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LETM1 is a potential cancer stem-like cell marker and predicts poor prognosis in colorectal adenocarcinoma

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ABSTRACT

The leucine zipper-EF-hand containing transmembrane protein 1 (LETM1) is highly expressed in many human malignancies and is correlated with poor prognosis. However, LETM1 has rarely been explored as a cancer stem-like cell marker for the prognostic evaluation of colorectal adenocarcinoma (CRA). Herein, we assessed the expression of LETM1 and its relationship with cancer stemness genes, cell cycle markers, PI3K/Akt/NFκB signaling pathway genes, and HIF1α in 102 paraffin-embedded CRA tissue samples using immunohistochemistry (IHC). Additionally, we further confirmed the correlation between LETM1 and cancer stemness genes in CRA cell lines using immunofluorescence (IF) imaging and Western blotting. LETM1 expression was remarkably upregulated in human fetal sagittal sections and CRA tissues. The expression of LETM1 in CRA tissue was correlated with clinical stage, lymph node metastasis, distant metastasis, and microvessel density. LETM1 expression was significantly associated with lower overall survival and disease-free survival. Moreover, the expression of LETM1 positively correlated with SOX9, LSD1, CD44, CD133, LGR5, SOX2, and HIF1α. IF revealed that LETM1 co-localized with CD44, SOX9, and LGR5 in HCT116. Moreover, LETM1 expression was also strongly linked to the expression of cell cycle regulators (cyclinD1, CDK4, p27) and PI3K/Akt/NFκB pathway genes (pPI3K-p85, pAkt-Ser473, pAkt-Thr308, pNFκB-p65). LETM1 may therefore be a cancer stem-like cell marker and an indicator of poor prognosis in patients with CRA.

1. Introduction

In China, there are 191,000 new colorectal adenocarcinoma (CRA) cases and an estimated 376,300 CRA-related deaths annually [1,2]. Although recent diagnostic and therapeutic advances have significantly improved survival, approximately 50% of patients with CRA have overt metastasis [3]. Therefore, it is important to discover novel biomarkers for the early detection and potential targets for the treatment of CRA.

Leucine zipper-EF-hand containing transmembrane protein 1 (LETM1), which was first discovered in patients with Wolf-Hirschhorn syndrome, has been reported to enhance mitochondrial Ca^{2+} transport and cell proliferation [4,5]. LETM1 encodes the human homologue of yeast Mdm38p, which is involved in mitochondrial morphology [6]. Although LETM1 regulates mitochondrial translation and reduces mitochondrial biogenesis through its association with MRPL36, increasing evidence has shown that dysregulation of LETM1 is a key factor in tumorigenesis [7]. LETM1 has been reported to be a molecular biomarker associated with tumor progression and prognosis in breast, bladder, thyroid, and esophageal cancer [8–11].

Cancer stem-like cells (CSCs) can self-renew and undergo either asymmetric or symmetric cell division and are associated with cellular heterogeneity [12–15]. They are thought to be derived from mutations in stem or progenitor cells and hence tend to have the same stem cell markers [16]. Furthermore, promising evidence shows a positive association between the proportion of stem cells in tumors and disease progression, and that high levels of CSCs correlate with the risk of metastasis and poor clinical outcome [17]. Colon CSCs express several markers, including CD44, CD133, CD166, LGR5 and DCLK1 [18,19]. Therefore, a better understanding of the molecular mechanisms in CSCs that are involved in the process of CRA development and progression is needed to help develop more efficient anticancer strategies.

In this study, we aimed to explore whether LETM1 is a novel marker for CSCs in CRA. We specifically focused on the clinicopathological significance of LETM1 expression and evaluated the relationship

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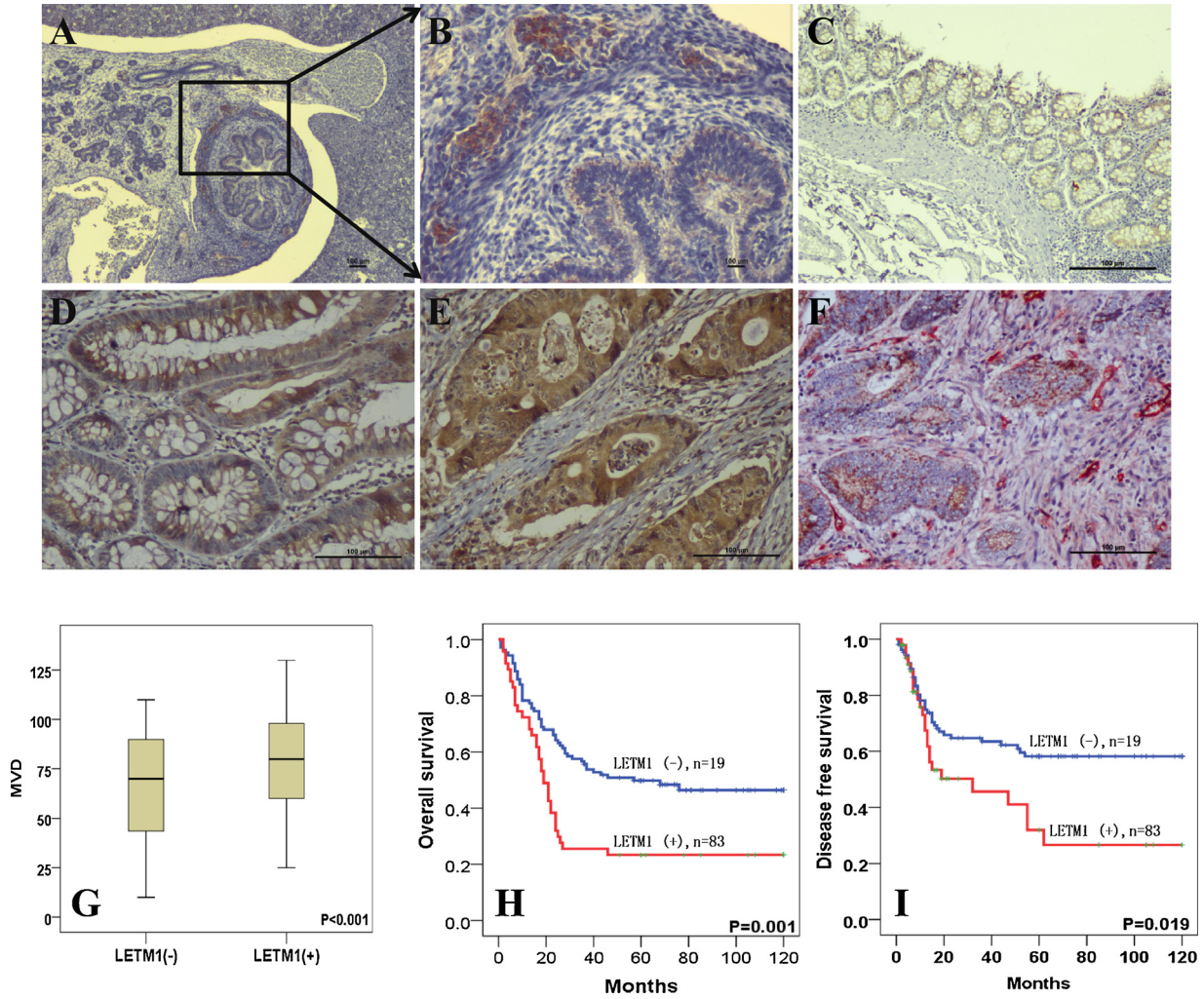

Fig. 1. LETM1 was associated with unfavorable clinicopathological parameters. (A, B) Immunohistochemical staining of LETM1 in the colon of the human fetus. B indicated the higher magnification of the selected area in A (original magnification left: × 40; right: × 200). (C, D, E) Immunohistochemical staining of LETM1 in normal tissue, adenoma, colorectal adenocarcinoma tissues (original magnification ×200). (F) Immunohistochemical staining of LETM1 (brown) in the cytoplasm, and CD105 (red) was expressed in new capillary blood vessels around the cancer cells in the host (original magnification ×200). (G) Expression of LETM1 in colorectal adenocarcinoma was significantly associated with increased microvessel density. (H, I) Kaplan-Meier analysis of overall survival and disease-free survival curves for LETM1 expression in colorectal adenocarcinoma.

between the expressions of LETM1 and cancer stemness proteins in CRA tissues. The relationship of LETM1 and the PI3K/Akt/NFκB pathway, which may affect the participation of LETM1 in maintaining the stem-like phenotype, was also examined.

2. Materials and methods

2.1. Tissue specimens

A total 145 formalin-fixed and paraffin-embedded samples including 102 of colorectal adenocarcinoma, 8 of adenoma, and 35 of normal mucosa tissues who underwent curative surgery and were obtained from the Department of Pathology at the Samsung Medical Center. Clinical and pathological reports were reviewed for sex, age, differentiation, primary tumor (pT) stage, lymph node metastasis, distant metastasis and clinical stage. The median follow-up period was 112 months (range 2–136 months). In addition, we used three unstained sagittal sections of human fetus (CRL 53 mm) from the collection of university.

This research complied with the Helsinki Declaration and was approved by the Human Ethics Committee, the Research Ethics Committee of Yanbian University and Sungkyunkwan University School (Seoul, Korea). All patients provided written informed consent according to institutional guidelines. Patients were informed that the resected specimens were stored by the hospital and potentially used for scientific research and that their privacy would be maintained. Follow-up survival data were collected retrospectively through medical record analyses.

2.2. Cell lines

HT29 and HCT116 (CRA cell lines), were maintained in RPMI-1640 with high glucose (Life Technologies, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Life Technologies, Grand Island, NY), 100 mg/ml penicillin G, and 50 mg/ml streptomycin (Life Technologies, Grand Island, NY) at 37 °C in a humidified atmosphere containing 5% CO2. All cell lines were purchased from ATCC (Manassas, USA).

2.3. Immunohistochemical (IHC) staining procedure

Sections on microslides were deparaffinized with xylene, hydrated using a diluted alcohol series, and immersed in 3% H_2O_2 in methanol to quench endogenous peroxidase activity. Sections were treated with TE

Table 1
Comparison of clinicopathologic characteristics according to the LETM1 expression in CRA.

Variable	n	LETM1 (-) n(%)	LETM1 (+) n(%)	χ^2	R	P-value
Sex				1.214	0.109	0.271
Male	62	9(14.5)	53(85.5)			
Female	40	10(25.0)	30(75.0)			
Age				0.241	0.049	0.623
≤65	46	10(21.7)	36(78.3)			
> 65	56	9(16.1)	47(83.9)			
Differentiation				3.117	0.010	0.374
Well	32	8(25.0)	24(75.0)			
Moderately	62	9(14.5)	53(85.5)			
Poorly	8	2(25.0)	6(75.0)			
pT stage				0.764	0.087	0.382
T1-2	11	1(9.1)	10(90.9)			
T3-4	91	18(19.8)	73(80.2)			
Lymph node metastasis				4.434	0.210	0.035*
Negative	59	15(25.4)	44(74.6)			
Positive	43	4(9.3)	39(90.7)			
Distant metastasis				11.357	0.335	0.001*
Negative	69	19(27.5)	50(72.5)			
Positive	33	0(0.0)	33(100.0)			
Clinical stage				9.882	0.313	0.002*
I	47	15(31.9)	32(68.1)			
II-IV	55	4(7.3)	51(92.7)			
Chemotherapy				0.356	0.059	0.551
Negative	21	3(14.3)	18(85.7)			
Positive	81	16(19.8)	65(80.2)			
Radiotherapy				2.237	0.149	0.135
Negative	85	18(21.2)	67(78.8)			
Positive	17	1(5.9)	16(94.1)			

Table 2
The correlation of LETM1 expression with cancer stem cell makers expression in CRA.

Variable	n	LETM1 (-) n(%)	LETM1 (+) n(%)	χ^2	R	P-value
Sox2				8.068	0.238	0.005*
Negative	12	4(33.3)	8(56.7)			
Positive	90	15(16.7)	75(83.3)			
Sox9				9.258	0.261	0.002*
Negative	18	7(38.9)	11(61.1)			
Positive	84	12(14.2)	72(85.7)			
LSD1				3.868	0.164	0.049*
Negative	49	11(22.4)	38(77.6)			
Positive	53	8(15.1)	45(84.9)			
CD44				9.491	0.256	0.002*
Negative	53	13(24.5)	40(75.5)			
Positive	49	6(12.2)	43(87.8)			
CD133				9.010	0.250	0.003*
Negative	28	9(32.1)	19(67.9)			
Positive	74	10(13.5)	64(86.5)			
LGR5				5.587	0.202	0.018*
Negative	26	7(26.9)	19(73.1)			
Positive	76	12(15.8)	64(84.2)			
HIF-1α				4.947	0.189	0.026*
Negative	11	4(36.4)	7(63.6)			
Positive	91	15(16.5)	76(83.5)			

buffer (10 mM Tris and 1 mM EDTA, pH 9.3) at 98 °C for 30 min. The sections were then incubated with anti-LETM1 (1:100, Abnova, Taipei, Taiwan), anti-CD133 (1:100, Abcam, Cambridge, UK), anti-CD44 (1:100, Abcam, Cambridge, UK), anti-LSD1 (1:250, Sigma, St. Louis, MO, USA), anti-SOX2 (1:100, R&D, Minneapolis, MN, USA), anti-SOX9 (1:100, Abnova, Walnut, CA, USA), anti-LGR5 (1:40, Abcam, Cambridge, UK), anti-p16 (1:50, Millipore, Bedford, MA, USA), anti-CDK4 (1:100, Millipore, Bedford, MA, USA), anti-cyclinD1 (1:100, Millipore, Bedford, MA, USA), anti-p21 (1:50, Millipore, Bedford, MA, USA), anti-p27 (1:80, Millipore, Bedford, MA, USA), anti-HIF1α (1:100, Millipore, Bedford, MA, USA), anti-phospho-NFκB p65 (1:80, Millipore, Bedford, MA, USA), anti-phospho-AKT (pAKT-Ser473, 1:100, Millipore, Bedford, MA, USA, and pAKT-Thr308, 1:80, Millipore, Bedford, MA, USA) and anti- phospho-PI3K p85 (1:50, Abcam, Cambridge, UK), for 2 h at room temperature, followed by three washes with TBST. Then sections were incubated with an anti-mouse/rabbit antibody (Envision Plus, Dako, Copenhagen, Denmark) for 30 min at room temperature. A chromogen was used to create red staining with ImmPACT AEC Peroxidase Substrate (Vector Laboratories, Burlingame, CA, USA) for 20 min. Counterstaining was performed with Mayer's hematoxylin for 1 min. Sections were counterstained with Meyer's hematoxylin. After reading and taking photograph of the slide, sections were then used stripping buffer (20% SDS, 0.5 M Tris, and mercaptoethanol) to removing the original antibody for 1 h at the water bath of 56 °C and then for 10 min dehydrated alcohol to removing the red reaction, so that the sections can be used again. Omitting the primary antibody provided negative controls for immunostaining. Double immunostaining procedure was performed using a two-step method with anti-LETM1 (1:80, Abnova, Taipei, Taiwan) developed with 3,3′-diaminobenzidine (a brown reaction product) and anti-CD105 (1:250, Abcam, Cambridge, UK) developed with ImmPACT AEC Peroxidase Substrate (a red reaction product) antibodies to observe the correlation between the expression of LETM1/CD105 in ESCC. First, for the LETM1 protocols, except for the chromogen reaction with the 3,3′-diaminobenzidine for 10 min, all steps were the same as before. Staining of the same section was performed after incubating the samples with an antibody to CD105 by ImmPACT AEC Peroxidase Substrate for 20 min.

2.4. Evaluation of the IHC analysis

Two pathologists (LHP & YHX) who did not possess knowledge of the clinical data examined and scored all tissue specimens. IHC scores was measured, as follows: [IHC score 1], weak staining in < 50% or moderate staining in < 20% of carcinoma cells; [IHC score 2], weak staining in ≧50%, moderate staining in 20–50% or strong staining in < 20% of carcinoma cells; [IHC score 3], moderate staining in ≧50% or strong staining in ≧20% of carcinoma cells. Cases with score 2 and 3 were regarded as positivity for each protein expression, the staining results were semi-quantitatively scored as negative and positive. In case of discrepancies, a final score was established by reassessment by both pathologists using a double-headed microscope.

2.5. Western blotting analysis

Cell lysates were produced in RIPA lysis buffer (50 mM Tris pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton x-100, 1% Na-Doc, 0.1% SDS) supplemented with protease inhibitor cocktail (Roche). Cell extracts were quantitated using a BCA protein assay kit (Thermo). Western blotting analysis was performed using standard techniques for anti-LETM1 (1:1000, Abnova, Taipei, Taiwan), anti-CD133 (1:1000, Abcam, Cambridge, UK), anti-CD44 (1:1000, Abcam, Cambridge, UK), anti-SOX2 (1:1000, R&D, Minneapolis, MN, USA), anti-SOX9 (1:500, Abnova, Walnut, CA, USA), anti-LGR5 (1:1000, Abcam, Cambridge, UK), anti-LSD1 (1:2000, Sigma, St. Louis, MO, USA) and anti-β-actin (1:1000, Abcam, Cambridge, UK). Membranes were then washed three times with TBST followed by incubation with horseradish peroxidase-conjugated goat anti-rabbit/mouse IgG secondary antibody (1:500, BIOSS, Beijing, China) for 1 h at room temperature. Finally, the membranes were washed three times with TBST, and the protein bands were detected using an ECL system (Merck) according to the manufacturer's instructions.

2.6. Immunofluorescence (IF) analysis

The autoclaved cover glasses were placed in the 6-well plate with flate bottoms. HCT116 cells were grown on cover-slips to 50%

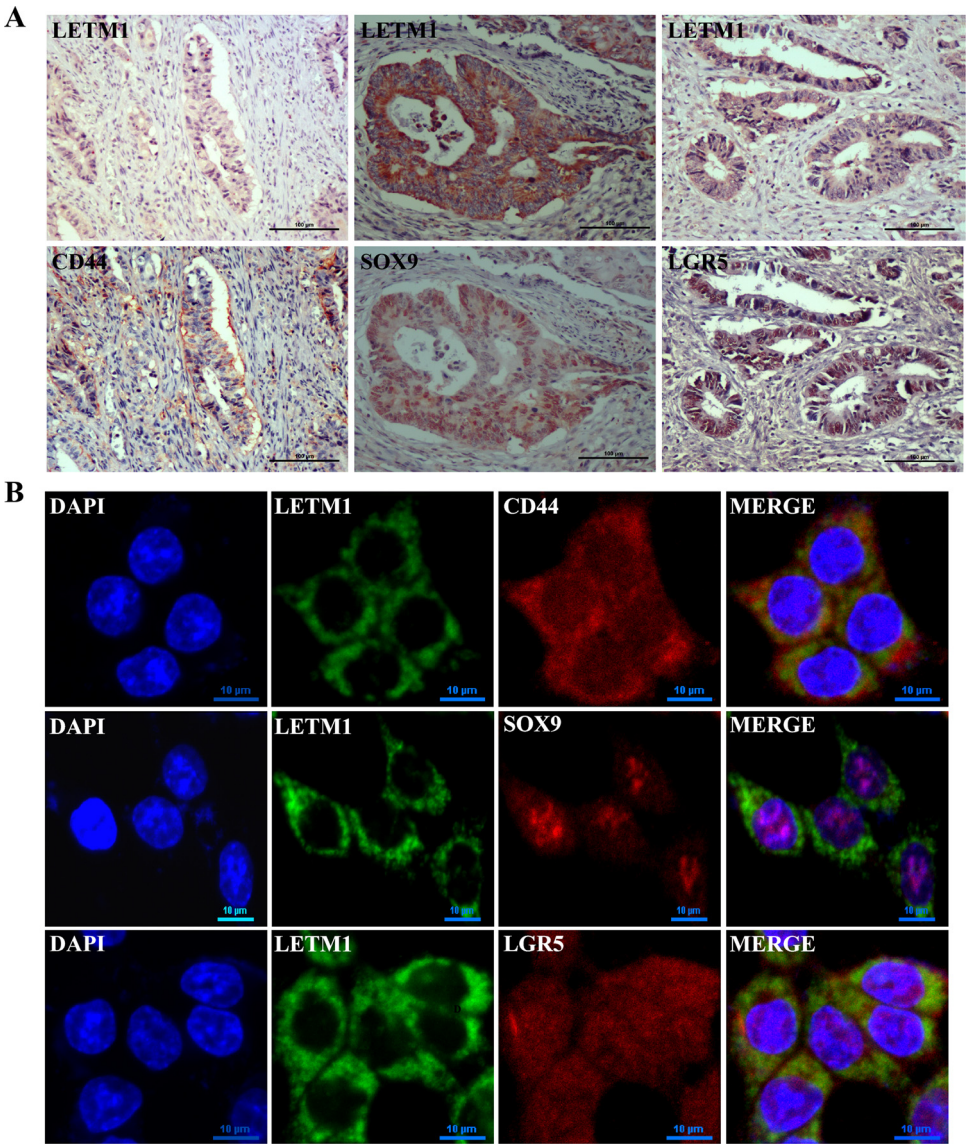

Fig. 2. LETM1 may be a potential cancer stem-like cell marker in colorectal adenocarcinoma. (A) Immunohistochemical multi-staining revealed that LETM1/CD44, LETM1/SOX9 and LETM1/LGR5 co-localized in the same colorectal adenocarcinoma tissues (original magnification × 200). (B) Immunofluorescence staining for LETM1/CD44, LETM1/SOX9 and LETM1/LGR5 in the HCT116 cells. Blue for DAP1; green for LETM1; red for CD44, SOX9 and LGR5; double labeling for Merge.

confluence. Cells were washed with PBS, and then with 4% paraformaldehyde fixation. Washing with cold PBS, cells were permeabilized with 0.5% Tri-ton X-100 for 20 min. And then cells were blocked in 3% BSA for 1 h at RT. After washing with PBS, the cover-slips were then incubated with the primary antibodies antibodies in 3% BSA at 4 °C overnight. After three washes, the cover-slips were followed by incubation with secondary antibodies in 3% BSA at RT. The cells were counter stained with DAPI (Vector Laboratorise, Burlingame, CA, USA). Finally, the location of proteins in cell were observed in a confocal laser scanning microscope (Carl Zeiss, Thornwood, New York).

2.7. Statistical analysis

Correlations were examined using Pearson's chi-square test as appropriate. The cumulative survival time was calculated using the Kaplan-Meier method and analyzed by the log-rank test. Survival was measured from the date of surgery. Univariate and multivariate analysis were based on the Cox proportional hazards regression model. The hazard ratio (HR) and its 95% confidence interval (CI) were assessed for each factor. Two-tailed $P < 0.05$ was considered to be significant. The statistical analysis was performed using SPSS 25.0 statistical software (SPSS Inc, Chicago, IL, USA).

3. Results

3.1. Association between the expression of LETM1 and clinicopathological characteristics in CRA

Positive signals of LETM1 are mainly localized in the cytoplasm and membrane of cancer cells and normal mucosa cells (Fig. 1A–E). LETM1 was expressed in the colon of the human fetus (Fig. 1A, B). LETM1 was significantly higher in CRA tissue samples (81.4%, 83/102) than in adjacent non-tumor tissues including the normal mucosa (31.4%, 11/35) and adenoma tissues (62.5%, 5/8) (Fig. 1C–E; Supplementary Table 1). The positive LETM1 expression was associated with clinical stage ($P = 0.002$), lymph node metastasis ($P = 0.035$), and distant metastasis ($P = 0.001$) (Table 1). As the double IHC staining results showed (Fig. 1F), CD105 was expressed in the new capillary blood vessels around the cancer cells, and microvessel density(MVD) was significantly higher in LETM1-positive tissues than in LETM1-negative

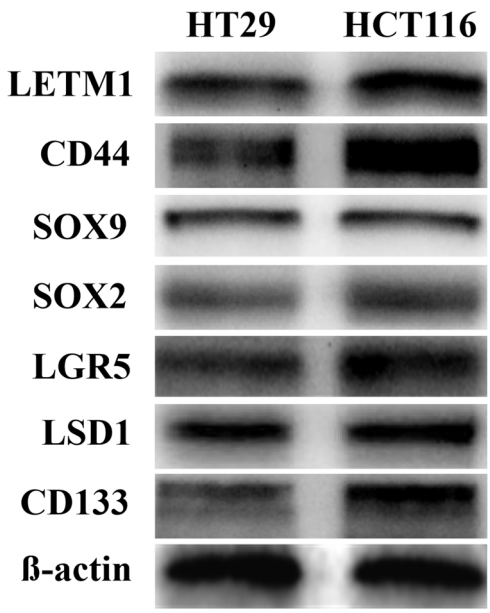

Fig. 3. Western blotting analysis of LETM1 and stemness related proteins CD44, SOX9, LGR5, LSD1, SOX2 and CD133 in colorectal adenocarcinoma cells.

tissues ($P < 0.001$; Fig. 1G).

The Kaplan-Meier survival analysis revealed that LETM1-positive expression in CRA was significantly associated with both poor overall survival (OS) ($P = 0.001$) and disease-free survival (DFS) ($P = 0.019$) (Fig. 1H, I). Univariate and multivariate Cox regression analyses confirmed that LETM1 is a prognostic indicator of unfavorable clinical outcomes of CRA. Upon univariate Cox regression analysis, the following were significant poor prognostic indicators of OS and DFS: pT stage (OS, $P = 0.002$), lymph node metastasis (OS, $P = 0.002$), distant metastasis (OS, $P = 0.012$; DFS, $P = 0.003$), and LETM1 expression status (OS, $P < 0.001$; DFS, $P = 0.005$) (Supplementary Table 2). Multivariate survival analysis using the Cox proportional hazards model revealed that pT stage ($P = 0.049$) and LETM1 expression status ($P = 0.017$) were independent prognostic indicators for OS, and distant metastasis ($P = 0.033$) and LETM1 expression status ($P = 0.023$) were independent prognostic indicators for DFS (Supplementary Table 3).

3.2. The expression of LETM1 and its relation with cancer stemness proteins in CRA

We investigated the association between LETM1 and cancer stemness-related proteins in CRA tissues. The results showed that LETM1 expression was significantly associated with the stemness-related proteins such as SOX9 ($P = 0.002$), LSD1 ($P = 0.049$), CD44 ($P = 0.002$), CD133 ($P = 0.003$), SOX2 ($P = 0.005$), and LGR5 ($P = 0.018$) expression (Table 2). Immunohistochemical multi-staining revealed that LETM1/CD44, LETM1/SOX9 and LETM1/LGR5 co-localized in the same CRA tissues. Positive signals of CD44 were mainly localized in the cell membranes of cancer cells, and SOX9 and LGR5 were localized in the nucleus and cytoplasm of cancer cells (Fig. 2A). The co-expression of LETM1/CD44, LETM1/SOX9 and LETM1/LGR5 was examined in HCT116 cells using confocal microscopy (Fig. 2B). To verify the above results, the expressions of LETM1 and stemness-related proteins were also detected using Western blotting. As a result, LETM1 and stemness-related proteins were more highly expressed in poorly differentiated CRA cell line HCT116 than in the moderately differentiated cell line HT29. Additionally, LETM1 expression in CRA cell lines (HCT116, HT29) was concordant with the expression of stemness-related proteins (Fig. 3). Moreover, LETM1 expression was positively correlated with the expression of HIF1α in CRA tissues (P = 0.026) (Table 2; Supplementary Fig. 1).

3.3. LETM1 was significantly associated with enhanced expression of cell cycle regulating proteins and PI3K/Akt/NFκB signaling proteins in CRA

The expression of LETM1 in CRA tissue was associated with the expression of cell cycle markers cyclinD1 ($P = 0.003$), CDK4 ($P = 0.048$), and p27 ($P = 0.001$) (Fig. 4A; Table 3). Moreover, there was significant association between LETM1 expression and pPI3K-p85 ($P = 0.001$), pAkt (pAkt-Ser473: $P = 0.004$; pAkt-Thr308: $P = 0.033$), and NFκB-p65 ($P = 0.018$) (Fig. 4B; Table 4). Positive signals of cyclinD1, CDK4, p27, pPI3K-p85, pAkt-Thr308, pAkt-Ser473, and NFκB-p65 are localized in the cytoplasm and nucleus of cancer cells.

4. Discussion

LETM1 regulation of mitochondrial biogenesis is an important feature of human cancer [20,21]. Data from our present study suggest that LETM1 may also play a significant role in stem-like cells in CRA. We showed that high LETM1 expression not only indicates poor prognosis but also is a potential CSC marker in CRA. To our knowledge, this is the first study to investigate the expression and significance of LETM1 in CRA and its association with CSC-related markers.

Previous studies have shown that the strongly positive rate of LETM1 protein expression was significantly higher in breast cancer than in ductal carcinoma in situ, hyperplasia, and adjacent normal breast tissues [8]. In our study, LETM1 is transiently expressed in the colon of the human fetus and is reexpressed in pathologic conditions such as CRA. Furthermore, LETM1 expression was significantly higher in CRA tissue than in normal colorectal tissue and adenoma tissues, which was highly consistent with the findings in breast cancer. Therefore, LETM1 may be a potential diagnostic biomarker for the detection of CRA. Huang et al. [9] demonstrated that LETM1 was expressed in bladder cancer tissues and bladder cancer cell lines, and knockdown of LETM1 significantly inhibited cell migration and invasion. Moreover, Yang et al. [11] demonstrated that LETM1 plays an important role in the development of ESCC, as high levels of LETM1 protein are significantly associated with cancer at an advanced clinical stage. Similarly, our data showed that LETM1 expression in CRA was positively correlated with advanced clinical stage, lymph node metastasis, and distant metastasis. Furthermore, angiogenesis is an important characteristic of cancer. Switching from the avascular phase to the vascular phase is a necessary process for tumor growth. CD105 was used as an endothelial cell marker and expressed in the new capillary blood vessels around the cancer cells. In our study, MVD was significantly higher in CRA in the LETM1-positive group than in the LETM1-negative group, suggesting that LETM1 may contribute to angiogenesis. Overexpression of LETM1 is correlated with reduced OS and DFS in patients with triple-negative breast cancer and ESCC [11,22]. Highly consistent with these reports, the Kaplan-Meier curves showed that LETM1 overexpression was strongly associated with reduced OS and DFS in patients with CRA. Moreover, multivariate Cox proportional hazards regression analysis also showed that LETM1 was an independent predictor for poor OS and DFS in patients with CRA. Overall, our results suggest that the upregulation of LETM1 expression in CRA may play a key role in tumor growth, leading to poor prognosis. Therefore, we believe that LETM1 could be a new biomarker to predict CRA prognosis and could also be used as a therapeutic target.

Our previous study also found that LETM1 was positively associated and co-localized with the CSC markers LSD1, CD44, and OCT4 in ESCC tissues, suggesting that LETM1 may serve as a novel CSC marker for the evaluation of poor prognosis in ESCC [11]. Highly consistent with these reports, we found that, in CRA, LETM1 expression was significantly correlated with the expression of stemness-related proteins, including SOX9, LSD1, CD44, CD133, SOX2, and LGR5. This result indicates that LETM1 is a potential cancer stemness gene in CRA. A hypoxic

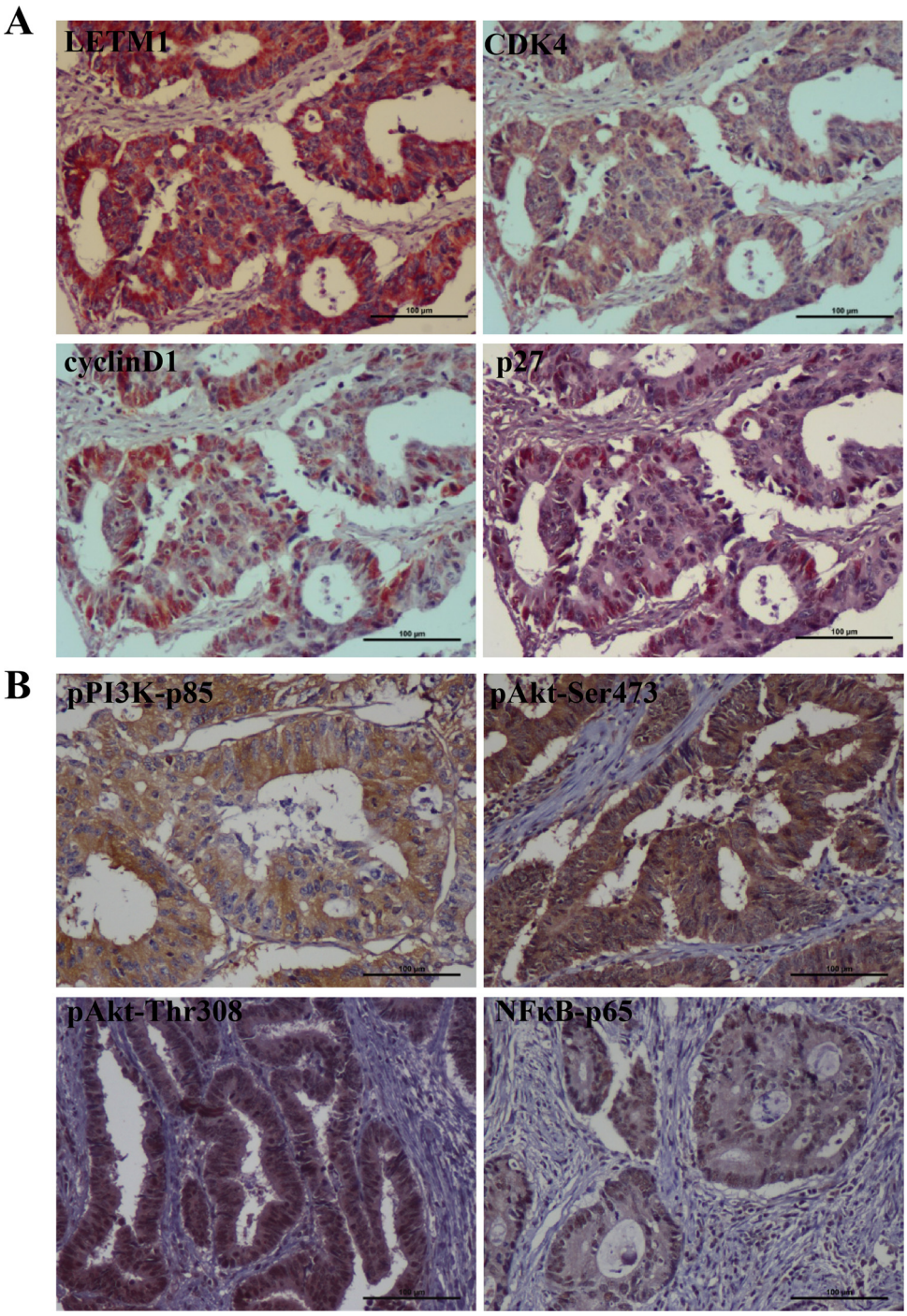

Fig. 4. Cell cycle regulator proteins and PI3K/Akt signaling proteins expressed in colorectal adenocarcinoma. (A) Immunohistochemical multi-staining of LETM1, CDK4, p27 and cyclinD1 in the same of colorectal adenocarcinoma tissues (original magnification × 200). (B) Immunohistochemical staining of pPI3K-p85, pAkt-Thr308, pAkt-Ser473 and NFκB-p65 in colorectal adenocarcinoma tissues (original magnification ×200).

microenvironment is an important stem cell niche that promotes the persistence of CSCs in tumors. In our study, we showed that LETM1 expression was positively correlated with HIF1α expression, suggesting that hypoxia induces LETM1 expression. However, further investigation is required to elucidate the mechanism of LETM1 regulation and its relationship to cancer stemness proteins in CRA.

Silencing of LETM1 expression influences autophagy and promotes AMPK activation and cell cycle arrest [5]. However, in the present study, expression of LETM1 was positively correlated with cyclinD1, CDK4, and p27 in CRA. The results revealed that LETM1 could stimulate cell cycle progression through the regulation of cell cycle-related proteins. The PI3K/Akt pathway is known to promote invasion and metastasis through the regulation of stem cell properties [23]. Lee et al. [10] have shown that the upregulation of LETM1 induces sustained activation of proliferative signaling pathways, such as PDGF signaling via Akt-induced YAP1 transactivation, resulting in aggressive thyroid cancer phenotypes [24]. Furthermore, LETM1 promotes Akt/PKB activation by the inhibition of C-terminal modulator protein [24]. We found that the expression of LETM1 in CRA tissue was associated with the expression of pPI3K-p85, pAkt-Thr308, pAkt-Ser473 and NFκB-p65, indicating that activation of PI3K/Akt/NFκB signaling is important for the oncogenic effect of LETM1 on cell progression and stem-like

Table 3
The correlation of LETM1 expression with cell cycle genes expression in CRA.

Variable	n	LETM1 (-) n(%)	LETM1 (+) n(%)	χ^2	R	P-value
p21				0.616	0.069	0.432
Negative	93	18(19.4)	75(80.6)			
Positive	9	1(11.1)	8(88.9)			
cyclinD1				9.084	0.250	0.003*
Negative	40	11(27.5)	29(72.5)			
Positive	62	8(12.9)	54(87.1)			
CDK4				3.912	0.164	0.048*
Negative	74	16(21.6)	58(78.4)			
Positive	28	3(10.7)	25(89.3)			
P27				10.609	0.271	0.001*
Negative	49	13(26.5)	36(73.5)			
Positive	53	6(11.3)	47(88.7)			
P16				0.494	0.060	0.482
Negative	41	7(17.1)	34(82.9)			
Positive	61	12(19.7)	49(80.3)			

Table 4
The correlation of LETM1 expression with PI3K/Akt/NF-κB signaling in CRA.

Variable	n	LETM1 (-) n (%)	LETM1 (+) n (%)	χ^2	R	P-value
pPI3K-p85				11.287	0.284	0.001*
Negative	53	14(26.4)	39(73.6)			
Positive	49	5(10.2)	44(89.8)			
pAkt-Ser473				8.129	0.238	0.004*
Negative	33	9(27.3)	24(72.7)			
Positive	69	10(14.5)	59(85.5)			
pAkt-Thr308				4.522	0.182	0.033*
Negative	44	11(25.0)	33(75.0)			
Positive	58	8(13.8)	50(86.2)			
NFκB-p65				5.629	0.201	0.018*
Negative	18	6(33.3)	12(66.7)			
Positive	84	13(15.5)	71(84.5)			

properties of cancer cells. However, the specific mechanism still needs to be further elucidated.

In conclusion, the present study provides evidence that LETM1 may be a potential cancer stemness gene, and its increased expression could predict poor prognosis in patients with CRA. Moreover, LETM1 may be a novel therapeutic target in the treatment of CRA.

Conflict of interest

The authors declare that they have no conflict of interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prp.2019.152437.

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