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# Overexpression of a Novel Tumor Metastasis Suppressor Gene TMSG1/LASS2 Induces Apoptosis via a Caspase-dependent Mitochondrial Pathway

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## ABSTRACT

The tumor metastasis suppressor gene 1 (TMSG1), also designated homo sapiens longevity assurance homologue 2 of yeast LAG1 (LASS2), is a novel tumor metastatic suppressor gene. Although its effects on metastasis have been reported, its biological functions remain unclear. The purpose of this study was to investigate the effects of TMSG1/LASS2 protein on apoptosis and proliferation in human embryonic kidney cell lines HEK293 and 293 T and explore the potential mechanisms. Cell growth, morphology, expressions of apoptotic-related proteins and cell cycle distribution were evaluated in HEK293 and 293 T cells transfected with TMSG1/LASS2 expression plasmids or vector controls. MTT assays showed that overexpression of TMSG1/LASS2 inhibited cell proliferation; and morphological observations and flow cytometric assays with Annexin V/propidium iodide showed TMSG1/LASS2 overexpression increased apoptosis in these cells. Western blot analysis demonstrated that overexpression of TMSG1/LASS2 resulted in the downregulation of Bcl-2, release of cytochrome c from mitochondria, activation of procaspase-9 and procaspase-3, and the cleavage of PARP. Subsequent cell cycle analysis showed that TMSG1/LASS2 overexpression inhibited cell proliferation by mediating the induction of G0/G1 cell cycle arrest. Together, these results confirmed that *TMSG1/LASS2* is a potential metastasis suppressor gene, and suggested that the mechanism involved the induction of apoptosis and inhibition of cell proliferation via a caspase-dependent mitochondrial pathway. J. Cell. Biochem. 116: 1310–1317, 2015. 

**KEY WORDS:** TMSG1/LASS2; TUMOR METASTASIS; APOPTOSIS; PROLIFERATION; CASPASE

Apoptosis is essential in multiple biological events, including embryonic development, tissue homeostasis, and immune responses against tumorigenic or virus-infected cells [Wang and Youle, 2009]. However, excessive or insufficient apoptosis contributes to various diseases including ischemia, neurodegeneration, and autoimmunity. Aberrant apoptosis has been implicated in the growth and regression of tumors. The two major signaling pathways that trigger apoptosis in most tumor cells are the intrinsic and extrinsic pathways. Mitochondria play a key role in the intrinsic pathway by releasing soluble proteins, such as cytochrome c, from the intermembrane space into the cytosol. The extrinsic pathway is related to the activation of death receptors, such as TNF, CD95/Fas, and TRAIL-related death receptors. The apoptotic process is executed by a family of cysteine proteases, known as caspases, which cleave their target substrates at aspartic acid residues in response to intrinsic and extrinsic signals [Pradelli et al., 2010].

The tumor metastasis suppressor gene 1 (TMSG1) was first cloned in our laboratory (GeneBank accession number: AF189062) in 1999 as a novel tumor metastasis suppressor gene [Ma et al., 2002]. It was also identified from the human liver cDNA library and designated as homo sapiens longevity assurance homologue 2 of yeast LAG1 (*LASS2*; GeneBank accession number: AF 177338) by researchers at Shanghai Fudan University in 2001 [Pan et al., 2001]. The *TMSG1/LASS2* gene is located on chromosome 1q21.2 and encodes 380 amino acids with a homeodomain at amino acids 71–128 and a TLC domain at amino acids 131–332. Previous studies have shown that TMSG1/LASS2 protein is mainly located in the endoplasmic reticulum and has a ubiquitous tissue

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distribution primarily in the liver and kidneys [Mizutani et al., 2005].

The *TMSG1/LASS2* gene has been found to play an important role in the suppression of metastasis and tumor invasion in various cancers. It was negatively correlated with tumor metastatic potential in prostate and breast cancer cell lines [Su et al., 2007, 2008]. It exhibited high expression in non-metastatic tumors and low expression in metastatic tumors in breast cancer and colon cancer, demonstrating negative correlation with metastasis [Fei et al., 2004]. Furthermore, expression of LASS2/TMSG1 mRNA has been significantly negatively correlated with clinical stage, depth of tumor invasion and recurrence in bladder cancer [Wang et al., 2012; Zhao et al., 2013]; and was found to inhibit colony formation in human hepatocellular carcinoma cells in vitro, indicating that this gene may play a prominent role in the growth of hepatocellular carcinoma [Tang et al., 2007, 2010; WenXin, 2009].

All of these reports focused on the relationship between TMSG1/LASS2 and tumor progression. Less is known about the underlying molecular mechanisms. Therefore, we investigated the biological roles of TMSG1/LASS2 by examining its growth-suppressive activity and effects on proliferation and apoptosis in human embryonic kidney cell lines, HEK293 and 293 T, expressing FLAG-tagged TMSG1/LASS2 protein. We also measured the levels of apoptotic-associated proteins to explore the potential mechanisms by which *TMSG1/LASS2* regulates apoptosis in 293 T cells.

## MATERIALS AND METHODS

### MATERIALS

Dulbecco's Modified Eagle's medium (DMEM), penicillin, streptomycin, pcDNA3.0-FLAG vector, and Lipofectamine 2,000 were purchased from Invitrogen (Carlsbad, CA). Hoechst 33346, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), aprotinin and monoclonal antibodies against FLAG-tag were purchased from Sigma-Aldrich (St. Louis, MO). Monoclonal antibodies against cytochrome c, Bax and Bcl-2 were purchased from Santa Cruz (California, CA). Monoclonal antibodies against caspase-3, caspase-9 and PARP were obtained from Cell Signaling Technology (Danvers, MA). Monoclonal antibodies against β-tubulin were purchased from Neomarkers (Fremont, CA). Enhanced chemiluminescence (ECL) Western blotting reagents were purchased from Pierce (Rockford, IL). Bicinchoninic acid (BCA) protein assay kit was purchased from Beyotime (Haimen, Jiangsu, China). Horseradish peroxidase (HRP)-conjugated secondary antibodies (mouse and rabbit) were purchased from ZhongShan JinQiao Bioscience (Beijing, China). FITC-conjugated Annexin V was purchased from Beijing Biosea Biotechnology (Beijing, China). The pGEM-T Easy vector was purchased from Promega (Madison, WI). All other reagents were standard laboratory stocks.

### CDNA CLONING AND PLASMID CONSTRUCTION

The following PCR primers were designed according to the sequence of human TMSG1/LASS2, using the cDNA of human prostate cancer cells PC-3M-2B4 (a subclone of PC-3M cells with low metastatic potential) as a template: 5′-CGCGAATTCGCCGCCACCATGCTCCA-GACCTTGTATGATTA-3′ (forward); and 5′-CGCCTCGAGGT-CATTCTTACGATGGTTGTTATT-3′ (reverse). The PCR product containing a Kozak sequence and full-length open reading frame of *TMSG1/LASS2* was cloned into the TA cloning vector pGEM-T Easy. After purification, the 1.14 kbp *TMSG1/LASS2* fragment was subcloned into the overexpression vector pcDNA3.0-FLAG. The entire length of the insert strands was sequenced to confirm correct fusion between FLAG-tag and TMSG1/LASS2.

### CELL CULTURE AND TRANSFECTION

Human embryonic kidney cell lines HEK293 and 293 T were cultured in DMEM containing 10% fetal bovine serum (FBS) supplemented with penicillin (100 units/mL) and streptomycin (100 μg/mL). All cells were grown at 37°C in a humidified atmosphere of 5% $CO_2$. Cells were transfected with the specified plasmids using Lipofectamine 2,000 according to the manufacturer's instructions.

### PROTEIN EXTRACTION AND WESTERN BLOT ANALYSIS

Cells were harvested at the specified times following transfection, washed twice with pre-chilled phosphate buffered saline (PBS) and lysed in lysis buffer (20 mM Tris–HCl, pH 7.4; 150 mM NaCl; 1 mM EDTA; 0.5% NP-40; 1% Triton X-100; 1 mM PMSF; 1 μg/mL freshly added aprotinin). After being incubated on ice for 30 min, the cells were centrifuged at 10,000 × *g* for 10 min at 4°C. The supernatant was removed and the protein concentration was measured using a BCA protein assay kit according to the manufacturer's instructions. Equal amounts of heat denatured protein samples were separated by SDS-PAGE and electroblotted onto nitrocellulose membranes. After blocking in Tris-buffered saline containing 0.05% Tween 20 (TBST) and 5% non-fat milk for 1 h at room temperature, the membranes were incubated with the specified primary antibodies at 4°C overnight. They were then incubated with their respective HRP-conjugated anti-mouse or anti-rabbit secondary antibodies (1:6,000) for 1 h at room temperature. Signal detection using ECL Western blotting reagents was performed to evaluate the protein levels.

### HOECHST 33342 STAINING

Hoechst 33342 fluorescent dye was used to highlight morphological and nuclear changes in transfected HEK293 and 293 T cells. Following transfection, the cells were incubated in Hoechst 33342 solution (10 μg/mL in PBS) for 15 min in the dark. They were washed twice with PBS and immediately visualized under fluorescence microscopy.

### ANNEXIN V/PROPIDIUM IODIDE APOPTOSIS ASSAY

Transfected cells were collected at the specified times, washed twice with PBS and resuspended in 200 μL binding buffer (10 mM HEPES, PH 7.4; 140 mM NaCl; 1 mM $MgCl_2$; 5 mM KCl; 2.5 mM $CaCl_2$). FITC-conjugated Annexin V (10 μL) was added according to the manufacturer's protocol, and the cells were incubated for 15 min at room temperature or for 30 min at 4°C in the dark. After adding 300 μL binding buffer and 5 μL propidium iodide (PI), the cells were immediately analyzed using a FACSCalibur flow cytometer (Becton Dickinson; San Jose, CA). Unstained cells were classified as "live"; cells stained with Annexin V only were classified as "early

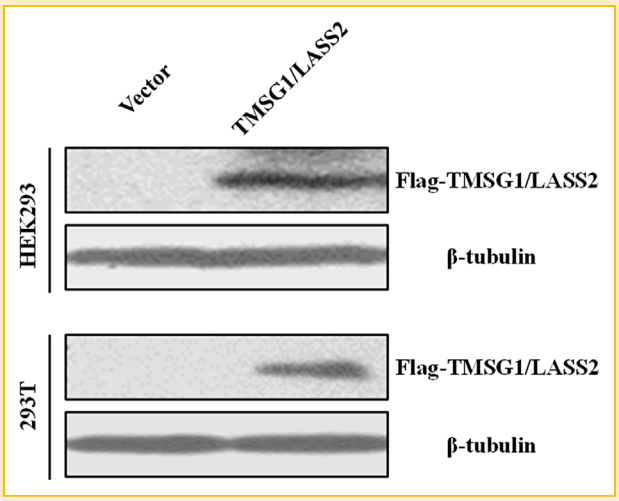


**Fig. 1.** Transfection of TMSG1/LASS2 in HEK293 and 293 T cells. Western blot analysis shows the expression of FLAG-tagged TMSG1/LASS2 fusion protein in HEK293 and 293 T cells transfected with the indicated plasmids. Analysis was performed 24 h post-transfection; β-tubulin was used as a protein loading control.

apoptotic"; cells stained with both Annexin V and PI were classified as "late apoptotic"; and cells stained with PI only were classified as "dead".

### CYTOSOLIC PROTEIN EXTRACTION

Cells were harvested 30 h post-transfection, washed twice with ice-cold PBS and resuspended in 0.2 mL PT-1 buffer (250 mM sucrose; 20 mM Hepes, pH7.5; 0.1 mM EDTA; 0.1% fatty acid-free BSA; and 1 μg/mL freshly added aprotinin). The cell suspensions were homogenized on ice and centrifuged at 4,000 × *g* for 10 min at 4°C. The supernatants were removed and centrifuged at 12,000 × *g* for 30 min at 4°C. The final supernatants were gently collected and used for identification of cytosolic cytochrome c by Western blot analysis.

### MTT ASSAY

HEK293 and 293 T cells were transfected with either TMSG1/LASS2-expression plasmids or control vectors. The cells were trypsinized 24 h post-transfection and seeded into 96-well plates ($1 \times 10^3$ cells/well). At the specified times, 20 μL MTT solution (50 μg/μL in PBS) was added to each well and the cells were incubated at 37°C in a humidified atmosphere of 5% $CO_2$ for 4–6 h. The supernatants were gently removed and the cells were lysed in dimethyl sulfoxide (DMSO). The absorbance was measured at 570 nm. Each experiment was performed in triplicate.

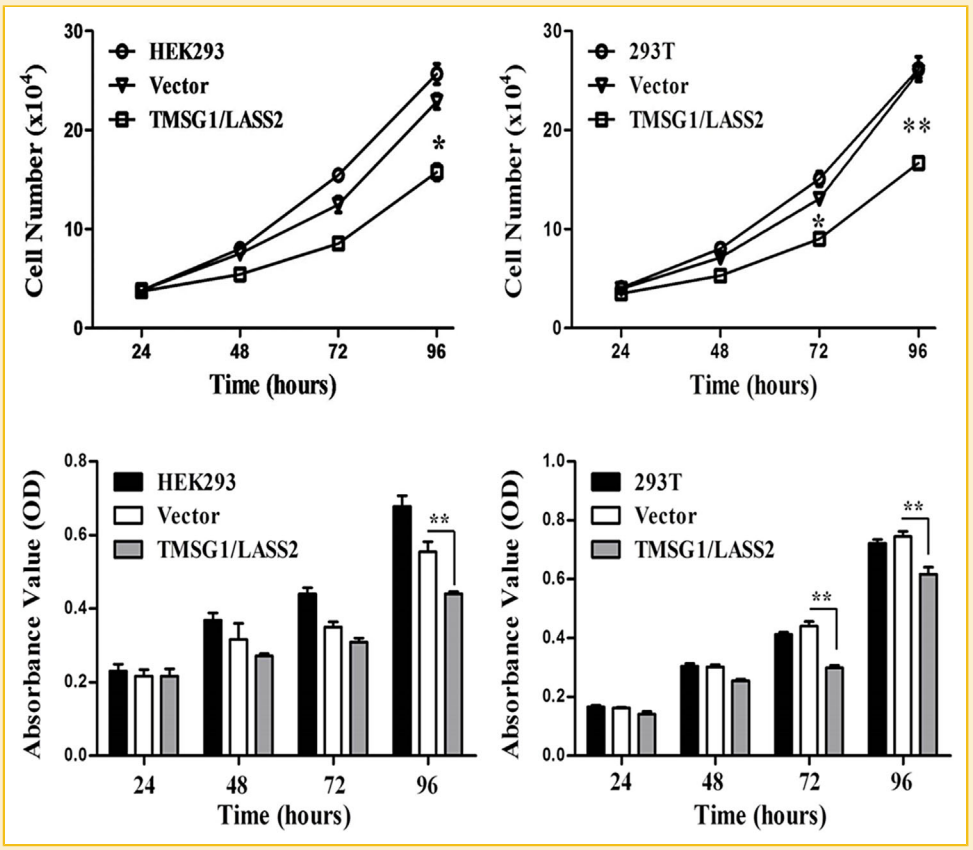


**Fig. 2.** Overexpression of TMSG1/LASS2 inhibits cell proliferation. HEK293 and 293 T cells were transiently transfected with the indicated plasmids. Analysis of cell growth curves and MTT assays show significantly decreased proliferation in TMSG1/LASS2-transfected cells compared to the control cells. * $P < 0.05$; ** $P < 0.01$, relative to vector control.

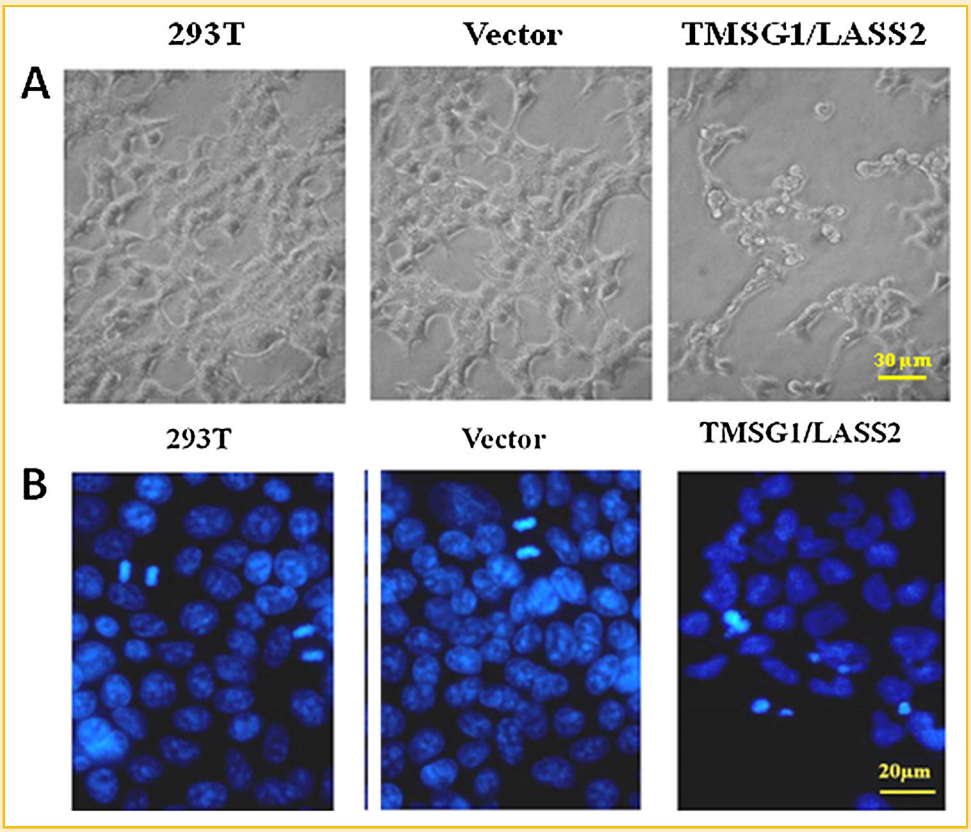


Fig.3. Overexpression of TMSG1/LASS2 induces morphological changes of apoptosis. A: Micrographs show the morphological changes induced by TMSG1/LASS2 in 293 T cells 30 h post-transfection with the indicated plasmids. B: Fluorescence microscopy of Hoechst 33342-stained 293T cells 30 h post-transfection show nuclear changes induced by TMSG1/LASS2.

### CELL CYCLE ANALYSIS

Cells were collected 24 h post-transfection and fixed in 75% ethanol at 4°C for 24 h. After washing with PBS, the cells were incubated in 0.2 mg/mL DNase-free RNase A at 37°C for 30 min and then stained with 20 μg/mL PI solution in PBS containing 0.1% Triton X-100. Cell cycle distribution was analyzed using a FACSCalibur flow cytometer.

### STATISTICAL ANALYSIS

Results were expressed as mean ± SEM. Student's *t*-test was used for statistical comparisons. *P*-values $< 0.05$ were considered statistically significant.

## RESULTS

### OVEREXPRESSION OF TMSG1/LASS2 INHIBITS PROLIFERATION IN HEK293 AND 293T CELLS

To determine whether the TMSG1/LASS2 eukaryotic plasmid could express FLAG-tagged TMSG1/LASS2 protein, HEK293, and 293 T cells were transiently transfected with TMSG1/LASS2 expression plasmids or control vectors. Western blot analysis was performed 24 h post-transfection. The TMSG1/LASS2 transfected cells showed a strong band at 45 kDa, which was consistent with the predicted size of the FLAG-TMSG1/LASS2 fusion protein (Fig. 1).

To examine the effect of TMSG1/LASS2 overexpression on cell proliferation, the cell growth curves and MTT assays were performed. The cell growth curves and MTT assays showed that HEK293 and 293 T cells transfected with TMSG1/LASS2 expression plasmids exhibited significantly reduced proliferation compared to those transfected with control vectors or untransfected cells ($P < 0.05$ and $P < 0.01$ ; Fig. 2A–D). However, cells transfected with control vectors also showed marginally lower growth compared to untransfected cells, suggesting that transfection may affect cell viability. These data demonstrated that overexpression of TMSG1/LASS2 could inhibit cell growth in HEK293 and 293 T cells indicating that TMSG1/LASS2 protein may play a negative role in cell proliferation.

### TMSG1/LASS2 INDUCES APOPTOSIS IN HEK293 AND 293T CELLS

Morphological changes typical of apoptosis were observed in TMSG1/LASS2 transfected HEK293 and 293 T cells. These included marked rounding, shrinkage, blebbing, and detachment from the culture dish. These changes were not exhibited by cells transfected with control plasmids or normal cultured cells (Fig. 3A).

Hoechst 33342 acts as a probe of membrane permeability, therefore to confirm whether overexpression of TMSG1/LASS2

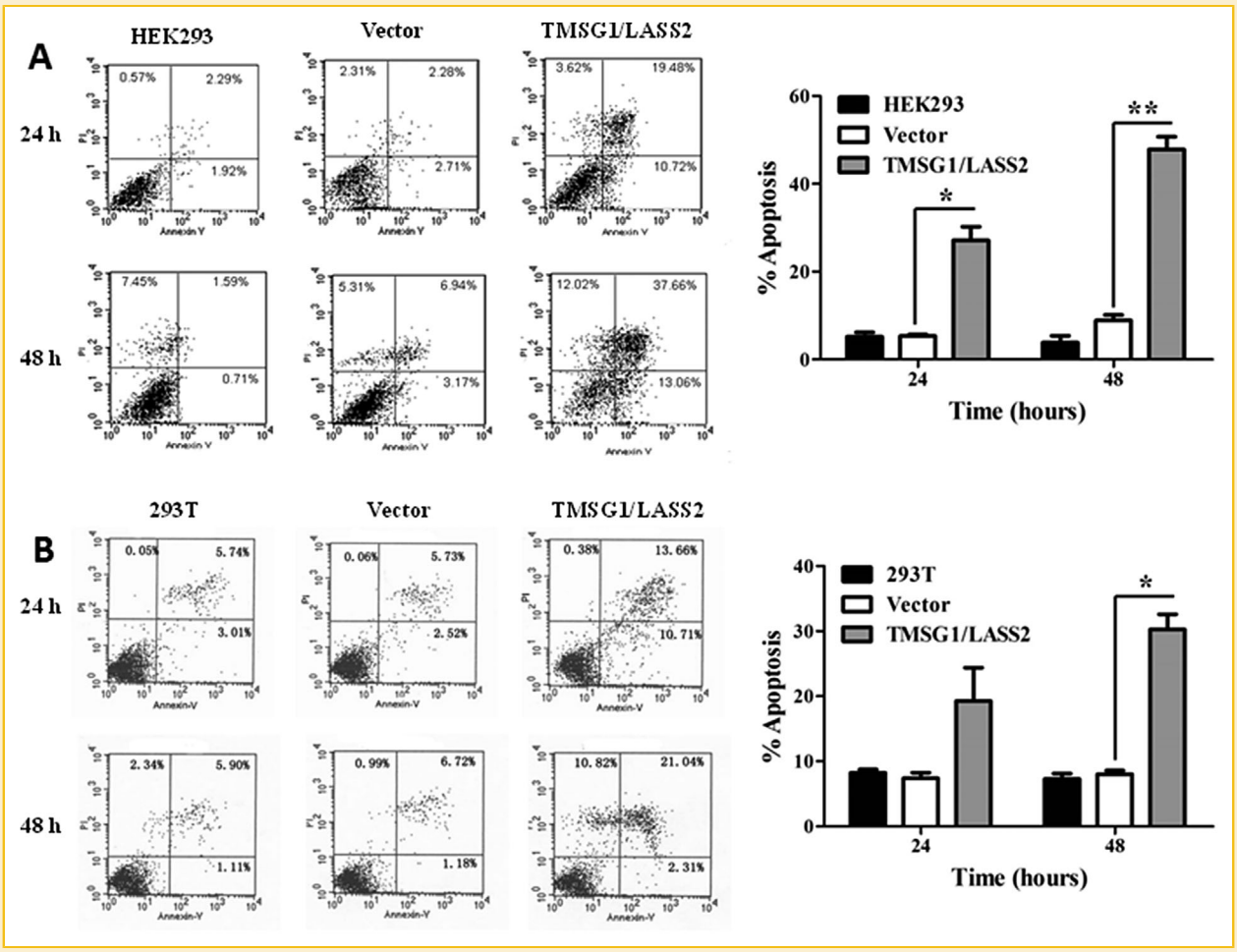


**Fig. 4.** Overexpression of TMSG1/LASS2 induces apoptosis. Representative flow cytometric plots of HEK293 and 293 T cells 24 h and 48 h post-transfection with the indicated plasmids. The plots show the percentages of Annexin V and/or PI positive cells in each sample. Analysis of the flow cytometric data represent the mean ± SEM of three independent experiments. * $P < 0.05$; ** $P < 0.01$, relative to vector control.

induces apoptosis, HEK293 and 293 T cells were stained with Hoechst 33342 and examined under fluorescent microscopy. As shown in Figure 3B, the TMSG1/LASS2-transfected cells displayed chromatin condensation, margination and rupture, which are characteristic of apoptosis.

Exposure of phosphatidylserine (PS) on the surface of apoptotic cells can be detected by flow cytometry using fluorescently labeled Annexin V, which specifically binds to PS. Plasma membrane integrity can be assessed simultaneously using two-color fluorescence-activated cell sorting (FACS) with propidium iodide (PI) staining. These assays showed that PS was exposed on the surface of cells 24 h and 48 h post-transfection. The proportions of Annexin V and/or PI positive cells in the TMSG1/LASS2 transfected samples were significantly increased compared to cells transfected with control vectors or untransfected cells (Fig. 4A and B). Furthermore, the increase was greater at 48 h than at 24 h post-transfection ($P < 0.05$; $P < 0.01$, respectively). These results indicated that TMSG1/LASS2 overexpression induced apoptosis in HEK293 and 293 T cells.

### CASPASE-3 AND CASPASE-9 ARE ACTIVATED BY TMSG1/LASS2

Activation of effector caspases, such as caspase-3 and caspase-9, is responsible for proteolytic cleavage in a range of structural and regulatory proteins involved in apoptosis. To examine whether TMSG1/LASS2 played a role in the activation of caspase-3, we performed Western blot analysis in transfected 293 T cells to detect the cleavages of caspase-3 and poly(ADP-ribose) polymerase (PARP), which is a specific substrate of activated caspase-3. TMSG1/LASS2 overexpressing cells exhibited both cleavage of caspase-3 and cleavage of PARP compared to the control cells (Fig. 5). Additionally, the cleavage of caspase-9 was detected in TMSG1/LASS2 transfected cells (Fig. 5). These results demonstrated that the caspase cascade was activated during TMSG1/LASS2-induced apoptosis.

### TMSG1/LASS2 PROMOTES THE RELEASE OF CYTOCHROME C FROM MITOCHONDRIA TO CYTOSOL

The release of cytochrome c from mitochondria to the cytosol is one of the key events in apoptosis induced by the mitochondrial

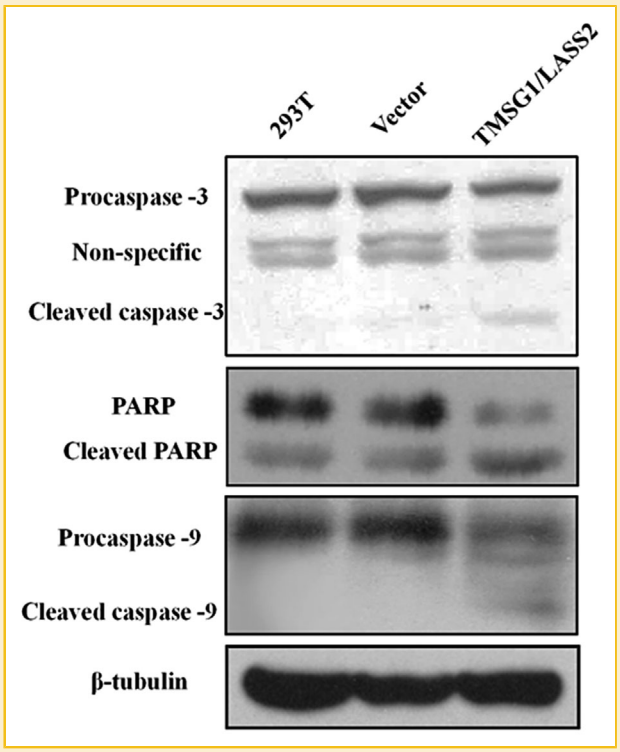


Fig. 5. Overexpression of TMSG1/LASS2 activates caspase-3 and caspase-9. Western blot analysis shows the the levels of cleaved caspase-3, PARP, caspase-9 and their precursor proteins 30 h post-transfection with the indicated plasmids. β-tubulin was used as a protein loading control.

pathway. Western blotting was performed 30 h post-transfection to assess the levels of cytosolic cytochrome c in 293 T cells transfected with TMSG1/LASS2 expression plasmids, control vectors, and untransfected control cells. The results showed that TMSG1/LASS2 overexpression led to a marked increase in cytosolic cytochrome c compared to that in control cells (Fig. 6). These

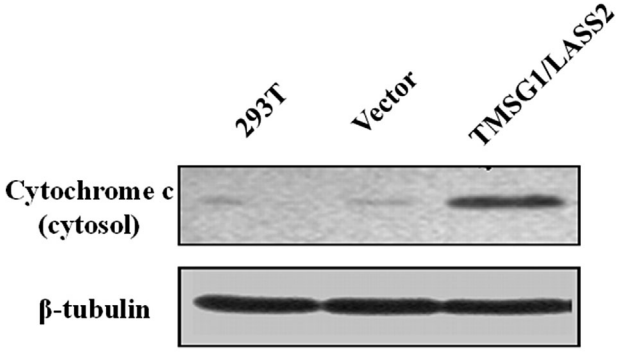


Fig. 6. Overexpression of TMSG1/LASS2 induces the release of cytochrome c from mitochondria to cytosol. Western blot analysis shows the level of cytochrome c in the cytosol of 293 T cells 30 h post-transfection with the indicated plasmids. β-tubulin was used as a protein loading control.

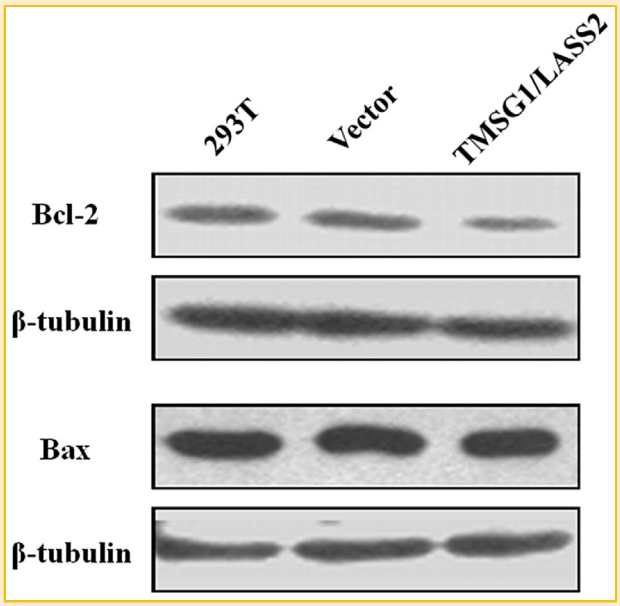


Fig. 7. Overexpression of TMSG1/LASS2 alters expression of Bcl-2 family proteins. Western blot analysis shows the expression levels of Bax and Bcl-2 in 293 T cells 30 h post-transfection with the indicated plasmids. β-tubulin was used as a protein loading control.

observations indicated that TMSG1/LASS2 induced the release of cytochrome c from mitochondria to cytosol, suggesting that TMSG1/LASS2-induced apoptosis acted through a mitochondrial-cytochrome c pathway.

### TMSG1/LASS2 INDUCES A DECREASE IN THE EXPRESSION OF BCL-2 PROTEIN

Whether TMSG1/LASS2 mediated pro-apoptotic effects via modulation of Bcl-2-family proteins, the expressions of Bax and Bcl-2 were assayed by Western blotting. The results indicated that TMSG1/LASS2 overexpression induced a marked decrease in the level of anti-apoptotic Bcl-2, but had little effect on the level of pro-apoptotic Bax (Fig. 7). These observations suggested that down-regulation of Bcl-2 might be involved in TMSG1/LASS2-induced apoptosis.

### OVEREXPRESSION OF TMSG1/LASS2 INDUCES G0/G1 CELL CYCLE ARREST

In order to determine whether the anti-proliferative effect of TMSG1/LASS2 was associated with cell cycle regulation, we performed flow cytometric analysis to assess the effect of TMSG1/LASS2 overexpression on cell cycle distribution in HEK293 and 293 T cells. The results showed a significant increase in the proportion of G0/G1 phase cells, and concurrent decrease in the proportion of S and G2/M phase cells, 24 h post-transfection in TMSG1/LASS2 overexpressing cells compared to control cells, ($P < 0.05$, $P < 0.01$, respectively; Fig. 8). However, there was also a slight increase in the proportion of control-vector HEK293 cells in G0/G1 phase compared to untransfected HEK293 cells. This was consistent with the findings of the MTT assay and may be a consequence of transfection. These

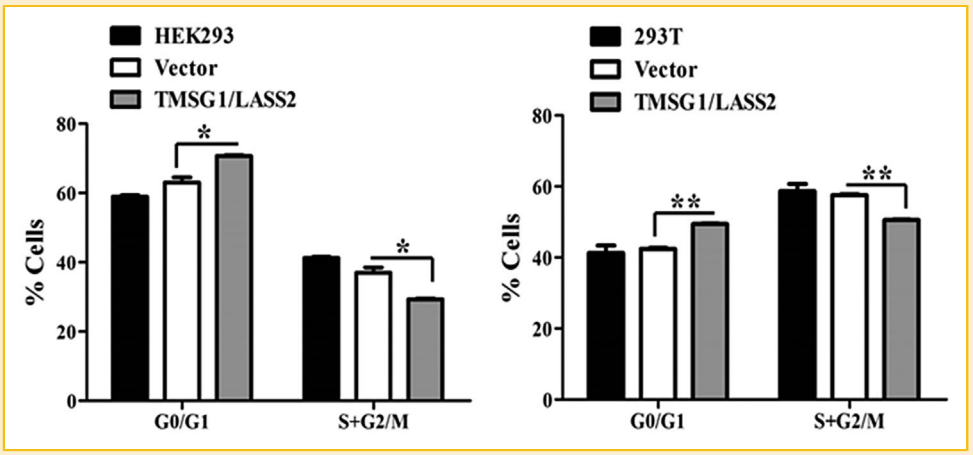


**Fig. 8.** Overexpression of TMSG1/LASS2 induces G0/G1 cell cycle arrest in HEK293 and 293 T cells. Flow cytometric analysis shows the cell cycle distribution of propidium iodide-stained HEK293 and 293 T cells 24 h post-transfection with the indicated plasmids. The data represent the mean ± SEM of three independent experiments. * $P < 0.05$; ** $P < 0.01$, relative to vector control.

results indicated that TMSG1/LASS2 inhibited cell proliferation by arresting cell cycle progression at G0/G1 phase.

## DISCUSSION

*TMSG1/LASS2* was first cloned as a tumor metastasis suppressor gene. More than ten years after its discovery, data from our group and others demonstrated its close association with carcinogenesis, tumor invasion, and metastasis in different types of malignant tumors. However the biological properties of TMSG1/LASS2 were poorly understood. In this study, we found that overexpression of TMSG1/LASS2 could inhibit cell growth and induce apoptosis in HEK293 and 293 T cells. This discovery provided an important insight into the potential function of TMSG1/LASS2.

Tumor metastasis is a multistep biological process controlled by the acquisition of genetic and epigenetic alterations in tumor cells. A tumor metastasis suppressor gene may be involved in one or more of these steps. The *TMSG1/LASS2* gene has been shown to play a prominent role in the suppression of tumor metastasis. We previously reported that overexpression of TMSG1/LASS2 could inhibit proliferation, growth, and invasion in highly metastatic prostate cancer cell lines (PC-3M-1E8) and breast cancer cell lines (MDA-MB-231) [Su et al., 2007, 2008]. Further study found that TMSG1/LASS2 might inhibit the invasion and metastasis through regulating the function of V-ATPase [Xu et al., 2012, 2014]. Other reports have shown that overexpression of TMSG1/LASS2 could inhibit secretion and activation of MMP-2, and inhibit cell migration and invasion in highly metastatic HCCLM3 human hepatocellular carcinoma cells. Conversely, siRNA silencing of *TMSG1/LASS2* increased activation of MMP-2 and promoted cell invasion by facilitating proton secretion in the low metastatic MHCC97-L human hepatocellular carcinoma cell line [Tang et al., 2007, 2010; WenXin, 2009]. These reports demonstrated that *TMSG1/LASS2* acted as a metastasis suppressor gene in a variety of tumor types via the inhibition of cell proliferation and invasion. In this study, we demonstrated that TMSG1/LASS2 could also inhibit cell growth in HEK293 and 293 T human embryonic kidney cell lines.

Apoptosis has been shown to play an important role in regulating metastasis [Mehlen and Puisieux, 2006]. In vivo studies have demonstrated that increased levels of apoptosis correspond to a decrease in metastasis [Wong et al., 2001]. Tumor cells are subjected to multiple apoptotic stimuli during the metastatic process, which results in very few cells that leave the primary tumor giving rise to secondary tumors. Therefore alterations in the genes that induce apoptosis can suppress the metastatic ability and efficiency of tumor cells. We were able to confirm that overexpression of TMSG1/LASS2 induced apoptosis in HEK293 and 293 T cells by detecting distinct morphological and nuclear changes, and alterations in biochemical and molecular markers associated with apoptosis. TMSG1/LASS2 overexpressing cells appeared small, round, and detached, and displayed chromatin condensation around the nuclear margins. The characteristic biochemical indicators of apoptosis that were induced by overexpression of TMSG1/LASS2 included translocation of PS to the outer plasma membrane, activation of caspase-3 and caspase-9, and cleavage of the caspase-3 substrate, PARP. These observations suggested that TMSG1/LASS2 plays an important role in regulating apoptosis and that this may be related to its ability to suppress metastatic activity.

Further investigations revealed that overexpression of TMSG1/LASS2 could alter the expression levels of Bcl-2 protein and promote cytochrome c release from the mitochondria to the cytosol. Anti-apoptotic members the Bcl-2 family, such as Bcl-2, Bcl-XL, and Mcl-1, preserve the integrity of the outer mitochondrial membrane; whereas pro-apoptotic members, such as Bax and Bak, promote permeabilization which allows efflux of cytochrome c. Cytochrome c plays a central role in the caspase-dependent mitochondrial-mediated pathway of apoptosis [Pradelli et al., 2010]. When cytochrome c is released from mitochondria, cytosolic cytochrome c binds to Apaf-1, a cytosolic protein containing a caspase-recruitment domain (CARD) and a nucleotide-binding domain. The

binding of cytochrome c increases Apaf-1 affinity for dATP/ATP by approximately 10-fold. The binding of nucleotides to the Apaf-1/cytochrome c complex forms the apoptosome, a multimeric Apaf-1 and cytochrome c complex. This in turn initiates caspase-activation cascades and recruits multiple procaspase-9 molecules to the complex, facilitating their autoactivation [Rodriguez and Lazebnik, 1999; Zou et al., 1999]. When caspase-9 binds to the apoptosome, downstream executioner caspases, such as caspase-3 are efficiently cleaved and activated. The executioner caspases subsequently cleave many important intracellular substrates, leading to characteristic changes associated with apoptosis including chromatin condensation, nuclear fragmentation, nuclear membrane rupture, externalization of PS, and formation of apoptotic bodies.

Western blot analysis revealed that overexpression of TMSG1/LASS2 resulted in the downregulation of Bcl-2, release of cytochrome c from mitochondria, activation of procaspase-9 and procaspase-3, and cleavage of PARP. Taken together, these results suggested that TMSG1/LASS2 overexpression induced apoptosis via a caspase-dependent mitochondrial pathway. However apoptosis is a complex process involving multiple pathways and molecules. In this study, only a few representative molecules were examined, therefore further research is required to determine whether other molecules or pathways are involved in this process.

Effective anti-metastatic agents can induce cell cycle arrest, impair cell proliferation in established metastases, and elicit metastatic regression. In this study, flow cytometric analysis revealed that overexpression of TMSG1/LASS2 in transfected HEK293 and 293 T cells induced cell cycle arrest at G0/G1 phase, suggesting that the inhibition of cell proliferation may be mediated via the induction of cell cycle arrest. However, details on the underlying mechanisms will require further study.

In conclusion, our experimental data indicated that TMSG1/LASS2 might inhibit cell proliferation and induce apoptosis via a caspase-dependent mitochondrial pathway. As apoptosis is implicated in the inhibition of metastasis, these findings may provide an important insight into the mechanism by which *TMSG1/LASS2* acts as a metastasis suppressor gene. As such, TMSG1/LASS2 may be a novel therapeutic target in anticancer gene therapy.

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