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LASS2 overexpression enhances early apoptosis of lung cancer cells through the caspase-dependent pathway

YAMEI WANG[1,2*], SHIRONG LI[1*], LIXIN WENG[1], HUA DU[1], JINGYUAN WANG[1] and XIAOYAN XU[1,3]

[1]Department of Pathology, College of Basic Medical Sciences, Inner Mongolia Medical University, Hohhot, Inner Mongolia Autonomous Region 010059; [2]Department of Pathology, China Northeast International Hospital, Shenyang, Liaoning 110000; [3]Department of Pathology, The Affiliated Hospital of Inner Mongolia Medical University, Hohhot, Inner Mongolia Autonomous Region 010059, P.R. China

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Abstract. In a previous study by the authors, the longevity assurance homolog 2 (*LASS2)* gene was determined to inhibit activity of vacuolar H^+-ATPase (V-ATPase) by combining with the C subunit (ATP6L) of V-ATPase. However, the influence of *LASS2* overexpression and silencing on apoptosis of human lung cancer cells 95D or 95C remains unclear. Thus, the effect of *LASS2* on apoptosis and its potential mechanisms were investigated in 95D and 95C cells. Using the lentiviral transfection method, lentiviral vectors of *LASS2* overexpression and silencing were transfected into 95D and 95C cells, respectively. The apoptotic ability of tumor cells was observed by flow cytometry. The expression levels of *LASS2*, Bcl-2, Bax, cytochrome *c*, caspase-9, and caspase-3 were detected by western blotting. CCK-8 assay was used to detect the growth ability of tumor cells *in vitro*. Flow cytometric analysis revealed that *LASS2* overexpression could promote the early apoptosis of lung cancer cells 95D. CCK-8 assay demonstrated that *LASS2* overexpression inhibited the proliferation of 95D cells. Additionally, *LASS2* overexpression decreased the expression of Bcl-2, induced the release of cytochrome *c* from mitochondria, and promoted the activation of caspase-9 and caspase-3. There was a significant difference in the expression of Bcl-2, cytochrome *c*, caspase-9 and caspase-3 in the *LASS2*-overexpression group compared with the normal and negative control groups. Alternatively, the aforementioned experiments in lung cancer cells 95C following *LASS2* silencing produced the opposite effects. *LASS2* may induce early apoptosis of lung cancer cells by influencing the caspase-dependent mitochondrial pathway.

Correspondence to: Dr Xiaoyan Xu, Department of Pathology, College of Basic Medical Sciences, Inner Mongolia Medical University, 1 Tongdaobei Street, Huimin, Hohhot, Inner Mongolia Autonomous Region 010059, P.R. China
E-mail: xxyfpc@126.com

[*]Contributed equally

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Introduction

Apoptosis, a basic process of programmed cell death, is necessary for homeostasis of all organisms. The occurrence and development of cancer is not only associated with cell proliferation, but also caused by dysregulated cell apoptosis (1). To a certain extent, most cancer treatment is based on a process to accelerate tumor cell apoptosis. A thorough understanding of the apoptotic mechanisms is critical for the development of potential therapeutic targets using gene therapy techniques (2). According to global cancer statistics of 2018, lung cancer was the most common and leading cause of cancer in males (3). Therefore, the research of apoptotic mechanisms has become a popular and urgent topic in lung cancer research.

Tumor metastasis suppressor gene 1 (*TMSG-1*) was discovered by Ma *et al* at Peking University in 1999 (4). Subsequently, Pan *et al* cloned a new gene highly homologous to the longevity support gene of yeast [longevity assurance homolog 1 (*LAG1*)] in the human liver cDNA library (5). This gene, termed *LASS2*, is highly homologous with *TMSG-1* (5). The *LASS2*-encoded protein contains two structural domains: Homeodomain structural domain, and TRAM, LAG1 and CLN8 (TLC) structural domain (6). Reportedly, homeodomains play a vital role in the regulation of the cell cycle and may inhibit tumor invasion, metastasis, growth, and apoptosis by combining with C subunit (ATP6L) of vacuolar H^+-ATPase (V-ATPase) (7). The TLC structural domain is predominantly involved in lipid metabolism and synthesis of ceramide, which can induce apoptosis in tumor cells, thereby inhibiting tumor growth (8).

Previous studies have shown that the *LASS2* gene is negatively correlated with the metastasis capacity of prostate, breast, liver, and gastrointestinal cancers (9-12). Recently, it was reported that *LASS2* suppressed proliferation and promoted apoptosis of HepG2 cells by regulating the NF-κB signaling pathway (13). However, the ability of *LASS2* to induce apoptosis of lung cancer cells, and the mechanism thereof remain unclear.

The typical mechanism of apoptosis is the caspase activation pathway. The precise role that *LASS2*, mediated by the caspase pathway, plays in the apoptosis of lung cancer cells is unclear. In a previous study by the authors, it was determined that the expression and gene positive rate of *LASS2* in non-small cell lung cancer tissues were significantly reduced compared with those in para-carcinoma tissues (14). Additionally, it was revealed that the mRNA and protein expression levels of LASS2 in lung cancer 95C cell lines with low metastatic potential were significantly higher than that in 95D cells with high metastatic potential (15). Therefore, the aim of the present study was to determine the influence of LASS2 on apoptosis and proliferation of 95D cells transfected with the *LASS2* overexpression vector, and 95C cells transfected with *LASS2*-RNAi to elucidate the underlying mechanism. *LASS2* was silenced and the protein expression levels of cytochrome *c*, Bax, Bcl-2, caspase-3, and caspase-9 were detected in 95D and 95C cells. The present study may reveal certain theoretical mechanisms for lung cancer apoptosis, and provide some potential targets for guiding lung cancer treatment.

Materials and methods

Cell lines and cell culture. The human lung cancer cell lines, 95D (cat. no. XF0039; low expression of *LASS2* and high metastatic potential), and 95C (cat. no. XF0041; high expression of *LASS2* and low metastatic potential) were purchased from Shanghai Fuxiang Biotechnology Co., Ltd (http://www.xiangbio.com/). Cells were cultured in RPMI-1640 medium with 10% fresh fetal bovine serum (both Gibco; Thermo Fisher Scientific, Inc.), and 1% penicillin and streptomycin. Cells were maintained at 37˚C with 5% CO_2. For the selection of the cell lines, the expression levels of LASS2/TMSG1 mRNA and protein in 95C cells were significantly higher than that of 95D cells ($P<0.01$). In addition, wound healing assay also confirmed that the migration ability of 95D cells was higher than that of 95C cells ($t=23.56$; $P<0.05$) (16).

Lentivirus transfection experiment. The 2nd generation system was used in the lentivirus transfection experiment. The interim cell line used in this study was 293T cells (cat. no. SCSP-502; Cell Bank of Chinese Academy of Sciences, Shanghai, China). The cells ($1x10^5$) were seeded on a 6-well plate wherein 95D and 95C cells were transfected with *LASS2* overexpression lentiviral vectors (LV-LASS2 8649-2) and *LASS2*-RNAi (LV-LASS2-RNAi 30011-1; both from Shanghai Genechem Co., Ltd., China), respectively, according to the transfection instructions (Polybrene and Enhanced Infection Solution; Shanghai Jikai Gene Medical Technology Co., Ltd). The quantity of plasmids, Helper 1.0 (packaging) and Helper 2.0 (envelope) were 20, 15, and 10 μg, respectively. Transfection was performed at room temperature for 12 h. Subsequently, the transfection solution was replaced with complete medium, and the stable strain selection experiment was carried out after culturing for 48-72 h. The stable transfectants with *LASS2* overexpression plasmid in 95D cells and *LASS2* shRNA in 95C cells were designated as *LASS2* overexpression, and silencing, respectively. The sequence of LASS2-RNAi (*LASS2* shRNA) was 5'-AGTATTGGTACTACATGAT-3', and the sequence of the negative control (NC) was 5'-TTCTCCGAACGTGTCACGT-3'. The stable transfectants with scrambled plasmid in 95D and 95C cells (LV-CON054; Shanghai Genechem Co., Ltd., China) were used as negative control, while the untransfected cells were designated as normal controls. Preheated complete medium (500 μl) was added to preheated transfection cells, followed by lentivirus (virus titer was $1x10^8$ TU/ml and multiplicity of infection was two times), and polybrene infection solution (with concentration of 5-10 μg/ml), and enhanced infection solution were added to make the final volume up to 1 ml. Fresh medium was then added after 8-12 h, the culture medium was replaced by the complete medium with puromycin after 48 h. The puromycin concentration for selection was 2 μg/ml, while for maintenance it was 0.5 μg/ml.

Flow cytometric analysis. Cells ($1x10^5$) were seeded in a 25 cm^2 ventilated culture bottle and cultured to 70% cell confluency. Following centrifugation at 187 x g at 4˚C for 10 min, the cell precipitation was washed with 1 ml precooled Dulbecco's phosphate-buffered saline (DPBS; Biosharp Life Sciences). The cell precipitation was suspended in 200-μl buffer solution with 10 μl Annexin V-FITC and incubated at 4˚C for 30 min in the dark. Then, 300-μl buffer solution, and 5 μl prodium iodide (PI) were added in the mixed solution. by adding. The cells were then immediately detected by BD FACSCalibur flow cytometer (Becton, Dickinson and Company). NovoExpress software version 1.3.0 (Agilent Technologies, Inc.) was used to analyze samples. The flow cytometric assay was performed using three biological replicates with three technical repetitions each.

Cell Counting Kit-8 (CCK-8) assay. The proliferation activity of cells in different treatment groups was measured by CCK-8 assay (cat. no. C0038; Beyotime Institute of Biotechnology). Briefly, the stable transfected cells in logarithmic phase were digested with 1 ml 0.25% trypsin. Once the cells became round, the digestion process was terminated using 1 ml complete medium. The cells were then centrifuged at 187 g for 5 min, and resuspended by adding 1 ml complete medium. Following dilution of the suspension to 500 cells per 100 μl, 100 μl cell suspension was added into the 96-well plate. After 48 h of preincubation, 10 μl CCK-8 solution was added into each well. Finally, the cells were incubated for 4 h, and the absorbance was measured at 450 nm using a microplate reader (ELX800TM; Bio-Tek Instruments, Inc.). This experiment was repeated independently three times.

Western blotting. The total protein of the cell was extracted using RIPA Lysate and PMSF (RIPA: PMSF, 100:1; Beyotime Institute of Biotechnology), and the protein concentration was determined using the BCA method (Pierce; Thermo Fisher Scientific, Inc.). Cells were washed twice with pre-chilled phosphate-buffered saline (PBS) and lysed in lysis buffer. After being incubated on ice for 30 min, the cells were centrifuged at 10,000 x g for 10 min at 4˚C. The protein concentration was measured using a BCA protein assay kit according to the manufacturer's instructions. The sample was then mixed with 5X SDS sample buffer solution in equal volumes, and placed in a boiling water bath for 5 min. Thereafter, 10 μg of protein from each sample was separated in 10% SDS-PAGE, and transferred to nitrocellulose

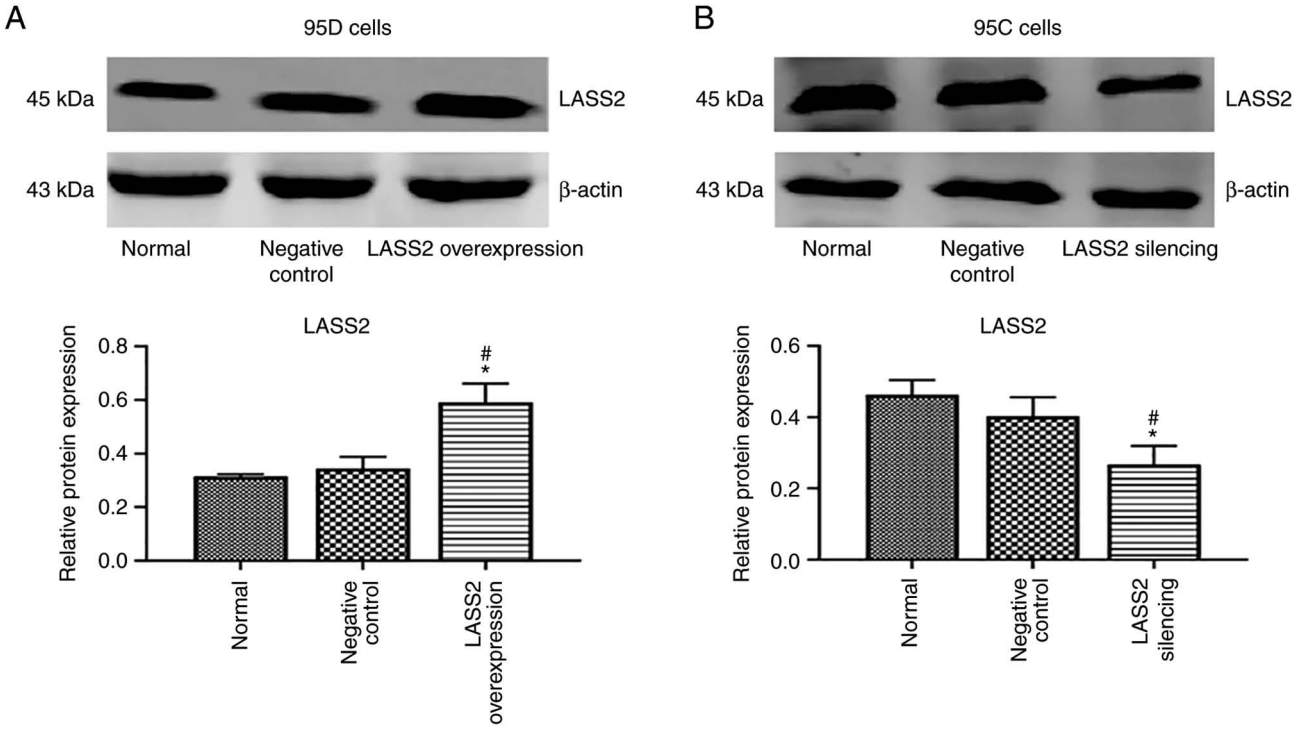

Figure 1. Expression of *LASS2* in human lung cancer cells. The expression of *LASS2* in human lung cancer (A) 95D cells and (B) 95C cells in three groups was detected using western blotting. *P<0.05 vs. the control; and #P<0.05 vs. the negative control. *LASS2*, longevity assurance homolog 2.

membranes for sealing. The nitrocellulose membranes were then blocked with 5% skim milk which included 2.5 g skim milk and 50 ml TBST (BioFroxx; neoFroxx GmbH) at room temperature for 1 h. The primary antibody was then added, in which the working concentration was 1:1,000 for β-actin (cat. no. AA128-1; Beyotime Institute of Biotechnology), 1:300 for LASS/TMSG-1 (cat. no. bs-5077R), 1:200 for Bcl-2 (cat. no. bs-4563R), 1:200 for Bax (cat. no. bs-0127R), 1:200 for cytochrome *c* (cat. no. bs-0013R), 1:200 for caspase-9 (cat. no. bs-0050R), and 1:200 for caspase-3 (cat. no. bs-0081R). The antibodies of LASS, Bcl-2, Bax, cytochrome *c*, caspase-9 and caspase-3 were purchased from Beijing Biosynthesis Biotechnology Co., Ltd.; BIOSS). The membranes were incubated for 12 h at 4˚C, and then they underwent PBST membrane cleaning. The membranes were then incubated for 1 h at room temperature with a secondary antibody (working concentration was 1:15,000), and another PBST membrane cleaning was performed. The secondary antibody used was Dylight 800 AffiniPure goat anti-rabbit IgG (cat. no. A23920; Abbkine Scientific Co., Ltd.). Finally, the NC membranes were scanned on the Odyssey CLX infrared laser imaging system (Image Studio 3.1; LI-COR Biosciences) in the dark. Results were observed, and the gray value was recorded. Three biological replicates were prepared for each experiment.

Statistical analysis. SPSS 21.0 (IBM Corporation) was used for statistical analyses and the experimental results were expressed as the mean ± standard deviation (M ± SD). One-way ANOVA followed by Tukey's post hoc test was used. P<0.05 was considered to indicate a statistically significant difference.

Results

LASS2 overexpression and silencing confirmation. The highly metastatic 95D cells were transfected with *LASS2* gene overexpression vector, and 95C cells with low metastaticity were transfected with *LASS2* gene silencing vector. The results revealed that the level of LASS2 protein in the *LASS2*-overexpressed group was significantly increased compared with the normal and negative control groups (P<0.05; Fig. 1A). Additionally, the level of *LASS2* protein in the *LASS2*-silenced group was significantly reduced compared with the normal and negative control groups (P<0.05; Fig. 1B).

Effects of LASS2 overexpression on apoptosis of 95D cells and silencing on apoptosis of 95C cells. As revealed in Fig. 2A, the percentage of early apoptotic cells in the *LASS2*-overexpressed group was significantly increased compared with the normal and negative control groups (P<0.01), while there was no significant difference in late apoptosis among the three groups. Conversely, compared with the normal and negative control groups, the percentage of early apoptotic cells in the *LASS2*-silenced group was significantly reduced (P<0.01). Similarly, no significant difference was detected in late apoptosis among these groups (Fig. 2B).

Effects of LASS2 overexpression and silencing on proliferation of 95D and 95C cells. After inoculation from day 1 to 5, the proliferation ability of 95D cells in the *LASS2*-overexpressed group was significantly decreased after day 3, compared with the normal and negative control groups (P<0.05; Fig. 3A). Additionally, the proliferation ability of 95C cells in *LASS2*-silenced group was significantly increased after

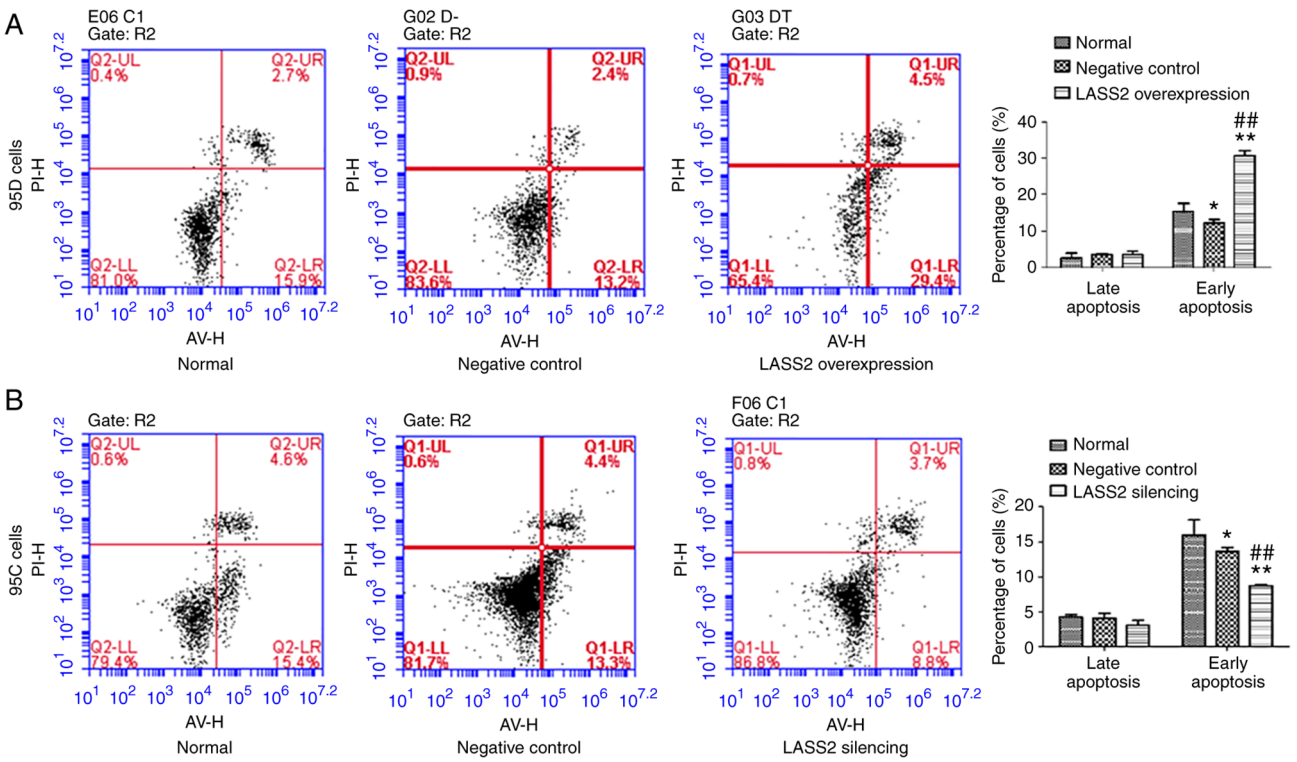

Figure 2. Effect of *LASS2* overexpression and silencing on the apoptosis of human lung cancer cells. (A) The effect of *LASS2* overexpression on the apoptosis of 95D cells. (B) The effect of silencing of *LASS2* on the apoptosis of 95C cells. In the UL quadrant, cellular debris is identified; late apoptotic cells are presented in the UR quadrant, while the normal cells and early apoptotic cells are presented in the LL and LR quadrants, respectively. *P<0.05 and **P<0.01 vs. the control; and $^{\#\#}$P<0.01 vs. the negative control. *LASS2*, longevity assurance homolog 2; UL, upper left; LL, lower left; UR, upper right; LR, lower right.

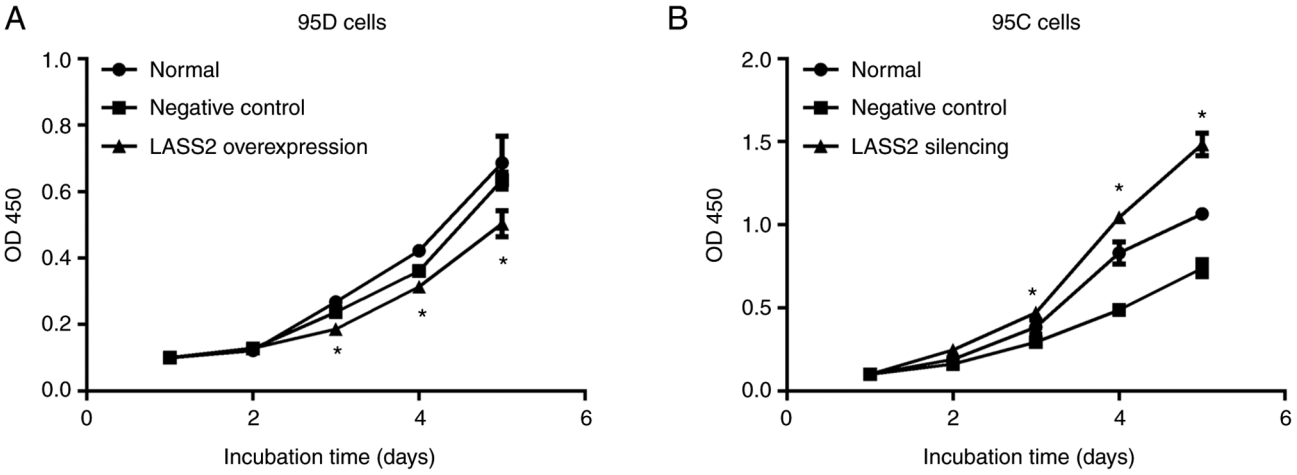

Figure 3. Effect of *LASS2* overexpression and silencing on the proliferation of human lung cancer cells. Changes in the proliferation abilities of (A) 95D cells following *LASS2* overexpression and (B) 95C cells following *LASS2* silencing from day 1 until day 5 as detected by CCK-8 assay. *P<0.05 vs. the control. *LASS2*, longevity assurance homolog 2; CCK-8, Cell Counting Kit-8.

day 3, compared with the control and negative control groups (P<0.05; Fig. 3B).

Protein expression of Bcl-2 and Bax following LASS2 overexpression in 95D cells and silencing in 95C cells. In comparison with the normal and negative control groups, the expression level of Bcl-2 in 95D cells was significantly decreased in the *LASS2*-overexpressed group (P<0.01), while that of Bax underwent no change (Fig. 4A). In addition, the ratio of Bax/Bcl-2 was increased. Conversely, the expression level of Bcl-2 in the *LASS2* gene-silenced group was significantly increased (P<0.05; Fig. 4B), and no significant change in the level of Bax was detected. The ratio of Bax/Bcl-2 was decreased in 95C cells following silencing of *LASS2*.

Protein expression of cytochrome c after LASS2 overexpression in 95D cells and silencing in 95C cells. As revealed in Fig. 5A, following *LASS2* overexpression, the protein level of cytochrome *c* in 95D cells was significantly increased as compared with the control and negative control groups

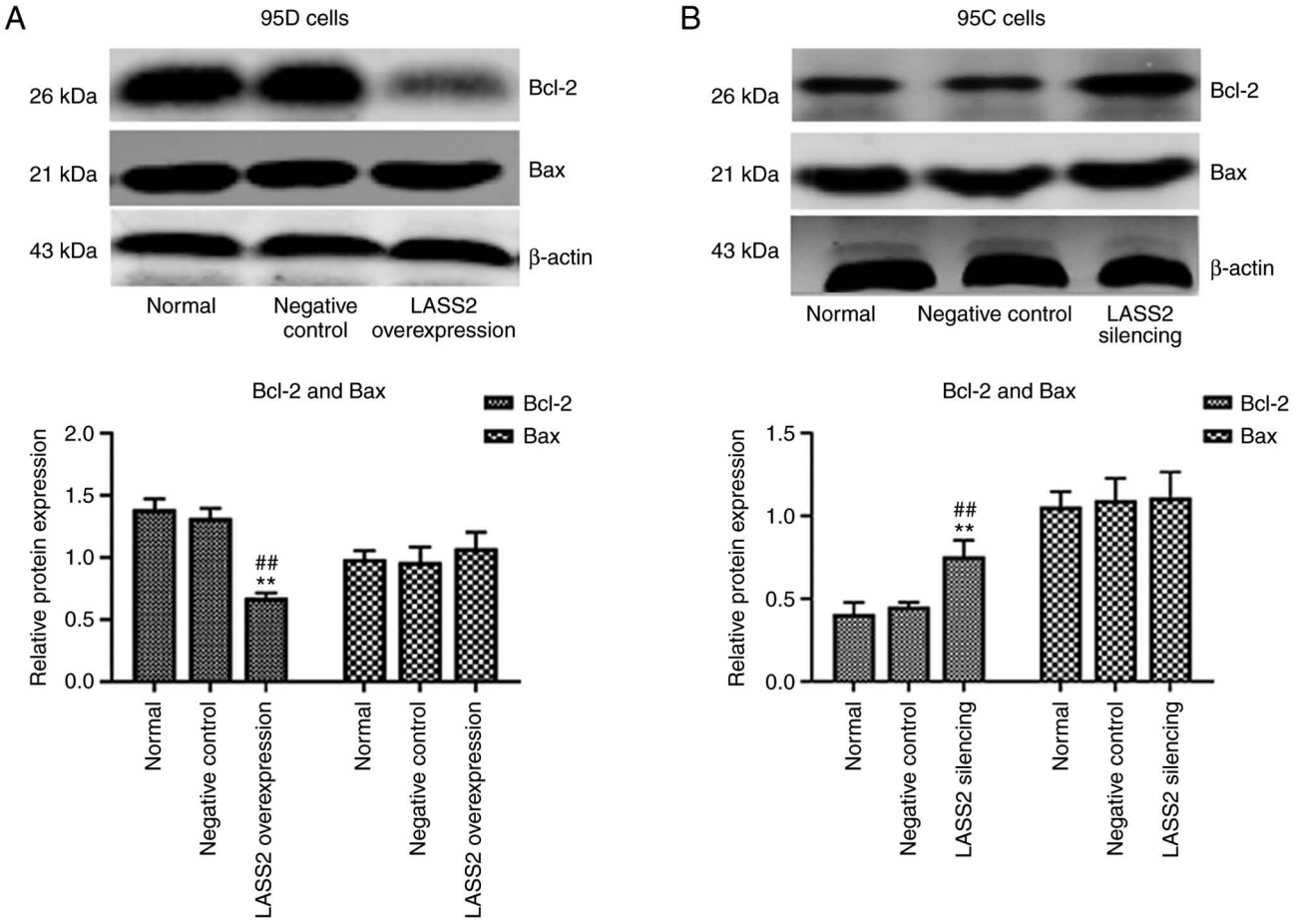

Figure 4. Effects of *LASS2* overexpression and silencing on the protein levels of Bcl-2 and Bax. (A) The effects of *LASS2* overexpression on the protein levels of Bcl-2 and Bax in 95D cells. (B) The effects of *LASS2* silencing on the protein levels of Bcl-2 and Bax in 95C cells. **P<0.01 vs. the control; and $^{\#\#}$P<0.01 vs. the negative control. *LASS2*, longevity assurance homolog 2.

(P<0.05). Additionally, the protein level of cytochrome *c* in 95C cells with silenced *LASS2* was significantly reduced than the control and negative controls (P<0.05; Fig. 5B).

Expression of procaspase-9 and activated caspase-9 after LASS2 overexpression and silencing. The effects of *LASS2* overexpression and silencing on procaspase-9 and activated caspase-9 expression levels in 95D and 95C cells were detected, respectively. The results revealed that the level of procaspase-9 protein in the *LASS2* overexpression group was significantly lower than that in the normal and negative control groups (P<0.01), while the activated caspase-9 expression in 95D cells was significantly increased (P<0.05; Fig. 6A). As revealed in Fig. 6B, the expression of procaspase-9 in 95C cells with silenced *LASS2* was significantly enhanced (P<0.05), while the activated caspase-9 was significantly reduced compared with the normal and negative control groups (P<0.05).

Expression of procaspase-3 and activated caspase-3 after LASS2 overexpression and silencing. The effects of *LASS2* overexpression and silencing on procaspase-3 and activated caspase-3 expression levels in 95D or 95C cells were detected, respectively. As revealed in Fig. 7A, the procaspase-3 protein level in 95D cells with *LASS2* overexpression was significantly decreased compared with the normal (P<0.05) and negative control groups (P<0.05). Furthermore, activated caspase-3 protein was significantly increased (P<0.01), which indicated that the number of active fragments converted from the procaspase-3 protein increased to participate in the process of tumor cell apoptosis. Notably, the expression levels of procaspase-3 and activated caspase-3 in 95C cells with silenced *LASS2* gene were the opposite of those observed in the 95D cells (Fig. 7B).

Discussion

In the present study, the effect of *LASS2* overexpression and silencing on proliferation and apoptosis of human lung cancer 95D and 95C cells were investigated. The changes of apoptotic-related molecular markers (cytochrome *c*, Bax, Bcl-2, caspase-3 and caspase-9) were detected using western blotting. It was confirmed that the overexpression of *LASS2* in 95D cells promoted the early apoptosis and suppressed the proliferation of tumor cells. The overexpression of *LASS2* could decrease the expression level of Bcl-2 protein, increase that of Bax/Bcl-2, promote the release of cytochrome *c*, and activate downstream caspase-9 and caspase-3. Following *LASS2* silencing in 95C cells, the opposite effects were observed.

LASS2 gene is a new tumor suppressor gene, which plays an important role in inhibiting tumor metastasis. A previous study reported that transfection of LASS2 using

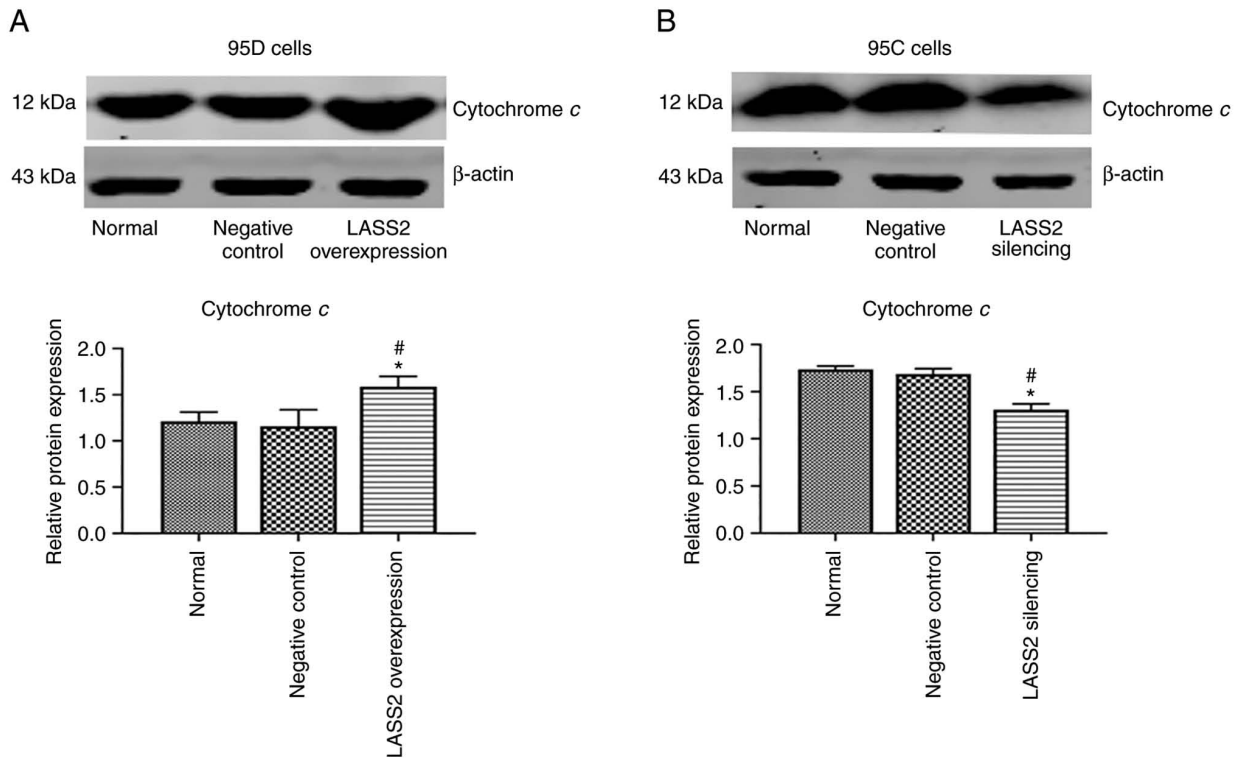

Figure 5. Effects of *LASS2* overexpression and silencing on the protein levels of cytochrome *c*. (A) The effects of *LASS2* overexpression on the protein levels of cytochrome *c* in 95D cells. (B) The effects of *LASS2* silencing on the protein levels of cytochrome *c* in 95C cells. *P<0.05 vs. the control; and $^{\#}$P<0.05 vs. the negative control. *LASS2*, longevity assurance homolog 2.

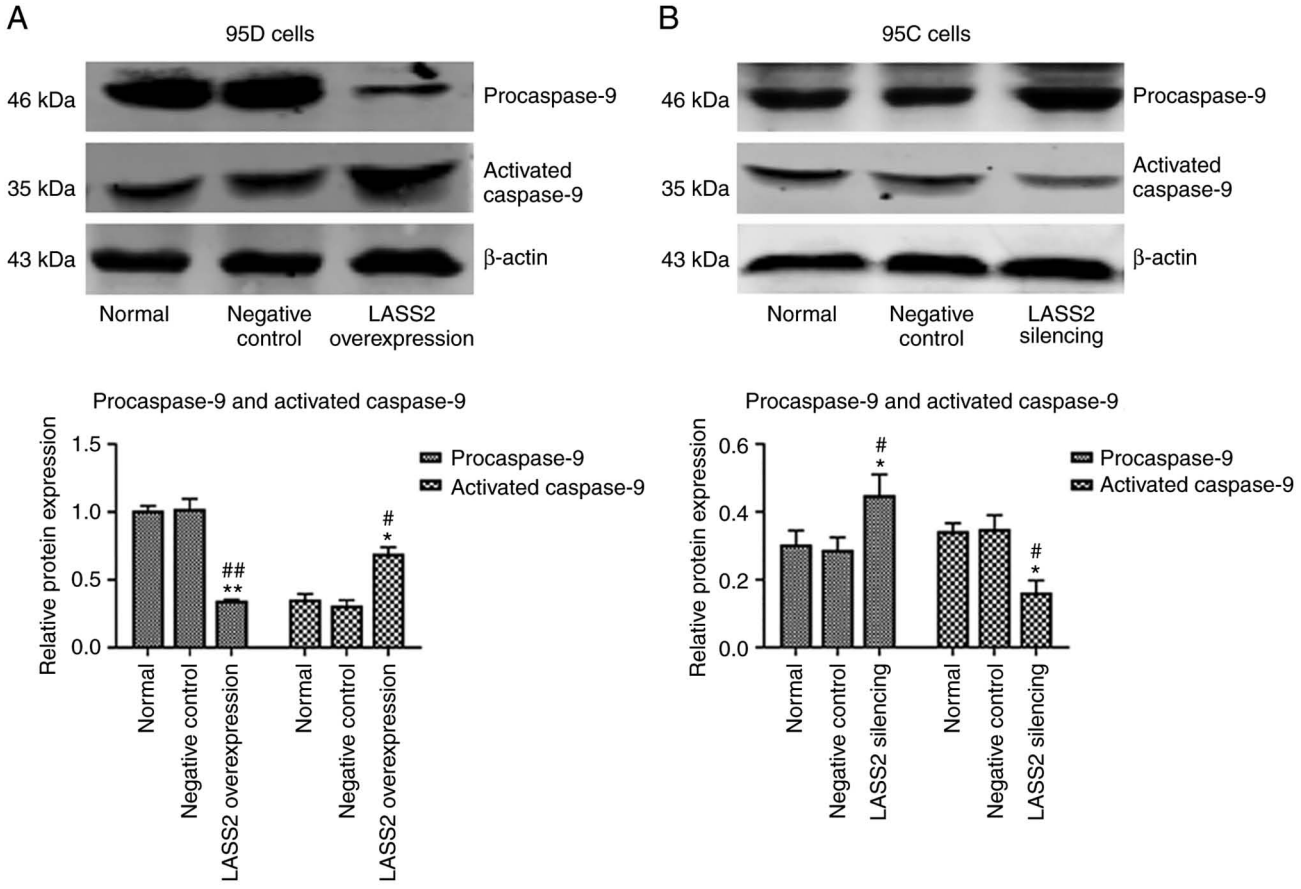

Figure 6. Effects of *LASS2* overexpression and silencing on the protein levels of procaspase-9 and activated caspase-9. (A) The effects of *LASS2* overexpression on the protein levels of procaspase-9 and activated caspase-9 in 95D cells. (B) The effects of *LASS2* silencing on the protein levels of procaspase-9 and activated caspase-9 in 95C cells. *P<0.05 and **P<0.01 vs. the control; and $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. the negative control. *LASS2*, longevity assurance homolog 2.

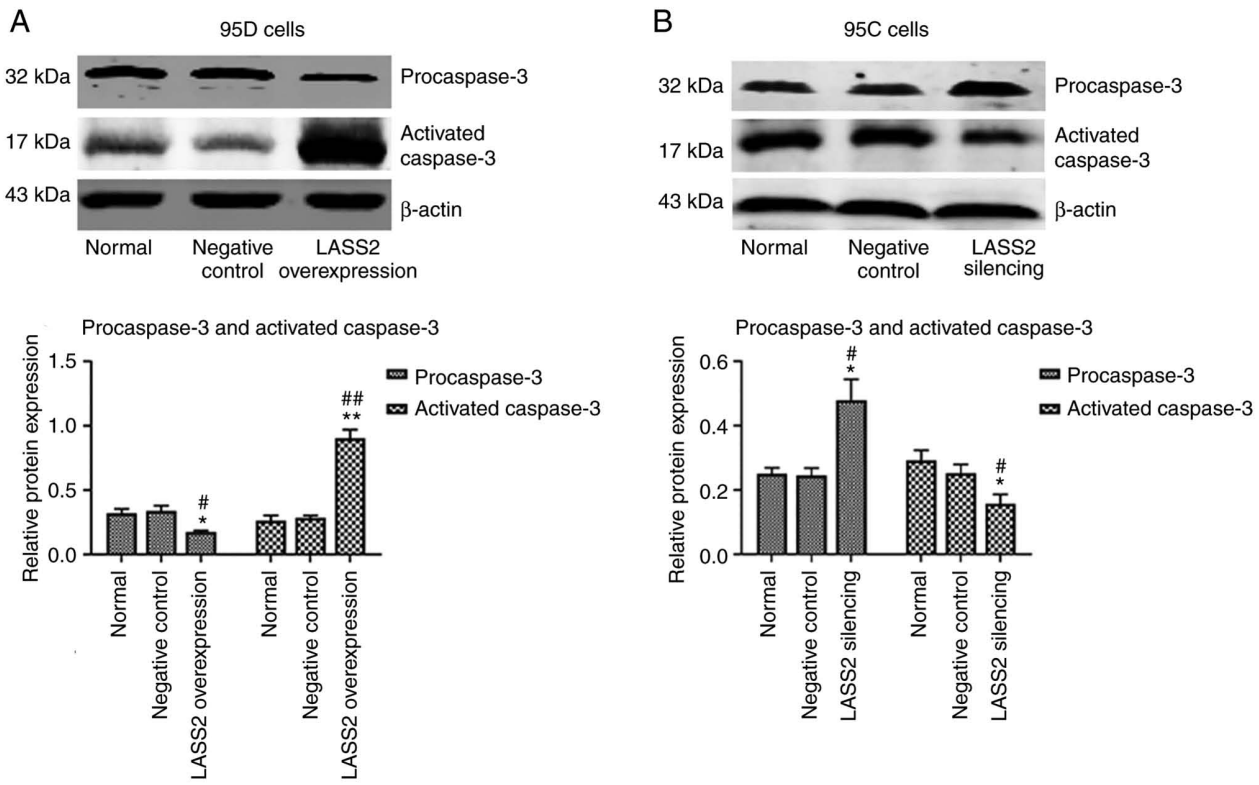

Figure 7. Effects of *LASS2* overexpression and silencing on the protein levels of procaspase-3 and activated caspase-3. (A) The effects of *LASS2* overexpression on the protein levels of procaspase-3 and activated caspase-3 in 95D cells. (B) The effects of *LASS2* silencing on the protein levels of procaspase-3 and activated caspase-3 in 95C cells. *P<0.05 and **P<0.01 vs. the control; and $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. the negative control. *LASS2*, longevity assurance homolog 2.

Lipofectamine inhibited the invasion and metastasis of the highly metastatic liver cancer cell line, HCCLM3 (17). Overexpression of *LASS2* was also determined to inhibit the proliferation, growth, and invasion of the highly metastatic prostate cell line, PE-3ME8 (18). In another previous study on liver cancer, downregulating the expression of *LASS2* in liver cancer cells, MHCC97-L, increased the extracellular hydrogen concentration, demonstrating that inhibiting the binding of LASS2 to V-ATPase promotes the hydrogen transport of the transmembrane proton pump and increases tumor cell invasion (19). It has been previously shown that apoptosis plays an crucial role in the regulation of metastasis (20). A previous *in vivo* study revealed that an increase in the level of apoptosis reduced the occurrence of metastasis in breast carcinoma cell lines (21). Tumor cells undergo a variety of apoptotic processes during metastasis, and their corresponding metastases decrease (22). Therefore, alteration of induced apoptotic genes can inhibit the metastatic ability and efficiency of tumor cells (23).

Recently, studies have focused on *LASS2* inhibition of growth, and metastasis through inhibition of V-ATPase activity in various types of cancer such as prostate (24), and bladder cancer (25). Similarly, previous studies by the authors revealed that the *LASS2* gene inhibited V-ATPase activity by binding the C subunit, ATP6L of V-ATPase. This resulted in an increase of intracellular H^+ concentration, and a decrease of extracellular H^+ concentration, thereby reducing the activity of extracellular matrix metalloproteinase which affects the invasion and migration of lung cancer cells (26,27). It has been reported that *LASS2* overexpression significantly inhibits proliferation, and promotes apoptosis of papillary thyroid cancer (28). Moreover, *LASS2* was revealed to inhibit proliferation and induce apoptosis in HepG2 cells by affecting mitochondrial dynamics, the cell cycle, and the NF-κB pathway (14). However, the ability of *LASS2* to affect the proliferation and early apoptosis of lung cancer cells has not previously been reported. V-ATPase is a transmembrane protein that can transport H^+ against an inverse concentration gradient to maintain the acid homeostasis in the intracellular environment (29). It remains unclear whether intracellular pH affects the microenvironment of tumor cells, and causes tumor cell growth and apoptosis. V-ATPase inhibitor treatment inhibited proliferation and increased cell death in melanoma cells by reducing the pH gradient across the plasma membrane, increasing the external pH and decreasing the internal pH (30). Furthermore, it has been demonstrated that the increase of H^+ concentration in cells affects the tumor microenvironment, which can induce the cascade reaction of mitochondria, and stimulate apoptosis of tumor cells (31). Thus, it was inferred that the increase of intracellular H^+ concentration and decrease of extracellular H^+ concentration mediated by *LASS2* overexpression may be responsible for proliferation and early apoptosis of 95D cells. More experiments need to be performed to confirm the aforementioned hypothesis.

The most important apoptotic pathway mediated by H^+ is considered to be dependent on the mitochondrial apoptotic pathway (32), which cannot only transmit the energy needed for life, but also regulate the central location of cell apoptosis (33). In mitochondrion-dependent apoptosis, the release of cytochrome *c* from mitochondria to the cytoplasm, and activated caspases (such as caspase-9 and caspase-10) are

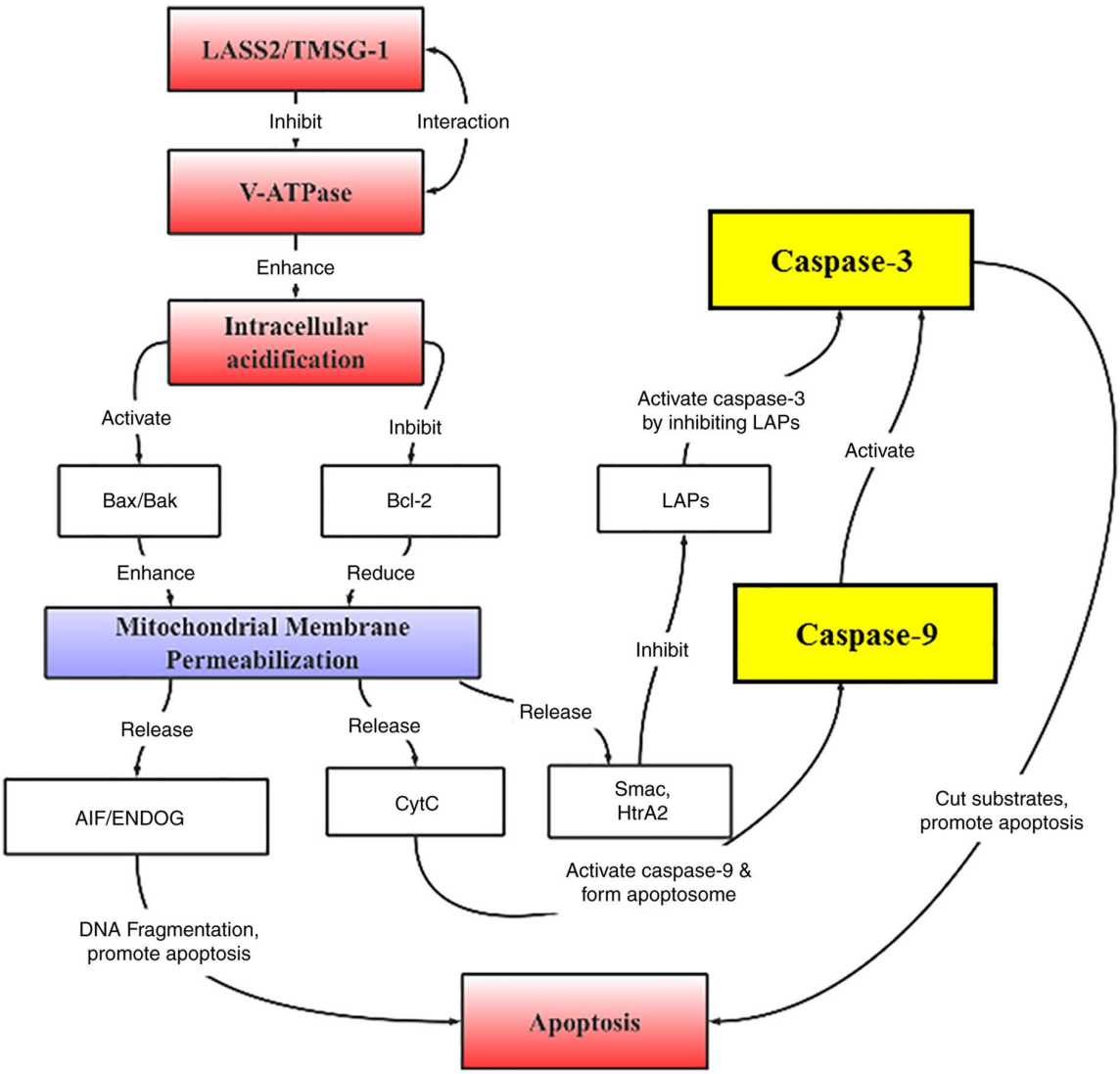

Figure 8. Proposed caspase-dependent mitochondrial pathway for the role of *LASS2* on apoptosis. LASS2 overexpression enhances intracellular acidification via the inhibition of V-ATPase activity, and then triggers dysregulation of Bcl-2 family members to induce mitochondrial membrane permeabilization and initiate caspase-regulated apoptosis. LASS2, longevity assurance homolog 2; LAPs, lectin adhesive-like proteins; AIF, apoptosis-inducing factor; ENDOG, endonuclease G; HtrA2, high-temperature-regulated A2.

the initiators at the top of the caspase signaling cascade (34). Additionally, Bax and Bcl-2 as two main members of the Bcl-2 family, are the upstream regulators of cytochrome *c* that affect caspase expression (35). Bcl-2 is a membrane binding protein that binds to Bax to inhibit opening of the mitochondrial membrane permeability transition pore, and thereby prevent the release of cytochrome *c*, while Bax elicits the opposite effect (36). The ratio of apoptotic protein and inhibitor of apoptotic protein (Bax/Bcl-2) in the Bcl-2 family determines cell survival and death. In the present study, it was determined that overexpression of *LASS2* decreased Bcl-2 expression, increased the Bax/Bcl-2 ratio, promoted the release of cytochrome *c*, and activated downstream caspase-9 and caspase-3 in 95D cells.

A previous study has suggested that cytosolic acidosis leads to apoptosis of coronary endothelial cells by activating caspase-12 and caspase-3 (33). Furthermore, an acidic extracellular environment increases the Bax/Bcl-2 ratio to induce apoptosis and inhibit proliferation of BM-EPCs in tumor microenvironments (37). Additionally, it has been reported that V-ATPase inhibitor activates the caspase-independent apoptotic pathway via inhibiting Bcl-2 and Bcl-xL (38). Moreover, the V-ATPase inhibitor induces tumor cell death through rapid intracellular acidification and activation of several caspases (30). A low pH was demonstrated to change the Bax/Bcl-2 ratio in placental cells (39). Conversely, the Bcl-2-like protein 13 knockdown in 3T3-L1 cells, revealed a higher extracellular acidification rate than the control groups (40). The Bcl-2 family members are important regulators of the mitochondrial pathway of apoptosis, and can commit a cell to programmed death by permeabilizing the outer mitochondrial membrane, followed by initiation of the caspase cascade (41). In light of previous findings by the authors and the results of the present study, it is hypothesized that *LASS2* may promote the early apoptosis of lung cancer via caspase-dependent mitochondrial pathway (Fig. 8).

In the present study, only Annexin V (FITC) staining was selected to detect apoptosis. Although Annexin V (FITC) can

label the entire cell population, and makes it easy to distinguish live cells from dead cells (42), double-stained Annexin V + PI is more suitable for suspension cells, while the 95C and 95D cells lines were adherent cells. Thus, the specimen collection may easily lead to cell membrane damage to improve the false positive rate of apoptosis. In future research, more methods of detecting apoptosis are required to mutually confirm the experimental results. Certainly, more *in vitro* and *in vivo* experiments are needed to confirm the results in the future. Whether the expression of LASS2 affects the activity of lung cancer cells by affecting other pathways is also a topic worthy of discussion in the future. The main focus of the present study, was the caspase-dependent apoptotic pathway. The effects of LASS2/TMSG1 gene intervention on metastatic ability of 95D or 95C cells will be examined in a future study. To observe the release of cytochrome *c*, detection of the expression of cytochrome *c* in the cytoplasm and mitochondria may be an effective method to be studied in the future. In subsequent experiments, the target site will also be investigated in depth.

In summary, *LASS2* was revealed to enhance the expression of Bax/Bcl-2 ratio, cytochrome *c*, caspase-9, and caspase-3 to activate the caspase pathway to induce early apoptosis of tumor cells, thereby inhibiting tumor cell proliferation. Therefore, *LASS2* may be a new therapeutic target in anticancer gene therapy.

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Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Authors' contributions

YW and XX conceived and designed the research as well as coordinated the study, helped draft the manuscript and revised it for important intellectual content. SL and XX participated in the acquisition of the data. LW and XX carried out the analysis and interpretation of data. JW and HD participated in the design of the study and performed the statistical analysis. XX contributed to funding acquisition. YW and XX confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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