

Lysine-specific demethylase 2 contributes to the proliferation of small cell lung cancer by regulating the expression of TFPI-2

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Abstract. The present study aimed to investigate the effect of lysine-specific demethylase 2 (LSD2) in small cell lung cancer (SCLC) and explore its underlying regulatory mechanism. Cell growth was tested by MTT assay and mRNA and protein expression was determined by quantitative polymerase chain reaction (q-PCR) and western blot analysis, respectively. Chromatin immunoprecipitation (ChIP) was used to investigate the degree of H3K4me2 enrichment in the promoter region of tissue factor pathway inhibitor-2 (TFPI-2). SCLC tissues and cell lines presented significantly higher expression of LSD2 and DNA methyltransferase 3B (DNMT3B) and lower expression of TFPI-2 compared with the controls. In H1417 cells LSD2 overexpression increased the mRNA and protein expression of DNMT3B, while inhibiting the mRNA and protein expression of TFPI-2. Following transfection with short interfering (si) RNA-DNMT3B, the expression of TFPI-2 increased in H1417 cells. The results of ChIP demonstrated that compared with the controls, H3K4me1 enrichment in the TFPI-2 promoter region was to a lower degree in the H1417 cells with LSD2 overexpression and a higher degree in the H1417 cells with LSD2 silencing. MTT assays revealed that LSD2 overexpression significantly promoted the growth of H69, DMS-114 and H1417 cells, which was contradictory to the effect on LSD2 silencing. Compared with the LSD2 overexpression cells, SCLC cells with simultaneous overexpression of LSD2 and TFPI-2 demonstrated a decreased proliferation. These results suggest that LSD2 achieves a promoting effect on SCLC by indirectly regulating TFPI-2 expression through the mediation of DNMT3B expression or through the regulation of the demethylation of H3K4me1 in the promoter region of the TFPI-2 gene.

Introduction

For small cell lung cancer (SCLC) patients, the two-year survival rate is below 5%, with a five-year survival rate below 2%, and curative resection is currently the main therapy (1). SCLC has a significant early propensity to metastasize and is very sensitive to initial systemic cytotoxic chemotherapy. Therefore, systemic chemotherapy combined with radiotherapy and surgery is the main treatment for SCLC (2). At the genetic level, SCLC is a heterogeneous disease associated with a large number of genetic changes. Many cancer-associated genes are likely prone to somatic mutations, including oncogenes, tumor suppressor genes, enzymes involved in chromatin modification, tyrosine kinase receptors and their downstream signaling components (3). As a result, more and more targeted drugs are expected to be developed for the treatment of SCLC. For example, anti-angiogenesis drugs have been previously used to treat extensive-stage SCLC (ES-SCLC). In a phase II clinical trial, basic chemotherapy using bevacizumab combined with platinum was adopted for the treatment of ES-SCLC patients. The results illustrated that progression-free survival (PFS) was significantly increased in the experimental group compared to the control group, suggesting that bevacizumab had a curative effect on ES-SCLC patients (4). External environmental factors also have an important impact on the occurrence of SCLC. Smoking is recognized as the most important risk factor for SCLC, and most SCLC patients have a history of smoking or being in a smoking environment. So far, many studies have (5-7) confirmed the correlation between the progression of SCLC and gene methylation, providing a theoretical and experimental foundation for further research to better understand the occurrence and development of SCLC.

Lysine-specific demethylase 2 (LSD2) is encoded by a gene on human chromosome 6p22, which has an extremely high incidence of genetic abnormalities in cancer patients, such as substitutions, deletions, and DNA amplifications. Therefore, LSD-2 is likely involved in the occurrence and development of carcinogenesis. Similar to its homologue LSD-1, LSD-2 promotes the demethylation of mono- and dimethylated H3K4 and H3K9 (8,9). It has been found that the overexpression of LSD2 in breast cancer promotes cancer cell growth and endows cancer cells with similar characteristics to stem cells, while the inhibition of LSD2 blocks the growth of breast cancer cells (10). Research has also found that

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inhibiting the mRNA expression of LSD2 suppresses clonal formation and migration of MDA-MB-231 cells, while LSD2 knock-down (LSD2 KD) promotes the expression of tumor proliferation-related genes (such as CLDN1, CDH11, CASP5) and tumor suppressor genes (such as ERBB2IP, PR, ER α) and can also enhance the sensitivity of breast cancer cells to DNA methyltransferase (DNMT) inhibitors (11). In non-small cell lung cancer (NSCLC), LSD2 was found to have E3 ubiquitin ligase function, and it directly promoted the ubiquitination and protein degradation of O-GlcNAc transferase (OGT). Moreover, the inhibitory effects of LSD2 on NSCLC cell line A549 depended on this E3 ligase activity (12). However, related research on the role of LSD2 in SCLC has not yet been reported.

We found that LSD2 presented high expression in SCLC tissue and cell lines. Importantly, we show that LSD2 can indirectly regulate tissue factor pathway inhibitor-2 (TFPI-2) expression by mediating DNMT3B expression or by regulating the demethylation of H3K4me2 in the promoter region of the TFPI-2 gene.

Materials and methods

SCLC clinical samples and cell lines. From 2012 to 2016, 40 patients with SCLC were chosen to undergo surgical treatment, during which cancer and cancer-adjacent tissues (>3 cm from the edge of the tumor) were collected. These SCLC patients comprised 30 males and 10 females with an average age of 57 years, and most of them (32 cases) had a history of smoking. The inclusion criteria for SCLC consisted of the following: Cytologically or histologically diagnosed as SCLC, age of at least 18 years, complete clinical data. The exclusion criteria included mixed cancers, patients without clear postoperative pathological diagnosis and patients with detectable inflammatory disease or liver disease. All cases were diagnosed and divided by their stages according to the 7th TNM staging system proposed by the International Association for the Study of Lung Cancer. All of the patients signed informed consent forms. All experiments were approved by the Medical Ethics Association of Shandong Provincial Qianfoshan Hospital (Jinan, China).

The normal human bronchial epithelial cell line BEAS-2B and SCLC cell lines (H69, DMS-114 and H1417) were purchased from the American Type Culture Collection (ATCC). After cell recovery, all cells were seeded in RPMI-1640 medium containing 10% fetal bovine serum (FBS; both Sigma-Aldrich, St. Louis MO, USA), and then cultured in a humidified chamber with 5% CO₂ at 37°C. The medium was replaced, and cells were passaged once every 3-4 days.

Vector construction for gene silencing and overexpression. Based on the sequences of the LSD2 and TFPI-2 genes, primers were designed to amplify their full-length cDNA (Table I). The cDNA of each gene was subcloned into the pCDH-CopGFP vector (System Biosciences, Mountain View, CA, USA) to construct overexpression plasmids, which were then transfected into the cell lines using Lipofectamine 3000 (Invitrogen Inc., Carlsbad, CA, USA). Empty vectors were used as controls. Subsequently, the cells were cultured for 48 h and then screened with DMEM supplemented with

400 μ g/ml G418 (Sigma-Aldrich). After 1 month of screening, stable gene silencing and overexpression strains (LSD1, LSD2, and DNMT3B) were obtained, cultured in DMEM with 10% FBS+P.S. medium and cryopreserved. Meanwhile, short interfering (si) RNA to target LSD2 (si-LSD2) and corresponding control siRNA were designed according to their sequences (Table I), and transfected into the cell lines using Lipofectamine 3000.

RNA extraction and fluorescence quantitative polymerase chain reaction (Fq-PCR). Total RNA was extracted from cells using TRIzol (Invitrogen Inc.) according to the manufacturer's protocol. Complementary DNA (cDNA) was synthesized from 1 μ g of total RNA using Superscript III reverse transcriptase (Invitrogen Inc.). Fq-PCR was performed using an ABI StepOne real-time PCR system as the following steps: 95°C for 10 min, followed by 50 cycles of 95°C for 15 sec and 68°C for 45 sec. Primers used for quantitative polymerase chain reaction (q-PCR) are shown in Table I. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal reference.

Western blot analysis. The SCLC cells were washed 3 times with cold phosphate buffered saline (PBS), and then lysed with RIPA lysis buffer (both Sigma-Aldrich) to extract total protein. The total protein concentration was determined using a BCA kit (Thermo Fisher Scientific, Inc., San Jose, CA, USA). For each sample, 20 μ g protein was added to a gel, separated by 10% SDS-PAGE, and then transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore Corp., Bedford, MA, USA). Each membrane was blocked for 1 h with 5% skim milk at room temperature, washed once with PBS, and incubated overnight with primary antibodies at 4°C. The primary antibodies were as follows: Anti-DNMT3B (ab16049; 1:1,000), anti-TFPI2 (ab86933; 1:1,000), and anti-GAPDH (1:2,500; Abcam, Cambridge, MA, USA). Then, after being washed 2-3 times with TBS with Tween-20 (TBST), the membranes were incubated for 1 h with secondary anti-rabbit IgG antibodies (ab205718; 1:2,000; Abcam) at room temperature. Finally, an Odyssey Infrared Imaging System (LI-COR Inc., Superior St. Lincoln, NE, USA) was used to detect immune responses. GAPDH was used as an internal reference.

MTT assays. Cell proliferation was quantified using MTT assays. Cells were plated into 96-well plates in triplicate using 100 μ l cell suspension fluid with a density of 2x10⁴/ml and cultured for 24, 48 and 72 h in 5% CO₂ incubator at 37°C. At each time point, 50 μ l MTT solution (1 g/l in normal saline) was added into each well. After a 4-h incubation at 37°C, the supernatant was removed and 100 μ l dimethylformamide (DMSO) was added. The plates were shaken for 10 min to fully resolve the MTT pyrolysis products. Then, the optical density (OD) at 590 nm was measured on an immunoassay analyzer, and the cell growth curve was determined based on the OD values.

Chromatin immunoprecipitation (ChIP). ChIP was performed according to the method described in a previous study (13). In brief, 1x10⁶ NSCLC cells were cross-linked with 1% formaldehyde and washed with PBS in the presence of protease inhibitors. Cells were homogenized and their

Table I. Primers for the clone and q-PCR of genes.

Gene	Forward primer	Reverse primer
Clone		
LSD2 ⁺	CAGTAATCATATGGCAACTCCACG GGGGAGGACAAAG	TAGTCTCGAGTTAAAATGCTGCAATC TTGCTTG
si-LSD2	ATCGATGCGGTATGAAACCAA	
TFPI-2 ⁺	GCTTTCTCGGACGCCTTGC	GAATACGACCCCAAGAAATGAGTGA
q-PCR		
LSD2	CCTGGCTTTGAGAAACCTCATC	TCTCCACTTCCTGAACGCATC
TFPI-2	GTCGATTCTGCTGCTTTTCC	CAGCTCTGCGTGTACCTGTC
GAPDH	GGTCGGTGTGAACGGATTTG	GGGGTCGTTGATGGCAACA
DNMT3B	GTGCTCGCTTCGGCAGCACA	TGGAACGCTTCACGAATTTG

q-PCR, quantitative polymerase chain reaction; LSD2, lysine-specific demethylase 2; si, short interfering RNA; TFPI-2, tissue factor pathway inhibitor-2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; DNMT3B, DNA methyltransferase 3B.

chromatin was subjected to ChIP using magnetic Dynabeads (Invitrogen Inc.) and antibodies against dimethylated lysine 4 of histone H3 (H3K4) (Millipore Corp., Temecula, CA, USA). The fold enrichment of the DNAs amplified by ChIP was assessed using previously described protocols. In accordance with the promoter region of the TFPI-2 gene, the specific primers were designed as follows: S1, 5'-ATAAAGCGGGTA TTCGGGTC-3' and AS1, 5'-CTCCGCCGATTAATAAAAAA-3'.

Statistical analysis. Quantitative data are expressed as the mean \pm standard error. Statistical analysis was performed by using one-way analysis of variance followed by the least significant difference test or two-way analysis of variance followed by Tukey's multiple comparisons test. Statistical analysis was performed with GraphPad prism 7 software (GraphPad Software, Inc., La Jolla, CA, USA). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

LSD2 presented high expression SCLC tissues and cell lines. To investigate the role of LSD2 in SCLC, q-PCR was performed to measure LSD2 expression in SCLC samples. The results suggested that LSD2 is expressed relatively highly in cancer tissues compared with normal cancer-adjacent tissues ($P < 0.05$) (Fig. 1A). Similarly, compared to the normal cell line BEAS-2B, LSD2 expression was significantly increased in H69, DMS-114, and particularly in H1417 cells (Fig. 1B).

LSD2 overexpression promoted the growth of SCLC cell lines. The high expression of LSD2 observed in SCLC clinical samples and cell lines indicated that LSD2 may participate in the progression of SCLC. Therefore, we assessed the effect of LSD2 on the growth of H69, DMS-114 and H1417 cells by MTT assays. The results demonstrated that, compared to that of control cells transfected with empty vectors, the growth of H69, DMS-114 and H1417 cells was significantly promoted by LSD2 overexpression (Fig. 2A-C), while it was inhibited by LSD2 silencing (Fig. 2D-F).

LSD2 negatively regulated the expression of TFPI-2 through DNMT3B. Previous studies have shown that LSD2 regulates the expression of DNMT3B and TFPI-2 in breast cancer (14,15), and TFPI-2 also plays a tumor suppression role in a variety of tumors (16,17). We first detected the expression of TFPI-2 and DNMT3B in SCLC. The results of q-PCR demonstrated that DNMT3B was significantly higher expressed in SCLC tissues (Fig. 3A) and cell lines (H69, DMS-114, and H1417) (Fig. 3B) than in cancer-adjacent tissues and the control cell line BEAS-2B, respectively ($P < 0.05$). In contrast, the expression of TFPI-2 was significantly decreased in SCLC tissues (Fig. 3C) ($P < 0.01$) and cell lines (Fig. 3D) ($P < 0.05$).

We then investigated the effects of LSD2 overexpression or inhibition on the expression of DNMT3B and TFPI-2. q-PCR and western blots suggested that compared with the control cells, LSD2-overexpressing H1417 cells showed a remarkable increase in the mRNA and protein expression of DNMT3B and a strong decrease in the mRNA and protein expression of TFPI-2. After LSD2-overexpressing H1417 cells were transfected with siRNA-DNMT3B, the mRNA and protein expression of TFPI-2 were elevated to a different degree than in the cells only overexpressing LSD2 (Fig. 3E and F). These results suggested that LSD-2 indirectly represses the expression of TFPI-2 by mediating the expression of DNMT3B.

LSD2 regulated TFPI-2 expression by mediating the demethylation of H3K4me1 in the promoter region of TFPI-2. To determine whether LSD2 regulates TFPI-2 expression by regulating the demethylation of H3K4me1 in the promoter region of TFPI-2, ChIP was performed. The results showed that H3K4me1 enrichment in the promoter region of TFPI-2 was reduced in H1417 cells compared to that of BEAS-2B cells (Fig. 4A) ($P < 0.05$). Compared to the control cells transfected with empty vectors, the TFPI-2 promoter region showed a lower level of H3K4me1 enrichment in H1417 cells overexpressing LSD2 (Fig. 4B) ($P < 0.05$). Contrarily, the enrichment of H3K4me1 in the TFPI-2 promoter region of H1417 cells was significantly increased after LSD2 was inhibited (Fig. 4C) ($P < 0.01$). These findings indicated that in the H1417 cell line,

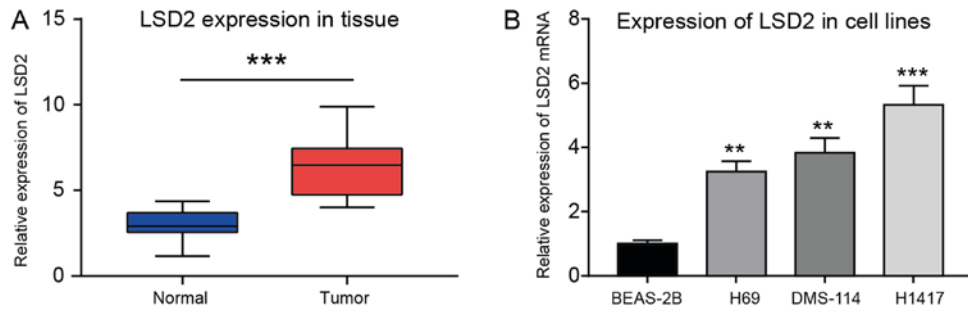


Figure 1. Expression of LSD2 in SCLC. (A) Expression of LSD2 mRNA in 40 cases of clinical samples of SCLC. *** $P < 0.001$. (B) Expression of LSD2 mRNA in SCLC cell lines. LSD2 expression significantly increased in H69, DMS-114, and H1417 cells, particularly in H1417 cells when compared with BEAS-2B. ** $P < 0.01$ and *** $P < 0.001$ vs. the BEAS-2B group. LSD2, lysine-specific demethylase 2; SCLC, small cell lung cancer.

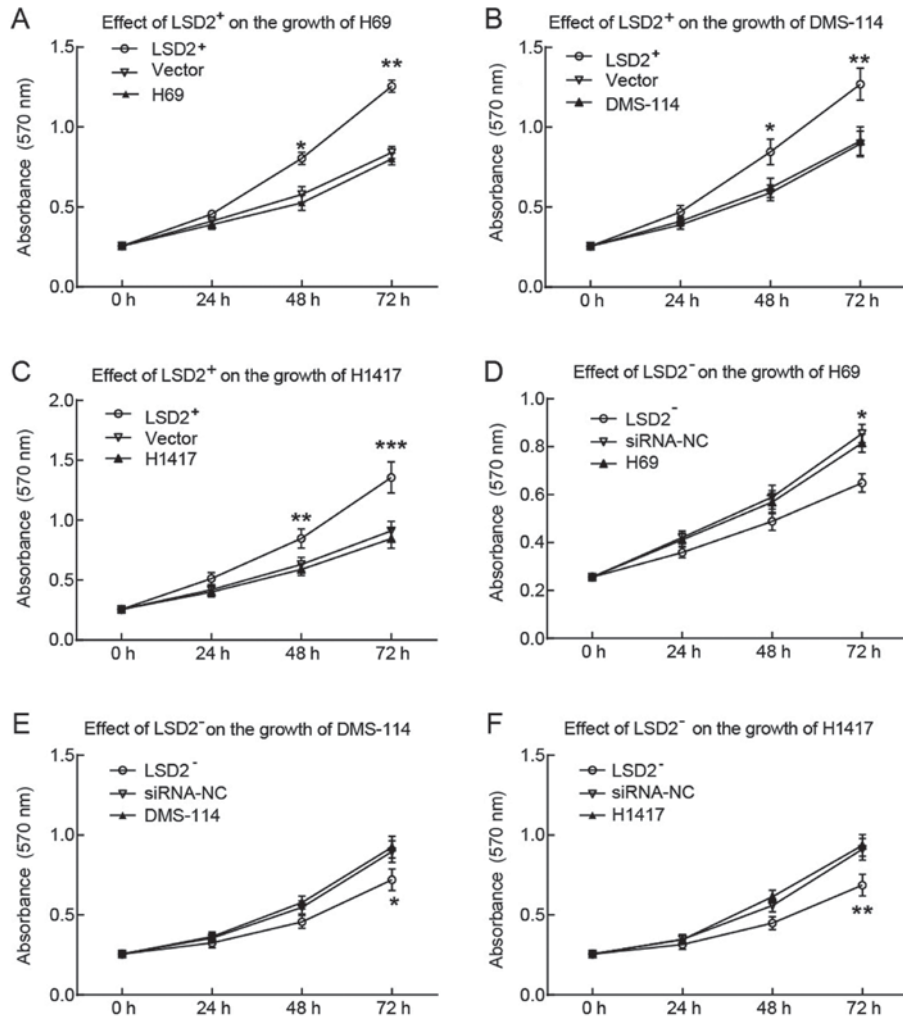


Figure 2. Effect of LSD2 on the growth of SCLC cells. Cells were plated into 6-well plates in triplicate using 100 μ l cell suspension fluid with a density of 2×10^4 /ml and cultured for 24, 48 and 72 h. Then cell growth was detected via MTT assays. The growth of (A) H69, (B) DMS-114 and (C) H1417 cells was significantly promoted by LSD2 overexpression when compared with the control groups. The growth of (D) H69, (E) DMS-114 and (F) H1417 cells was significantly inhibited by LSD2 silencing when compared with the control groups. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs. the vector group. LSD2, lysine-specific demethylase 2; SCLC, small cell lung cancer; si-LSD2, siRNA to target LSD2; siRNA, short interfering RNA.

LSD2 positively regulates the demethylation of H3K4me1 in the promoter region of TFPI-2 and thereby controls its transcription.

TFPI-2 overexpression inhibited the promoting effect of LSD2 on the proliferation of SCLC cells. To further investigate whether

the effect of LSD2 on the proliferation of SCLC cells is related to the expression of TFPI-2, a rescue experiment for TFPI-2 was conducted. MTT assays revealed that, compared with cells only overexpressing LSD2, SCLC cells (H69, DMS-114 and H1417) overexpressing both LSD2 and TFPI-2 showed significantly reduced proliferation (Fig. 5). This implied that the promoting

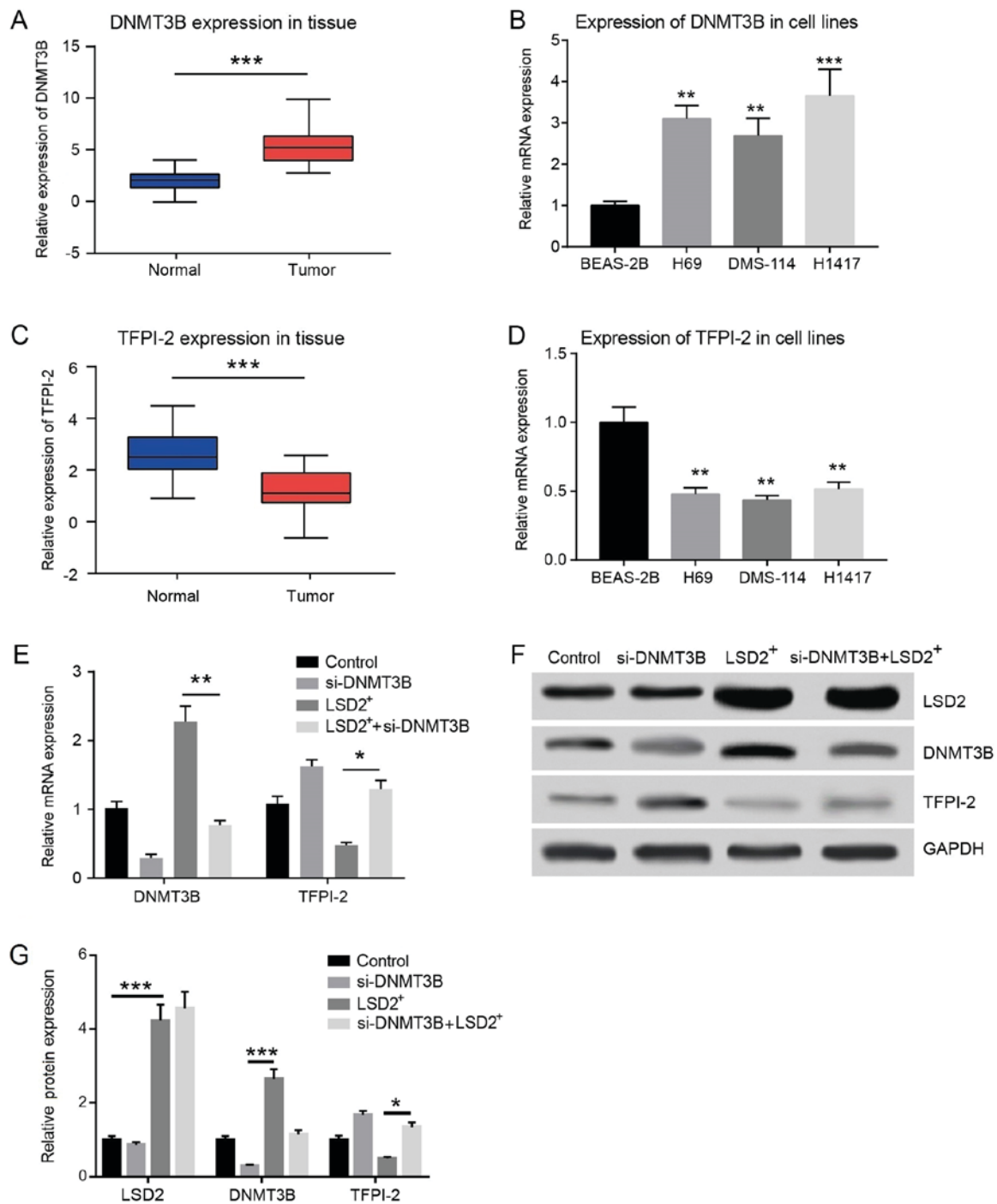


Figure 3. LSD2 negatively regulates the expression of TFPI-2 through DNMT3B. (A) Expression of DNMT3B mRNA in 40 cases of clinical samples of SCLC. (B) Expression of DNMT3B mRNA in SCLC cell lines. BEAS-2B was used as the control group. (C) Expression of TFPI-2 mRNA in 40 cases of clinical samples of SCLC. (D) Expression of TFPI-2 mRNA in SCLC cell lines. BEAS-2B was used as the control group. (E) mRNA expression of DNMT3B and TFPI-2 in LSD2⁺ and/or si-DNMT3B H1417 cells. (F and G) Effect of LSD2⁺ and/or si-DNMT3B on the protein expression of TFPI-2. Control, H1417 cells; si-DNMT3B, H1417 cells transfected with si-DNMT3B; LSD2⁺, H1417 cells transfected with LSD2 overexpression plasmids; si-DNMT3B+LSD2⁺, H1417 cells transfected with si-DNMT3B and LSD2 overexpression plasmids. *P<0.05; **P<0.01 and ***P<0.001 vs. the BEAS-2B group or as indicated. DNMT3B, DNA methyltransferase 3B; TFPI-2, tissue factor pathway inhibitor-2; LSD2, lysine-specific demethylase 2; SCLC, small cell lung cancer; siRNA, small interfering RNA; si-DNMT3B, siRNA to target DNMT3B.

effect of LSD2 on the proliferation of SCLC cells may be partially achieved through the inhibition of TFPI-2.

Discussion

DNA methylation is one of the major causes of epigenetic changes in organisms. It exerts its effects by differentially regulating gene

expression based on the number and distribution of methylated cytosines in the genome, without changing the DNA sequence. Methylation plays a critical role in embryonic development, cell differentiation, and in the occurrence of a variety of human diseases (18). Although methylation levels are generally reduced in cancer, hypermethylation occurs periodically in the course of cancer progression, which is associated with the activation

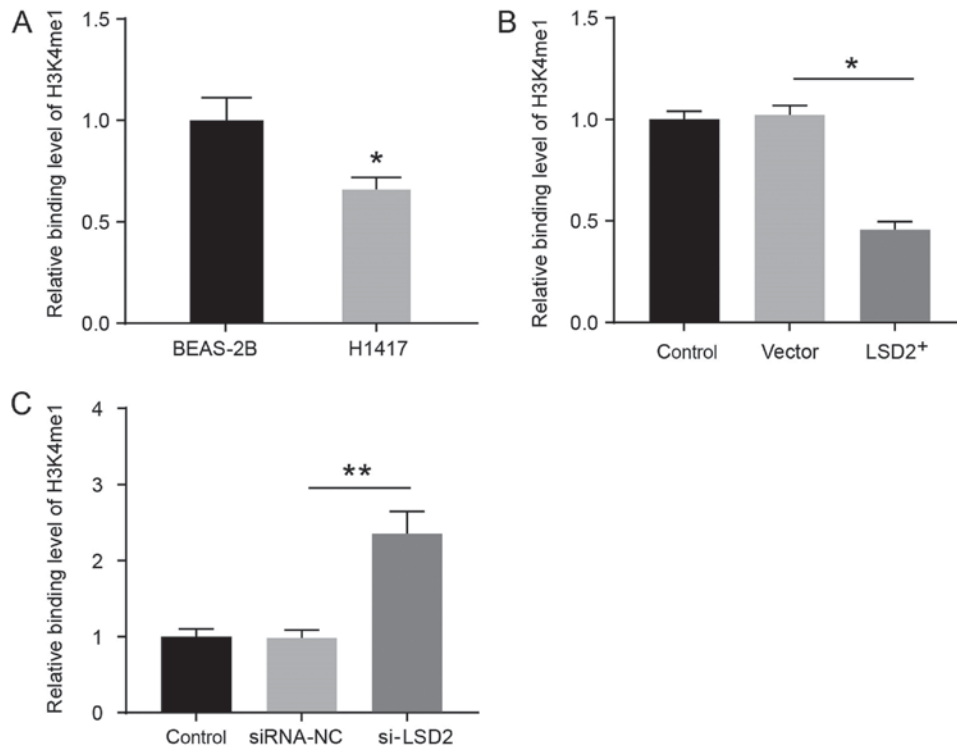


Figure 4. Histone modification H3K4me1 at the promoter region of TFPI-2 in SCLC cells. ChIP assays were performed with antibodies against H3K4me1 in SCLC cells. Region of the TFPI-2 promoter for quantitative ChIP-PCR assay were indicated via primers S1 and AS1. The binding of H3K4me1 antibody to region of the TFPI-2 promoter was measured by the quantification of ChIP DNA against a standard curve generated from input DNA. (A) H3K4me1 enrichment level in the promoter region of TFPI-2 showed lower degree in H1417 cells than that in BEAS-2B cells, * $P < 0.05$ vs. the BEAS-2B group. (B) TFPI-2 promoter region showed a significantly lower enrichment level of H3K4me1 in the cell line H1417 with LSD2 overexpression. (C) TFPI-2 promoter region showed a significantly higher enrichment level of H3K4me1 in the cell line H1417 with siRNA-LSD2. * $P < 0.05$ and ** $P < 0.01$ as indicated. Control, H1417 cells; si-LSD2, H1417 cells transfected with si-LSD2; TFPI-2, tissue factor pathway inhibitor-2; siRNA-NC, H1417 cells transfected with siRNA-NC; si, small interfering; SCLC, small cell lung cancer; si-LSD2, siRNA to target LSD2; siRNA-NC, control siRNA of LSD2; H3K4me1, monomethylated histone H3 lysine 4; ChIP, chromatin immunoprecipitation.

of proto-oncogenes, thereby promoting the occurrence and metastasis of cancer (19). In addition, covalent modification of histones is a key mechanism for regulating gene expression, including methylation, phosphorylation, and acetylation. (20). Histone modification has been recognized as being involved in chromatin-related processes, including DNA replication, repair, and transcription. The association between histone modification and transcription has been intensively studied (21). Methylation of H3K4 is one type of histone modification, which is generally believed to mediate the transcriptional activation of genes (22).

Meanwhile, previous studies have claimed that knocking out LSD2 can promote the expression of both tumor proliferation-associated genes and tumor suppressor genes (23). LSD2 overexpression in MDA-MB-231 cells significantly altered the expression of key epigenetic modifiers such as DNMT3B, HDAC1/2, and LSD1; augmented colony formation in soft agar; and promoted cellular proliferation (14). To investigate whether LSD2 plays a role in suppressing or promoting SCLC, we measured the expression of LSD2 in SCLC and tested its effect on the growth of cancer cells. The results demonstrated that LSD2, compared with controls, was more highly expressed in SCLC clinical tissues or SCLC cell lines (including H69, DMS-114 and H1417), while the results of MTT assays revealed that the growth of SCLC cell lines was promoted by LSD2 overexpression and inhibited by LSD2 gene silencing. These results suggest that the up-regulation of

LSD2 in SCLC participates in the tumor progression of SCLC and that its expression in SCLC may be beneficial for SCLC tumorigenesis. In addition, a previous study claimed that knockout of LSD1 promotes the expression of TFPI-2, which is accompanied by increased levels of H3K4me2 in the promoter region of TFPI-2, suggesting that TFPI-2 expression can be regulated by LSD1-mediated transcriptional initiation (24). Thus, we hypothesized that the homologous gene, LSD2, may regulate H3K4 demethylation in the promoter region of TFPI-2 in SCLC. In the present study, the level of H3K4me1 enrichment in the TFPI-2 promoter region was reduced in H1417 cells compared to that in normal BEAS-2B lung cells. In H1417 cells, LSD2 overexpression reduced the H3K4me2 enrichment level in the TFPI-2 promoter region. In breast cancer, LSD2-KD led to accumulation of H3K4me1/2 without changing the methylation levels of other key histone lysine residues, indicating that LSD2 serves as a bona fide H3K4 demethylase (23). Therefore, the results of this study indicated that LSD1/2 suppresses TFPI-2 expression by promoting the demethylation of H3K4me1 in the TFPI-2 promoter region.

TFPI-2 plays an important role as a tumor suppressor gene. For instance, TFPI-2 acts as an independent prognostic factor for NSCLC patients (25). In cervical cancer, TFPI-2 expression shows a decreasing trend with tumor progression and has a close association with tumor cell apoptosis and angiogenesis (26). The methylation level of TFPI2 is significantly

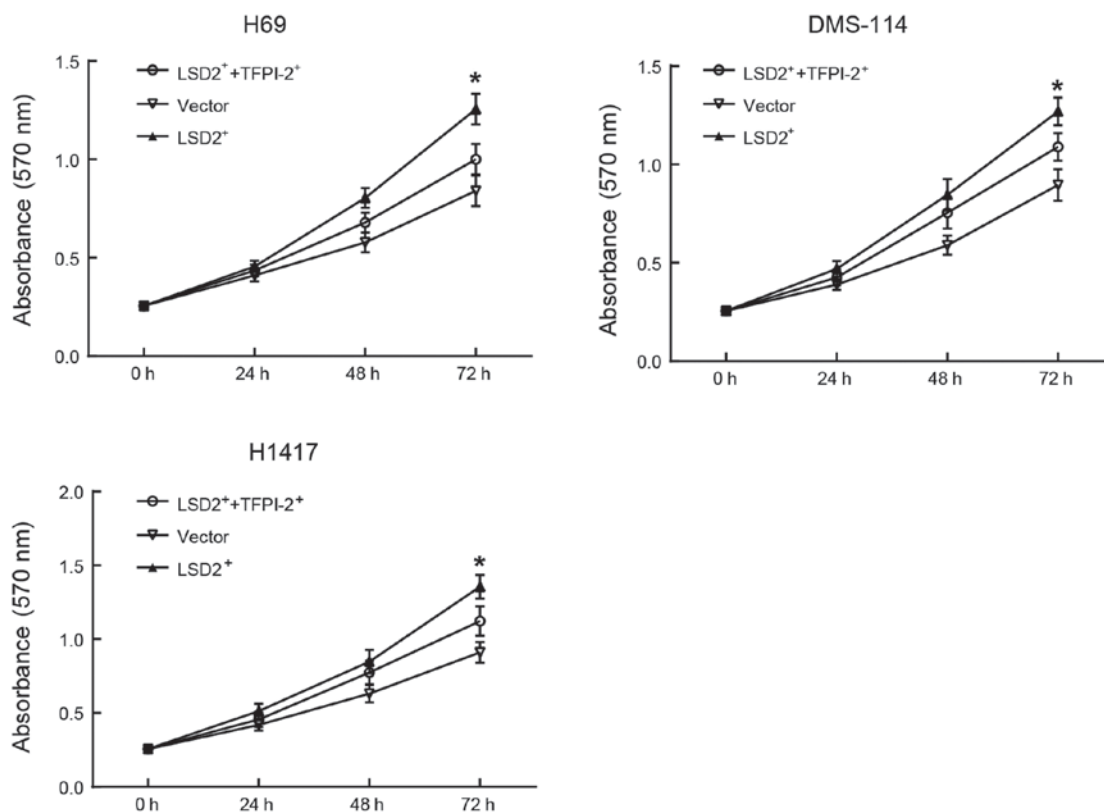


Figure 5. TFPI-2 overexpression inhibited the promoting effect of LSD2 on the proliferation of SCLC cells. Cell proliferation was assessed with an MTT assay. H69, DMS-114 and H1417 cells with overexpression of LSD2 and TFPI-2 showed significantly reduced proliferation compared with the LSD2⁺ group. *P<0.05 vs. the LSD2⁺ group. SCLC, small cell lung cancer; TFPI-2, tissue factor pathway inhibitor-2; LSD2, lysine-specific demethylase 2.

increased in colorectal tumor tissues compared with that in colorectal normal tissues, and TCGA data also supported the hypothesis that TFPI2 hypermethylation is a promising diagnostic marker for CRC and GC (27). In the present study, our results suggested that TFPI-2 expression in SCLC tissues was significantly lower than that in normal tissue. One of the primary reasons for the low expression of TFPI-2 in tumors is hypermethylation of its promoter region (28-31). The depletion of DNMT1 or DNMT3B expression in lung, esophageal carcinoma and malignant pleural mesothelioma cells increases the expression of the tumor suppressor genes p16, p21 and TFPI-2. In the present study, after DNMT3B in H1417 cells was inhibited by siRNA-DNMT3B, the mRNA and protein expression of TFPI-2 were significantly increased, suggesting that TFPI-2 expression was also affected by the methylation of its promoter region. Further experiments demonstrated that in H1417 cells overexpressing LSD2, the mRNA and protein expression of DNMT3B were remarkably increased compared with that in the control group, in contrast to the expression of TFPI-2. This indicated that LSD2 indirectly represses TFPI-2 expression by regulating DNMT3B expression. It has been reported that LSD2 overexpression can significantly alter the expression of epigenetic modifying enzymes such as LSD1, HDAC1/2 and DNMT3B in the breast cancer cell line MDA-MB-231, thereby promoting cell proliferation and colony formation in soft agar (14). Interestingly, in SCLC cells overexpressing both LSD2 and TFPI-2, the growth of cancer cells was reduced compared to that in cells only overexpressing LSD2. This result indicated that the role of LSD2 in promoting the proliferation

of SCLC cells may partially be due to the inhibition of TFPI-2 expression. In the future clinical experiments, researchers should focus on the role of the LSD2/DNMT3B/TFPI-2 axis in SCLC, and identify suitable drugs or inhibitors targeting them to provide a new path for SCLC treatment.

Collectively, we have shown that LSD2 can indirectly mediate TFPI-2 expression in SCLC by regulating DNMT3B. On the other hand, LSD2 can negatively mediate TFPI-2 expression by regulating the demethylation of H3K4me2 in the TFPI-2 promoter region, further leading to the promotion of SCLC.

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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.

Authors' contributions

YFC, CHG and YHY designed the present study and conducted experiments, analysis and interpretation of data.

XL and LZ were involved in the experimental analysis and data acquisition. All authors have read and approved the final submitted manuscript.

Ethics approval and consent to participate

All patients provided written informed consent. All experiments were approved by the Medical Ethics Association of Shandong Provincial Qianfoshan Hospital.

Consent for publication

All patients provided written informed consent for the publication of their data.

Competing interests

The authors declare that they have no competing interests.

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