Overexpression of LASP1 is associated with proliferation, migration and invasion in esophageal squamous cell carcinoma

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Received October 9, 2012; Accepted November 23, 2012

DOI: 10.3892/or.2012.2199

Abstract. LIM and SH3 protein 1 (LASP1) is an actin-binding protein which is overexpressed in many types of cancers and plays important roles in cancer progression. However, the role of LASP1 in esophageal squamous cell carcinoma (ESCC) is still unknown. We sought to analyze the expression level of LASP1 in ESCC, and the role of LASP1 in the development of ESCC was further investigated. We evaluated the expression levels of LASP1 in 89 ESCC tissues and two ESCC cell lines using quantitative real-time polymerase chain reaction, western blotting and immunohistochemistry. The effects of LASP1 depletion on tumor cell behavior were investigated using gene transfection and small interfering RNA (siRNA) in ESCC cell lines *in vitro*. The expression levels of LASP1 at the mRNA and protein levels were significantly higher in ESCC tissues and ESCC cell lines compared to adjacent tissues. Immunohistochemistry showed that LASP1 was localized in the cytoplasm and nuclei of tumor epithelia. Silencing of LASP1 in ECA109 and KYSE510 cell lines significantly inhibited cell proliferation, migration and invasion when compared with the negative control cells *in vitro*. LASP1 may play an important role in the pathogenesis of ESCC and shows promise as a treatment target in ESCC.

Introduction

The incidence and mortality of esophageal cancer (EC) ranks eighth and sixth among all cancers and affects more males than females (1). Esophageal squamous cell carcinoma (ESCC) and adenocarcinoma (EAC) are two main subtypes of EC in regards to their pathological characteristics. ESCC remains the dominant subtype of EC. However, ESCC is usually diagnosed locally at an advanced stage or with lymph node metastases. The characteristics dictating the potential for invasion and metastasis of esophageal carcinoma cells are important prognostic factors. The overall 5-year survival rate of ESCC patients is extemely poor despite advanced treatment (1,2). To develop new treatment strategies and diagnostic methods, a better understanding of the biological behavior of ESCC is needed.

The Lim and SH3 domain protein (LASP1) is an actin-binding protein. It was initially identified from a cDNA library of breast cancer metastases. The gene was mapped to human chromosome 17q21 a region that is altered in 20-30% of human breast cancers (3,4). LASP1 encodes a membrane-bound protein of 261 amino acids containing an N-terminal LIM domain, followed by two actin-binding domains in the core of the LASP1 protein mediating an interaction between LASP1 and the actin cytoskeleton at cell membrane extensions (5-9). The exact functions of LASP1 are still not clear, yet it appears to be involved in the dynamic actin assembly, such as focal contacts, focal adhesions, lamellipodia, membrane ruffles and pseudopodia (3,6,10-12). Recently, high LASP1 expression has been observed in many types of human cancers, including breast, ovarian, colorectal, liver and bladder (13-17). Furthermore, silencing of LASP1 by siRNA was found to suppress cell proliferation and migration of breast cancer cells *in vitro* (13), arrest ovarian cancer cells at the G2/M phase, reduce cell proliferation and affect zyxin localization (14). In conclusion, previous studies suggest that LASP1 is involved in cell migration, invasion and proliferation and may play an important role in carcinogenesis and cancer progression. However, to our knowledge, expression of LASP1 in ESCC and its role in the progression of this disease have not yet been reported.

In the present study, to investigate the roles of LASP1 in ESCC, we used quantitative real-time polymerase chain reaction (qRT-PCR) to evaluate the expression of LASP1, and found that LASP1 was overexpressed in ESCC tissues and ESCC cell lines when compared with adjacent normal esophageal tissues. This result was confirmed by western blot analysis and immunohistochemistry (IHC). We also studied the potential roles of LASP1 in ESCC cell growth and migration by siRNA

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Key words: LIM and SH3 protein 1, esophageal squamous cell carcinoma, proliferation, migration, invasion

transfection. It was found that the LASP1 gene may mediate cell proliferation, migration and invasion of ESCC cells.

Materials and methods

Tissue samples and cell culture. Pairs of primary ESCC and matching adjacent normal esophageal tissues (5 cm above the upper margin of the ESCC) were obtained from 89 patients after esophagectomy at The Second Xiangya Hospital of Central South University between 2008 and 2010. The patients who underwent adjuvant chemotherapy or radiotherapy preoperatively were excluded in this study. Tissue samples were immediately snap-frozen in liquid nitrogen and then stored at -80˚C. Serial paraffin-wax sections from the specimens were stained with hematoxylin and eosin, and the slides of all the cases were reviewed by two pathologists to confirm the diagnosis based on UJCC criteria. All information regarding the clinical and histopathological data was collected. The Ethics Commitee of The Second Xiangya Hospital approved this study, and written consent was obtained from each patient.

Two human esophageal squamous cell lines, ECA109 and KYSE510, were obtained from the American Type Culture Collection (ATCC) and the German Collection of Microorganisms and Cell Cultures (DSMZ), respectively, and were grown in HyClone RPMI-1640 medium (Thermo Scientific, Beijing, China) supplemented with 10% fetal bovine serum (FBS), 100 U/ml of penicillin sodium, and 100 mg/ml of streptomycin sulfate and cultured at 37˚C in a humidified air atmosphere containing 5% carbon dioxide.

RNA extraction and qRT-PCR. Total RNA was extracted from 56 pairs of frozen tissue (ESCC and matching adjacent normal tissues) and two cell lines using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. The final elution volume was 30-50 μl, and all RNA samples were quantified using the DU 800 UV spectrophotometer (Beckman Coulter, Japan). All samples had an OD value of 1.8-2.0 and an RNA integrity number >C 5.0.

To determine the expression of LASP1 in ESCC tissues and ESCC cell lines, SYBR-Green qRT-PCR assay was used. In brief, total RNA was polyadenylated by poly(A) polymerase and reverse transcribed to cDNA using the OneStep PrimeScript® cDNA Synthesis kit (Takara, Dalian, China) according to the manufacturer's instructions. Primer sets for LASP1 were sense, 5'-GGTGCGGCAAGATCGTGTA-3' and antisense, 5'-TGCAGGTCTCGCAATGGAA-3'. Real-time quantitative polymerase chain reaction (RQ-PCR) was performed using the SYBR® PrimeScript™ RT-PCR II kit (Takara) in the ABI PRISM StepOnePlus real-time PCR system (Applied Biosystems, Foster City, CA, USA). Each amplification reaction was performed in a final volume of 20 μl containing 40 ng of cDNA, 0.8 μl of each primer and 1X SYBR-Green PCR Master Mix. LASP1 expression data were normalized to β-actin, and the experiments were performed in triplicate. Data are expressed as the means ± SD. Relative quantification of LASP1 expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Western blotting. For western blotting, proteins were extracted from 13 pairs of frozen tissue (ESCC and matching adjacent normal tissues) and two cell line lysates. Equal amounts of protein were resolved by 12% SDS-PAGE. After transferring the protein to a nitrocellulose membrane and blocking with 3% non-fat dry milk in 10 mM Tris, pH 7.5, 10 mM NaCl, 0.1% (w/v) Tween-20, the membrane was first incubated with the antibody raised against LASP1 (1:4,000) followed by incubation with HRP goat anti-rabbit IgG (Proteintech, Chicago, IL, USA), which were diluted to 1:3,00. Visualization was carried out using ECL (Thermo Scientific). Quantification of ECL signals was carried out by densitometry using the Image-Pro Plus software 6.0 (Media Cybernetics, Inc., Silver Spring, MD, USA). The LASP1 protein was normalized to β-actin.

Immunohistochemistry (IHC). IHC was performed to investigate the expression of LASP1 protein in 20 pairs of human ESCC and matching adjacent normal tissues. Sections (4 μm) were dewaxed in xylene and rehydrated in graded ethanol. Sections were subjected to heat pretreatment by boiling in 0.01 M of sodium citrate buffer (pH 6.0) for 3 min in a pressure cooker for antigen retrieval. Endogenous peroxidase was blocked by incubation in 0.1% hydrogen peroxide in PBS for 10 min, and then sections were incubated with primary antibodies against LASP-1 (1:400) (BT, USA) overnight at 4˚C. Aliphatic alcohol-polyoxyethylene ether carboxylic acid sodium salt was used as the chromogen, and sections were counterstained with hematoxylin.

siRNA transient transfection. The day before transfection, cells in the exponential phase of growth were seeded into 24-well plates at a density of 2-8x10^4 cells per well in 0.5 ml RPMI-1640 medium, grown for 24 h at 50-80% cell confluence and then transiently transfected with 75 ng (10 nM) of siRNA-LASP1 (cat. no. SIO2654855; Qiagen, Germany) (sense strand: 5'-GGUUCUUGCCUUUCUUAATT-3'; antisense, 5'-AUUA AGAAAGGCAAGAACCTG-3') or negative control siRNA (AllStars negative nontrol siRNA, cat. no. SI03650318; Qiagen, Germany) using HiPerFect transfection reagent (1.5 μ) (Qiagen) according to the manufacturer's protocol. Cells were incubated with the transfection complexes under their normal growth conditions and gene silencing was monitored after an appropriate time.

Cell proliferation assay. To evaluated the viability of ESCC cells after transfection with siRNA-LASP1, cell proliferation was determined using the MTT assay. In brief, the day before transfection, cells were seeded into 96-well plates at a density of 5x10^3 cells/well. siRNA-LASP1 (75 ng) and negative-control siRNA were transfected using 0.75 μl HiPerFect transfection reagent. At 24, 48 and 72 h after transfected with siRNA-LASP1, 10 μl 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Roche Diagnostics GmbH, Mannheim, Germany) was added to each well and incubation was carried out at 37˚C for 4 h. The medium and MTT were removed carefully with a 1-ml inoculator, and 100 μl DMSO was added to each well. Cell proliferation was determined at 490 nm according to the manufacturer's protocol. Five wells were detected for cell viability in each experimental group.

Cell migration and invasion assays. To investgated the motility of ESCC cells after tansfection with siRNA-LASP1,

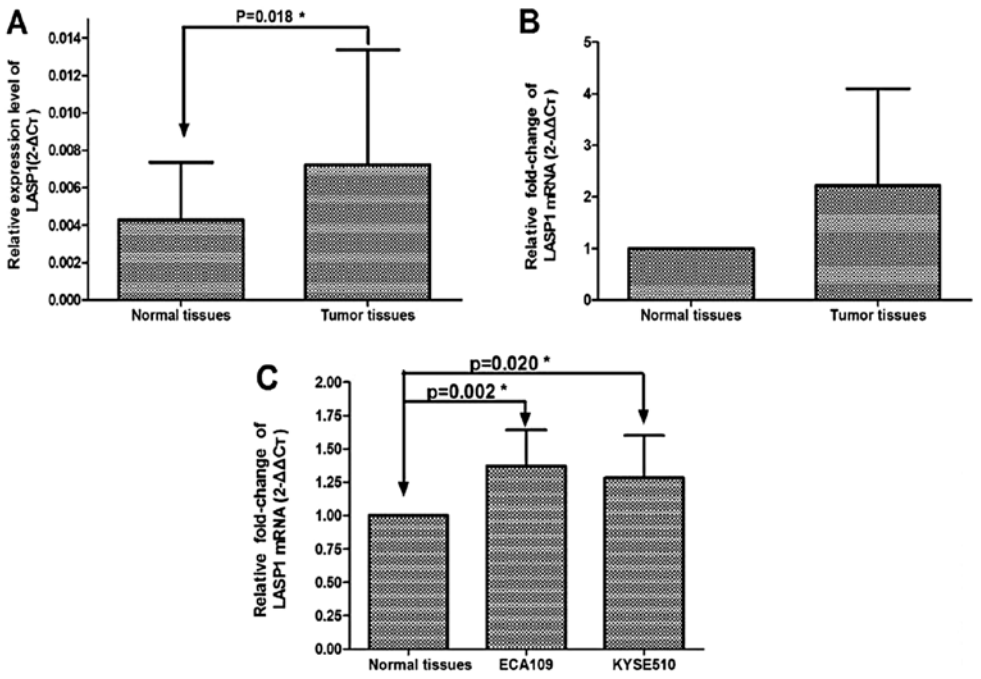

Figure 1. mRNA expression of LASP1. (A) Relative expression level of LASP1 was measured by qRT-PCR in 56 pairs of ESCC and adjacent normal esophageal tissues. The expression level of LASP1 was higher in ESCC tissues than that in the adjacent normal tissues; *P<0.05. (B) Fold-change of LASP1 expression level in ESCC tissues compared with the adjacent normal tissues. (C) Fold-change of LASP1 expression level in two ESCC cell lines (ECA109 and KYSE510) compare with the normal esophageal tissues. LASP1 expression data were presented as the means ± SD and were normalized to β-actin expression. Relative quantification of LASP1 expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

cell migration assay was carried out using 24-well Millicell hanging cell culture insert chambers. Upper and lower culture compartments were separated by polycarbonate membranes with 8-μm-sized pores (BD, USA) according to the manufacturer's instructions. In brief, 72 h after transfection, the siRNA-LASP1-transfected, negative-control siRNA-transfected and non-transfected cells were seeded into the upper chamber at $3x10^5$ cells/well in 100 μl RPMI-1640 medium without serum, and 600 μl RPMI-1640 medium was placed into the lower chamber and incubation was carried out for 6 h at 37˚C in 5% CO_2 humidified air. The cells at the upper surface of the membrane were removed with a wet cotton swab, and the cells that adhered to the lower surface of the membrane were fixed using methanol. Cells were then stained with 0.1% crystal violet for visualization. Migration was evaluated by the number of cells which penetrated the membrane per field. Five wells were assayed for cell migration in each experimental group.

The cell invasion assay was performed using 24-well Millicell hanging cell culture inserts chambers. The upper and lower culture compartments were separated by polycarbonate membranes with 8-μm-sized pores coated with MaxGel ECM (Sigma, USA). Seventy-two hours after transfection with siRNA-LASP1 or negative-control siRNA, 100 μl of medium without serum containing $3x10^4$ cells was seeded into the upper chamber per well. RPMI-1640 medium (600 μl) was placed in the lower chamber, and cells were incubated for 24 h at 37˚C in 5% CO_2 humidified air. The remaining steps were the same as for the migration assay. Five wells were assayed for cell invasion in each experimental group.

Statistical analysis. Data are presented as the means ± SD and were analyzed using the software package SPSS 17.0 for Windows. Unless otherwise noted, the differences between groups were analyzed using the independent-samples t-test. To assess the significant differences in LASP1 mRNA between ESCC and adjacent normal tissues, a paired-samples t-test was used. P-value <0.05 was considered to indicate a statistically significant difference.

Results

LASP1 is overexpressed in ESCC tissues and cell lines as determined by qRT-PCR. To evaluated the role of LASP-1 in ESCC, the mRNA expression levels of LASP1 were measured in 56 pairs of tumor tissues of ESCC and adjacent normal esophageal tissues using qRT-PCR. The relative expression of LASP1 mRNA was significantly higher in the tumor tissues compared to the adjacent normal tissues (P=0.018) (Fig. 1A). LASP1 expression was 2.66-fold upregulated in ESCC compared to that of the adjacent normal tissues (Fig. 1B). The expression of LASP1 mRNA was also evaluated in cell lines. Consistent with ESCC tissues, the expression of LASP1 in the ECA109 and KYSE510 cell lines was also relatively higher than that in the adjacent normal esophageal tissues, and was 1.37- and 1.28-fold upregulated in esophageal cancer cell lines compared to normal esophageal tissues, respectively (P=0.002, P=0.020) (Fig. 1C).

Verification of the differential expression of LASP1 by western blot analysis. To confirm the qRT-PCR results, western blot

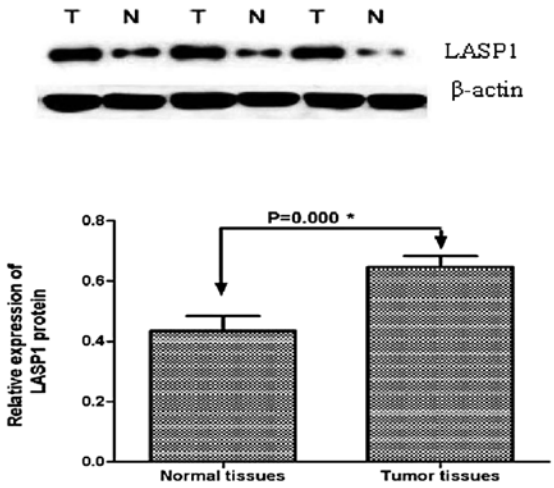

Figure 2. The protein expression level of LASP1 by western blot analysis revealed that LASP1 expression was upregulated in 13 pairs of ESCC tissues (T) compared to that in the adjacent normal esophageal tissues (N). The LASP1 protein was normalized to β-actin.

analysis was used to detect the LASP1 expression in another 13 pairs of primary ESCC and adjacent normal esophageal tissues. The LASP1 protein was normalized to β-actin. Western blot analysis revealed that LASP1 expression was upregulated in the ESCC tissues (P=0.000) (Fig. 2) compared to that of the adjacent normal esophageal tissues. This result was consistent with that found in the previous qRT-PCR.

Increased expression of LASP1 as detected by immunohistochemistry. To further confirm the above-mentioned results, IHC was performed to evaluate the expression of LASP1 in 20 pairs of paraffin wax slices of ESCC and adjacent normal tissues. IHC showed positive staining of LASP1 in 18 out of 20 (90%) ESCC tissues. Strong immunoreactivity was observed in 13 cases, whereas 5 samples showed a medium to low LASP1 expression and 2 (10%) specimens were LASP1 negative. In contrast, all of the adjacent normal esophageal tissues were LASP1-negative. Furthermore, we found that positive staining of LASP1 was localized not only in the cytoplasm but also in the nucleolus (Fig. 3).

Efficiency of the transient transfection of siRNA-LASP1. Using a knockdown gene technique, we investigated the function of LASP1 in ECA109 and KYSE510 cells. To evaluate the efficiency of the transient transfection of siRNA-LASP1, qRT-PCR and western blot analysis were performed at 24, 48 and 72 h after transfection. Results showed that the mRNA expression of LASP1 was obviously repressed at 72 h after transfection with siRNA-LASP1, and was reduced by 30.5±3.33 and 48±2.18% in ECA109 and KYSE510 cells compared with the negative-control siRNA-transfected and non-transfected cells (P=0.006, P=0.001 and P=0.039, P=0.047, respectively) (Fig. 4). The expression of LASP1 protein was also inhibited at 72 h after transfection with siRNA-LASP1 in the ECA109 and KYSE510 cells compared with the negative-control siRNA-transfected cells (P=0.003 and P=0.001, respectively). These results demonstrated that the expression of LASP1 in

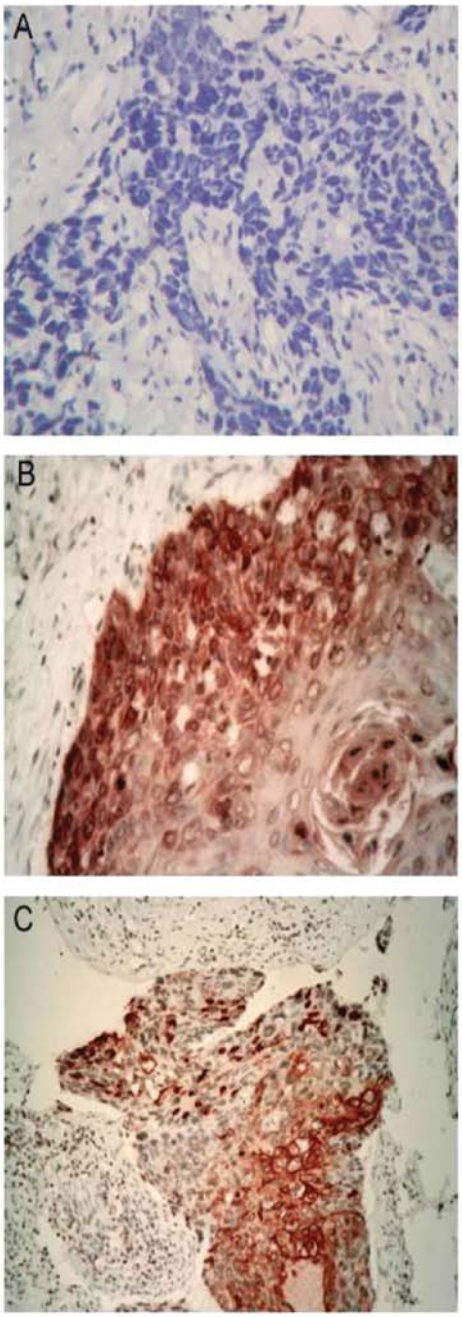

Figure 3. Histological expression pattern of LASP1 by IHC. Immunoreactivity for the positive staining of LASP1 was localized in the cytoplasm and nuclei. (A) Negative control in ESCC (PBS instead of the primary antibody as negative control); (B) strong expression of LASP1 in ESCC; (C) weak expression of LASP1 in ESCC (magnification, x400).

the ECA109 and KYSE510 cells was effectively suppressed following transfection with specific siRNA-LASP1.

Silencing of LASP1 in ESCC cells inhibits proliferation in vitro. To evaluate the effect of LASP1 on cell growth, ECA109 and KYSE510 cells were transiently transfected with either the siRNA-LASP1 or negative-control siRNA. At 24, 48 and 72 h after transfection, MTT was performed to detect the cell numbers. The results showed marked cell growth inhibition in the ECA109 and KYSE510 cell lines transfected with siRNA-LASP1 compared to the negative-control siRNA-

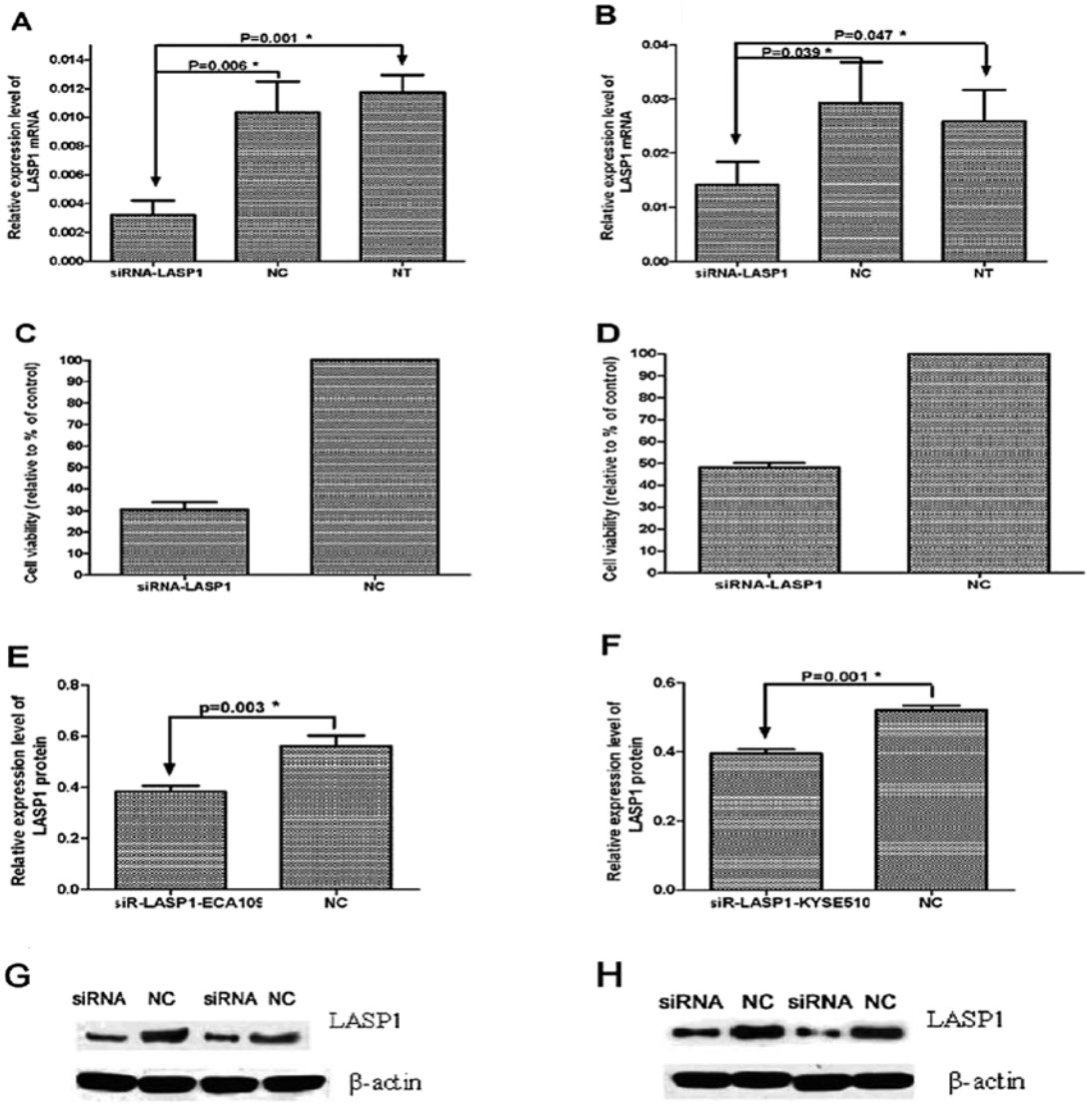

Figure 4. Expression of LASP1 in the ECA109 and KYSE510 cells was effectively suppressed by transfection with siR-LASP1. (A and C) LASP1 mRNA level was inhibited in ECA109 cells transfected with siR-LASP1 compared with NT and NC cells. (B and D) LASP1 mRNA level was inhibited in KYSE510 cells transfected with siR-LASP1 compared with NT and NC cells. (E and G) LASP1 protein level was inhibited in ECA109 cells transfected with siR-LASP1 compared with NC cells. (F and H) LASP1 protein level was inhibited in KYSE510 cells transfected with siR-LASP1 compared with NC cells. The LASP1 expression level was normalized to β-actin; *P<0.05. NT, non-transfected; NC, negative-control; siR-LASP1, siRNA-LASP1.

transfected cells. The percentage of growth suppression of the cells was 23.6% in ECA109 and 27.1% in KYSE510 cells at 72 h following transfection with siRNA-LASP1, respectively [optical density (OD), 0.674±0.019 vs. 0.882±0.033; P=0.001 and 0.847±0.017 vs. 1.162±0.079; P=0.017] (Fig. 5). These results indicated that inhibition of LASP1 prevents the proliferation of ECA109 and KYSE510 cells.

Silencing of LASP1 in ESCC cells inhibits migration and invasion in vitro. Migration and invasion experiments were performed to evaluate the role of LASP1 in ESCC cell motility and invasiveness. Cell migration assay demonstrated that silencing of LASP1 by siRNA-LASP1 in ECA109 and KYSE510 cells strongly reduced cell migration compared to the negative-control siRNA-transfected and non-transfected cells (12±3.46 vs. 28±8.76 and 28.17±9.56; P=0.002 and P=0.007; 11±3.41 vs. 23.5±6.89 and 23.67±5.79; P=0.005 and P=0.001, respectively) (Fig. 6). Cell invasion assay showed significant reduction in cell invasion in siRNA-LASP1-transfected ECA109 cells (41±3.03 vs. 69.83±10.68 and 70.67±13.74; P=0.001, P=0.003) and KYSE510 cells compare to the negative-control siRNA-transfected and non-transfected cells (20.83±4.31 vs. 53±8.81 and 54.8±6.18; P=0.000 and P=0.000) (Fig. 7). These results suggest that the inhibition of LASP1 expression inhibits the migratory ability and invasiveness of ECA109 and KYSE510 cell lines *in vitro*.

Discussion

EC is the leading cause of cancer-related death worldwide. ESCC is the most common subtype of EC in China. Since ESCC patients are associated with an increase incidence of relapse and metastasis, the prognoses of these patients are still poor despite improvements in therapeutic techniques. Yet, the

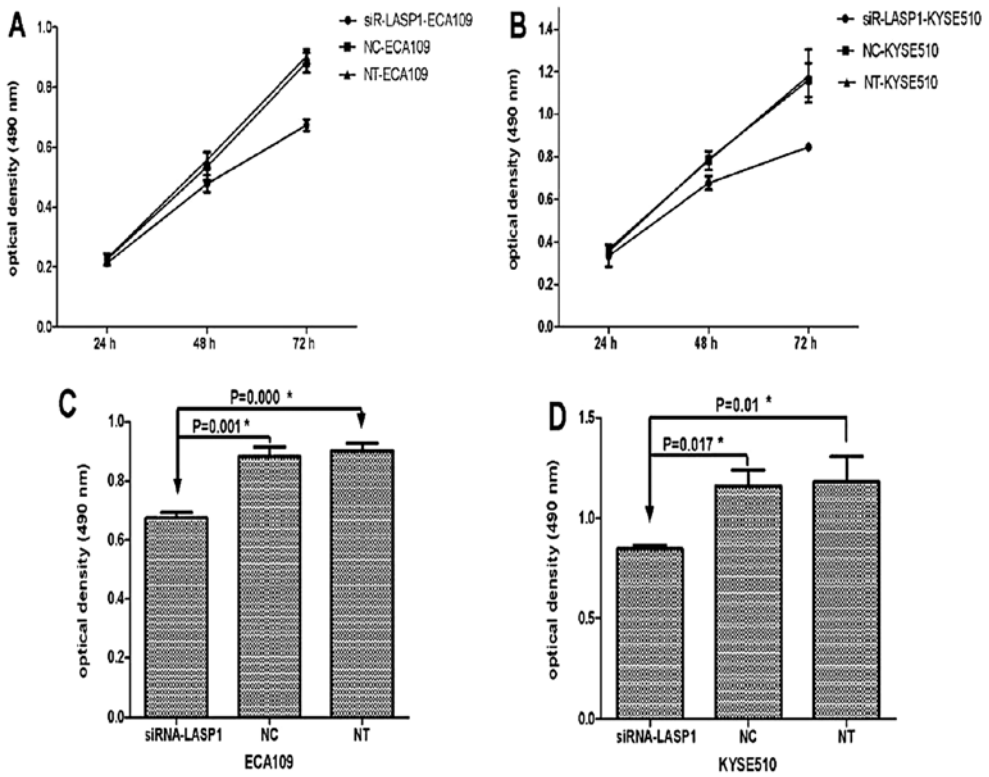

Figure 5. Silencing of LASP1 in ESCC cells inhibits proliferation *in vitro*. Two ESCC cell lines (ECA109 and KYSE510) were transfected with 75 ng of siR-LASP1 or negative-control siRNA. Cell proliferation was determined using the MTT assay at 24, 48 and 72 h after transfection in 3 different independent experiments.ve-control-siR and cells. There was significant difference in the OD at 24, 48 and 72 h in (A) ECA109 and (B) KYSE510 cells following transfection with siR-LASP compared with NT and NC cells. Marked cell growth inhibition was noted in (C) ECA109 and (D) KYSE510 cell lines after transfection with siR-LASP1 compared to NC and NT cells at 72 h. Data are presented as the means ± SD of the absorbance value [optical density (OD)] of cells. *P<0.05. NT, non-transfected; NC, negative-control; siR-LASP1, siRNA-LASP1.

mechanisms involved in the relapse and metastasis in ESCC to date have not been fully elucidated.

LASP1 is an actin-binding protein. Previous studies have revealed that the expression of LASP1 is higher in several types of cancers, and the overexpression of LASP1 plays significant roles in carcinogenesis and cancer progression. In this study, we evaluated the expression level of LASP1 in 89 ESCC tissues at the mRNA and protein levels. Results showed that LASP1 expression at the mRNA and protein levels was obviously higher in human ESCC tissues and ESCC cell lines than that in adjacent normal esophageal tissues. Simultaneously, consistent with the results of studies in other types of cancers (18), IHC demonstrated that positive staining of LASP1 was noted in the cytoplasm and nucleolus of the tumor cells. This observation indicates that LASP1 is not only a cytosolic protein, but is also a nuclear protein. This result is consistent with a previous study (18). Recent data showed that cytosolic overexpression and nuclear localization significantly correlates with tumor size and nodal-positivity in many cancers, and the postoperative relevance of LASP1 expression for prediction of nodal-positivity has a sensitivity of approximately 85% (15,18); Cserni found that LASP1 could be used as a predictive marker for lymph node metastasis together with other markers such as the superior method of sentinel lymph node biopsy with an average sensitivity of approximately 95% (19). Consequently, we hypothesized that overexpression of LASP1 plays an important role in the progression of ESCC.

Tumor cell motility is a sign of invasiveness and an essential step in metastasis (20-22). Several studies have indicated that LASP1 is an important factor for increasing the viability and motility of tumor cells and it plays important roles in proliferation, migration and invasion of several types of cancers. Inhibition of highly expressed LASP1 using siRNA in human breast and ovarian cancer cells induced a reduction in cell proliferation and migration (13,14). Furthermore, Zhao *et al* demonstrated that use of gain-of-function analysis with gene transfection-mediated overexpression of LASP-1 in SW480 CRC cells resulted in an aggressive phenotype of cancer cells and promoted cancer growth and metastasis *in vitro* and promoted an aggressive phenotypes of CRC cells *in vivo* (15). In this study, we investigated the function of LASP1 in ECA109 and KYSE510 cells using a knockdown gene technique. Our study showed that the proliferation, migration and invasion of ESCC cell lines, ECA109 and KYSE510, folowing transfection with siRNA-LASP1 were obviously reduced compared to the negative control cells. These results suggest that LASP1 may be an important factor for promoting cell viability, migratory ability and invasiveness in ESCC progression, and it may function as an oncogene. Moreover, alterations in LASP1 levels can have an impact on cell growth. Therefore, further studies with a large sample size are needed to comfirm these findings and establish the role of LASP1 in the prognosis of ESCC.

Migration and invasion are central features of the molecular pathology of malignant tumors and are the main causes

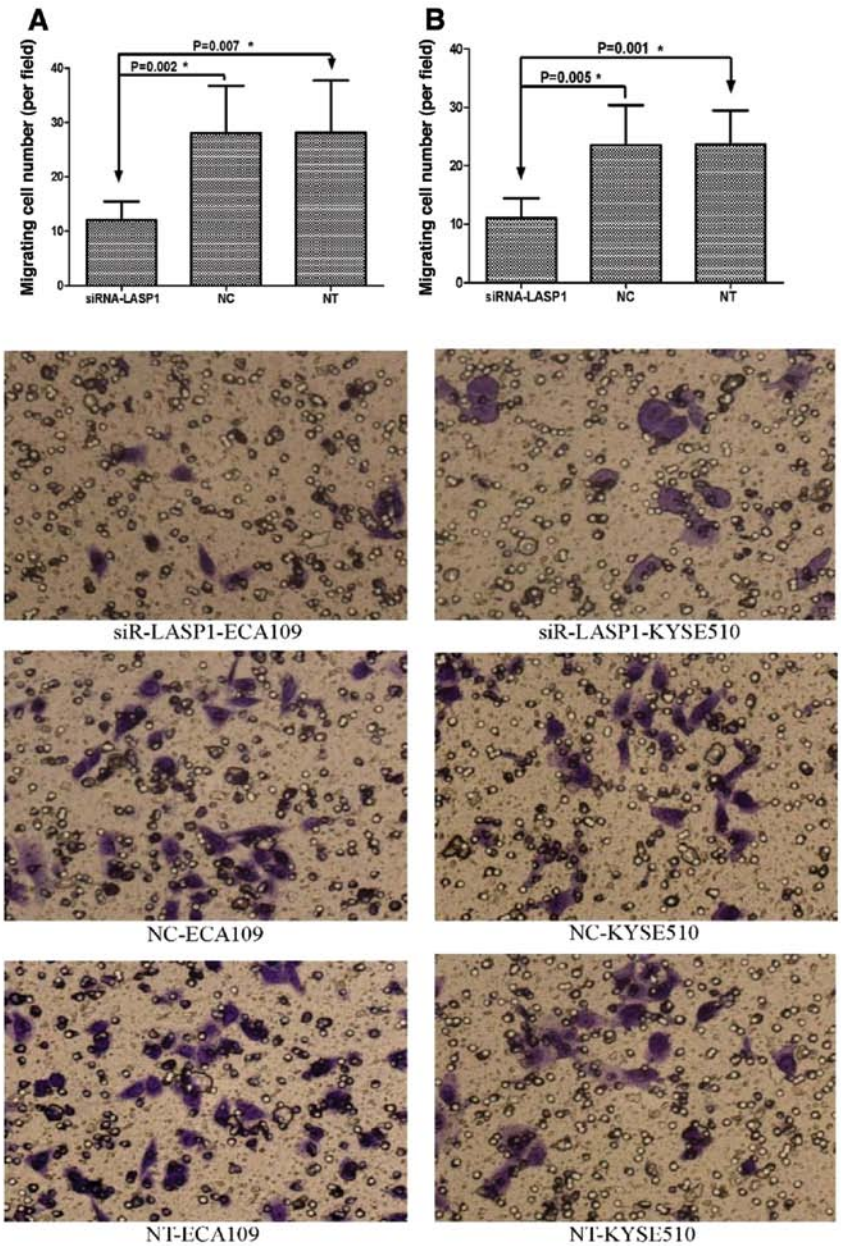

Figure 6. Silencing of LASP1 in ESCC cells inhibits migration *in vitro*. Two ESCC cell lines (ECA109 and KYSE510) were transfected with 75 ng of siR-LASP1 or control-negative-siRNA. Cell migration was evaluated by the migration assay 6 h after transfection in three different independent experiments. (A) An obvious reduction was noted in cell migration of ECA109 cells after transfection with siR-LASP1 when compared to NC-siRNA-transfected and NT cells. (B) Obvious inhibition of cell migration of KYSE510 cells was noted after transfection with siR-LASP1 when compared to NC-siRNA-transfected and NT cells. Results are expressed as the means ± SD of five randomly selected x200 magnification fields. *P<0.05. NT, non-transfected; NC, negative-control; siR-LASP1, siRNA-LASP1.

of death from malignant tumors. Alterations in cell shape are crucial in the first step of migration and invasion in the early stage of tumor progression. The changes are closely correlated with parapodium formation by cytoskeleton rearrangements and concentrations of actin-binding protein. Adhesion proteins have a crucial role in tumor growth and metastasis. To data, more than 50 different adhesion proteins that regulate the rate and organization of actin polymerization and focal adhesion turnover in protrusions have been identified. LASP1 is an actin-binding protein, of which the C-terminal SH3 domain has an important function that is involved in protein-protein interactions through binding to proline-rich sequences, specifically to lipoma preferred partner (LPP), zyxin, palladin and vasodilator stimulated phosphoprotein (VASP) (8,23,24). However, the potential molecular mechanism of LASP1 in the promotion of cell viability, migratory ability and invasiveness in carcinogenesis is unclear. The zinc-finger containing LIM domain of LASP1 is a morphologically and perhaps functionally independent folding-unit offering the possibility of direct binding to DNA (25) and it is known to be a nuclear shuttle protein involved in cell cycle control and cell migration (26,27). LASP1 silencing is accompanied by reduced binding of the LASP1-binding partner zyxin which is necessary for proper cell migration

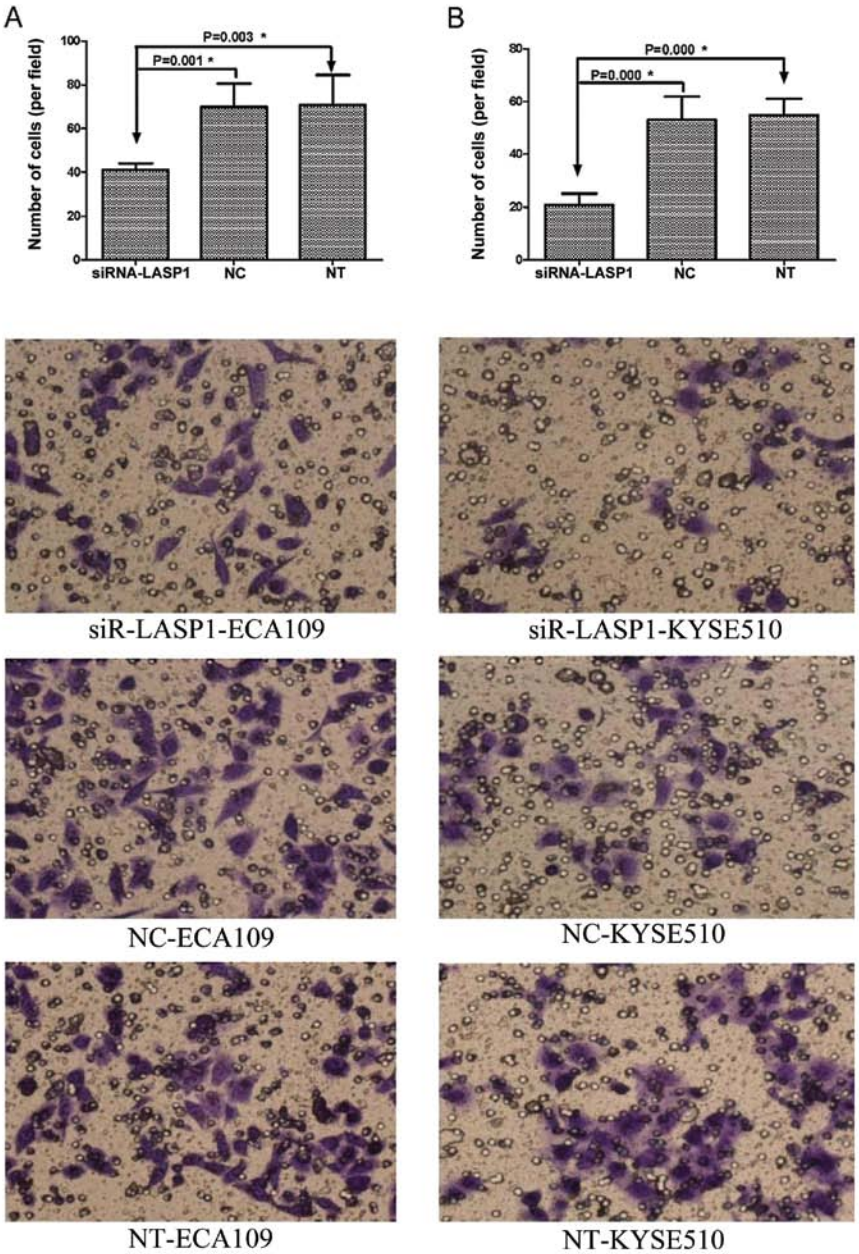

Figure 7. Silencing of LASP1 in ESCC cells inhibits invasion *in vitro*. Two ESCC cell lines (ECA109 and KYSE510) were transfected with 75 ng of siR-LASP1 or control negative-siRNA. Cell invasion was evaluated by the invasion assay 18 h after transfection in three different independent experiments. (A) An obvious inhibition was noted in the cell invasiveness of ECA109 cells after transfection with siR-LASP1 when compared to NC-siRNA-transfected and NT cells. (B) An obvious inhibition was noted in cell invasiveness of KYSE510 cells after transfection with siR-LASP1 compared to NC-siRNA-transfected and NT cells. Results are expressed as the means ± SD of five randomly selected x200 magnification fields *P<0.05. NT, non-transfected; NC, negative-control; siR-LASP1, siRNA-LASP1.

and growth possibly through influencing zyxin localization (13,14). Mutation analysis of LASP1 demonstrated that its SH3 domain is necessary for pseudopodial extension and invasion (28,29). However, in the present study, we did not investigate the mechanism of inhibition of cell growth and motility after LASP1 silencing. Based on the LASP1 molecular mechanism and previous data, we presumed that inhibition of LASP1 may change the assembly of cytoskeletal proteins, causing a reduction in cell proliferation, migration and invasion in particular actin microfilaments and in filopodia. The potential molecular mechanisms of LASP1 in carcinogenesis require further research.

Our study firstly observed that LASP1 was overexpressed in ESCC. Using a knockdown gene technique, we found that silencing of LASP1 reduced cell proliferation, migration and invasion in ESCC cell lines *in vitro*. Our study contributes to the growing understanding of the role of LASP1 in the pathogenesis of ESCC. Further prospective studies are necessary to define the potential of migration and invasion of LASP1 in carcinogenesis.

Acknowledgements

The authors would like to thank Yerong Hu for her help. We appreciate support from the Hunan Provincial Health Department Science Foundation (B2007041 to N.Y.), Hunan Provincial Nature Science Foundation (2011TT2058 to N.Y.) and Natural Science Foundation of Changsha (K0803130-31 to NY).

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