Tumor-Suppressive Functions of Leucine Zipper Transcription Factor–Like 1

Qun Wei1,3, Wen Zhou3, Weining Wang3, Boning Gao4, Linbo Wang1, Jiang Cao2, and Zhi-Ping Liu3,5

Abstract

Human leucine zipper transcription factor–like 1 (LZTFL1) is a novel gene with unknown biological functions. It is located in the chromosome region 3p21.3, a hotspot for tumor suppressor genes. To understand the biological functions of LZTFL1, we surveyed the expression level of LZTFL1 in tumor and normal samples in tissue microarrays and a clinical archive of 84 gastric cancer specimens using immunohistochemistry. We found that LZTFL1 is expressed highly in the epithelial cells of normal tissues and is significantly downregulated in the corresponding tumor samples. The expression level of LZTFL1 correlated significantly with the survival outcomes of the patients and had significant inverse correlation with tumor metastasis. Overexpression of LZTFL1 in tumor cells inhibited anchorage-independent cell growth and cell migration in vitro and repressed tumor growth in vivo. Furthermore, we show that LZTFL1 expression is upregulated on epithelial cell differentiation and is graded along the crypt-villus axis of the intestine, with weakest expression level in the proliferative zone of the crypt and highest expression level at the apex of the differentiation zone in the villus. Expression of LZTFL1 overlaps with that of E-cadherin at the plasma membrane. Our results indicate that LZTFL1 is a tumor suppressor and that loss of LZTFL1 expression has significant clinical outcomes. LZTFL1 expression may serve as an independent prognostic marker for survival outcome of gastric cancer patients. We propose that LZTFL1 may inhibit tumorigenesis by stabilizing E-cadherin–mediated adherens junction formation and promoting epithelial cell differentiation.

Introduction

Chromosomal abnormalities at the 3p21.3 region are frequent and early events in the formation of human tumors of the lung, kidney, head and neck, breast, cervix, and gastrointestinal tract (1, 2). These abnormalities range from homozygous deletions and loss of heterozygosity to loss of protein expression, suggesting the presence of tumor suppressor genes (TSG) in this region. As TSGs offer opportunities for molecular cancer therapy, there have been intense efforts during the past decades to identify candidate 3p21.3 TSGs and to characterize their biological functions. A critical region of ∼120 kb in the 3p21.3 centromeric border (also called LUCA region) has been identified and contains nine candidate TSGs: HYAL2, HYAL1, FUS1, RASSF1, BLU, NPRL1, HYAL3, LRDC, and C15orf20. The functions of many of these gene products are being elucidated and they seem to be involved in a wide spectrum of biological processes, including cell proliferation, cell cycle kinetics, signaling transduction, ion exchange and transportation, and cell death. The 3p21.3 region also contains other candidate TSGs outside the LUCA region, but they are less well studied.

In an effort to discover potential TSGs in the 3p21.3 region, Kiss and colleagues (3) identified and cloned leucine zipper transcription factor–like 1 (LZTFL1) through an elimination test and subsequent genomic sequencing and cDNA cloning. LZTFL1 is located ∼5 Mb from the LUCA region on the telomeric end of the 3p21.3 region. Northern blot analysis indicates that LZTFL1 mRNA is expressed ubiquitously in both human and mouse. The open reading frame from human and mouse cDNAs revealed a protein of 299 amino acids with a molecular weight of 34.6 kDa. The sequence analysis suggested that LZTFL1 shares 90.6% sequence identity between human and mouse. The open reading frame from human and mouse cDNAs revealed a protein of 299 amino acids with a molecular weight of 34.6 kDa. The sequence analysis suggested that LZTFL1 shares 90.6% sequence identity between human and mouse. LZTFL1 contains a basic region, a coil domain, and a leucine zipper domain, suggesting that LZTFL1 may be a transcription factor (3, 4). However, the biological and molecular function of LZTFL1 remains to be determined.

The loss of differentiation in cancer cells is often associated with tumor progression, but the underlying causes and mechanisms remain poorly understood. The majority of human solid tumors are carcinomas that originated from various epithelial cell types. Differentiated carcinomas are composed of cohesive polarized epithelial cells connected to one another...
by intercellular adherens junctions. E-cadherin is the core molecule of adherens junctions (5). The cytoplasmic tail of E-cadherin is indirectly linked to the actin cytoskeleton through catenins, including α- and β-catenin, and other associated proteins. The attachments of E-cadherin to the cytoskeleton, hence associated proteins in the adherens junction, are essential for maintaining the differentiated state of epithelial cells and the apico-basal polarity of the epithelium. Disruption of the adherens junction can generate invasive mesenchymal cells through a process called epithelial-mesenchymal transition (EMT), which converts polarized, immotile epithelial cells to motile invasive mesenchymal cells. EMT has been proposed to be a potential mechanism for carcinoma metastases (6, 7). Loss of membranous E-cadherin can also increase the cytoplasmic pool of β-catenin, which can then translocate to the nucleus and activate genes that promote cell proliferation and EMT.

In the present study, we sought to test whether LZTFL1 functions as a tumor suppressor. We asked three experimental questions. First, is LZTFL1 expression downregulated in tumors and does the loss of LZTFL1 expression have any clinical significance? Second, can LZTFL1 gain-of-function inhibit tumor growth? Finally, what is the potential mechanism(s) behind LZTFL1 inhibition of tumor cell growth? Our results reveal that LZTFL1 is a tumor suppressor and may inhibit tumor growth and metastases by stabilizing E-cadherin–mediated adhesive function, thereby inhibiting EMT.

Materials and Methods

Plasmids. The expression vector of LZTFL1 (pcDNA-Flag-LZTFL1) was constructed by subcloning a PCR-amplified insert corresponding to the mouse LZTFL1 open reading frame (Invitrogen). The pTRE2-LZTFL1-ires-enhanced green fluorescent protein (EGFP) plasmid was constructed by a two-step cloning through PCR and restriction enzyme digestion; the Flag-LZTFL1 fragment from pcDNA-Flag-LZTFL1 was first subcloned into the pIres-EGFP vector (Clontech) to yield the pLZTFL1-ires-EGFP vector (Clontech). The glutathione S-transferase (GST)-LZTFL1 construct (pGEX-kg-LZTFL1) was constructed by subcloning the PCR-amplified LZTFL1 fragment into the pGEX-kg vector. Construction details are available on request. The sequences of all cloning products were verified using an automated sequencer.

Generation of LZTFL1-specific antibody. Recombinant GST-LZTFL1 protein was produced and purified according to a standard protocol (8). After being cleaved and separated from the GST protein, full-length LZTFL1 protein was used as the antigen to immunize rabbits (Cocalico Biological).

Cell lines, transfection, and siRNA knockdown. Human intestinal epithelial cell line HT-29 and human breast cancer cell line MCF-7 (American Type Culture Collection) were maintained in complete medium [DMEM supplemented with 10% fetal bovine serum (FBS), 2 mmol/L glutamine, and penicillin-streptomycin]. Cells were transfected with a combination of plasmids indicated for each experiment using Lipofectamine 2000 according to the manufacturer’s protocol (Invitrogen). To induce cell differentiation, HT-29 cells were cultured in complete medium supplemented with 3 mmol/L sodium butyrate (NaB) for 3 d. For stable transfection, HT-29 cells were transfected with the pLZTFL1-ires-EGFP and pIres-EGFP control vectors. Cells were selected with G418 for 3 to 4 wk.

Three independent LZTFL1-specific siRNA duplexes in TriFecta Kit were purchased from IDT Technology and transfected into C2C12 cells using Lipofectamine 2000 (Invitrogen). FITC-labeled nonsilencing duplex siRNA was used as the negative control.

Generation of HeLa-Tet-On cells with doxycycline-inducible expression of LZTFL1. HeLa-tet-on cells expressing the rTA (Invitrogen) were maintained in complete medium containing 0.5 mg/mL G418. The cells were transfected with either pTRE2-LZTFL1-ires-EGFP or control pTRE2-ires-EGFP vector using Lipofectamine 2000. Green fluorescent protein (GFP) was used here as a reporter for the ease of selection. Transfected cells were double selected with hygromycin (0.1 mg/mL) and G418 (0.5 mg/mL). The resistant clones were screened for the induction of GFP expression on addition of 1 μg/mL doxycycline by live fluorescence imaging.

Tissue microarray, immunohistochemistry, and data analysis. Tissue microarrays were from IMAGEA. Slides IMH-326 and IMH-327 contained a total of 118 samples of common tumors/cancers. IMH-336 and IMH-337 contained corresponding matched normal/normal adjacent tissues of IMH-326 and IMH-327, respectively. Each individual tumor array contained 8 to 10 cases. Clinical and pathologic information for individual cancer samples was provided by the array manufacturers. Immunohistochemical (IHC) staining was carried out following the manufacturer’s protocol using LZTFL1 antibody as the primary antibody followed by biotinylated secondary antibody and streptavidin-horseradish peroxidase. Diaminobenzidine was used as the substrate chromagen and slides were counterstained with hematoxylin. Staining intensity (0, no staining; 1, weak staining; 2, moderate staining; and 3, intense staining) and the proportion of stained cells (0, no staining; 1, <10% staining; 2, between 11% and 33%; 3, between 34% and 66%; and 4, >67%) were semiquantitatively determined following published protocols (9). All slides were scored by two observers blinded to the pathology and clinical features. Student’s t-test was used for statistical analysis. P < 0.05 was considered statistically significant.

Tumor samples, immunohistochemistry, patients’ data, and statistical analysis. Paraffin-embedded tumor and corresponding normal tissue sample slides from 84 gastric cancer patients were stained with LZTFL1 antibody using immunohistochemistry (IHC) and the slides were scored as described above. Normal tissue structures near the cancerous ones in the same histologic section or normal corresponding tissue from the same individual in a separate slide was used as a positive control. The clinical data were obtained from the Department of Surgical Oncology, Sir Run Run Shaw Hospital, China. The patients were enrolled...
between July 1995 and March 2007. All patients underwent a curative gastrectomy and none of the patients received pre-operative treatments. Total gastrectomy was performed in 10 patients and subtotal gastrectomy was performed in 74 patients. Post-surgery pathologic examination showed various tumor types including papillary, mucinous, signet ring, and mucinous adenocarcinoma. All clinical pathologic profiles were evaluated in accordance with the criteria of WHO. Tumor stage was evaluated according to the tumor-node-metastasis (TNM) classification of the 6th edition criteria of the International Union Against Cancer.

The patients were followed up until death or until the date of last follow-up on November 30, 2007, and no patient had been lost to follow-up. Thirty-three of 84 patients (39.2%) died during the follow-up period, and the median follow-up interval was 50.6 mo (range, 2.8–119.2 mo). Informed consent from patients was obtained for the use of paraffin-embedded tumor and normal tissues. This study was approved by the ethics committee of Sir Run Run Shaw Hospital.

For the correlation between LZTFL1 IHC score and clinical parameters, a Pearson r correlation coefficient was calculated using the GraphPad Prism Software (GraphPad Software, Inc.). Two-tailed parametric analysis was considered significant when \( P < 0.05 \). Survival curves were estimated by the Kaplan-Meier method with log-rank test using the GraphPad Prism program.

**Soft-agar assays and tumorigenicity assays in athymic nude mice.** For soft-agar assays, cell aliquots in growth medium mixed with 0.35% soft agar were plated in triplicate onto a 12-well plate with a 0.5% semisolid agar basal layer. For HT-29 and MCF-7 cells, fresh medium was added every 7 d. For HeLa-tet-on cells, fresh media with and without

![Figure 1](cancerres.aacrjournals.org/a requeriments. This study was approved by the ethics committee of Sir Run Run Shaw Hospital.

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doxycycline were added every 3 d. After 4 wk of incubation at 37°C, colonies in the soft agar were photographed and scored under an inverted microscope. For tumorigenicity assays in nude mice, we injected a suspension of 1 × 10^7 cells in 0.2-mL PBS s.c. into the flank of 6-wk-old female BALB/C athymic nude mice (National Cancer Institute). Mice were scored for tumor development and tumor size at each site of injection. Mice were sacrificed and the tumors were weighed when the largest tumor reached a maximal diameter of 2 cm (at the end of 5 wk after injection). All animal experiments were done in accordance with institutional guidelines.

Western blot and immunofluorescence analyses and antibodies. Western blot and immunofluorescence were done according to standard protocols. Antibodies used in this study are anti-Flag (Sigma), anti-E-cadherin (Cell Signaling), anti–β-catenin and anti–glyceraldehyde-3-phosphate dehydrogenase (Santa Cruz Biotechnology), and anti-LZTFL1 (in house). Confocal immunofluorescence images were taken on a Zeiss LSM510.

In vitro migration assay. Parental, EGFP-expressing, and LZTFL1-expressing Heta-tet-on cells were cultured in the presence and absence of doxycycline for 48 h, washed, trypsinized, and suspended in DMEM plus 0.1% bovine serum albumin (BSA). Approximately 1 × 10^5 cells in 100 μL of DMEM/0.1% BSA were placed into the upper chamber of a 24-well Transwell cell culture plate (Corning Costar). The lower chamber contained DMEM with 5% FBS as a chemoattractant. After 8 h of incubation, migrated cells on the lower surface were stained with hematoxylin and counted under a microscope.

Results

LZTFL1 expression is downregulated in human tumors. To study the biological function of LZTFL1, we first generated and affinity purified a rabbit polyclonal antibody against LZTFL1 using bacterially derived full-length mouse LZTFL1 as the antigen. This antibody recognized endogenous and overexpressed LZTFL1 specifically in both Western blot and IHC (Supplementary Fig. S1). Using this antibody, we surveyed the expression of LZTFL1 in various normal human tissues and their corresponding cancer samples by IHC analysis of tissue microarrays. Intense LZTFL1 staining was visible in epithelial cells of normal tissues of breast, esophagus, pancreas, stomach, ovary, prostate, lung, colon, thyroid, kidney, bladder, and liver (Fig. 1A). In almost all the corresponding invasive carcinoma samples, only diffused, low levels of LZTFL1 staining were observed. Analysis of multiple cases in each individual type of normal and matched cancer samples in the tissue microarray showed that LZTFL1 was significantly downregulated in the aforementioned human tumors (Fig. 1B).

Table 1. Patients and tumor characteristics stratified by LZTFL1 expression

<table>
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<th>Feature</th>
<th>No. of patients</th>
<th>Pearson r</th>
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<td>LZTFL1 IHC score</td>
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<td>&gt;4</td>
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<td>Death</td>
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Abbreviation: mLN, metastasized lymph nodes.
Downregulation of LZTFL1 expression in gastric cancer patients correlates with poorer survival outcome. To address the clinical significance of downregulation of LZTFL1 in cancers, tissue samples from a cohort of 84 patients diagnosed with stomach cancer between the ages of 31 and 79 years were screened by IHC for LZTFL1 expression. Patients’ characteristics are summarized in Table 1. IHC staining was quantitatively scored on a 0 to 12 scale based on the product of the staining intensity (0–3; Fig. 2A) and the percentage of stained area (0–4; data not shown) as described in Materials and Methods.

LZTFL1 IHC scores and the clinical parameters for the cohort under study were tabulated and their correlations were analyzed for their statistical significance (Table 1). The loss of LZTFL1 expression was found to have significant inverse correlation with the TNM stage of the tumor (Pearson $r = -0.4117$; $P = 0.0001$, two-tailed; Fig. 2B, left) and with the number of metastasized lymph nodes (Pearson $r = -0.3229$; $P = 0.0027$, two-tailed; Fig. 2B, middle). No significant differences in LZTFL1 IHC scores were found for age, gender distribution, or tumor classifications (Table 1).

LZTFL1 expression level correlated significantly with survival time as well (Pearson $r = 0.3805$; $P = 0.0004$; Fig. 2B, right). The overall survival was significantly better for patients with tumors showing moderate or strong LZTFL1 expression (IHC score $>4$) than for those whose tumors showed negligible or weak expression (IHC score $\leq 4$; $P = 0.0002$, log-rank test; Fig. 2C, left). The median survival for patients with an IHC score of $<4$ was 32.8 months. Although the median survival was not achieved for patients with an IHC score of $>4$, patients in this group have a lower risk of death with hazard ratio of 0.22 (95% confidence interval, 0.1052–0.4175). When analyzed according to the stratified expression levels, patients with weak LZTFL1 expression (IHC score $<2.5$) were found to have the worst median survival (29.3 months; $P < 0.0001$, log-rank test). There is a significant trend toward longer survival times with higher LZTFL1 expression levels ($P < 0.0001$, log-rank test; Fig. 2C, right).

LZTFL1 inhibits tumor cell growth in vitro and in vivo. To determine whether LZTFL1 plays a direct role in tumorigenesis, we performed gain-of-function studies to test whether the increased level of LZTFL1 expression in tumor cells can inhibit tumor cell growth. We used an inducible expression system to induce LZTFL1 expression through the addition of doxycycline in cultured HeLa-tet-on cells that constitutively produce the reverse tetracycline transactivator. Three clonal cell lines were obtained (Fig. 3A). Clones LZTFL1-29 and LZTFL1-32 had minimal basal expression of LZTFL1, whereas clone LZTFL1-10 showed weak LZTFL1 expression. Clones LZTFL1-10, LZTFL1-32, and LZTFL1-29 had highest, modest, and weakest inducible LZTFL1 expression on addition of doxycycline, respectively. Clones LZTFL1-32 and LZTFL1-10 were chosen for further experiments.

Figure 2. A, representative micrographs showing typical examples of the four intensity grades of LZTFL1 staining in tumor samples of the cohort of 84 gastric cancer patients. B, correlations between LZTFL1 IHC score and TNM stage (left; Pearson $r = -0.4117$; $P = 0.0001$, two-tailed; bars, average IHC score for each individual TNM stage), number of metastasized lymph nodes (mLN; middle; Pearson $r = -0.3229$; $P = 0.0027$, two-tailed; not all the data points are in view because the majority of the patients have $<10$ metastasized lymph nodes), and survival times (right; Pearson $r = 0.3805$; $P = 0.0004$, two-tailed) in the cohort of patients in this study. C, Kaplan-Meier survival curves according to LZTFL1 IHC scores for negligible and weak (IHC score $\leq 4$) versus moderate and strong expression (IHC scores $>4$; left; $P = 0.0002$, log-rank test) and the individual stratified IHC scores (right; $P = 0.0002$, log-rank test). $n$, number of patients.
We first investigated the effect of LZTFL1 expression on the growth phenotype of HeLa-tet-on cells under adherent conditions in monolayer cultures using MTT and flow cytometry assays. No significant differences were observed between HeLa-tet-on-LZTFL1 cells in the absence and presence of doxycycline and between LZTFL1-expressing cells and parental HeLa-tet-on cells (data not shown). We next asked whether LZTFL1 has any effect on tumor cell growth under anchorage-independent conditions in soft-agar assays. A large number of colonies of uninduced cells were visible within 4 weeks (Fig. 3B, top). The LZTFL1-expressing cells in both LZTFL1-32 and LZTFL1-10 clones showed dramatically reduced numbers of colonies on addition of doxycycline (Fig. 3B, bottom). As controls, the numbers of colonies in HeLa-tet-on and HeLa-tet-on-EGFP cells with and without doxycycline and in HeLa-LZTFL1 cells without doxycycline were similar, suggesting that LZTFL1 indeed specifically inhibited the anchorage-independent growth of tumor cells. We also tested whether overexpression of LZTFL1 inhibits the colony formation ability of other tumor cells, including intestinal epithelial carcinoma HT-29 cells and breast carcinoma MCF-7 cells. We observed similar inhibitory effects of LZTFL1 in these cells (data not shown; Fig. 3C).

Because downregulation of LZTFL1 in human gastric tumors correlated with tumor metastases in patients, we next
investigated a role for LZTFL1 in cell migration. Upregulation of LZTFL1 in HeLa-LZTFL1-10 and HeLa-LZTFL1-32 on doxycycline induction significantly reduced the migration properties of HeLa cells in Transwell assays (Fig. 3D, lanes 6 and 8). In negative controls, doxycycline had no effect on the migration of parental or EGFP-expressing HeLa-tet-on cells. The number of migrated cells is similar among parental or EGFP-expressing HeLa-tet-on cells with or without doxycycline, and HeLa-LZTFL1-10 and LZTFL1-32 cells without doxycycline (Fig. 3D, lanes 1-4, 5, and 7).

To further test whether overexpression of LZTFL1 results in the suppression of tumor growth in vivo, we injected s.c. HeLa-tet-on and LZTFL1-32 cells into the flank of nude mice. We chose the LZTFL1-32 clone to avoid any artifact that might arise due to overexpression because the level of induction of LZTFL1 in this clone is similar to the physiologic level of LZTFL1 in differentiated HT-29 cells (see Fig. 5 for details). As expected, at the end of 5 weeks, mice injected with HeLa-tet-on cells developed large tumors (Fig. 4A). The tumor size in LZTFL1-32–injected mice that were given doxycycline in drinking water for induction of LZTFL1 was significantly reduced compared with that in mice that were not given doxycycline (Fig. 4B). As doxycycline in drinking water had little effects on the tumor size of the mice injected with HeLa-tet-on cells, our results suggest that LZTFL1 inhibited tumor growth significantly in vivo. It is noted that tumor sizes in mice with uninduced LZTFL1-32 cells, although not statistically significant, are smaller compared with those in mice with HeLa-tet-on cells. This is probably due to the leaky expression of LZTFL1, as shown in the Western blot of tumor lysates with long exposure time (Fig. 4C, middle).

**LZTFL1 expression is upregulated on epithelial cell differentiation and colocalizes with E-cadherin at the plasma membrane.** It has been shown that many TSGs are inactivated in cancer by epigenetic silencing induced by aberrant methylation of CpG islands in the promoter region of the TSG or by overexpression of histone deacetylases (HDAC; refs. 10–13). To understand the mechanism of LZTFL1 inactivation in tumor cells, we treated HT-29 cells with 5′-aza-2′-deoxycytidine, a DNA methylation inhibitor, and NaB, a HDAC inhibitor, respectively. No difference of LZTFL1 expression was observed between 5′-aza-2′-deoxycytidine–treated and nontreated cells (data not shown), whereas NaB treatment increased the level of LZTFL1 expression (Fig. 5A, left). Other HDAC inhibitors had similar effects on the upregulation of LZTFL1 expression in HT-29 cells (Fig. 5A, right). These results suggest that LZTFL1 is inactivated in HT-29 cells by alterations in chromatin structure.

NaB is a naturally occurring compound in the intestine and induces differentiation of epithelial cells in culture (14, 15). Upregulation of LZTFL1 in NaB-treated HT-29 cells suggests that the expression level of LZTFL1 may be correlated with the differentiation status of the cell. To test this hypothesis in vivo, we stained the mouse small intestine with anti-LZTFL1 antibody along the crypt-villus axis. The intestinal epithelium undergoes a constant self-renewing process. The stem cells in the crypt give rise to an intermediate cell population that undergoes rapid proliferation and differentiation as they migrate toward the apex of the villus (16). Indeed, a graded expression of LZTFL1 along the crypt-villus axis was observed, with minimal staining of LZTFL1 in the crypt and maximum staining at the apex of the villus (Fig. 5B). Next, we performed colocalization studies of LZTFL1 with E-cadherin/β-catenin using confocal immunofluorescence microscopy. Expression of LZTFL1 overlaps with that of E-cadherin at the plasma membrane in differentiated normal colonic epithelial cells (Fig. 5C). This colocalization was absent in colorectal carcinomas due to a loss of LZTFL1 protein expression.

Colocalization of LZTFL1 with E-cadherin suggested that LZTFL1 may stabilize E-cadherin–mediated adherens junction. Indeed, we observed that, when treated with phorbol 12-myristate 13-acetate (PMA), a known scatter factor to break down the epithelial tight junction (17), the LZTFL1-expressing HT-29 cells are more resistant to PMA-induced cell scattering than EGFP-expressing or parental HT-29 cells (Fig. 5D).
LZTFL1 is a novel gene with unknown biological function. Although it was predicted to have a tumor-suppressive function based on its genomic location at the 3p21.3 region, there was no experimental evidence for this hypothesis. In this study, we present the first biochemical and functional evidence supporting a function for LZTFL1 in tumor suppression. LZTFL1 is highly expressed in epithelial cells of a wide array of normal tissues. Its expression is downregulated significantly in the corresponding tumor samples (Fig. 1). Clinically, we found that downregulation of LZTFL1 correlated significantly with tumor metastases and predicted poorer survival outcome in gastric cancer patients (Fig. 2). Restoration of LZTFL1 expression in tumor cells inhibited anchorage-independent cell growth and cell migration in vitro (Fig. 3) and tumor growth in vivo (Fig. 4). These data define LZTFL1 as a tumor suppressor. How does LZTFL1 inhibit tumorigenesis?

LZTFL1 may inhibit cell proliferation by promoting its differentiation. This is based on the experimental observations in Fig. 5; LZTFL1 is upregulated in differentiated epithelial cells and colocalizes with E-cadherin at the plasma membrane. The epithelium is composed of epithelial cells that are polarized and cohesively connected through E-cadherin–mediated adherens junctions. E-cadherin is attached to the actin cytoskeleton through a protein complex containing α- and β-catenin and other associated proteins. The stability of the attachment is essential for maintaining the epicol-basal polarities and, therefore, the fully differentiated state of epithelial cells. We observed that LZTFL1 can bind actin in vitro. Thus, LZTFL1 could serve as a component of the protein complex at the adherens junction and bridge E-cadherin and the actin cytoskeleton. Loss of LZTFL1 could destabilize the E-cadherin–mediated adhesive complex and promote epithelial cell dedifferentiation. In support of this hypothesis, we observed that LZTFL1-overexpressing HT-29 cells are more resistant to PMA-induced cell scattering than HT-29 cells. A detailed biochemical analysis of the interaction between LZTFL1 and E-cadherin–associated protein complex at adherens junction is needed in the future to validate this hypothesis.

LZTFL1 may inhibit carcinoma metastases by inhibiting the EMT. For carcinoma to metastasize, the tumor epithelial

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Unpublished results.
cell has to go through EMT to break away from its neighbors, lose E-cadherin–mediated cell-cell contacts, and gain migratory properties and other mesenchymal cell traits. Destabilization of E-cadherin–mediated adherens junction due to a loss of LZTFL1 would subject the tumor cells more susceptible to EMT in response to signals from host stroma, whereas upregulation of LZTFL1 could enable the cells to resist it. Consistent with this idea, we found that down-regulation of LZTFL1 in gastric tumors correlated with carcinoma metastases, whereas upregulation of LZTFL1 in tumor cells inhibited cell migration and anchorage-independent growth (Figs. 2 and 3), a hallmark of tumor cell transformation.

Although LZTFL1 has several structural features that are shared by many transcription factors (4), the biochemical evidence remains to be established. It is conceivable that LZTFL1 may translocate into the nucleus in response to certain external signals and function as a transcriptional cofactor. The membrane and cytoplasmic localization of LZTFL1 favors the hypothesis that LZTFL1 is a cytoplasmic adaptor that participates in cell proliferation/differentiation.

In summary, our studies underscore the importance of LZTFL1 as a human tumor suppressor protein and provide mechanistic insights into the role LZTFL1 in tumor suppression. In the future, it will be worthy to validate its prognostic value in a separate group of patients and to study the effect of the loss of function of LZTFL1 in tumorigenesis and its interplay with other oncogenic signaling pathways.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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