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Original Articles

The tumor-suppressor gene LZTS1 suppresses colorectal cancer proliferation through inhibition of the AKT–mTOR signaling pathway

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ABSTRACT

The Leucine zipper tumor suppressor gene 1 (LZTS1/FEZ1) gene was originally identified as a potential tumor suppressor. However, the expression pattern and the role of LZTS1 in the progression of colorectal cancer (CRC) have not been well characterized. Herein, we reported that LZTS1 was markedly reduced in CRC tissues compared with matched adjacent normal intestine epithelial tissues. In analysis of 160 CRC specimens, we revealed that decreased expression of LZTS1 was correlated to aggressive characteristics and poor survival of patients with CRC. Moreover, we found that expression of LZTS1 in CRC cells significantly inhibited cell proliferation in vitro and prohibited tumor growth in vitro. On the contrary, silence of LZTS1 promoted cell proliferation and tumor growth in CRC cells. Furthermore, we demonstrated that LZTS1 inhibited cell proliferation and tumor growth in CRC in part via suppression of AMT–mTOR, subsequently down-regulating p27Kip and up-regulating cyclin D1. These findings suggest that LZTS1 plays a potential tumor suppressor role in CRC progression and represents a valuable clinical prognostic marker of this disease.

Introduction

Colorectal cancer (CRC) is the third most prevalent malignancy worldwide. Although improvements have been made in diagnosis and synthetic therapy methods, the outcome of patients with late stage CRC remains pessimistic [1]. Genetic and epigenetic alterations of oncogenes and tumor suppressors have been found in CRC [2]. However, the power of many existing biomarkers in early diagnosis or predicting the clinical outcome of individual tumors is limited due to the great heterogeneity of this cancer. Thus, identification of the molecular mechanism that is responsible for the initiation and progression of CRC could help to identify potential biomarkers which may facilitate efficient predictive and therapeutic strategies.

Chromosome 8p is considered to be a strong candidate for the location of a tumor suppressor gene inactivated in colorectal cancer and the 8p22-p21.3 region is frequently deleted in CRC [3,4]. The Leucine zipper tumor suppressor gene 1 (LZTS1/FEZ1) gene is located at 8p22 and encodes a 67 kDa leucine zipper protein [5,6]. Studies have suggested that LZTS1 plays an important role in human carcinogenesis by suppressing tumor formation in mice since the LZTS1 deficient mice develop cancers with diverse histogenetic backgrounds [7,8]. LZTS1 was ubiquitously expressed in normal tissues, while its expression was frequently downregulated or deficient in different epithelial originated tumors, including those derived from prostate, stomach, lung, bladder, oral, and breast [5,6,9–15]. More importantly, the reintroduction of LZTS1 suppresses cell growth and tumorigenicity in breast, prostate, and bladder cancer cells probably through inhibition of cell cycle progression by regulating cell cycle regulators CDC25C and cdk1 [6,11,16]. On the contrary, the absence of LZTS1 leads to impaired chromosome segregation, cell growth, cell transformation, and cancer progression [8]. These findings suggest that LZTS1 acts as a tumor suppressor gene. However, the expression pattern and biological function of LZTS1 in CRC progression have not been characterized. In this study, we investigated

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the expression and potential role of LZTS1 in the development and progression of CRC.

Materials and methods

Patients and specimens

For the use of clinical materials for research purposes, prior approval was obtained from the Southern Medical University Institutional Board (Guangzhou, China). A total of 160 archived, formalin-fixed paraffin-embedded human colorectal carcinoma samples were obtained from the Department of Pathology, NanFang hospital, Southern Medical University, China. All of the cases were clinically and histologically diagnosed between 2004 and 2007. The stage of disease was determined according to the lymph node and metastasis (pTNM) classification system [17]. The patients included 92 males and 68 females, ranging in age from 25 to 69 years-old (mean, 54 years). The median follow-up time for overall survival was 71 months for patients still alive at the time of analysis (ranged, 2–87 months). A total of 52/160 (32.5%) patients died during follow-up. The 11 freshly collected colorectal cancer tissues and paired normal mucosal tissue specimens taken from sites distant to the cancerous lesion were obtained from patients with CRC who underwent surgical resection, frozen and stored in liquid nitrogen till further use.

Cell cultures

The human CRC cell lines SW480, SW620, HCT116, HCT15, HT29 and Ls174t were purchased from American Type Culture Collection. SW620 and HT29 were cultured in DMEM medium (Gibco) supplemented with 10% FBS (Gibco). SW480, HCT116, HCT15 and Ls174t were cultured in RPMI 1640 medium (Gibco) with 10% FBS (Gibco).

Vector construction and retroviral infection

The LZTS1 construct was generated by subcloning PCR amplified full-length human LZTS1 cDNA into pEZ-Lv105. For deletion of LZTS1, 2 short hairpin RNA (shRNA) sequences were cloned into the GV248 vector to generate GV248-RNAi(s), respectively. Retroviral production and infection were performed as previously described [18]. Stable cell lines expressing LZTS1 or shLZTS1 were selected for 10 days with 0.5 mg/mL puromycin, respectively.

Real-time RT-PCR and western blotting analyses

Total RNA extraction and real-time RT-PCR were performed as previously described, using the ABI PRISM 7500 Sequence Detection System (Applied Biosystems) [18]. Real-time PCR primers were designed using the Primer Express. Sequences of the real-time PCR primers were: *LSZT1*, sense, 5′-ATGGGCAGCGTCAGTAGC-3′; anti-sense, 5′-CCTGGGAGAAGCCAAACCT-3′; *GAPDH*, sense, 5′-GACTCATGACCAC AGTCCATGC-3′; anti-sense, 5′-AGAGGCAGGGATGATGTTCTG-3′. Reverse Transcription was carried out with the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Real-time RT-PCR was carried out using SYBR Green I (Applied BioSystems, Foster, CA). The data were normalized to the geometric mean of housekeeping gene *GAPDH* and calculated using the $2^{-\Delta\Delta CT}$ method.

Western blotting was performed according to standard methods as described previously [19], using anti-LZTS1 (Sigma-Aldrich), anti-phospho-AKT, anti-AKT, anti-Cyclin D1, and anti-p27Kip antibodies (Bioworld Technology Inc., St. Louis Park, MN, USA), anti-p70S6K at Thr-389 (p-p70S6K) and anti-4EBP1 at Thr-37/46 (p-4EBP1) (Cell Signaling Technology, Danvers, MA, USA). A mouse monoclonal anti-a-Tubulin antibody (Sigma, St. Louis, MO, USA) was used as inner control to confirm equal loading of proteins.

Immunohistochemistry

Immunohistochemistry (IHC) staining and scoring were done as previously described [20]. The sections were reviewed and scored independently by two observers, based on both the proportion of positively stained tumor cells and the intensity of staining. The proportion of positive tumor cells was scored as follows: 0 (no positive tumor cells), 1 (<10% positive tumor cells), 2 (10–50% positive tumor cells), and 3 (>50% positive tumor cells). The intensity of staining was graded according to the following criteria: 0 (no staining); 1 (weak staining = light yellow), 2 (moderate staining = yellow brown), and 3 (strong staining = brown). The staining index (SI) was calculated as staining intensity score × proportion of positive tumor cells. Using this method of assessment, the expression of LSZT1 was scored as 0, 1, 2, 3, 4, 6 and 9. The scores of 0 and 1 were used to define tumors as negative LSZT1 expression, and the scores of 2, 3, 4, 6, 9 as positive expression of LSZT1.

MTT assay

3×10^3 cells were seeded on 96-well plates and cultured for 24 hours. 20 μL of 5 g/L 3-(4,5-dimethylthiazol-z-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma, St. Louis, MO, USA) was added to each well and incubated for 4 hours. After MTT was removed, 150 μL dimethyl sulfoxide (DMSO, Sigma, St. Louis, MO, USA) was added to the wells. The absorbance was measured at 450 nm with a Microplate Autoreader (Bio-Rad, Hercules, CA, USA). The experiment was repeated three times.

Colony formation assays

Cells were trypsinized and plated on 6-well plates (200 cells/well) and cultured for 2 weeks. The colonies were stained with 1% crystal violet for 30 s after fixation with 4% paraformaldehyde for 30 minutes. The number of colonies, defined as >50 cells/colony, was counted. Three independent experiments were performed. The data were calculated using paired t test.

Soft agar assays

Cells (1×10^4) were resuspended in 2 mL RPMI 1640 containing 10% fetal bovine serum with 0.33% agarose. Then the agar–cell mixture was plated on top of 0.66% agar in a medium supplemented with 20% fetal bovine serum on 60 mm plates. The plates were incubated at 37 °C in a humid atmosphere of 5% CO_2. After 10 days, the colonies were measured with an ocular micrometer. Only colonies larger than 0.1 mm in diameter were counted. The experiment was performed 3 times for each cell line.

Tumorigenesis in nude mice

Xenograft tumors were generated by subcutaneous injection of CRC cells (2×10^6), including HCT15/Vector and HCT15/LZTS1, SW480/Sramble and SW480/LZTS1 RNAi#1, on the hind limbs of 4–6 week-old Balb/C athymic nude mice (nu/nu; Animal Center of Southern Medical University, Guangzhou, China. n = 6 for each group). All mice were housed and maintained under specific pathogen-free conditions, and all experiments were approved by the Use Committee for Animal Care and preformed in accordance with institutional guidelines. Tumor size was measured using a slide caliper and tumor volume was determined by the formula $0.44 \times A \times B^2$ (A represents the base diameter of the tumor and B represents the corresponding perpendicular value).

Statistical analysis

All statistical analyses were carried out using SPSS version 13.0. Mann–Whitney U tests were used to analyze the relationship between LZTS1 expression and the clinicopathologic features of CRC. Survival curves were plotted by using the Kaplan–Meier method and compared using the log-rank test. The Cox proportional hazard model was used to calculate relative risk ratios. Univariate and multivariate survival distributions were compared using the log-rank test. $P < 0.05$ was considered significant.

Results

LZTS1 is downregulated in CRC

Real-time PCR and western blotting analysis were performed to detect the expression of LZTS1 in 6 CRC cell lines, including SW480, SW620, HCT116, HCT15, HT29, and Ls174t. LZTS1 expression was relatively high in SW480, while negative or only weak expression of LZTS1 was observed in other 5 CRC cell lines (Fig. 1A). Comparative analysis indicated that LZTS1 was significantly downregulated in 11 examined tumor samples paired with adjacent non-cancerous tissues from the same patients (Fig. 1B–D). The normal/tumor (N/T) ratio of LZTS1 mRNA expression was >2-fold in all samples, and the highest ratio was up to 36-fold, as analysis by Real-time PCR (Fig. 1D).

Decreased LZTS1 expression was associated with progression and poor prognosis in CRC

IHC analysis was performed to determine the expression of LZTS1 in 160 paraffin-embedded archived CRC tissues. LZTS1 was highly expressed in the adjacent non-cancerous tissues (Fig. 2A, left). In contrast, LZTS1 expression was significantly decreased or not detected in CRC tissues (Fig. 2A, middle and right). Among the CRC tissues, 66.25% (106/160) of the cases showed negative expression of LZTS1, whereas among the remaining samples 33.75% (54/160) had positive staining for LZTS1. We further analyzed the correlation between LZTS1 expression and clinicopathologic features of CRC by Mann–Whitney U tests. As summarized in Supplementary Table S1, LZTS1 expression was significantly

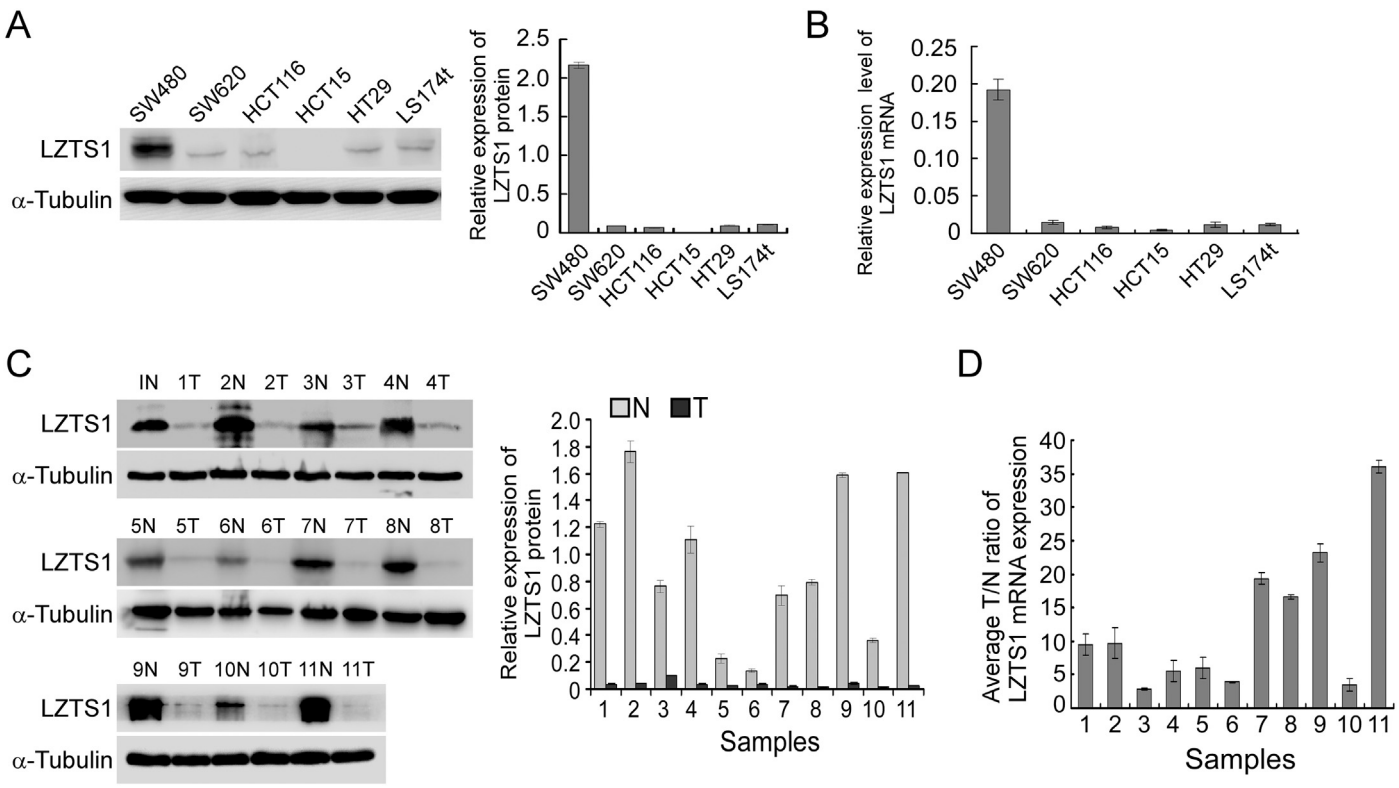

Fig. 1. LZTS1 is downregulated in CRC. (A) Expression of LZTS1 protein by western blotting (left). The protein expression levels were quantified by comparing the gray level of each band using Quantity One Software (right). (B) Expression of LZTS1 mRNA by Real-time PCR in six CRC cell lines. Expression levels of LZTS1 mRNA were normalized for GAPDH. Error bars represent mean ± SD calculated from 3 parallel experiments. (C) Expression of LZTS1 protein in each of the primary CRC (T) and adjacent noncancerous tissues (N) paired from the same patient by western blotting (left) (n = 11). The protein expression levels were quantified by comparing the gray level of each band using Quantity One Software (right). (D) Average N/T ratios of LZTS1 expression were quantified by Real-time PCR and normalized against GAPDH (n = 11). Error bars represent mean ± SD calculated from 3 parallel experiments.

associated with differentiation ($P = 0.017$), clinical stage ($P = 0.013$), T classification ($P = 0.009$) and distant metastasis ($P = 0.04$). These data were further confirmed by Spearman correlation analysis (Supplementary Table S2), and the coefficients for the correlations between LZTS1 expression and differentiation, clinical stage, T classification and distant metastasis were –0.188 ($P = 0.017$), –0.196 ($P = 0.013$), –0.206 ($P = 0.009$) and –0.136 ($P = 0.017$), respectively. Kaplan–Meier survival analysis indicated that LZTS1 expression is associated with good prognosis in CRC cancer patients. Patients with positive LZTS1 level had significantly well overall survival ($P = 0.001$, Fig. 2B) than patients with negative LZTS1 level. Cox regression analyses revealed that in this study, lymph node involvement, distant metastasis and LZTS1 expression were recognized as independent prognostic factors.

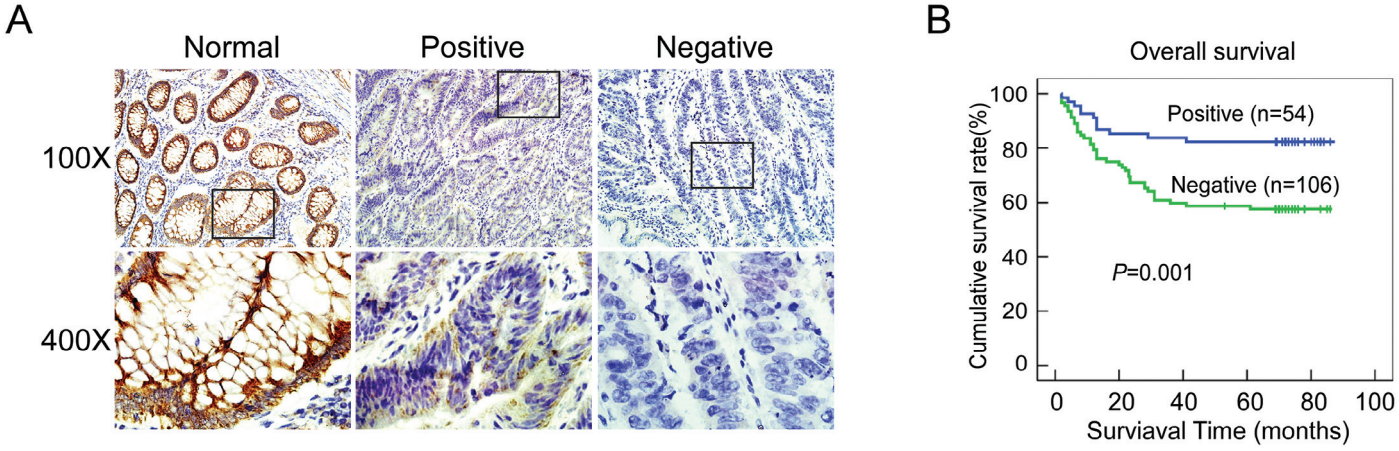

Fig. 2. Expression of LZTS1 in paraffin-embedded CRC specimens (n = 106). (A) Representative images of LZTS1 expression in normal intestinal epithelium and CRC specimens examined by IHC. LZTS1 was positively detected in normal intestinal epithelial cells (left), whereas it was only weakly (middle) or negatively (left) detected in CRC cells. (B) Kaplan–Meier analysis of survival in patients with CRC. Green, patients with negative LZTS1 expression (n = 106, 5-year survival rate 58%, median survival 57.45 months); blue, patients with positive expression of LZTS1 (n = 54, 5-year survival rate 87%, median survival 77.93 months; $P = 0.001$, log-rank test). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

LZTS1 inhibits cell proliferation and tumor growth in CRC cells

To evaluate the possible role of LZTS1 in the proliferation of human CRC cells, stable LZTS1 expressed cell line was made (Fig. 3A).We selected HCT15 for stable LZTS1 expression because HCT15 was a highly aggressive cell line and displayed the lowest endogenous LZTS1 level among the six cell lines examined. MTT assay showed that LZTS1 overexpression decreased the proliferation of HCT15 cells as compared with the control cells (Fig. 3B; $P < 0.05$). Colony formation assay revealed that HCT15/LZTS1 cells formed fewer colonies than that of control cells (Fig. 3C; $P < 0.01$). We next examined the effect of LZTS1 on the tumorigenic activity of CRC cells using anchorage-independent growth assays. The results showed that overexpression of LZTS1 significantly decreased the anchorage-independent growth of HCT15 cells, as indicated by the decrease in colony number and size on soft agar (Fig. 3D). To confirm this effect in vivo, we performed tumorigenesis assays in nude mice. HCT15/Vector and HCT15/LZTS1 cells were inoculated in nude mice. All mice developed Xenograft tumors at the injection site. As shown in Fig. 3E, tumors formed from HCT15/LZTS1 cells implanted in nude mice grew more slowly than those in the HCT15/Vector group (n = 6; $P < 0.01$).

Inhibition of endogenous LZTS1 promotes cell proliferation and tumor growth ability of CRC cells

To further investigate the impact of LZTS1 on CRC proliferation, we knocked down endogenous LZTS1 in SW480 CRC cells by expressing short hairpin RNAs (RNA#1 and RNi#2; Fig. 4A). MTT assay and colony formation assay (Fig. 4B and C; $P < 0.05$) showed

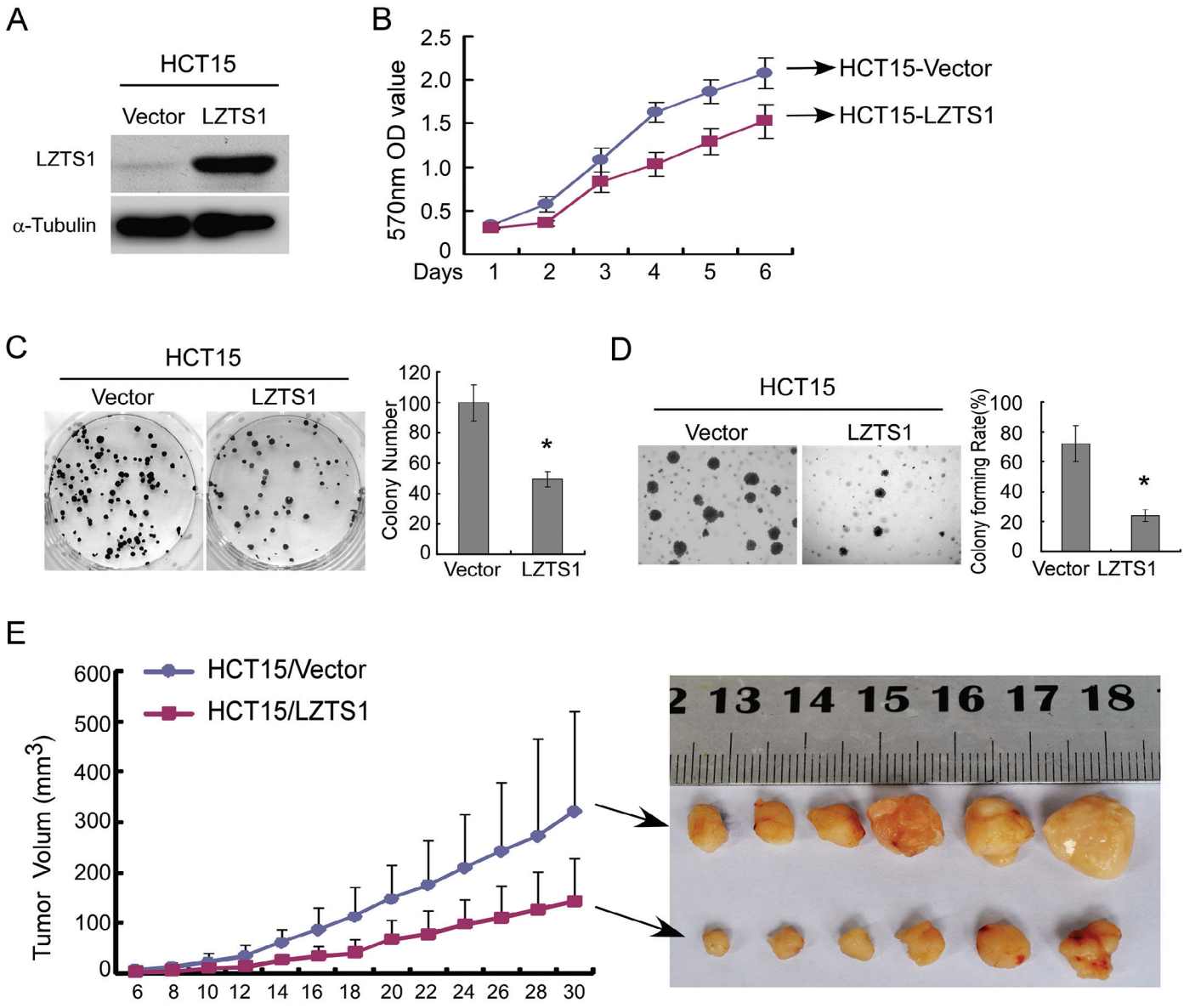

Fig. 3. Upregulation of LZTS1 inhibits cell growth and tumor-promoting activity of CRC cells. (A) Ectopic expression of LZTS1 in HCT15 cells analyzed by western blotting. α-Tubulin was used as a loading control. (B and C) Ectopic expression of LZTS1 inhibits cell proliferation as determined by MTT assays (B, Factorial analysis, $P < 0.001$) and colony formation assays (C). (D) Overexpression of LZTS1 inhibits anchorage independent growth ability of HCT15 cells in Soft agar colony formation assays. Colonies containing >50 cells were scored. Each bar represents the mean ± SD of three independent experiments. *: $P < 0.01$. (E) HCT15/Vector and HCT15/LZTS1 cells (2×10^6) were injected in the hind limbs of nude mice (n = 6 for each group).Tumor volumes were measured on the indicated days. Data points are the mean tumor volumes ± SD (left panel). Ectopic expression of LZTS1 inhibits tumor growth in nude mice (the left panel, Factorial analysis, $P < 0.001$). The right panel shows tumors after inoculation.

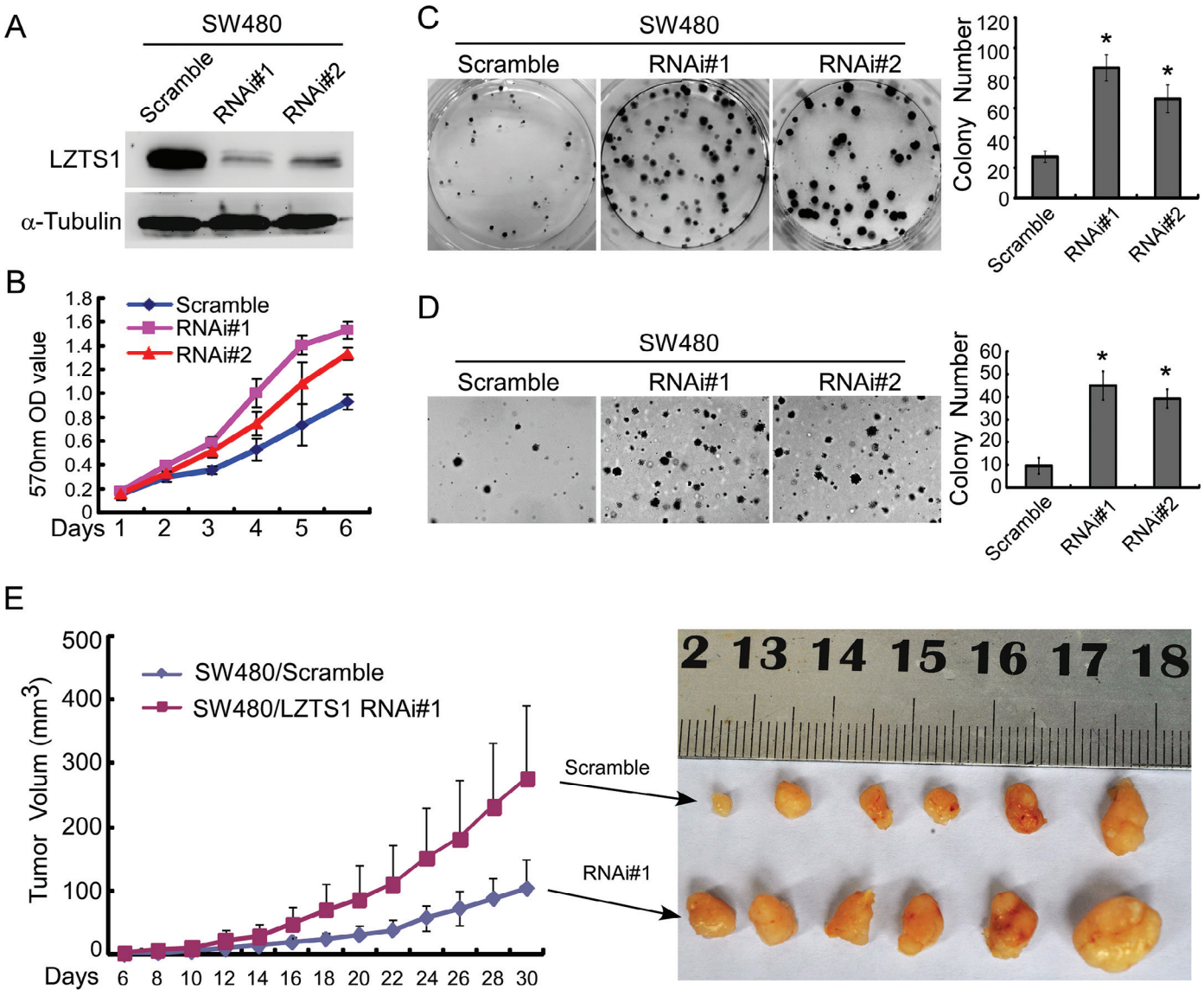

Fig. 4. RNAi-silencing of LZTS1 promotes cell growth and tumor-promoting activity. (A) RNAi-silencing of LZTS1 in shRNA-transduced stable SW480 cells. α-Tubulin was used as a loading control. (B and C) Silencing endogenous LZTS1 promoted cell growth as determined by MTT assays (B, Factorial analysis, $P < 0.001$) and colony formation assays (C). (D) Silencing of LZTS1 promotes anchorage independent growth ability of SW480 in Soft agar colony formation assays. Colonies containing >50 cells were scored. Each bar represents the mean ± SD of three independent experiments. (E) SW480/Sramble and SW480/LZTS1 RNAi#1 cells (2×10^6) were injected in the hind limbs of nude mice (n = 6 for each group).Tumor volumes measured on the indicated days. Data points are the mean tumor volume ± SD (left panel). Silencing of LZTS1 promoted tumor growth in nude mice (the left panel, Factorial analysis, $P < 0.001$).The right panel shows tumors after inoculation.

that knockdown of LZTS1 expression caused evident elevated viability in SW480 cells. In addition, depletion of endogenous LZTS1 in SW480 cells caused a significant increase in colony number and colony size on soft agar (Fig. 4D; $P < 0.01$). Tumorigenesis assays in nude mice showed that depletion of endogenous LZTS1 in SW480 cells caused significant inhibition of tumor growth (Fig. 4E; n = 6; $P < 0.01$).

LZTS1 regulates cell proliferation through the AKT–mTOR signaling pathway in CRC cells

Next, we observed that up-regulation of LZTS1 dramatically decreased the levels of phosphorylated AKT (p-AKT). In addition, overexpression of LZTS1 decreased the phosphorylation levels of p70S6K at Thr-389 (p-p70S6K) and 4EBP1 at Thr-37/46 (p-4EBP1), which represent the activity of p70S6K and 4EBP1, respectively, in HCT15 cells (Fig. 5A). Moreover, knocking down of LZTS1 in SW480 cells increased p-AKT, p-p70S6K and p-4EBP1 levels (Fig. 5A). Furthermore, we examined the expression of two representatives of mTOR targets, p27Kip1and Cyclin D1. Significant increases in the expression of p27Kip1 but decreases of Cyclin D1 were shown in LZTS1-overexpressing cells. In contrast, significant decreases in the expression of p27Kip1 but increases of Cyclin D1 were shown in LZTS1 knockdown cells.

The AKT pathway activates the mTOR pathway [21], which raises the possibility that loss of LZTS1 activates the mTOR pathway through AKT. To address this, we treated LZTS1 knockdown cells with a specific AKT inhibitor (Wortmannin). As shown in Fig. 5B, blocking AKT activity by wortmannin largely diminished the elevation of p-p70S6K and p-4EBP1 levels in LZTS1 knocking down SW480 cells. In addition, blocking AKT activity by wortmannin partly recued the expression of p27 but reduced the expression of cyclin D1 in LZTS1 knocking down SW480 cells. To determine whether the activation of the AKT–mTOR pathway contributes to the effect of LZTS1 on cell

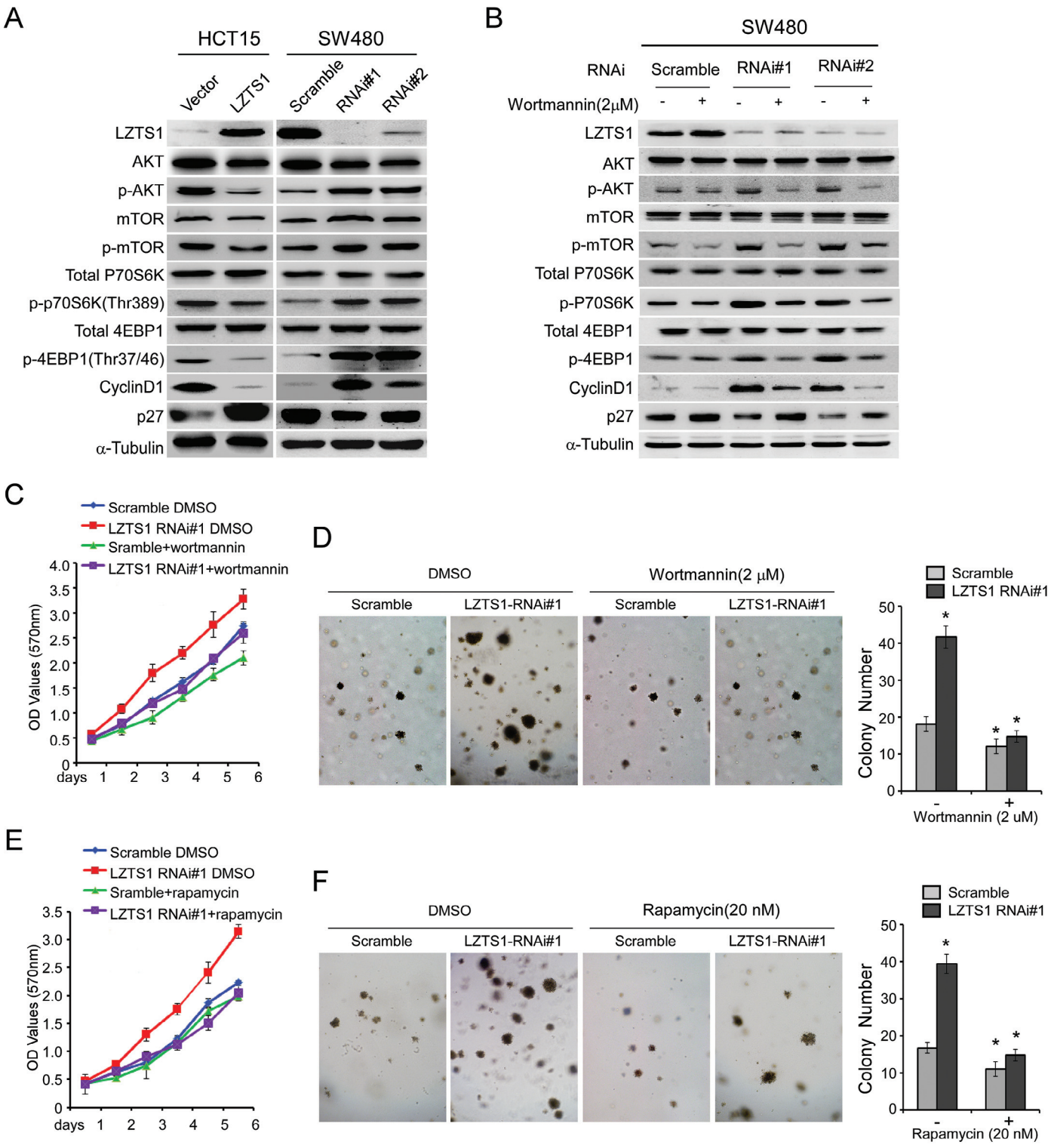

Fig. 5. LZTS1 regulates the cell proliferation factors though the AKT–mTOR pathway in CRC cells. (A) LZTS1 regulates the AKT–mTOR pathway activity and expression of Cyclin D1 and p27Kip in CRC cells. (B) Blocking the AKT signaling inhibits the promoting effect of LZTS1-silencing on AKT–mTOR activity in CRC cells. SW480-Scramble, SW480-LZTS1 RNAi#1 and SW480-LZTS1 RNAi#2 cells were treated with wortmannin (2 μM) for 24 h. (C and D) Blocking the AKT signaling inhibits the promoting effect of LZTS1-silencing on the cell proliferation of CRC cells. Cell proliferation was determined by MTT (C, Factorial analysis, $P < 0.001$) and soft agar assays (D) after treatment with wortmannin (2 μM). (E and F) Blocking the mTOR signaling largely abolishes the promoting effect of LZTS1-silencing on the cell proliferation of CRC cells. Cell proliferation was determined by MTT (E, Factorial analysis, $P < 0.001$) and soft agar assays (F) after treatment with Rapamycin (20 nM). Error bars represent mean ± SD from 3 independent experiments; *, $P < 0.01$.

proliferation and tumor growth ability of CRC cells, LZTS1 knocking down SW480 cells were treated with or without wortmannin to block the AKT pathway. MTT and soft agar assays showed that the growth of SW480/LZTS1-RNAi cells was significantly compromised by treatment with the AKT inhibitor compared to control cells treated with DMSO (Fig. 5C and D). To further investigate whether the activation of the mTOR pathway by loss of LZTS1 contributes to the role of LZTS1 in cell proliferation and tumor growth ability of CRC cells, rapamycin, a highly specific mTOR inhibitor, was employed to block the mTOR pathway. Rapamycin treatment largely blocked the promoting effect of cell proliferation and tumor growth ability of LZTS1 knocking down SW480 cells (Fig. 5E and F). Taken together, these results demonstrate that LZTS1 regulates the AKT–mTOR pathway, which contributes to the inhibiting effect of LZTS1 on cell proliferation and tumor growth in CRC cells.

Discussion

As a tumor suppressor gene identified at 8p22 [5], LZTS1 is ubiquitously expressed in normal tissues, while its expression is frequently reduced or absent in divers human cancers, including gastric, lung, bladder, breast, oral and kidney cancers [9–15,22]. In addition, loss of LZTS1 expression in lung cancer tissues was positively associated with higher tumor grading and poorer prognosis of patients [13]. Similarly, LZTS1 was significantly downregulated in breast cancer samples and low expression of LZTS1 was positively correlated to a higher tumor recurrence and a worse overall survival of patients [15]. However, the association between LZTS1 and prognosis of CRC has not been reported to date. In this study, we showed that the expression of LZTS1 was downregulated in CRC at both mRNA and protein levels in comparison with that in normal intestine epithelial tissues. Low expression level of LZTS1 is significantly associated with advanced clinical stage, T classification and poor survival of patients. This research implicates that downregulation or loss of LZTS1 protein expression might be served as a marker to identify patients with more aggressive CRC and poorer clinical outcomes.

Structurally, LZTS1 protein has a region of homology with protein kinase A (PKA)-activated leucine-zipper protein. Functionally, LZTS1 plays a role in mitosis and is involved in the stabilization of active CDK1 at late S-G2/M stage. Aberrant expression of LZTS1 results in early exit from mitosis [16]. It has been documented that restoration of LZTS1 expression in breast, prostate, and bladder cancer cells inhibited the growth in vitro and reduced their tumorigenicity in vivo [11,12,16]. In addition, LZTS1 deficient mice develop cancers that originated from different histological backgrounds [7,8]. Moreover, loss of LZTS1 increased docetaxel resistance of prostate cancer cells through its regulation of CDC25C, or the mitotic kinases CHEK1 and PLK1 [23]. Downregulation of LZTS1 significantly decreases sensitivity to paclitaxel in breast cancer cells in vitro [15]. These studies suggest that LZTS1 acts as a major tumor suppressor gene in multiple cell types. In the present study, we found that forced expression of LZTS1 in CRC cell lines led to repression of cell proliferation and tumor growth both in vitro and in vivo. On the contrary, RNAi-silencing of LZTS1 induced converse results. In the light of these findings, we considered that LZTS1 may play a tumor suppressor role in CRC and has the potential to be a novel predictor of CRC progression.

Although LZTS1 has been linked to regulation of proliferation and tumor growth, the molecular mechanisms remain poorly identified. It has been found that LZTS1 can interact with Disrupted-in Schizophrenia 1 (DISC1) in the regulation of neuronal development [24]. Interestingly, DISC1 is involved in the modulation of AKT–mTOR signaling through KIAA1212 [25]. mTOR plays an essential role in downstream signaling of the AKT pathway and is involved in diverse cellular processes including proliferation, differentiation and apoptosis, mainly via deregulated signal transduction and the subsequent hyper-activation of the critical effectors S6K1 and EIF4E [26,27]. Moreover, the AKT–mTOR pathway is frequently activated and plays a critical role in tumor growth and metastasis in various cancers, including CRC [27,28]. Thus, we hypothesize that LZTS1 affects CRC cell proliferation and tumor growth by controlling the AKT/mTOR signaling pathway. Results in the present study demonstrate that LZTS1 inactivates the mTOR pathway in CRC cells. Further investigation shows that blocking AKT activity largely abolishes the activation effect of mTOR by LZTS1-knocking down, and more importantly, largely abolishes the promoting effect of LZTS1-knocking down on proliferation and growth of CRC cells. Moreover, blocking mTOR activity largely abolishes the promoting effect of LZTS1-knocking down on CRC cell proliferation and growth, suggesting that the prohibiting effect of LZTS1 on CRC cell cancer tumorigenesis and progression is largely mediated by the inactivation of the mTOR pathway. These findings suggest that LZTS1 might inhibit proliferation and growth of CRC cells through the AKT–mTOR signaling pathway.

Cyclin D1 and p27Kip are key cell cycle regulators downstream of the AKT signaling. Activation of AKT pathway decreases the cellular levels of p27Kip1 and induces Cyclin D1, thereby promoting cell proliferation and contributing directly to tumor progression [29]. Cyclin D1 and p27Kip can be directly regulated by the AKT pathway at both transcriptional levels via AKT/FOXOs and posttranslational levels [29–31]. In addition, oncogenic mTOR activation also drives cell proliferation via modulation of cell cycle regulators Cyclin D1, Cyclin E, p21Cip and p27Kip [32–34]. In this study we showed that LZTS1 overexpression could downregulate Cyclin D1 but upregulate p27Kip1, while knocking down of LZTS1 could upregulate Cyclin D1 but downregulate p27Kip1. In combination with the observation that LZTS1 is involved in the modulation of the AKT–mTOR signaling pathway, the regulation of cyclin D1 and p27Kip1 by LZTS1 probably resulted from the alteration of the AKT–mTOR activity. Taken together, our observations link LZTS1 to the basic cell cycle regulation.

In summary, results from this study demonstrate that LZTS1 is downregulated in CRC and loss of LZTS1 might be a valuable prognostic marker of CRC progression. Loss of LZTS1 plays a vital role in promoting proliferation and growth of CRC. This function of LZTS1 in CRC is mainly mediated by the AKT–mTOR pathway. However, the biological function and possible mechanism of LZTS1 in human cancer and the regulatory mechanism of LZTS1 on the AKT–mTOR pathway will need to be investigated in detail.

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Conflict of interest

The authors of this manuscript have no conflict of interest.

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.canlet.2015.02.004.

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