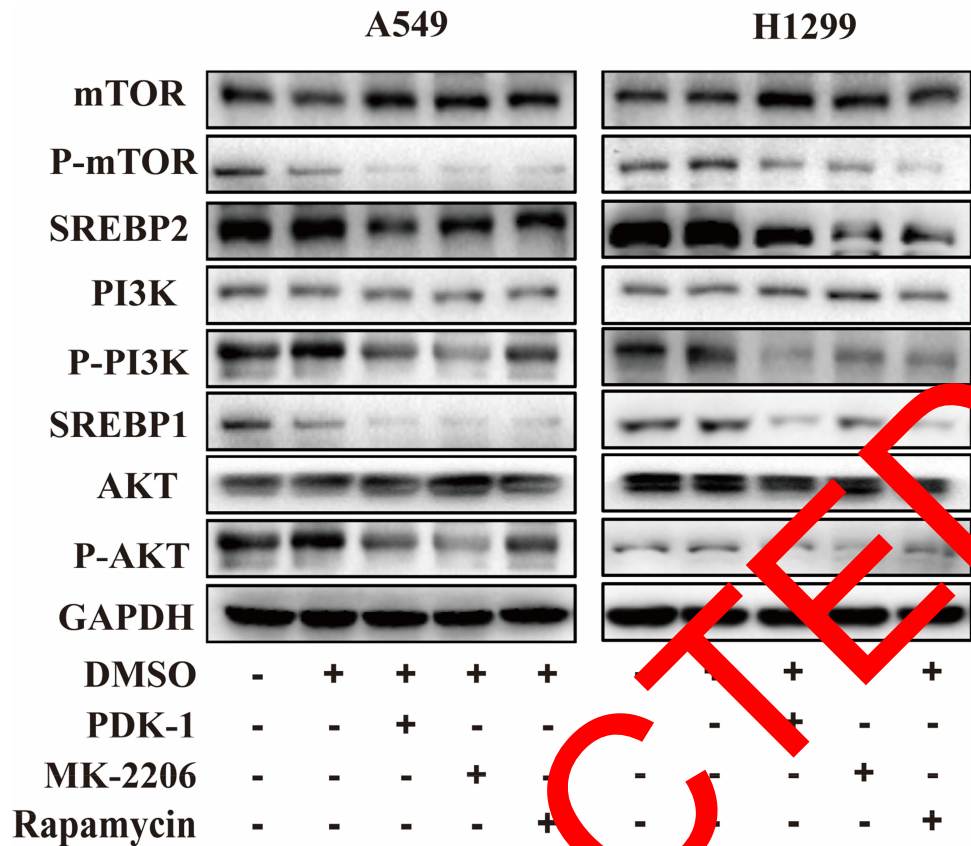


**Figure 4** Downregulation of SREBP suppressed the migration and invasion abilities of lung cancer cells. **(A)** Western blot analysis of the expression of epithelial marker protein E-cadherin was increased, and the expression of mesenchymal marker proteins N-cadherin and Vimentin were decreased. **(B)** Cell migration was detected using wound healing assay. This result was verified by Western Blot and immunofluorescence. **(C)** Scratch wound healing assay. 24 hours after the scratch culture, the extent of cell migration was photographed at the indicated times. **(D)** Cell invasion was detected using transwell assay (a: transwell assay; b: transwell invasion assay with matrigel).

the death of tumor cells and significantly inhibited the proliferation of tumor cells.<sup>21–23</sup> This study found that SREBP was also overexpressed in lung cancer cells and tissues, and affected the proliferation, migration and invasion of lung cancer cells. Therefore, using SREBP as a tumor treatment target and drug target has a little research potential and application prospect. For example, in the process of ripening, folding, and post-transcriptional modification of SREBP, protein phosphorylation, acetylation, saponization and ubiquitination are involved to regulate the expression and stability of SREBP,<sup>24</sup> so as to prevent and treat diseases.

Ki-67 is a nuclear antigen expressed in proliferating cells and is one of the most reliable indicators to detect the proliferating activity of tumor cells.<sup>25</sup> PCNA exists in various stages of the proliferation cycle of tumor cells and normal proliferating cells, and is closely related to the synthesis of cell DNA and it is also involved in the initiation process of cell proliferation and can reflect the state of cell proliferation.<sup>26</sup> Members of the bcl-2 family include Bax, which promotes apoptosis, and bcl-2, which fights apoptosis.<sup>27</sup> The ratio of Bax to bcl-2 determines the

state of apoptosis.<sup>28</sup> When the balance of Bax/bcl-2 in vivo is destroyed, its inhibitory effect on tumor cell proliferation is weakened.<sup>29</sup> P53 is a tumor suppressor gene and has an inhibitory effect on tumor growth.<sup>30</sup> In this study, the expression of proliferation-related genes ki-67 and PCNA decreased and the expression of apoptosis-related genes P53 and Bax/bcl-2 increased in the lung cancer cell lines with SREBP knockdown, indicating that the proliferation of lung cancer cells was inhibited and the apoptosis of lung cancer cells was promoted. EMT refers to the enhancement of cell movement and invasion after phenotypic change, weakened adhesion ability and loss of polarity of epithelial cells, which is considered as the initial stage of tumor metastasis.<sup>31</sup> EMT not only promotes tumor development and invasion and metastasis, but also enhances the anti-apoptotic ability of tumor cells.<sup>32</sup> The occurrence of EMT is often accompanied by the change of cell morphology (fusing/fibroblast morphology), the decrease of epithelial marker proteins such as E-cadherin, and the increase of mesenchymal marker proteins such as N-cadherin and Vimentin.<sup>33</sup> In this study, it was found that the expression of e-cadherin decreased and the expression



**Figure 5** Downregulation of KIF15 inhibited the growth of NSCLC cells via downregulation of the PI3K/AKT/mTOR signaling pathway. Expression levels of PI3K, p-PI3K, AKT, p-AKT, mTOR, p-mTOR, SREBP1, SREBP2 in A549 and H1299 cells was detected with Western blot.

of n-cadherin and vimentin increased in the lung cancer cell lines with the reduction of SREBP1 indicating that the lung cancer cells had invasion and metastasis initiated by epithelial mesenchymal transformation.

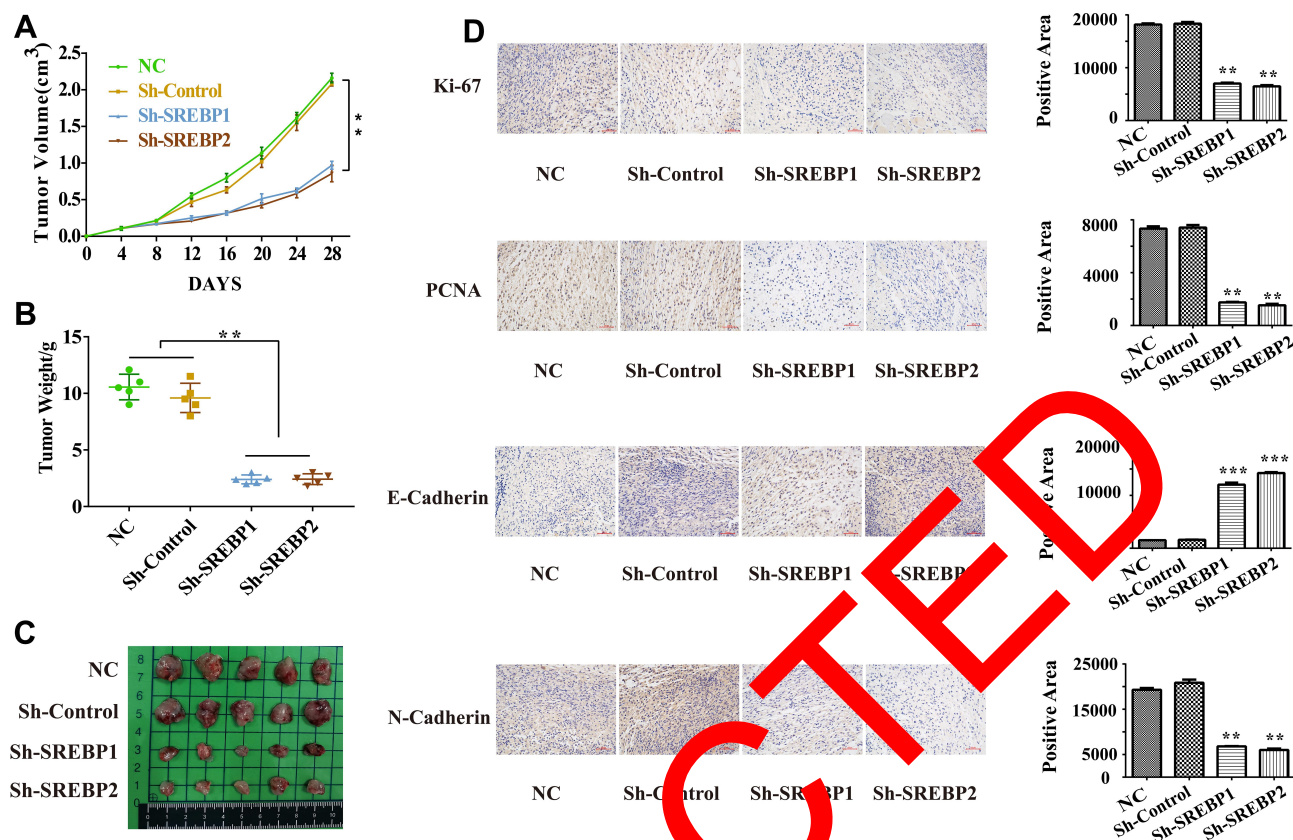
In a variety of human tumor tissues, the PI3K/Akt phosphorylation pathway is highly activated,<sup>34</sup> and the activation of Akt promotes the transcriptional activation of SREBP1, thus promoting the intracellular lipid synthesis of tumor cells.<sup>35</sup> mTOR is located in the downstream of PI3K/Akt pathway, can also regulate the expression of SREBP1 gene level to a certain extent and promote the intracellular localization of SREBP1 protein maturation.<sup>36</sup> Studies have shown that SREBPs is an important component in the downstream of the metabolic regulation network of PI3K/Akt/mTOR signaling pathway.<sup>37</sup> mTOR is an important downstream effector of AKT. Although the activity of mTOR is regulated by special amino acids, it also requires the enrichment of mature karyotype SREBP-1 in the nucleus.<sup>38</sup>

mTOR pathway is the key regulator of cell proliferation, metabolism and survival, and there is abnormal regulation of

mTOR signal in most cancers. Rapamycin is the inhibitor of mTOR pathway. Different doses of rapamycin affect the effect of action.<sup>39</sup> AKT phosphorylates ACLY and activates the expression of several genes involved in the synthesis of cholesterol and fatty acids.<sup>40</sup> mTOR also regulates the expression of sterol regulatory element binding protein gene 1 (SREBP1) and stimulates the production of lipids in the liver.<sup>41</sup> PI3K/AKT signal can not only up-regulate glucose uptake and glycolysis, but also promote the synthesis of fatty acids.<sup>42</sup> In this study, the method of piecewise inhibition was adopted to prove that SREBP is a downstream molecule of the PI3K/Akt/mTOR signaling pathway. Therefore, SREBP can be regarded as an important metabolic integrator in the process of regulating glucose production of fatty acids by PI3K/AKT/mTOR signaling.

Abnormal tumor metabolism is considered to be an important feature of tumors. In-depth study of the regulatory mechanism and biological functions of SREBP can help us have a deeper understanding of metabolic diseases and tumors, and at the same time will have a far-reaching impact on the guidance of clinical treatment. In the anticancer





**Figure 6** In vivo experiments of reducing SREBP lung cancer A549 cell. (A) Reduction of SREBP1 and SREBP2 inhibits the tumor growth and average tumor weight. (D) Immunohistochemistry was performed on subcutaneous transplanted tumor of mice, expressions of ki-67, PCNA, E-cadherin, N-cadherin and Vimentin were consistent with in vitro. \*\*P < 0.01 compared with NC group, \*\*\*P < 0.001 compared with NC group.

research targeted at lipid metabolism, it is worth further research to find and develop effective SREBP inhibitors, which can not only inhibit the synthesis of lipids at the source, but also inhibit the growth of tumor cells.

Regulation of SREBP through PI3K/AKT/mTOR pathway can affect the proliferation and invasion of lung cancer cells. Whether the changes in the expression of fatty acids or cholesterol downstream of SREBP affect the function of cells, or affect the expression of oncogenes, such as the bypass of advanced G1 glutamine (G1N),<sup>13</sup> is the subject of further study.

## Conclusion

In this study, we explored the mechanisms underlying the role of SREBP in NSCLC. Downregulation of SREBP inhibited cell proliferation, migration and invasion via PI3K/AKT/mTOR signaling pathway. These studies indicated an important role for SREBP as an oncogene in the NSCLC.

## Ethics Statement

All human studies have been approved by the Research Ethical Committee from Municipal Hospital of Traditional

Chinese Medicine Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai, China (2016SHL-KYYS-16). Informed consent was obtained from all study subjects.

All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Municipal Hospital of Traditional Chinese Medicine Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai, China. The maximal tumor measurements/volumes are in accordance with the IACUC. All procedures involving animal treatment followed the guidelines of the National Standardization Administration of China institutional committee of animal care and use (GB/T1.2-2016).

## Acknowledgments

This work is supported by grants to Yan Li from the National Natural Science Foundation of China (81973795, 81673947), the grant to Yingbin Luo from the Natural Science Foundation of Shanghai (17ZR1428000), the grant to Peng Guo from Innovation project of Shanghai University of Traditional Chinese Medicine (2019LK094),

the grant to Yi Wang from Science and technology project of Henan province (192102310374), and the grant to Jianchun Wu from Outstanding youth project of Shanghai Municipal Health Committee (2017YQ049).

## Author Contributions

Yan Li, Bo Zhang, Yingbin Luo and Jianchun Wu designed the experiments and interpreted data. Yi Wang provided tumor tissue. Peng Guo, Zhihong Fang, Jianhui Tian, Yongchun Yu gave advice on experimental design and paper writing. Bo Zhang, Peng Guo and Wenjing Teng performed the experiments. Bo Zhang, Jianchun Wu analyzed data. Bo Zhang and Yan Li wrote the paper. All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

## Disclosure

The authors report no conflicts of interest for this work.

## References

- Ward PS, Thompson CB. Metabolic reprogramming: a cancer hallmark even warburg did not anticipate. *Cancer Cell*. 2012;22(3):297–308. doi:10.1016/j.ccr.2012.02.014
- Griffiths B, Lewis CA, Bensaad K, et al. Sterol regulatory element binding protein-dependent regulation of lipid synthesis supports cell survival and tumor growth. *Cancer Metab*. 2013;1(1):3. doi:10.1186/2049-3002-1-3
- Briggs MR, Yokoyama C, Wang X, et al. Nuclear protein that binds sterol regulatory element of low density lipoprotein receptor promoter. I. Identification of the protein and delineation of its target nucleotide sequence. *J Biol Chem*. 1993;268(19):14481–14496.
- Sato R. Sterol metabolism and SREBP activation. *Arch Biochem Biophys*. 2010;501(2):177–181. doi:10.1016/j.abb.2010.06.004
- Huang WC, Li X, Lin J, et al. Activation of androgen receptor, lipogenesis, and oxidative stress converged by SREBP-1 is responsible for regulation of growth and progression of prostate cancer cells. *Mol Cancer Res*. 2012;20(1):133–142. doi:10.1158/1541-7786.MCR-11-0206
- Bao J, Zhu Y, Zhu Y, et al. SREBP-1 is an independent prognostic marker and promotes invasion and migration in breast cancer. *Oncol Lett*. 2016;12(4):209–2416. doi:10.3892/ol.2016.4988
- Shafiee MN, Monge N, Seedhouse C, et al. Sterol regulatory element binding protein-1 (SREBP1) gene expression is similarly increased in polycystic ovary syndrome and endometrial cancer. *Acta Obstet Gynecol Scand*. 2017;96(5):556–562. doi:10.1111/aogs.13106
- Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin*. 2020;70(1):7–30. doi:10.3322/caac.21590
- Dongre A, Weinberg RA. New insights into the mechanisms of epithelial-mesenchymal transition and implications for cancer. *Nat Rev Mol Cell Biol*. 2019;20(2):69–84. doi:10.1038/s41580-018-0080-4
- Lien EC, Dibble CC, Tokar A. PI3K signaling in cancer: beyond AKT. *Curr Opin Cell Biol*. 2017;45:62–71. doi:10.1016/j.ceb.2017.02.007
- Liu FH, Song JY, Shang XR, et al. The gene-gene interaction of INSIG-SCAP-SREBP pathway on the risk of obesity in Chinese children. *Biomed Res Int*. 2014;2014:538564.
- Eberlé D, Hegarty B, Bossard P, et al. SREBP transcription factors: master regulators of lipid homeostasis. *Biochimie*. 2004;86(11):839–848. doi:10.1016/j.biochi.2004.09.018
- Horton JD, Shimomura I, Ikemoto S, et al. Overexpression of sterol regulatory element-binding protein-1a in mouse adipose tissue produces adipocyte hypertrophy, increased fatty acid secretion, and fatty liver. *J Biol Chem*. 2003;278(38):36652–36660. doi:10.1074/jbc.M306540200
- Menendez JA, Lupu R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. *Nat Rev Cancer*. 2007;7(10):763–777. doi:10.1038/nrc2222
- Swinnen JV, Brusselmans K, Verhoeven G. Increased lipogenesis in cancer cells: new players, novel targets. *Curr Opin Clin Nutr Metab Care*. 2006;9(4):358–365. doi:10.1097/000062894.28674.30
- Wen YA, Xiong X, Zaytseva YY, et al. Downregulation of SREBP inhibits tumor growth and initiation by altering cellular metabolism in colon cancer. *Cell Death Dis*. 2018;9(3):e55. doi:10.1038/s41419-018-0330-6
- Guo D, Prins RM, Dong J, et al. EGFR signaling through an Akt-SREBP-1-dependent, rapamycin-resistant pathway sensitizes glioblastomas to a lipogenic therapy. *Sci Signal*. 2009;2(101):ra82. doi:10.1126/scisignal.2000446
- Yecies JL, Zhang HH, Menon R, et al. Akt stimulates hepatic SREBP1c and lipogenesis through parallel mTORC1-dependent and independent pathways. *Cell Metab*. 2011;14(1):21–32. doi:10.1016/j.cmet.2011.06.002
- Reed-Pastor WA, Mizuno H, Zhao X, et al. Mutant p53 disrupts primary tissue architecture via the mevalonate pathway. *Cell*. 2012;148(1–2):249–258. doi:10.1016/j.cell.2011.12.017
- Williams KJ, Angus JP, Zhu Y, et al. An essential requirement for the SCAP/SREBP signaling axis to protect cancer cells from lipotoxicity. *Cancer Res*. 2013;73(9):2850–2862. doi:10.1158/0008-5472.CAN-13-0582-T
- Krahmer N, Guo Y, Farese RV Jr, et al. SnapShot: lipid droplets. *Cell*. 2009;139(5):1024–1024.e1. doi:10.1016/j.cell.2009.11.023
- Nomura DK, Long JZ, Niessen S, et al. Monoacylglycerol lipase regulates a fatty acid network that promotes cancer pathogenesis. *Cell*. 2010;140(1):49–61. doi:10.1016/j.cell.2009.11.027
- Holm C, Osterlund T, Laurell H, et al. Molecular mechanisms regulating hormone-sensitive lipase and lipolysis. *Annu Rev Nutr*. 2000;20:365–393. doi:10.1146/annurev.nutr.20.1.365
- Dong Q, Giorgianni F, Beranova-Giorgianni S, et al. Glycogen synthase kinase-3-mediated phosphorylation of serine 73 targets sterol response element binding protein-1c (SREBP-1c) for proteasomal degradation. *Biosci Rep*. 2015;36(1):e00284. doi:10.1042/BSR20150234
- Gerdes J, Schwab U, Lemke H, et al. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. *Int J Cancer*. 1983;31(1):13–20. doi:10.1002/ijc.2910310104
- Lu Z, Li S, Zhao S, et al. Upregulated miR-17 regulates hypoxia-mediated human pulmonary artery smooth muscle cell proliferation and apoptosis by targeting mitofusin 2. *Med Sci Monit*. 2016;22:3301–3308. doi:10.12659/MSM.900487
- Piro F, Leonardi L. Expression of Bcl-2 in canine osteosarcoma. *Open Vet J*. 2015;5(1):27–29.
- Sharifi S, Barar J, Hejazi MS, et al. Doxorubicin changes Bax/Bcl-xL ratio, caspase-8 and 9 in breast cancer cells. *Adv Pharm Bull*. 2015;5(3):351–359. doi:10.15171/apb.2015.049
- Akazawa Y, Matsuda K, Isomoto H, et al. BH3-only protein Bim is associated with the degree of Helicobacter pylori-induced gastritis and is localized to the mitochondria of inflammatory cells in the gastric mucosa. *Int J Med Microbiol*. 2015;305(6):553–562. doi:10.1016/j.ijmm.2015.07.002

30. Contractor H, Zariwala M, Bugert P, et al. Mutation of the p53 tumour suppressor gene occurs preferentially in the chromophobe type of renal cell tumour. *J Pathol.* **1997**;181(2):136–139. doi:10.1002/(SICI)1096-9896(199702)181:2<136::AID-PATH766>3.0.CO;2-2
31. Nieto MA. Epithelial-mesenchymal transitions in development and disease: old views and new perspectives. *Int J Dev Biol.* **2009**;53(8–10):1541–1547. doi:10.1387/ijdb.072410mn
32. Nieto MA, Huang RY, Jackson RA, et al. EMT: 2016. *Cell.* **2016**;166(1):21–45. doi:10.1016/j.cell.2016.06.028
33. Das V, Bhattacharya S, Chikkaputtaiah C, et al. The basics of epithelial-mesenchymal transition (EMT): A study from a structure, dynamics, and functional perspective. *J Cell Physiol.* **2019**;234(9):14535–14555. doi:10.1002/jcp.28160
34. Cully M, You H, Levine AJ, et al. Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. *Nat Rev Cancer.* **2006**;6(3):184–192. doi:10.1038/nrc1819
35. Bengoechea-Alonso MT, Ericsson J. The phosphorylation-dependent regulation of nuclear SREBP1 during mitosis links lipid metabolism and cell growth. *Cell Cycle.* **2016**;15(20):2753–2765. doi:10.1080/15384101.2016.1220456
36. Porstmann T, Santos CR, Griffiths B, et al. SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth. *Cell Metab.* **2008**;8(3):224–236. doi:10.1016/j.cmet.2008.07.007
37. Düvel K, Yecies JL, Menon S, et al. Activation of a metabolic gene regulatory network downstream of mTOR complex 1. *Mol Cell.* **2010**;39(2):171–183. doi:10.1016/j.molcel.2010.06.022
38. Peterson TR, Sengupta SS, Harris TE, et al. mTOR complex 1 regulates lipin 1 localization to control the SREBP pathway. *Cell.* **2011**;146(3):408–420. doi:10.1016/j.cell.2011.06.034
39. Mukhopadhyay S, Frias MA, Chatterjee A, et al. The enigma of rapamycin dosage. *Mol Cancer Ther.* **2016**;15(3):347–353. doi:10.1158/1535-7163.MCT-15-0720
40. Porstmann T, Griffiths B, Chung YL, et al. PKB/Akt induces transcription of enzymes involved in cholesterol and fatty acid biosynthesis via activation of SREBP. *Oncogene.* **2005**;24(43):6465–6481. doi:10.1038/sj.onc.1208802
41. Li S, Brown MS, Goldstein JL. Bifurcation of insulin signaling pathway in rat liver: mTORC1 requires stimulation of lipogenesis, but not inhibition of gluconeogenesis. *Proc Natl Acad Sci U S A.* **2010**;107(8):3441–3446. doi:10.1073/pnas.0914710107
42. Cairns RA, Harris IS, Makris AM. Regulation of cancer cell metabolism. *Nat Rev Cancer.* **2011**;11(2):85–95. doi:10.1038/nrc2981
43. Mukhopadhyay S, Maqacena M, Foster D. Synthetic lethality in KRas-driven cancer cells created by glutamine deprivation. *Oncoscience.* **2015**;2(10):797–808. doi:10.18632/oncoscience.253

## OncoTargets and Therapy

### Publish your work in this journal

OncoTargets and Therapy is an international, peer-reviewed, open access journal focusing on the pathological basis of all cancers, potential targets for therapy and treatment protocols employed to improve the management of cancer patients. The journal also focuses on the impact of management programs and new therapeutic

agents and protocols on patient perspectives such as quality of life, adherence and satisfaction. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/oncotargets-and-therapy-journal>

Dovepress