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ORIGINAL ARTICLE

Knockdown of long non-coding RNA *LCPAT1* inhibits autophagy in lung cancer

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ABSTRACT

Objective: Long non-coding RNAs (lncRNAs) are involved in numerous biological processes in lung cancer cells. In our previous studies, we identified a lncRNA, *ENST00000439577*, which is highly expressed in lung carcinomas, and termed it lung cancer progression-associated transcript 1 (*LCPAT1*). To characterize the role of *LCPAT1* in lung cancer, we conducted the current study.

Methods: Expression of *LCPAT1* and autophagy-associated markers in tumor tissues and lung cancer cell lines was determined by real-time quantitative polymerase chain reaction (qPCR). Hematoxylin and eosin (HE) staining, qPCR, Western blot, and immunohistochemistry were performed to evaluate xenografted tumor tissues. Autophagy induced by rapamycin was detected by Western blot and immunofluorescence in lung cancer cell lines.

Results: Expression of *LCPAT1* and microtubule-associated protein 1 light chain 3 beta (*LC3B*) was positively correlated in lung cancer. Knockdown of *LCPAT1* inhibited tumor growth and suppressed cell autophagy *in vivo*. Moreover, *LCPAT1* knockdown in lung cancer cell lines resulted in decreased autophagy-associated gene expression and alleviated the cell autophagy induced by rapamycin.

Conclusions: We speculate that *LCPAT1* plays a crucial role in regulating autophagy in lung cancer.

KEYWORDS Lung cancer; autophagy; long non-coding RNA; *LCAPT1*; *ENST00000439577*

Introduction

Lung cancer is one of the leading causes of cancer deaths worldwide[1]. The most common type of lung cancer is non-small cell lung cancer (NSCLC) and its 5-year survival rate is < 15%[2]. Although extensive research has been carried out, the pathological process of lung cancer remains unclear. For the past few years, long non-coding RNAs (lncRNAs) have garnered increasing attention in lung cancer research, both as modulators of pathogenesis and as potential therapeutic targets[3-5].

LncRNAs are a class of non-protein coding transcripts that are 200 nucleotides or longer in length[6]. Though they do not code for proteins, lncRNAs control gene expression by functioning as guides, scaffolds, and decoys of many biomolecules. Also, transcription of lncRNAs is exquisitely regulated and the expression of lncRNAs reflects different developmental stages or responds to diverse extracellular signals[7]. LncRNAs are now recognized as novel regulators in tumor development and progression. It is well-known that the lncRNAs *H19*[8], *HOTAIR*[9], *MEG3*[10], *MALAT1*[11], and *ANRIL*[3] are dysregulated in cancer. In our previous study, we reported that expression of *LOC146880* and *ENST00000439577* was elevated in lung cancer[5].

ENST00000439577 is a 311-bp transcript and its gene is located on chromosome 1 (chr1: 17, 406, 760-17, 407, 382). We previously reported that *ENST00000439577* expression was associated with metastasis status and disease stage in lung cancer patients. Moreover, high expression of *ENST00000439577* was associated with poor overall survival of lung cancer patients[5]. Thus, we termed *ENST00000439577* lung cancer progression-associated transcript 1 (*LCPAT1*), suggesting that *LCPAT1* may act as an oncogene in lung cancer.

LncRNAs participate in tumorigenesis through multiple

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pathways, including autophagy[11-15]. Autophagy is an intracellular bulk degradation process through which proteins, lipids, and organelles are delivered to lysosomes for degradation[16]. In this process, autophagy related 5 (*ATG5*), autophagy related 14 (*ATG14*), *BECLIN1*, and other autophagy-related genes play an important role. Sequestosome 1 (p62) is incorporated into mature autophagosomes and degraded during autophagy. Microtubule-associated protein 1 light chain 3 beta (*LC3B*) levels increase and *LC3B*-encoded LC3-I is converted to LC3-II which accumulates and adsorbs on the autophagosome membrane. The degradation of p62 and increase of LC3-II are commonly used as markers of autophagy[17].

Autophagy acts as a double-edged sword in cell survival and cell death[18]. In the process of tumor development, autophagy suppresses chronic inflammatory reactions to prevent tumorigenesis, and enable tumor survival by recycling nutrients[19]. Genetic deletion of an autophagy protein, ATG5, accelerates the initiation of KRas (G12D)-driven lung tumors in mice, but impairs the progression of lung cancer, suggesting that autophagy may prevent oncogenesis, but promote tumor growth[20].

Several lncRNAs targeting autophagy have been described. The overexpression of the lncRNA *BANCR* results in an increase in the ratio of LC3II-to-LC3I, which indicates the formation of an autophagosome[21]. Our previous study revealed that the lncRNA *LOC146880* participates in $PM_{2.5}$ exposure-induced autophagy in lung cancer[4]. The purpose of the current study was to determine the role of *LCPAT1* in regulating autophagy *in vivo* and *in vitro*. We found that *LCPAT1* was positively correlated with *LC3B* in lung cancer. *In vitro* and *in vivo* evidence demonstrated that *LCPAT1* knockdown inhibits activation of autophagy in lung cancer. These findings indicate the pivotal role of lncRNA *LCPAT1* in lung cancer and the possible mechanisms involved with autophagy.

Materials and methods

Patients

Tumor samples and matched adjacent normal tissues were collected with informed consent from lung cancer patients during surgeries performed between May 2006 and July 2011 at the Tianjin Medical University Cancer Institute and Hospital (TMUCIH). RNA extraction and analysis were performed as previously described[4,5,22]. The current study was approved by the medical ethical review committees at TMUCIH and Shanghai Jiao Tong University School of Medicine.

Cell culture

HEK-293T and lung cancer cell lines, A549 and H1975, were obtained from the American Type Culture Collection (ATCC). All cell lines were cultured in Dulbecco's modified Eagle's medium (Invitrogen, Waltham, MA, USA) supplemented with 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO, USA) and 100 U penicillin-streptomycin under 5% CO_2 at 37 °C.

Lentiviral small hairpin (sh)RNA

Plasmids for shRNA targeting *LCPAT1* (sense, 5′-CAATGTTGTTGTTTATTTA-3′ and antisense, 5′-TAAAT AAACAACAACATTG-3′) and scrambled (sense, 5′-TTC TCCGAACGTGTCACGT-3′ and antisense, 5′-ACGTGACA CGTTCGGAGAA-3′) were obtained from Shanghai Integrated Biotech Solution Company and the interfering vector used to generate shRNA was pLKD-CMV-G & PR-U6-shRNA. Lentiviral vector DNA and package vectors were transfected into HEK-293T cells by Lipofectamine® 2000 transfection reagent (Invitrogen). At 48 and 72 h after transfection, lentivirus supernatants were harvested and used to infect H1975 cells. Stable shRNA cell lines were generated following selection with 1 μg/mL puromycin. The efficiency of gene knockdown was detected by real-time quantitative polymerase chain reaction (qPCR).

Animals

BALB/c mice, 4–6 weeks old, were obtained from Shanghai SLAC Laboratory Animal Co. Ltd. (Shanghai, China) and raised in a specific-pathogen-free grade environment. Three female and three male mice were used for a control experiment. H1975 cells transfected with scrambled shRNAs or *LCPAT1*-shRNA were suspended in phosphate-buffered saline with Matrigel matrix basement membrane extract (Bedford, MA, USA) in a 1:1 ratio. Then, the cell solutions (5×10^6 cells/100 μL/mouse) were injected subcutaneously in the left and right hind flanks of mice. Tumor growth was evaluated by measuring tumor diameters every 2 days with a vernier caliper, and tumor volume was calculated using the following formula: $V = 1/2 \times a \times b^2$, where V = tumor volume, a = the larger perpendicular diameter, and b = the smaller perpendicular diameter. The animals were sacrificed on day 21 after implantation and tumors were collected for analysis. All applicable guidelines of the Animal Care and Use Committee of Shanghai Jiao Tong University School of Medicine were followed.

Hematoxylin and eosin (HE) staining

Xenografted tumor tissues were fixed with 4% paraformaldehyde (BOSTER, Wuhan, China), embedded in paraffin, sectioned, and visualized at 100× and 200× magnification after HE staining.

Immunohistochemical (IHC) staining

For lung cancer patients' tissue, the sections (5 μm) from the tissue microarray blocks were labelled with antibodies. The LC3B antibody was diluted 1:2000. An HRP- conjugated secondary antibody (Beijing Sequoia Jinqiao Biological Technology Co., Ltd.) was used. The specific target was visualized with a 3, 3'-diaminobenzidine (DAB) detection kit (Beijing Sequoia Jinqiao Biological Technology Co., Ltd.) and counterstained with hematoxylin. Microphotographs from each arrayed tissue were taken with a fixed exposure time and color balance to ensure consistency. LC3B production was quantified using ImagePro Plus9.1 (Media Cybernetic, Silver Spring, MD).

For animal tumor tissue, paraffin sections were first stained with anti-ATG5 (#ab108327, 1:50; Abcam, Cambridge, MA, USA) and anti- LC3B (#ab48394, 1:50; Abcam). Horseradish peroxidase (DAKO, Glostrup, Denmark) was then used as a secondary antibody. When an antigen-antibody-antibody complex was formed, a substrate of the peroxidase, diaminobenzidine, was added as chromogen. The staining was performed according to the manufacturer's instructions. The pictures were taken at a magnification of 400 × and analyzed using Image Pro-plus 6.0 software (Media Cybernetics, Silver Spring, MD, USA). Five different pictures were measured for each sample.

Small interfering RNA (siRNA) transfectionss

Control and *LCPAT1*-specific small interfering RNA were obtained from Genepharma (Shanghai, China). Three specific siRNAs for *LCPAT1* were designed and mixed for transfection. The sequences of *LCPAT1* siRNAs are listed in **Table 1**. Cells (3×10^5) were seeded into six-well plates and transfected with siRNAs after overnight incubation at 37 °C. The transfection was performed with Lipofectamine® 2000 transfection reagent (Invitrogen) according to the manufacturer's protocol .

Real-time quantitative polymerase chain reaction (qPCR)

Total RNA was isolated from cells and tissues using TRIzol, and cDNA was synthesized using a FastQuant RT Kit with gDNase (TIANGEN, Beijing, China). The primers used for analyzing the expression of *LCPAT1*, *BECLIN1*, *LC3B*, *p62*, *ATG3*, *ATG5*, *ATG7*, *ATG12*, *ATG14*, and β-ACTIN were synthesized by Sangon Biotech (Shanghai, China) (**Table 2**).

Table 1 Sequences of siRNAs

LncRNA-siRNA		Sense/anti-sense
LCPAT1	siRNA-1	5′–CAAUGUUGUUGUUUAUUUAAA-3′/ 5′–UAAAUAAACAACAACAUUGUC -3′
	siRNA-2	5′–AGACAAUUACAGCACUAAACC-3′/ 5′–UUUAGUGCUGUAAUUGUCUUA -3′
	siRNA-3	5′–ACGCUAAGCCUCUUUCAAAUU-3′/ 5′–UUUGAAAGAGGCUUAGCGUUU -3′
Negative control	siRNA-NC	5′-UUCUCCGAACGUGUCACGUTT-3′/ 5′-ACGUGACACGUUCGGAGAATT-3

Table 2 Sequences of PCR primers

Gene	Forward primer 5′-3′	Reverse primer 5′-3′
LCPAT1	TACAGCACTAAACCAGGCAC	TCCAGCTCAGGAAAAAGAAT
BECLIN1	GGTGTCTCTCGCAGATTCATC	TCAGTCTTCGGCTGAGGTTCT
LC3B	AACATGAGCGAGTTGGTCAAG	GCTCGTAGATGTCCGCGAT
P62	GCACCCCAATGTGATCTGC	CGCTACACAAGTCGTAGTCTGG
ATG3	ACATGGCAATGGGCTACAGG	CTGTTTGCACCGCTTATAGCA
ATG5	AAAGATGTGCTTCGAGATGTGT	CACTTTGTCAGTTACCAACGTCA
ATG7	ATGATCCCTGTAACTTAGCCCA	CACGGAAGCAAACAACTTCAAC
ATG12	CTGCTGGCGACACCAAGAAA	CGTGTTCGCTCTACTGCCC
ATG14	GCAAATCTTCGACGATCCCAT	CACACCCGTCTTTACTTCCTC
β-ACTIN	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT

Quantitative RT-PCR was performed in the ABI Prism 7500 system (Applied Biosystems, Foster City, CA, USA) using the SYBR Green Supermix (Applied Biosystems). Relative gene expression was analyzed according to the formula: $2^{-\Delta\Delta CT}$.

Western blot analysis

Tissue samples and cultured cells were lysed with RIPA lysis buffer containing protease inhibitor. SDS-PAGE was used to detect the proteins of interest. Proteins were resolved on SDS gels and were electro-transferred to polyvinylidene fluoride membranes (Millipore, Boston, MA, USA). Then, the membranes were blocked with 5% non-fat milk and incubated overnight at 4°C with primary antibodies, including anti-*LC3B* (#ab48394, 1:500; Abcam), anti-p62 (#ab56416, 1:500; Abcam), anti-ATG5 (#ab108327, 1:500; Abcam), anti-BECLIN1 (#ab55877, 1:500; Abcam), and anti-β-ACTIN (#A8481, 1:4000; Sigma-Aldrich). After washing, the membranes were incubated with a secondary antibody and imaged with Odyssey SA (Gene Company Limited, Hong Kong, China).

Immunofluorescence assay

Cultured cells were grown on glass coverslips overnight to detect the LC3B puncta. Cells fixed with 4% paraformaldehyde (BOSTER) were treated with 0.2% Triton X-100 and blocked with 2% albumin from bovine serum (BSA). Then, cells were incubated with a primary anti- LC3B antibody (#ab48394, 1:200; Abcam) at 4 °C overnight. After the incubation of Alexa Fluor 568-conjugated goat anti-rabbit IgG (#ab175694, 1:1000; Abcam), cells were stained with 0.2 mg/mL DAPI and imaged with a confocal microscope (Zeiss LSM710, Carl Zeiss, Dresden, Germany). The LC3B puncta were measured using Image Pro-plus 6.0 software (Media Cybernetics). Five different pictures were measured for each group.

Statistical analysis

All data were analyzed using the SPSS software package, version 19.0 (SPSS, Chicago, IL, USA) and graphed by GraphPad Prism 5 (La Jolla, CA, USA). The results were presented as mean ± standard error based on three independent experiments. Statistical analysis was performed using correlation analysis and *t* test. P value < 0.05 was defined as statistically significant.

Results

Correlation between *LCPAT1* and *LC3B* in lung cancer

In lung cancer patients, tumor tissues showed higher *LCPAT1* and *LC3B* expression versus normal tissues at the gene expression level (**Figure 1A**). Also, IHC staining showed that the cytoplasmic*LC3B* protein was highly expressed in lung tumor tissues (**Figure 1B**). To investigate the relationship between *LCPAT1* and autophagy, we analyzed the correlation of *LCPAT1* and *LC3B* expression in tumor tissues. Our results showed that the correlation coefficient between these genes was high, $r = 0.783$ (**Figure 1C**), suggesting that *LCPAT1* may play a role in autophagy in lung cancer.

Suppression of tumor growth *in vivo* by *LCPAT1* knockdown

We evaluated the involvement of *LCPAT1* in tumor growth *in vivo*. H1975 cells transfected with scrambled shRNAs or *LCPAT1*-shRNA were injected subcutaneously into nude mice. Tumor growth was recorded and analyzed after the injection. We observed smaller tumor sizes in the right hind flanks of animals that were injected with *LCPAT1*-shRNA (**Figure 2A**). We also found that tumor growth was slower in the *LCPAT1*-shRNA tumors compared with that of the controls (**Figure 2B**). HE tissue staining revealed that tumors with *LCPAT1*-shRNA possessed less malignant morphology compared to the controls (**Figure 2C**). These findings suggest that *LCPAT1* may promote tumor growth.

Suppression of autophagy by *LCPAT1* knockdown *in vivo*

As shown in **Figure 3A** and **3B**, *LCPAT1* expression was reduced by nearly 60% in stable *LCPAT1* knockdown H1975 cells and 50% in *LCPAT1* knockdown tumors. We found that the expression of autophagy-associated genes was significantly reduced in *LCPAT1*-shRNA tumors compared to that in the controls, with an approximately 50% drop in *LC3B*, 59% drop in *ATG3*, 42% drop in *ATG5*, 54% drop in *ATG*7, 44% drop in *ATG*12, 36% drop in *ATG14*, and 59% drop in *BECLIN1* (**Figure 3C**). We further validated our findings using Western blot. We observed that *LCPAT1* knockdown significantly suppressed the expression of ATG5 (approximately 48% reduction) and LC3-II (approximately

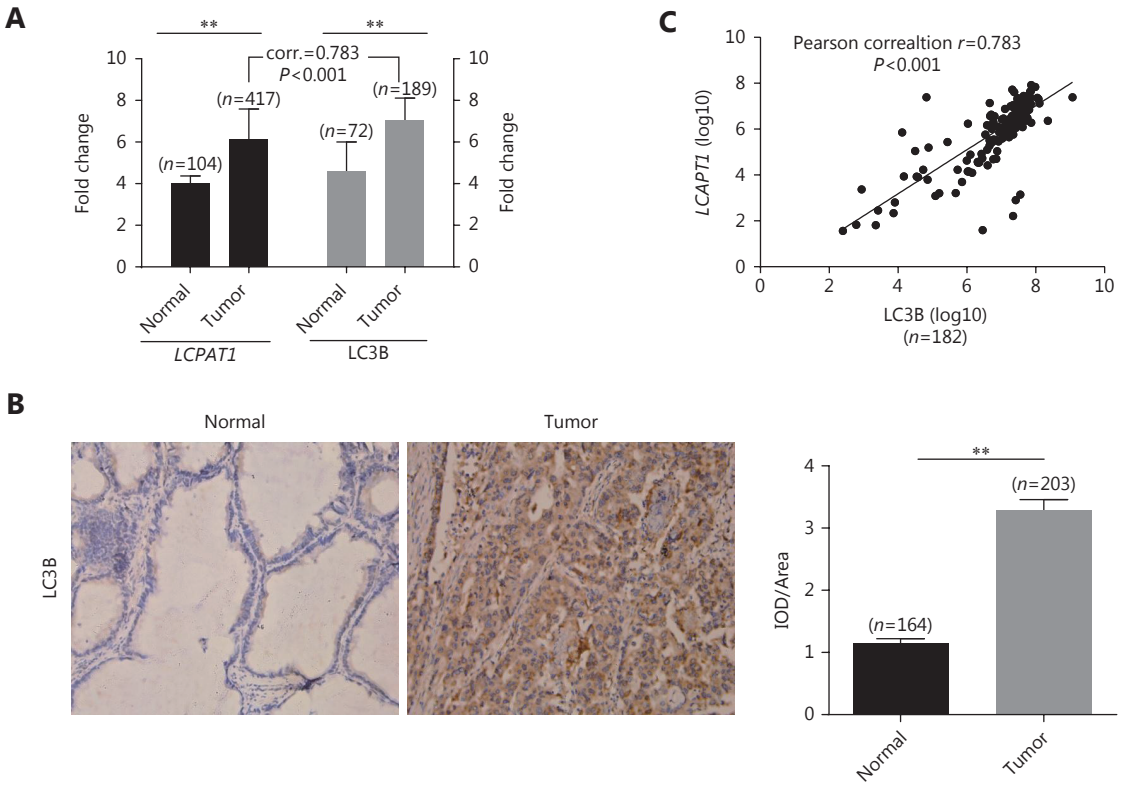


Figure 1 Correlation between *LCPAT1* and *LC3B* expression in lung cancer. (A) Gene expression of *LCPAT1* and *LC3B* in tumor tissues and matched adjacent normal tissues of lung cancer patients. Data are presented in mean ± SEM; $^{**}P < 0.01$. (B) Representative immunohistochemical staining images (400 ×) of cytoplasmic LC3B and its density (IOD/Aera) in normal and lung cancer tissues. (C) Correlation between *LCPAT1* and *LC3B* expression in tumor tissues of lung cancer patients.

57% reduction) and stimulated the expression of p62 (approximate 2.5-fold increase; **Figure 3D**). Additionally, the immunohistochemical analysis of LC3B and ATG5 showed a decreasing trend in sh*LCPAT1* xenografted tumor tissues (**Figure 3E**). Taken together, autophagy appeared to be suppressed after *LCPAT1* knockdown *in vivo*.

Suppression of autophagy by *LCPAT1* knockdown *in vitro*

Based on the above *in vivo* findings, we further investigated the role of *LCPAT1* in lung cancer cells. After detecting the expression of *LCPAT1* in the normal bronchial epithelial cell line HBE and lung cancer cell lines H520, H1299, A549, and H1975, we found that *LCPAT1* expression was higher in lung cancer cell lines than in HBE, especially in A549 and H1975 (**Figure S1**). It was observed that when *LCPAT1*-siRNA was transfected into A549 and H1975 cells (**Figure 4A** and **4B**), mRNA expression of autophagy-associated genes was markedly decreased. For example, in H1975 cell lines, *LC3B*, *ATG5*, *ATG7*, *ATG14*, and *BECLIN1* were decreased by 60%–80%, and *ATG3* and *ATG12* were reduced by 40%. In addition, upon treating cells with rapamycin, an autophagy inducer, we observed the accumulation of ATG5, BECLIN1, and LC3-II, and the degradation of p62. However, this effect was inhibited when *LCPAT1* was silenced (**Figure 4C** and **4D**). Furthermore, the assembly of rapamycin-induced *LC3B* puncta was inhibited in *LCPAT1* knockdown cells based on immunofluorescence assays (**Figure 4E** and **4F**). These experiments showed that *LCPAT1* regulated cell autophagy *in vitro*.

Discussion

Following our previous work on lncRNA *LCPAT1*, we further analyzed its relationship with autophagy in lung cancer in the current study. We found a strong positive correlation between *LCPAT1* and *LC3B*. Since high expression of *LC3B* indicates increased autophagy[17], we speculate that *LCPAT1* may promote autophagy in lung cancer. We investigated the role of *LCPAT1* in autophagy by knocking down *LCPAT1* expression in tumor cells and assessing its impact on

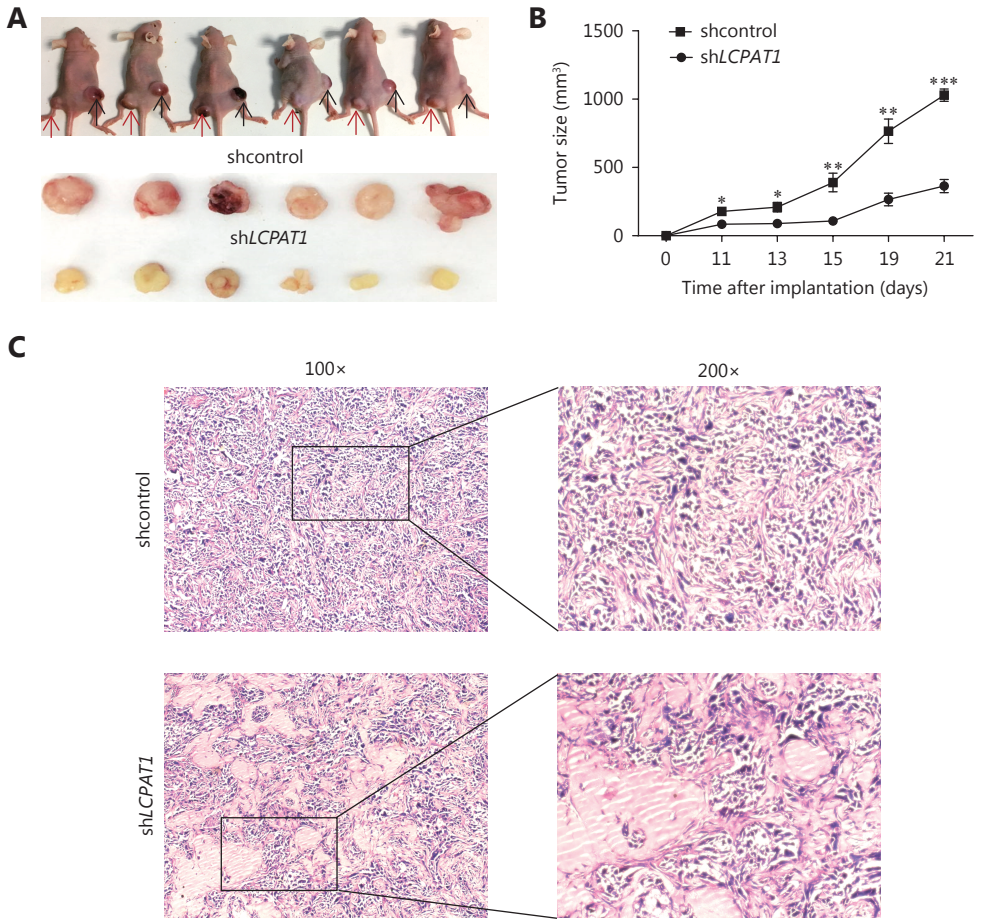


Figure 2 *LCPAT1* knockdown xenograft models. (A) Upper: *LCPAT1*-shRNA (sh *LCPAT1*)- and control shRNA (shcontrol)-transfected H1975 cells were implanted in flanks of mice (n = 6). The red arrow indicates the tumors implanted with sh*LCPAT1*-transfected H1975 cells on the left side, and the black arrow indicates the tumors implanted with shcontrol-transfected H1975 cells on the right side. Lower: tumors expressing sh*LCPAT1* or shcontrol. (B) Tumor growth curve. Data are presented as mean ± SEM; *P < 0.05, **P < 0.01, ***P < 0.001. (C) H & E staining of xenografted tumor tissues of sh*LCPAT1*- and shcontrol-transfected H1975 cells (Left: 100 ×. Right: 200 ×).

autophagy *in vitro* and *in vivo*. Our results demonstrated that *LCPAT1* could increase autophagy in lung cancer.

More and more lncRNAs are being found to be involved in tumorigenesis. In lung cancer, *HOTAIR* is up-regulated and the up-regulation promotes tumor cell invasion and metastasis[23]. In contrast to *HOTAIR*, *MEG3* is significantly down-regulated in lung cancer; increasing its expression in lung cancer cells could inhibit cell proliferation and induce apoptosis[24]. Considering that a large number of lncRNAs are known and only a few are recognized for their involvement in lung cancer, we previously performed an expression microarray analysis to identify dysregulated lncRNAs in lung cancer[5]. A methylation microarray chip was also used to determine the status of DNA methylation in the same samples. Among 8500 differentially-expressed lncRNAs, expression of *LOC146880* and *LCPAT1* was found to be elevated in tumors and the expression was negatively correlated with DNA methylation in the promoter. We further noticed that cell proliferation, invasion, and migration were inhibited when lowering the expression of *LOC146880* or *LCPAT1* in lung cancer cells[5]. In the present study, our animal experiments further confirmed that suppressing *LCPAT1* expression by shRNA could inhibit tumor growth in a xenograft model.

Autophagy plays a complicated role in tumorigenesis. Autophagy can suppress cancer initiation during the early stage of tumorigenesis. Karantza-Wadsworth et al.[25] reported that a defect in autophagy led to DNA damage, genomic instability, and further promoted the activation of proto-oncogenes and resulted in tumorigenesis. In contrast, autophagy promotes the growth of established cancer[25]. In malignant glioma, autophagy can be activated by hypoxia,

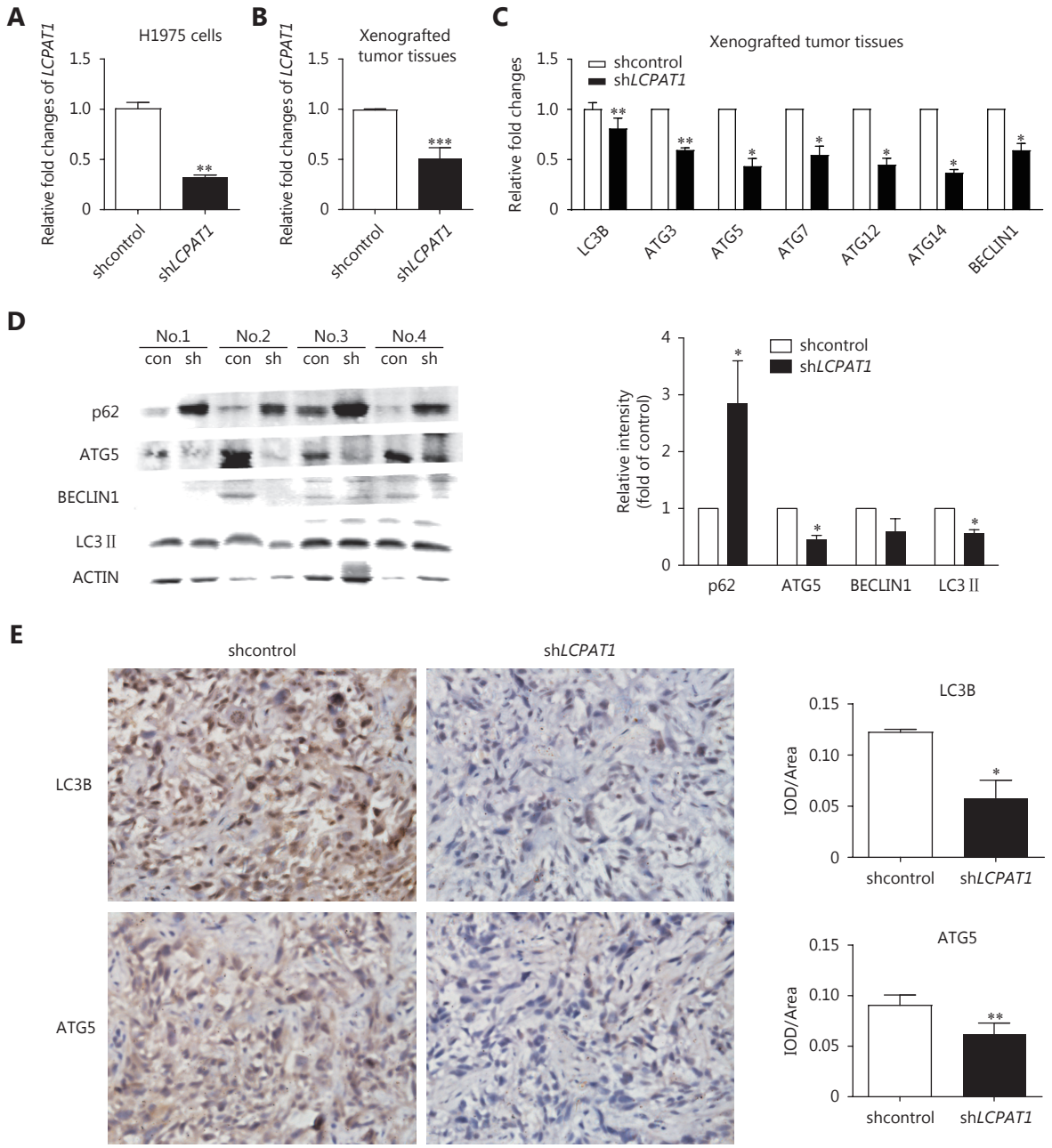
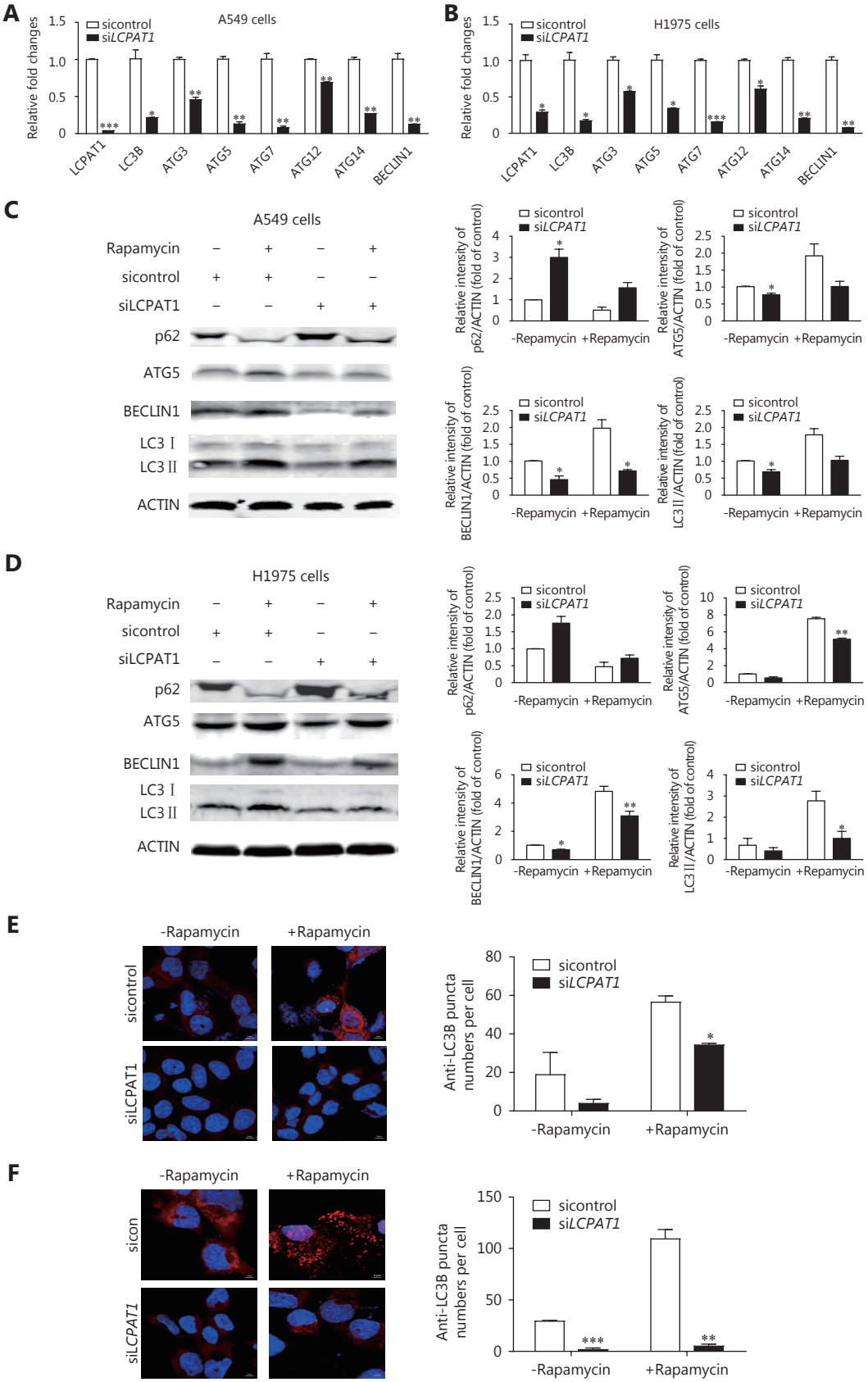


Figure 3 Autophagy regulation by *LCPAT1 in vivo*. (A) qPCR analysis of the expression of *LCPAT1* in stable-transfected H1975 cell line. Data are presented as mean ± SEM; $^{**}P < 0.01$. (B) qPCR analysis of the expression of *LCPAT1* in xenografted tumor tissues. Data are presented in mean ± SEM; $^{***}P < 0.001$ ($n = 6$). (C) qPCR analysis of the expression of *LC3B*, *ATG3*, *ATG5*, *ATG7*, *ATG12*, *ATG14*, and *BECLIN1* in sh*LCPAT1* and shcontrol xenografted tumor tissues ($n = 6$). Data are presented in mean ± SEM; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. (D) Representative image and quantification of Western blot assays for p62, ATG5, BECLIN1, and LC3B expression in sh*LCPAT1* and shcontrol xenografted tumor tissues ($n = 4$). Nos.1-4 represent the number of mice. Data are presented in mean ± SEM; $^{*}P < 0.05$. (E) Representative image (400 ×) and quantification of immunohistochemical assays for LC3B and ATG5 expression in sh*LCPAT1* and shcontrol xenografted tumor tissues using Image Pro-plus 6.0 software ($n = 4$). Data are presented in mean ± SEM; $^{*}P < 0.05$, $^{**}P < 0.01$.

thereby promoting tumor cell growth through the HIF-1α/AMPK signaling pathway[26]. Our finding of high expression of *LC3B* in lung cancer supports the notion that autophagy promotes cancer progression. We also found that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A549 cells
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B
H1975 cells
C
A549 cells
Rapamycin
sicontrol
siLCPAT1
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ATG5
BECLIN1
LC3 I
LC3 II
ACTIN
Relative intensity of p62/ACTIN (fold of control)
Relative intensity of ATG5/ACTIN (fold of control)
Relative intensity of BECLIN1/ACTIN (fold of control)
Relative intensity of LC3 II/ACTIN (fold of control)
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-Rapamycin
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Anti-LC3B puncta numbers per cell
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Figure 4 Autophagy regulation by *LCPAT1 in vitro*. (A, B) mRNA expression of autophagy-related genes in A549 cells (A) and H1975 cells (B) transfected with *LCPAT1* siRNA (si*LCPAT1*) and scrambled siRNA (sicontrol). Data are presented as mean ± SEM; **P* < 0.05, ***P* < 0.01, ****P* < 0.001. (C, D) Representative images and quantification of Western blot assays for p62, ATG5, BECLIN1, and LC3B expression in si*LCPAT1*- and sicontrol-transfected A549 cells (C) and H1975 cells (D), after exposure to 100 μM rapamycin for 48 h. Data are presented in mean ± SEM; **P* < 0.05, ***P* < 0.01. (E, F) Representative images and quantification of LC3 dots in si*LCPAT1*- and sicontrol-transfected A549 cells (E) and H1975 cells (F) after treatment with 100 μM rapamycin for 48 h. The LC3B puncta were quantified by Image Pro-plus 6.0 software. Data are presented in mean ± SEM; **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

LCPAT1 could increase autophagy in lung cancer, suggesting that *LCPAT1* may act as an oncogene by promoting autophagy in lung cancer.

The molecular mechanisms underlying how *LCPAT1* regulates autophagy are unclear. LncRNAs regulate the expression of genes through various mechanisms, including interacting with DNA, mRNAs, microRNAs, or proteins. In breast cancer, *linc-ROR* has been shown to suppress gemcitabine-induced autophagy by silencing miR-34a[27]. Through binding to EZH2, lncRNA *H19* down-regulates DIRAS3 expression, consequently inhibiting autophagy in cardiomyocytes[28]. Wu et al.[29] has reported that the lncRNA *HNF1A-AS1* promotes autophagy in hepatocellular carcinoma by composing a competitive endogenous RNAs system with miR-30b, which targeted *ATG5*[29]. We used starBase (http://starbase.sysu.edu.cn/)[30] and lncRNASNP (http://bioinfo.life.hust.edu.cn/lncRNASNP/)[31] databases to predict whether there were miRNA binding sites for *LCPAT1* and autophagy-related genes. The results showed that mir-186-5p binds to *LCPAT1*, *ATG7*, and *ATG12*. In addition, mir-206 can be combined with *LCPAT1* and *ATG14* at the same time, and mir-218-5p can be combined with *LCPAT1*, *ATG12*, and *ATG14* at the same time. Studies have shown that mir-186-5p, mir-206, and mir-218-5p are all tumor suppressors [32-34]. We suspect that *LCPAT1* can compete with autophagy-related genes for specific anti-cancer miRNAs, thereby elevating autophagy gene levels and exerting tumorigenic effects. The current study was an initial investigation into the role played by *LCPAT1* in lung cancer. Further studies are warranted to explore the mechanisms of *LCPAT1* in regulating autophagy in lung cancer.

In conclusion, we confirm that *LCPAT1* is a tumor-promoting lncRNA in lung cancer. We also found that *LCPAT1* could increase autophagy both *in vitro* and *in vivo* in lung cancer.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

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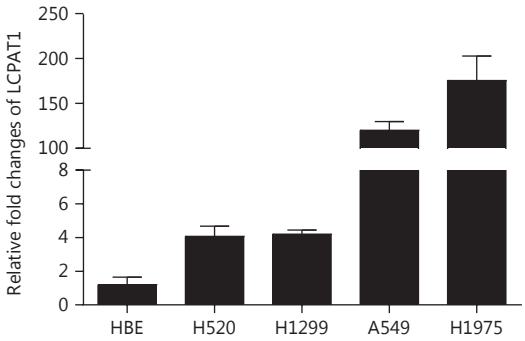

Figure S1 Expression of *LCPAT1* in normal bronchial epithelial cell line and lung cancer cell lines. The expression of *LCPAT1* in the normal bronchial epithelial cell line HBE and lung cancer cell lines H520, H1299, A549, and H1975 was detected by qPCR.