Article

J. Biol. Regul. Homeost. Agents. 2024; 38(6): 4663–4676
https://doi.org/10.23812/j.biol.regul.homeost.agents.20243806.371

LRPPRC Promotes Colorectal Cancer Cell Invasion and Metastasis through Tumor Cell Epithelial-Mesenchymal Transition

Shujing Wu[1], Yezhi Liang[2], Li Wang[1], Huaqiang Ruan[3], Xiaoxuan Wei[1], Zhengwen Cai[1], Li Liang[1,*]

[1]Departments of Oncology, The Second Affiliated Hospital of Guangxi Medical University, 530007 Nanning, Guangxi, China
[2]Departments of Pathology, The Second Affiliated Hospital of Guangxi Medical University, 530007 Nanning, Guangxi, China
[3]Departments of Gastroenterology, The Second Affiliated Hospital of Guangxi Medical University, 530007 Nanning, Guangxi, China
*Correspondence: 723491484@qq.com (Li Liang)
Submitted: 5 November 2023 Revised: 2 December 2023 Accepted: 18 December 2023 Published: 1 June 2024

Background: The leucine-rich pentatricopeptide repeat-containing protein (LRPPRC) functions to regulate cell cytoskeleton. This study assessed LRPPRC expression in colorectal cancer (CRC) for association with the clinicopathological features from patients and then investigated the impact of LRPPRC expression on CRC cells *in vitro* and *in vivo*.
Material and Methods: Tissue microarrays were built using 75 cases of each really normal and CRC tissues or 75-paired normal and CRC tissues for immunohistochemical analysis of LRPPRC expression. CRC cell lines were grown and assessed for tumor cell migration and invasion using wound healing and transwell assays. Changes in mRNA and protein expression in CRC cells were assayed using western blot and quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR), respectively. Knockdown or overexpression of LRPPRC was conducted using siRNA and cDNA transfections, respectively. Next, a nude mouse xenograft assay was performed to verify the impact of LRPPRC expressions *in vivo*.
Results: LRPPRC was overexpressed in CRC *vs.* real normal and paired normal tissues ($p < 0.05$), which was associated with CRC lymph node and distant metastases, and advanced clinical stages. *In vitro*, knockdown of LRPPRC expression inhibited CRC LOVO cell migration and invasion, whereas LRPPRC overexpression promoted CRC HCT116 cell migration and invasion. Moreover, LRPPRC overexpression upregulated Vimentin, N-cadherin, and Snail, and downregulated E-cadherin protein, whereas knockdown of LRPPRC expression had opposite results, suggesting increase in CRC cell epithelial-mesenchymal transition (EMT). In addition, knockdown of LRPPRC expression suppressed growth of colorectal cancer cell xenografts in mice.
Conclusions: LRPPRC could be an oncogene or had an oncogenic activity in CRC.

Keywords: CRC; LRPPRC; invasion; metastasis; EMT

Introduction

Colorectal cancer (CRC) is a common malignancy in the world, representing 10% of all newly detected 19.3 million cases of cancer and contributing to 9.4% of the 10.0 million deaths caused by cancer globally [1]. The CRC incidence and mortality rates were increased from 2018 to 2020 globally [2,3], although the rates were significantly reduced recently in the USA [4]. Clinically, when CRC is diagnosed at an advanced stage, the patient will lose the best opportunity for curative surgery [5]. CRC metastasis is the leading cause of CRC-associated death [6]. Therefore, further research and better understanding of CRC molecular mechanisms, like tumor initiation and progression, could lead to novel diagnosis and treatment options for CRC patients for better control and prognosis.

The pentatricopeptide repeat (PRR)-containing proteins are family of proteins that contain a 35-amino acid sequence motif as the RNA-binding proteins [7] and are widespread present in eukaryotes. This family of proteins functions to regulate transcription, post transcriptional processing, RNA stabilization, RNA editing, and translation of genes [8]. Leucine-rich pentatricopeptide repeat-containing protein (LRPPRC) is one of them, gene of which is localized in mammalian mitochondrial genome, and LRPPRC has multiple functions, such as regulation of energy metabolism, nuclear mRNA maturation, mRNA export, and gene signal transduction pathways as well as the mitophagy [9–11]. Altered LRPPRC expression in cells and tissues could lead to development and progression of various human cancers [12]. For example, a previous study [13] reported that LRPPRC protein was highly expressed and associated with advanced clinical stages, tumor de-differentiation, and poor prognosis of prostate cancer, while another study [14] showed that LRPPRC expression was aberrant in gastric cancer. LRPPRC expression

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

also induced bladder urothelial tumorigenesis [15]. Moreover, a poor prognosis for pancreatic cancer was associated with LRPPRC expression as an oncogene [16]. Regarding the role of LRPPRC in colorectal cancer, LRPPRC possesses anti-apoptosis activity and promoted CRC tumorigenesis [17]. A more recent study demonstrated that targeting of the LRPPRC-related gene signaling could collapse the chemoresistance of p53-inactive colorectal cancer *in vitro* [18]. An association was found between LRPPRC expression and poor prognosis of CRC [19].

During this study, we further assessed LRPPRC expression in paired normal and CRC tissues immunohistochemically for association with clinicopathological features from CRC patients. We then grew CRC cell lines to detect changes in the invasion and migration of tumor cells after knockdown or overexpression of LRPPRC using wound healing and transwell assays. We observed alterations in the expression of different mRNA and proteins associated with epithelial-mesenchymal transition (EMT) in CRC cells. To confirm the role of LRPPRC expressions *in vivo*, we conducted a xenograft assay using nude mice. We will conduct further study to verify the impact of LRPPRC on CRC.

Materials and Methods

Human CRC Tissue Microarray and Immunohistochemistry

This study involving human subjects was authorized by The Ethics Committee of Shanghai Outdo Biotech Co. Ltd. (Ethics number: SHXC2021YF01) in Shanghai, China and conducted in accordance with the principles outlined in the Declaration of Helsinki. Human CRC tissue microarrays consisting of either 75 normal and CRC tissues or 75 paired normal and CRC tissues were acquired from Shanghai Outdo Biotech Co. Ltd. (Cat. #HColA150CS02 and HRec-Ade150CS-02; Shanghai, China). These tissue microarray sections were used to detect LRPPRC expression immunohistochemically. Tissue microarray sections were deparaffinized in xylene, hydrated in alcohol, and then rehydrated in phosphate buffered saline (PBS). Afterwards, the segments underwent antigen restoration in a heated solution and were consistently heated with a low flame for 10 minutes. The sections were incubated in 3% H_2O_2 at room temperature for 15 min and then in 1% bovine serum albumin (BSA; Cat. #10711454001, Sigma, St Louis, MO, USA) at room temperature for 15 min. After that, the sections were incubated in the wet box with a diluted primary antibody (Cat. #ab259927; Abcam, Cambridge, MA, USA) in PBS at 1:100 at 4 °C overnight. The day after thrice washing with PBS, the sections were incubated with diluted secondary antibodies labeled with horseradish peroxidase (Cat. #31460; Thermo-Fisher, Waltham, MA, USA) in PBS at 1:500 at 37 °C for 60 min. A solution of 3,3′-diaminobenzidine (DAB) (Cat. #DAB-1031; Maixinshiji, Fuzhou, China) was used to visualize the sections, which were counterstained using hematoxylin solution (Cat. #H8070; Solarbio, Beijing, China). In the end, immunohistochemistry results were scored under a light microscope at 400× or 100× magnification after the sections had been dehydrated in ethanol, cleaned in xylene, and mounted with neutral gum (BX53, Olympus, Tokyo, Japan).

Data Acquisition from TCGA Database

The *LRPPRC* gene expression profile and other related clinical data of CRC patients were obtained from the The Cancer Genome Atlas (TCGA) database, accessible at https://www.cancer.gov/ccg/research/genome-sequencing/tcga. Since this study utilized the data from this database, which is open to public and freely available, there is no need to get the approval of the local ethics committee.

Cell Line, Culture, and Gene Transfection

Human CRC LOVO (CCL-229) and HCT116 (CCL-247) cell lines were originally obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA) and cultured in Roswell Park Memorial Institute medium-1640 (RPMI-1640) supplemented with 10% fetal bovine serum (FBS). The cells were incubated at 37 °C in an incubator with 95% air and 5% CO_2. Mycoplasma testing and STR validation has been performed on the cell lines used. For gene transfection, cells were grown in 6-well plates with a density of 3×10^4 cells per well [20]. Subsequently, the cells were transfected with *LRPPRC* siRNA (The siRNA sequences is CCGGGAGGAAGCAAACATTCAATTCAAGA GATTGAATG TTTGCTTCCTCCTTTTT) oligonucleotides or *LRPPRC* cDNA (purchased from China GenScript Gene Corporation, Nanjing, China) using Lipofectamine 2000 (Life Technologies Co., Carlsbad, CA, USA) according to the manufacturer's instructions.

Wound Healing Assay

LRPPRC-overexpressed and knocked down cells were changed to a serum-free medium and mitomycin C was added at 1 g/mL (Cat. #M0503; Sigma, St Louis, MO, USA) for 1 h. The cell scratches were then made with a 200-μL pipette tip and observed under an inverted microscope (Model IX53; Olympus, Tokyo, Japan) and photographed at a magnification of ×100. After 24 hours of growth in serum-free medium, the cells were photographed under a microscope at 100× magnification again after. The cell migration distance was calculated and compared using ImageJ software.

Transwell Assay

The invasion capacity of tumor cells was assessed using the transwell chamber (Cat. #14341; LABSELECT, Beijing, China) coated with Matrigel solution. Shortly, the transwell membranes were precoated with 100 μL Matrigel

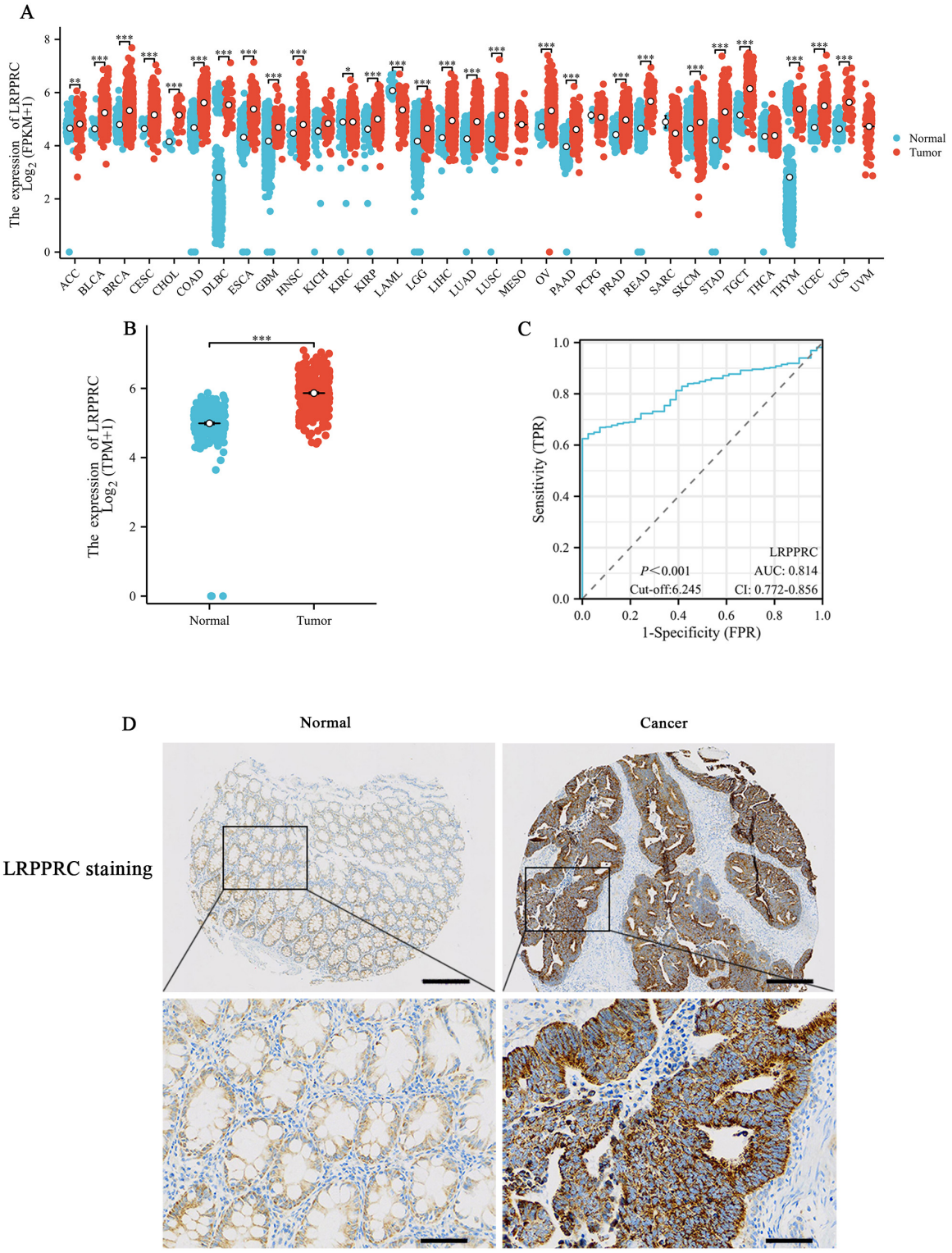

Fig. 1. LRPPRC overexpression in colorectal cancer tissue samples. (A) Level of LRPPRC expression in different cancers. (B) Level of LRPPRC expression in CRC and normal colon tissues. (C) ROC curve of LRPPRC expression in normal and tumor tissues. (D) Immunohistochemistry of LRPPRC in normal colon tissues and CRC. The upper panel, scale bar = 200 μm. Magnification, 100×. The bottom panel, scale bar = 50 μm. Magnification, 400×. LRPPRC, leucine-rich pentatricopeptide repeat-containing protein; ROC, receiver operating characteristic; CRC, colorectal cancer; TPR, true positive rate; FPR, false positive rate. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

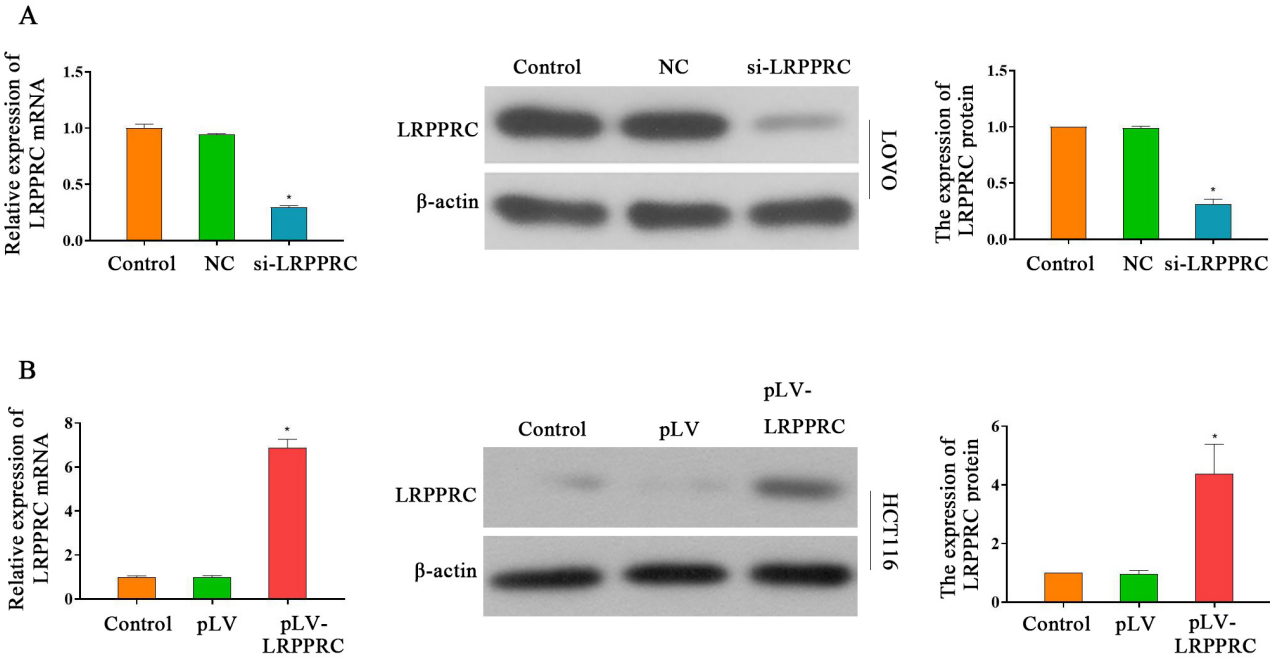

Fig. 2. Knockdown and overexpression of LRPPRC protein in CRC cell lines. (A) Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) and western blot assays. (B) qRT-PCR and western blot assays. Data are represented as the mean ± SD (n = 3). *$p < 0.05$. NC, negative control; si-LRPPRC, siRNA-mediated LRPPRC; pLV, pseudotyped lentiviral vector; SD, standard deviation.

(Cat. #356234; Corning, Corning, NY, USA), which had a concentration of 5 μg/mL. In 200 mL serum-free medium, 2×10^4 tumor cells were placed in each transwell chamber within 24-well plates, and a cell culture medium containing 10% FBS was placed in the lower chambers. The cells were incubated in a cell incubator at a temperature of 37 °C for a period of 24 hours. The cells that invaded the lower surface of the membranes were visualized by the crystal violet stain (Cat. #0528; Amresco, Solon, OH, USA), and an inverted microscope (Cat. #IX53, Olympus, Tokyo, Japan) at a 200× magnification was used to visualize and score cells that invaded the low surface of the membranes. Tumor cell migration capacity was also assayed in the transwell system without precoating with the matrigel. A total of 3–5 fields were captured in each group during the transwell assay and calculated using ImageJ software (Version 2.0.0, National Institute of Heath, Bethesda, MD, USA). The experiments were repeated three times.

Western Blot

The cultured cells were washed with ice-cold PBS and extracted for total cellular proteins using a cryogenic freezer centrifuge (Model H-2050R; Hunanxiangyi, Xiangtan, China) in a radioimmunoprecipitation assay buffer (RIPA buffer) containing a protease inhibitor. Subsequently, protein samples of 40 μg were electrophoresed in 10% polyacrylamide gel electrophoresis with sodium dodecyl sulfate (SDS–PAGE) and transferred to polyvinylidene difluoride membranes. For western blot, membranes were first incubated in 5% skim milk solution at room temperature for 1 hour and then with a primary antibody in PBS against LRPPRC (Cat. #ab259927; Abcam, Cambridge, MA, USA) at 1:1000 dilution, E-cadherin (1:1000; Cat. #WL01482; Wanleibio, Shenyang, China), N-cadherin (1:1000; Cat. #WL01047; Wanleibio, Shenyang, China), Snail (1:1000; Cat. #WL01863; Wanleibio, Shenyang, China), or β-actin (1:1000; Cat. #WL01372; Wanleibio, Shenyang, China) at 4 °C overnight. The following day, the membranes were rinsed three times with PBS-Tween 20 (PBS-T) and subsequently treated with a secondary sheep anti-rabbit antibody (1:5000; Cat. #WLA023; Wanleibio, Shenyang, China) at room temperature for 45 min. Subsequently, the membranes were briefly incubated with the chemiluminescence reagent (ECL; Cat. #WLA003; Wanleibio, Shenyang, China). With the gel image processing system (GEL-Pro-Analyzer software, Media Cybernetics, Rockville, MD, USA), target protein bands were captured and quantified in terms of optical density.

Quantitative Reverse Transcriptase-Polymerase Chain Reaction (qRT-PCR)

The total cellular RNA of LOVO cells was isolated after knockdown of LRPPRC, negative control, and control cells using a Trizol reagent (15596026, Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. RNA concentration was then measured using an ultraviolet spectrophotometer (NANO 2000, Thermo Company, Waltham, MA, USA) and then a Beyotime kit (D7160L, Beyotime, Shanghai, China) was used to reverse transcribe the

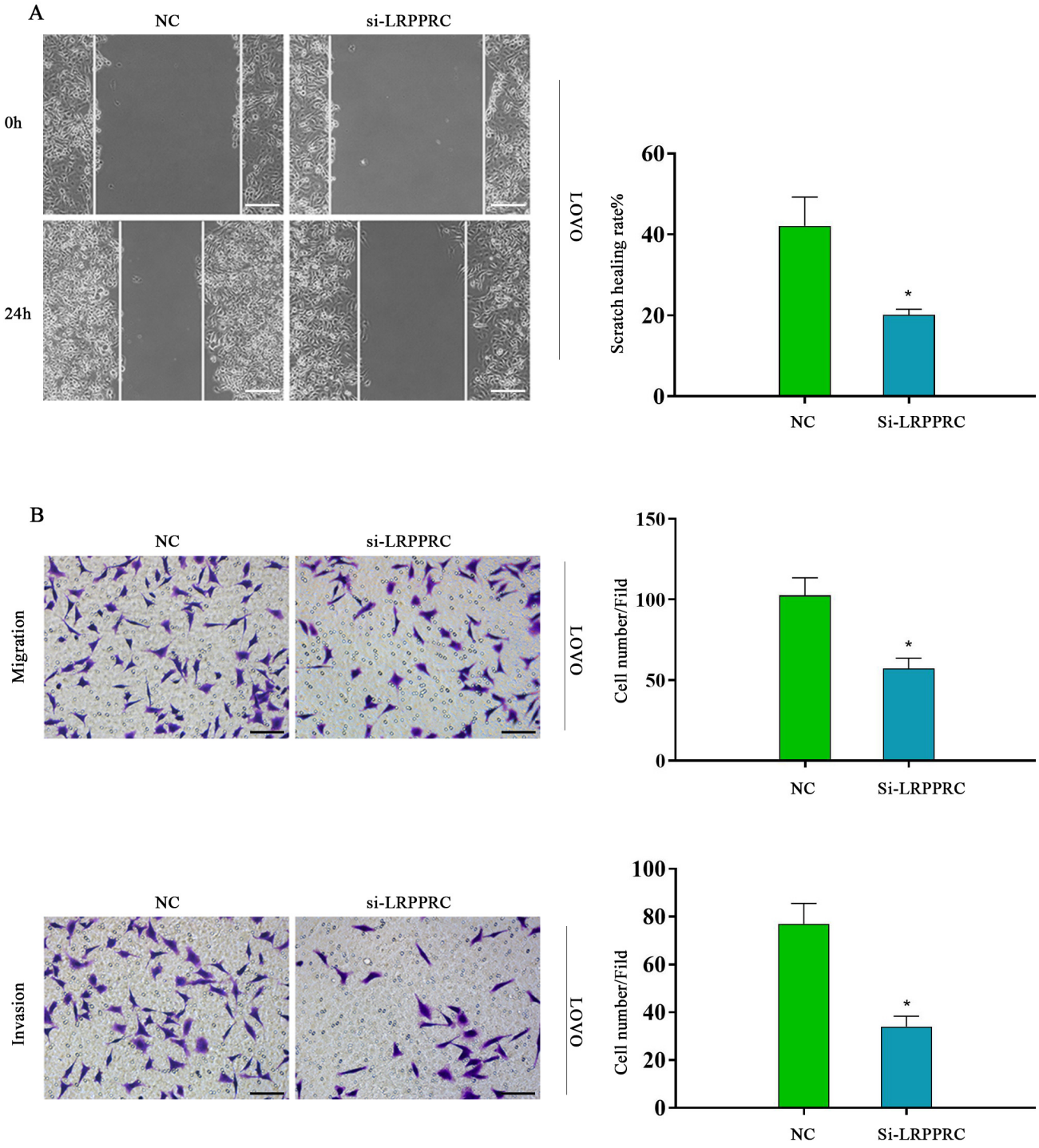

Fig. 3. Suppression of CRC cell migration and invasion after knockdown of LRPPRC expression in LOVO cells. (A) Wound healing assay of LOVO cells. Scale bar = 200 μm; Magnification, 100×. (B) Transwell assay of LOVO cells. Scale bar = 100 μm; Magnification, 200×. Data are represented as the mean ± SD (n = 3). *$p < 0.05$.

DNA into cDNA. For quantitative polymerase chain reaction (qPCR), 2 μL of each cDNA sample was added with 2× Taq PCR MasterMix (PC1150, Solarbio, Beijing, China) for amplification using LRPPRC primers in a BIONEER PCR machine (Exicycler 96, Bioneer, Daejeon, Korea). The reaction parameters included an initial heating at 94 °C for 5 minutes, followed by 40 repetitions of heating at 94 °C for 10 seconds, cooling at 60 °C for 20 seconds, and elongation at 72 °C for 30 seconds. The *LRPPRC* primers used were 5′-AAAATAAACGGTGACTGG-3′ and 5′-GTTGAGGCTGACGAAGAA-3′. An internal control was performed using *β-actin*, the primers of

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

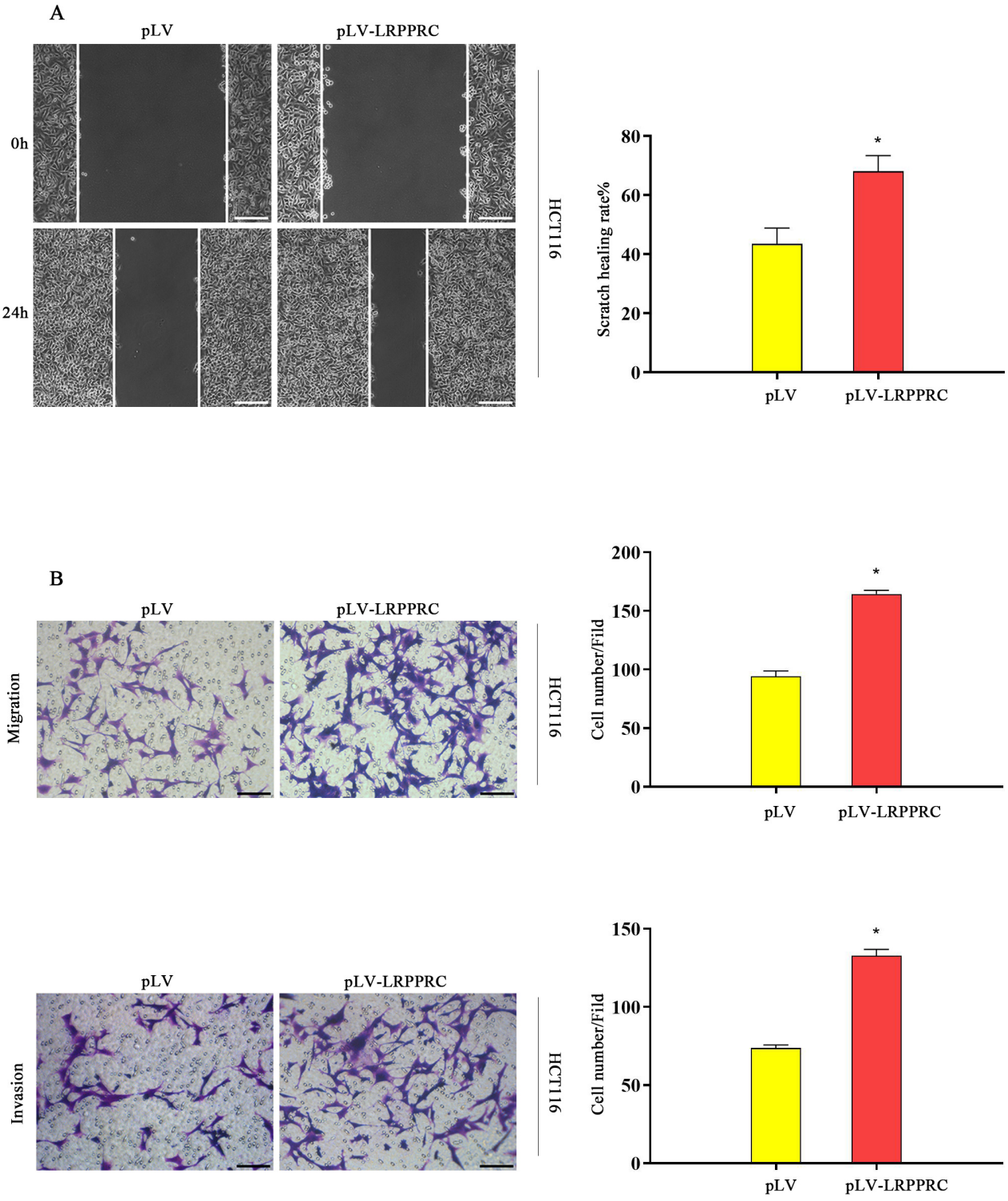

Fig. 4. After LRPPRC overexpression in HCT116 cells, the migration and invasion ability of CRC cell was upregulated. (A) Wound healing assay of HCT116 cells. Scale bar = 200 μm; Magnification, 100×. (B) Transwell assay of HCT116 cells. Scale bar = 100 μm; Magnification, 200×. Data are represented as the mean ± SD (n = 3). *$p < 0.05$.

which were 5′-GGCACCCAGCACAATGAA-3′ and 5′-TAGAAGCATTTGCGGTGG-3′. qPCR was conducted utilizing the $2^{-\Delta\Delta Ct}$ method, normalized against the level of *β-actin* mRNA.

Nude Mouse Tumor Cell Tumorigenicity Assay

Guangxi Medical University's Institutional Animal Care and Use Committee (IACUC) previously approved the animal experiments (Ethics Review number of #2020

key-0032). In brief, BALB/c nude mice (aged 4–5 weeks) were fed in a specifically pathogen-free (SPF) "barrier" facility and kept under controlled conditions. Nude mice were obtained from Charles River Laboratories (Beijing, China). SPF mouse chow and sterile water were provided to the mice. In our experiments, the nude mice were randomly divided into four groups (n = 6 mice per group). For pseudotyped lentiviral vector (pLV)-LRPPRC experiments, we first established CRC cell xenografts with 5×10^6 HCT116-Luc CRC cells in the right frank and 10 days later, the tumor mass was intratumorally injected with LRPPRC-overexpressed adenovirus [1×10^9 plaque-forming unit (PFU)] daily for 24 days. Moreover, for siRNA-mediated LRPPRC (si-LRPPRC) experiments, nude mice were injected with LOVO cells that were stably transfected with LRPPRC-siRNA or negative control (NC) (2×10^7cells). Tumor growth was checked every 3 days over the course of 24 days. After measuring the length and width, the tumor volume was determined by applying the formula (tumor xenograft volume (mm^3) = $\pi/6 \times$ (length) $\times$ (width) according to a previous study [21]. After the experiment had been completed, all mice were sacrificed by exposure of mice to CO_2 and neck dislocation and then tumor xenografts were collected, weighed, analyzed.

Statistical Analysis

The experimental data were expressed as mean ± standard deviation (SD). The one-way analysis of variance (ANOVA) and student's *t*-tests were used to analyze the data from two and multiple groups, respectively. SPSS (22.0, SPSS Inc., Chicago, IL, USA) was used to process all statistical analyses. For statistical significance, a *p*-value of 0.05 or less was required.

Results

LRPPRC Expression in CRC Tissues and Association with Clinicopathological Features

In these 33 cancer datasets of TCGA data, LRPPRC was differentially expressed in 27 cancers and highly expressed in 26 cancers (Fig. 1A). In the TCGA COAD RNA-Seq dataset, we analyzed the level of LRPPRC protein expression in 480 CRC and 41 normal colon tissues and found that LRPPRC was highly expressed in colorectal cancer tissues (Fig. 1B). The receiver operating characteristic (ROC) curve for LRPPRC expression in normal and tumor tissues demonstrated an area under the curve (AUC) value of 0.814 (Fig. 1C).

Then, we assessed LRPPRC expression in 75 cases of each really normal and CRC tissues immunohistochemically and found that LRPPRC protein was mainly expressed in the cytoplasm of tumor cells and was highly expressed in CRC *vs.* all normal mucosae (Fig. 1D). Furthermore, we associated LRPPRC expression with clinicopathological features from CRC patients and observed that LRPPRC expression was linked to lymph node involvement in CRC ($p = 0.011$), as well as distant metastasis ($p = 0.038$) and advanced clinical stages ($p = 0.002$; Table 1).

Table 1. The correlation between LRPPRC expression and clinicopathological data from patients with CRC.

Characteristics	LRPPRC expression		*p*-value
	Negative	Positive	
Sex			0.085
Male	16	15	
Female	14	30	
Age (years)			0.107
≥65	17	17	
<65	13	28	
T stage			0.003
T1+T2	10	3	
T3+T4	20	42	
N stage			0.011
N0	21	18	
N1+N2	9	27	
M stage			0.038
M0	29	36	
M1	1	9	
TNM stage			0.002
I+II	21	15	
III+IV	9	30	

T, primary tumor; N, lymph nodes; M, tumor distant metastasis; TNM, tumor-lymph node metastasis; LRPPRC, leucine-rich pentatricopeptide repeat-containing protein; CRC, colorectal cancer.

Impacts of LRPPRC Expression or Knockdown on CRC Cells

After transfection of LRPPRC cDNA or siRNA into HCT116 or LOVO cells, respectively, LRPPRC mRNA and protein levels were assessed in CRC LOVO cells or HCT116 cells before and after LRPPRC expression was knocked down or overexpressed. According to the western blot and qRT-PCR data, LRPPRC protein and mRNA levels were conspicuously lower in si-LRPPRC group of cells than that of the negative control siRNA-transfected cells ($p = 0.00$; $p = 0.00$; Fig. 2A). However, pLV-LRPPRC-transfected HCT116 cells showed upregulated level of LRPPRC protein and mRNA expression compared to those of pLV group of cells ($p = 0.01$; $p = 0.001$; Fig. 2B).

LOVO cells were grown and transfected with siLRPPRC or negative control siRNA, followed by performing a wound healing assay or transwell assay. HCT116 cells were grown and transfected with pLV-LRPPRC or a vector control, followed by performing the two identical experiments mentioned earlier. Our wound healing assay data revealed that si-LRPPRC transfection significantly reduced CRC cell migration ability compared to that of the negative control group ($p = 0.006$; Fig. 3A), while our transwell as-

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

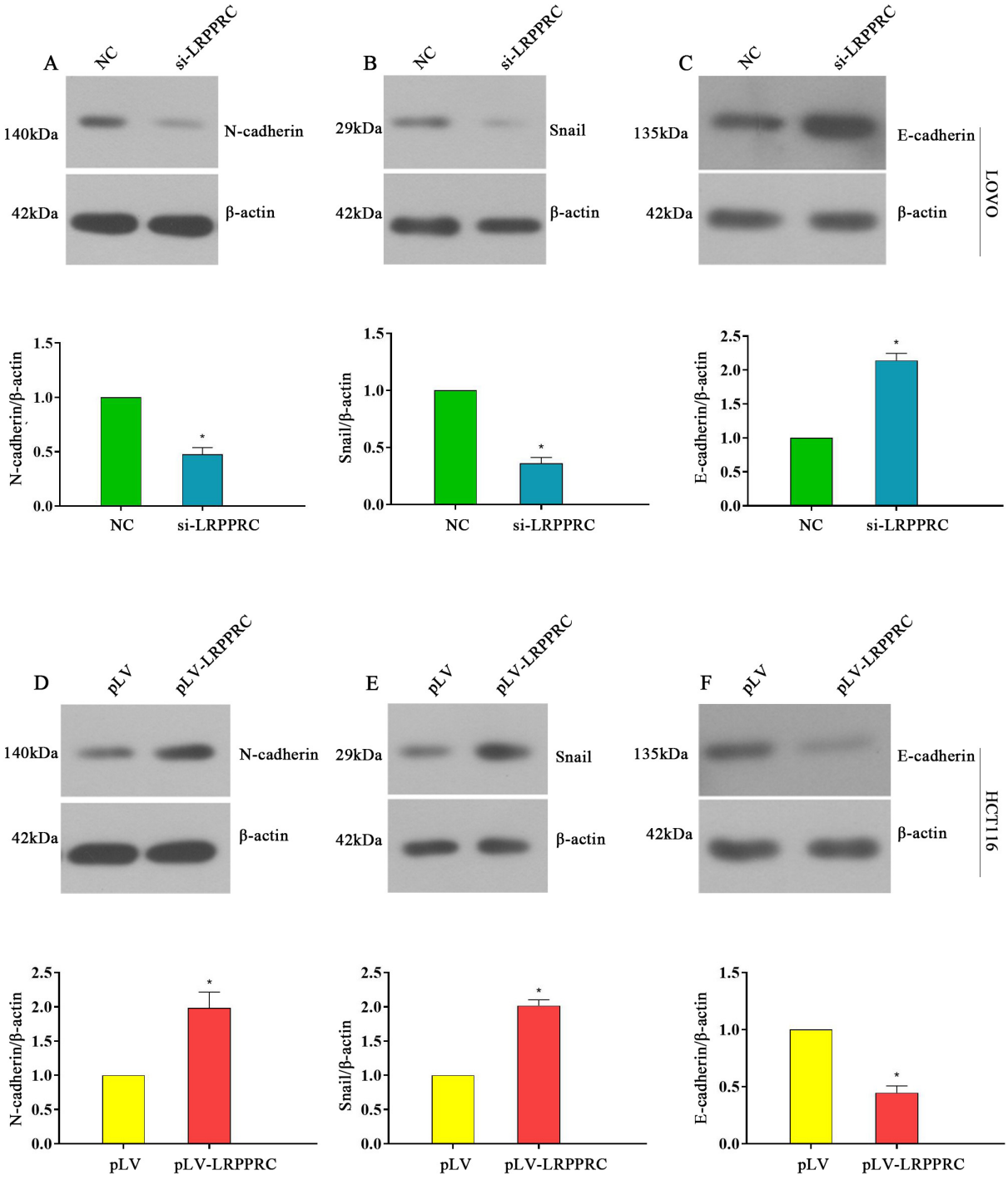

Fig. 5. LRPPRC promotion of CRC cell epithelial-mesenchymal transition (EMT). (A–C) Level of N-cadherin, Snail and E-cadherin expression after knockdown of LRPPRC expression. *$p < 0.05$. (D–F) Level of N-cadherin, Snail and E-cadherin expression after LRPPRC overexpression. The graphs show quantified data of the western blots. The data are represented as the mean ± SD (n = 3). *$p < 0.05$.

say data also showed that knockdown of LRPPRC expression suppressed CRC cell migration and invasion capacity ($p = 0.000$; $p = 0.000$; Fig. 3B). In contrast, LRPPRC overexpression promoted the migration and invasion of CRC cells vs. control cells ($p = 0.005$; $p = 0.000$; $p = 0.000$; Fig. 4A,B).

LRPPRC Promotion of CRC EMT in Vitro

LOVO cells were cultured and transfected into siLRPPRC or negative control siRNA, followed by western blot analysis. Western blot assay was performed on HCT116 cells that were cultured and transfected with pLV-LRPPRC

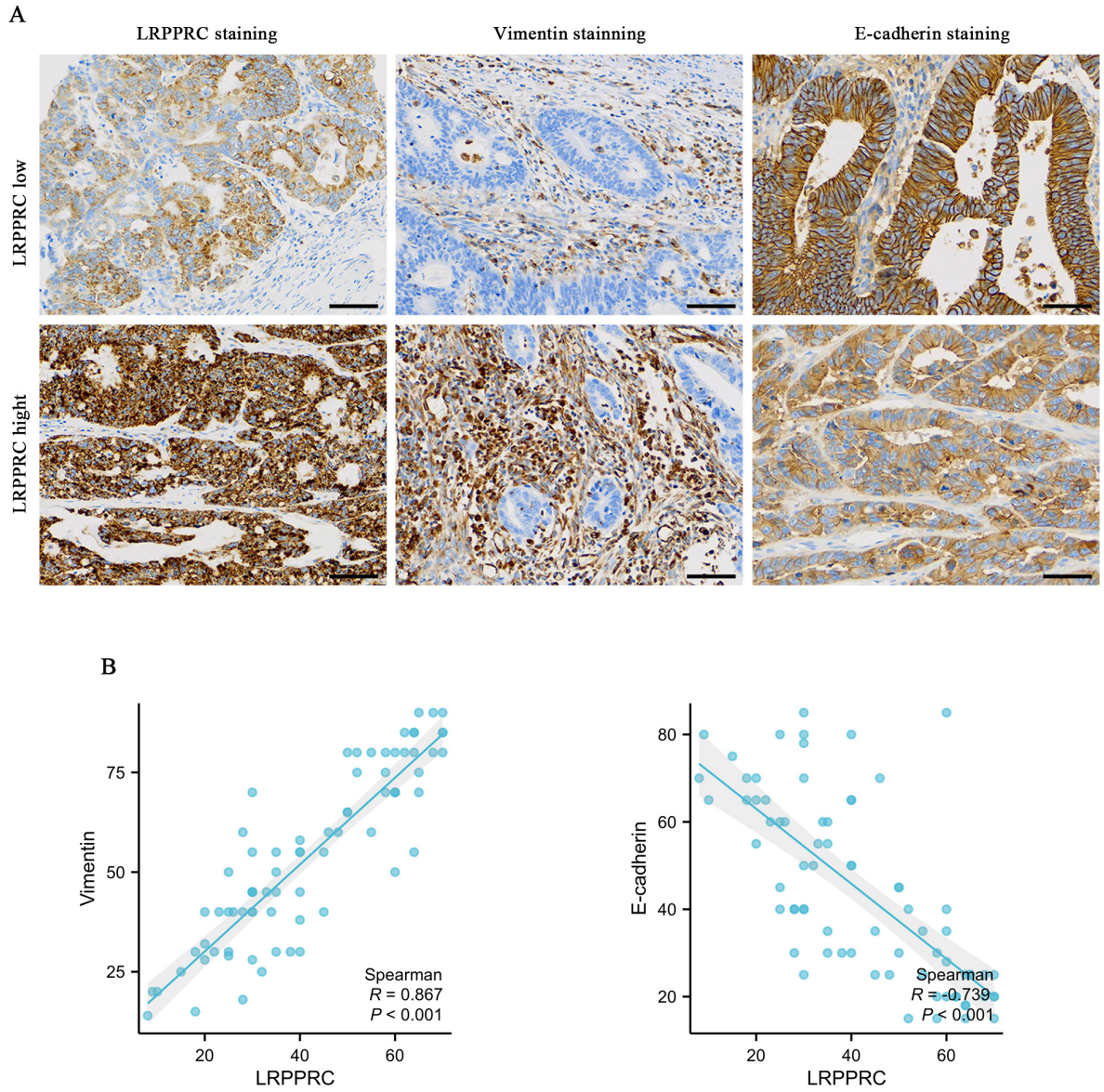

Fig. 6. Association of LRPPRC with Vimentin expression but inversely with E-cadherin in CRC tissue specimens. (A) Immunohistochemistry. Immunostaining of LRPPRC, Vimentin, and E-cadherin expression. Scale bar = 50 μm; Magnification, 400×. (B) Correlation analysis. Expression of Vimentin and LRPPRC or E-cadherin and LRPPRC (n = 75, Pearson's χ^2 test).

or a vector control. The underlying molecular events were then investigated after manipulation of LRPPRC expression and knockdown of LRPPRC expression was found to induce E-cadherin expression but reduce the expression of N-cadherin and Snail (p = 0.000; p = 0.000; p = 0.000; Fig. 5A–C). However, the the expressions of these proteins were affected in contrasting ways when LRPPRC was overexpressed (p = 0.000; p = 0.002; p = 0.000; Fig. 5D–F), suggesting that LRPPRC promoted CRC cell EMT to induce tumor cell migration and invasion *in vitro*.

Furthermore, we detected Vimentin/E-cadherin expression in CRC tissue specimens immunohistochemically and associated their expression with LRPPRC. Our data showed that Vimentin expression was high in CRC samples along with high LRPPRC expression, whereas E-cadherin was expressed in low LRPPRC expressed CRC tissues (Fig. 6A). Vimentin expression was positively correlated with LRPPRC expression in our correlation analysis (r = 0.867, Pearson's χ^2 test; Fig. 6B), but inversely associated with E-cadherin expression (r = –0.739, Pearson's χ^2 test; Fig. 6B).

Effect of LRPPRC Knockdown or Overexpression on CRC Xenograft Growth in Vivo

To assess the effect LRPPRC knockdown or overexpression on CRC xenograft growth *in vivo*, we first established nude mouse CRC cell xenografts using LOVO and HCT116 cells, respectively (see the methods section for de-

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

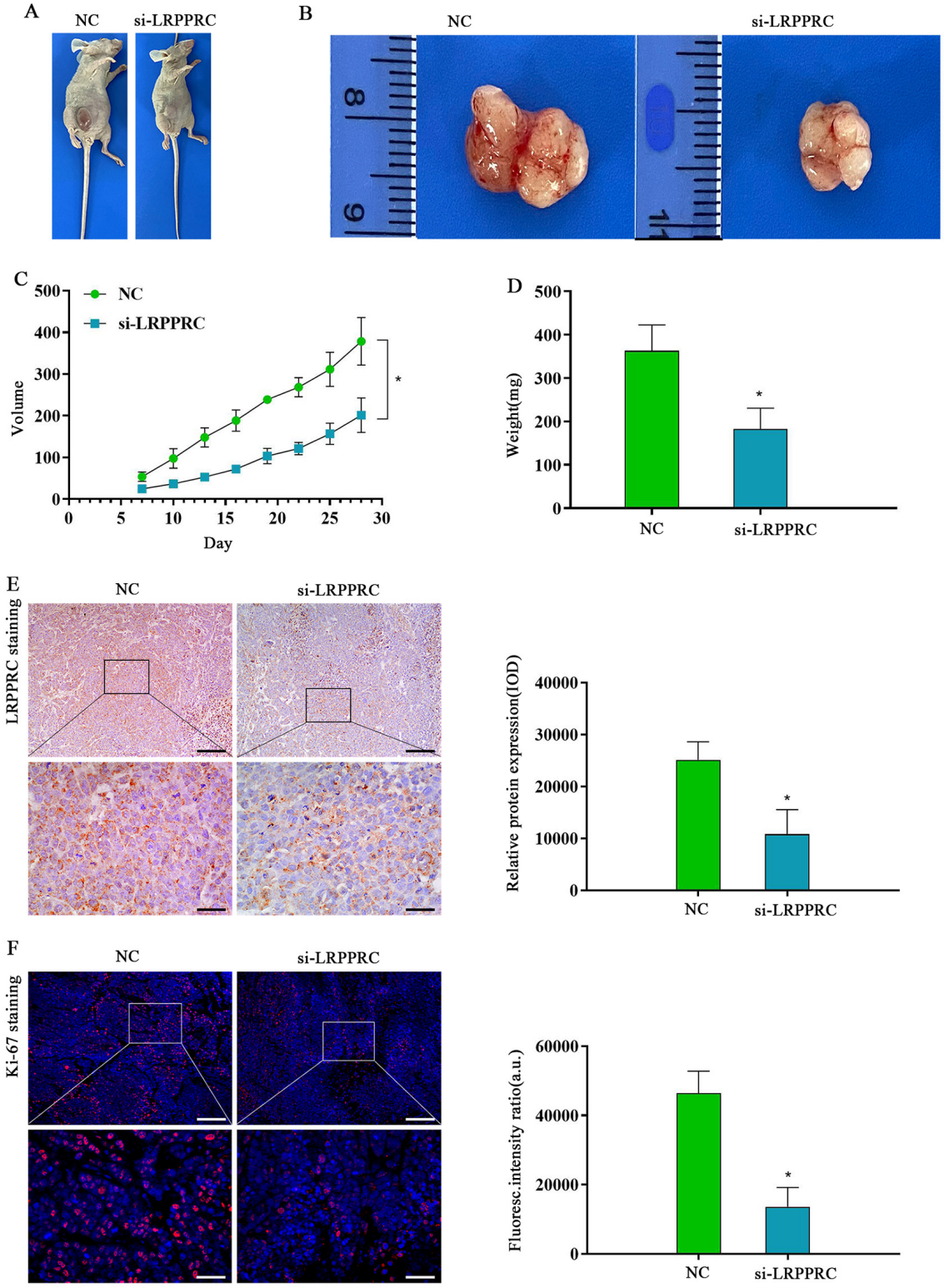

Fig. 7. Suppression of CRC cell xenograft growth in nude mice after knockdown of LRPPRC expression. (A) Nude mouse CRC cell xenograft assay and images. (B) CRC cell mouse xenografts. (C) Tumor growth curve diagram. (D) Tumor weight. (E) Immunohistochemical assay determining LRPPRC protein expression (left). The integral optical density (IOD) analysis of immunohistochemical data (right). The top section, scale bar = 200 μm. Magnification, 100×. The bottom section, scale bar = 50 μm. Magnification, 400×. (F) Immunofluorescence staining of Ki-67 used to assess proliferation (left). The top section, scale bar = 200 μm. Magnification, 100×. The bottom section, scale bar = 50 μm. Magnification, 400×. Quantitation of co-localization fluorescence intensity (right). Data are represented as the mean ± SD (n = 3). *$p < 0.05$.

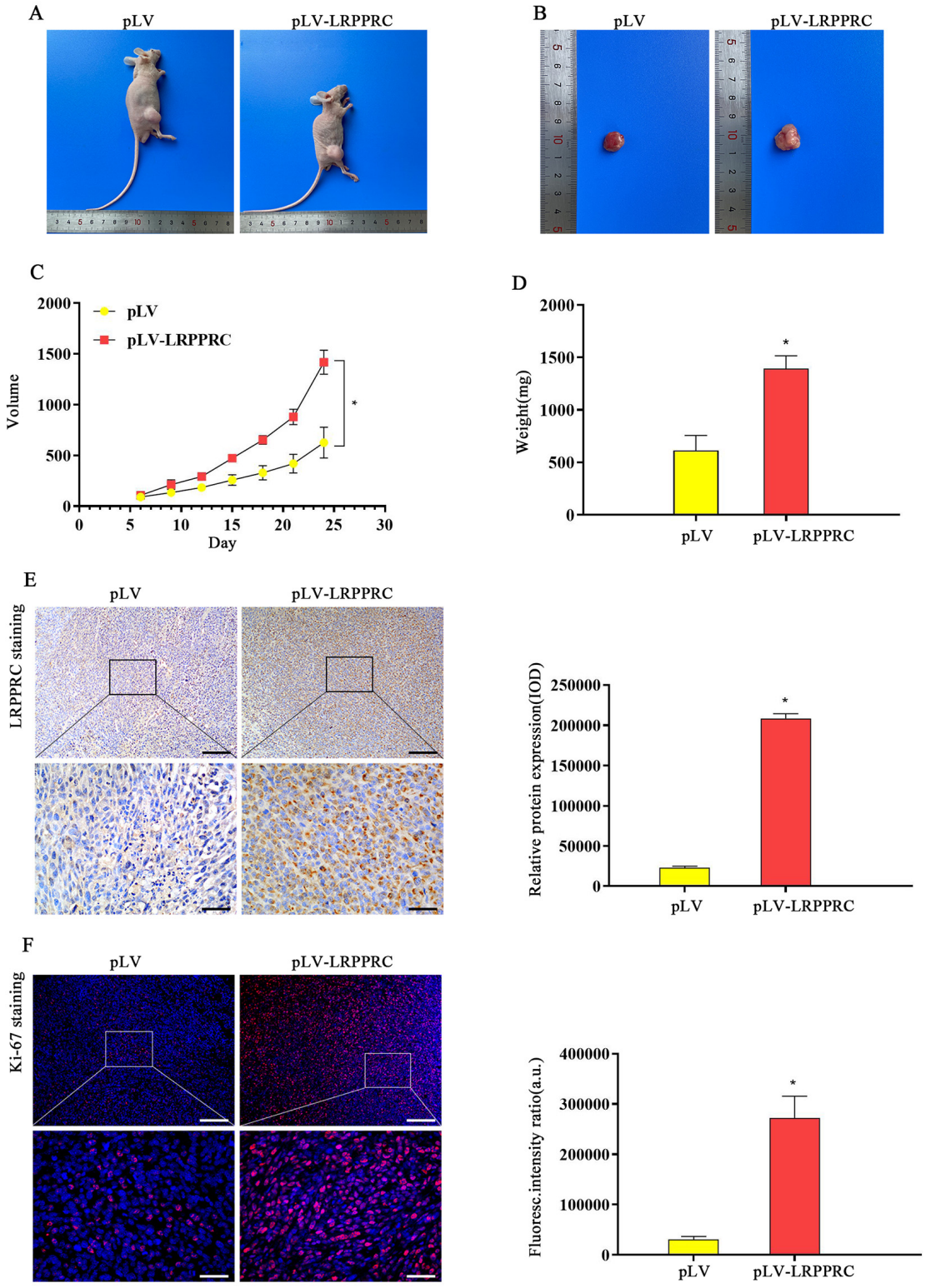

Fig. 8. Effect of LRPPRC knockdown on CRC xenograft growth *in vivo* in nude mice. (A) The nude mouse xenograft assay and imaging were performed on CRC cells. (B) CRC cell nude mouse xenografts. (C) Growth curves of tumors. (D) Tumor weights. (E) Immunohistochemical assay determining LRPPRC protein expression (left). IOD analysis of immunohistochemical data (right). The top section, scale bar = 200 μm. Magnification, 100×. The bottom section, scale bar = 50 μm. Magnification, 400×. (F) Immunofluorescence staining of Ki-67 was used to assess proliferation (left). The top section, scale bar = 200 μm. Magnification, 100×. The bottom section, scale bar = 50 μm. Magnification, 400×. Quantitation of co-localization fluorescence intensity (right). Data are presented as the mean ± SD (n = 3). *$p < 0.05$.

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

tails). Our *in vivo* data showed that knockdown of LRPPRC expression reduced CRC cell xenograft growth ($p = 0.012$; $p = 0.015$; Fig. 7A–D). Immunohistochemical data on CRC cell xenografts showed that LRPPRC and Ki67 expression was lower in si-LRPPRC group of xenografts than that in NC group ($p = 0.014$; $p = 0.003$; Fig. 7E,F). In contrast, pLV-LRPPRC-injected CRC xenografts showed the opposite effects, i.e., LRPPRC overexpression promoted subcutaneous tumor cell growth in nude mice ($p = 0.002$; $p = 0.002$; Fig. 8A–D). LRPPRC and Ki67 were all highly expressed in pLV-LRPPRC-injected CRC cell xenografts than those of the pLV control ones ($p = 0.000$; $p = 0.001$; Fig. 8E,F).

Discussion

In the current study, we demonstrated that LRPPRC protein was highly expressed in CRC compared to that of real normal and paired normal tissues. It was linked to advanced clinical stages, metastasis of the lymph nodes, and distant metastasis when LRPPRC was overexpressed. However, knockdown of LRPPRC expression suppressed CRC LOVO cell migration and invasion as well as expression of Vimentin, N-cadherin, and Snail, but upregulated E-cadherin expression. However, LRPPRC overexpression had opposite results, suggesting increase in CRC cell EMT. *In vivo*, knockdown of LRPPRC expression suppressed growth of CRC cell xenografts in mice, whereas LRPPRC overexpression had opposite effects on xenograft growth and LRPPRC and Ki67 expression. Thus, we can conclude that LRPPRC could be an oncogene or at least possesses an oncogenic activity in CRC by promoting CRC cell EMT. Future study will investigate LRPPRC as a tumor marker for CRC early detection, prognoses, or treatment response.

The PPR-motif-containing proteins are widespread expression in eukaryotes and play an important role in gene expression and regulation [8]. As one of them, LRPPRC can regulate cell energy metabolism, nuclear mRNA maturation and exporting, cell signaling, and mitophagy, a selective process in mitochondrial degradation [9–11]. Previous studies demonstrated that LRPPRC overexpression occurred in various cancers and associated with tumor progression or prognosis [13,15,17–19,22–24]. Our current study is surely consistent with these published data and further supported their findings that LRPPRC is an oncogene in CRC and other human cancers. Indeed, our current study also showed that a significant correlation was found between the expression of LRPPRC in lymph node metastasis and distant metastases in CRC, as well as advanced clinical stages, which indicates LRPPRC expression contributed to CRC progression. As a biomarker for timely detection and prediction of the prognosis of CRC, detection of LRPPRC expression may be useful.

Indeed, in a previous study, it was found that knockdown of LRPPRC expression induced the mitochondria-mediated apoptosis but reduced prostate cancer cell migration and invasion [13]. LncRNA SNHG17 and LRPPRC interact to stabilize c-Myc protein, promoting G1/S transition and proliferation of tumor cells in human hepatocellular carcinoma tissues [25]. Moreover, a relationship was found between LRPPRC expression and resistance to cisplatin among lung cancer patients [22]. The findings of our current study are as follows: knockdown of LRPPRC expression inhibited the migratory and invasive behavior of CRC cells, whereas LRPPRC overexpression promoted the migration and invasion of CRC HCT116 cells. LRPPRC overexpression had the same impact on CRC cells *in vivo*. Furthermore, LRPPRC-related cisplatin resistance occurred by regulating *MDR1* expression in lung cancer cells [22] and in p53-inactive colorectal cancer [18]. LRPPRC enhanced progression and immunity of HCC evasion by upregulation of m^6A modification of PD-L1 mRNA [26]. In our study, the findings were as follows: knockdown of LRPPRC expression induced E-cadherin expression but reduced N-cadherin and Snail expression, whereas these proteins were affected in the opposite way when LRPPRC was overexpressed. Taken altogether, LRPPRC may regulate different cell signaling pathway to facilitate cell processing and activities. However, in our current study, we just assessed these EMT-related proteins but we don't know the underlying molecular consequences of changes in expression of these EMT-related proteins in CRC cells, pending further investigation.

As we know, the EMT is to biologically convert the epithelial cells into mesenchymal cell types and the latter intend to be high mobility and invasion capacity, during which cells reduce expression of E-cadherin, an epithelial cadherin, but increase expression of Vimentin and N-cadherin, molecular characteristics of the mesenchymal cells [27,28]. Since the epithelial cells have unique cell polarity and cell-cell interactions and connection to the basement membrane, whereas the mesenchymal cells possess strong ability of migration and invasion by degradation of the extracellular matrix [29]. So the EMT phenomenon is one of the characteristics of cancer cells and the key step of cancer progression and metastasis [30]. In this regard, LRPPRC expression is phenotypically and molecularly associated with CRC progression and metastasis. Thus, targeting of LRPPRC expression could be useful in future control of CRC progression and metastasis.

However, our current study is just association assessment of LRPPRC expression in CRC *ex vivo*, *in vitro*, and *in vivo*. Lots of more need to do for better understanding of the role of LRPPRC in CRC.

Conclusions

In this study, our *in vitro* and *in vivo* experiments were performed to confirm the effect of knockdown or overexpression of the LRPPRC protein in colorectal cancer cells. Utilizing LOVO, HCT116 cells, and xenograft nude mice as models, we found that LRPPRC promoted the invasion and metastasis of colorectal cancer cells through EMT.

In conclusion, the data from our current research further verified the importance of LRPPRC in CRC progression as an oncogene or at least oncogenic activities in CRC. Further study is needed to verify LRPPRC expression as a biomarker for the early detection of CRC and as a prognostic prediction or molecular target for the control of CRC clinically in the future.

Abbreviations

AUC, area under the curve; CRC, colorectal cancer; EMT, epithelial-mesenchymal transition; FPR, false positive rate; IOD, integral optical density; LRPPRC, leucine-rich pentatricopeptide repeat-containing protein; NC, negative control; pLV, pseudotyped lentiviral vector; ROC, receiver operating characteristic; si-LRPPRC, siRNA-mediated LRPPRC; TPR, true positive rate.

Availability of Data and Materials

All data included in this study are available upon request by contact with the corresponding author.

Author Contributions

LL conceived and designed the experiments; SJW and YZL prepared the manuscript; YZL, LW, HQR and XXW performed the experiments; SJW, ZWC and LL analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study were previously approved by the Institutional Animal Care and Use Committee (IACUC) of Guangxi Medical University (Ethics Review number of #2020 key-0032). This study involving human subjects was authorized by The Ethics Committee of Shanghai Outdo Biotech Co. Ltd. (Ethics number: SHXC2021YF01) in Shanghai, China and conducted in accordance with the principles outlined in the Declaration of Helsinki.

Acknowledgment

Not applicable.

Funding

This work was supported in part by grants from The National Natural Science Foundation of China (#82060761), Natural Science Foundation of Guangxi Province (#2018GXNSFBA050072, #2023GXNSFBA026077), Guangxi Medical and health key discipline construction project (#2022049), National Natural Science Foundation of the Second Affiliated Hospital of Guangxi Medical University (#GJPY2023003), and The 2020 excellent reserve talents project of the Second Affiliated Hospital of Guangxi Medical University (#hbrc202003).

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al*. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: a Cancer Journal for Clinicians. 2021; 71: 209–249.

[2] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a Cancer Journal for Clinicians. 2018; 68: 394–424.

[3] Long F, Lin Z, Li L, Ma M, Lu Z, Jing L, *et al*. Comprehensive landscape and future perspectives of circular RNAs in colorectal cancer. Molecular Cancer. 2021; 20: 26.

[4] Xia C, Dong X, Li H, Cao M, Sun D, He S, *et al*. Cancer statistics in China and United States, 2022: profiles, trends, and determinants. Chinese Medical Journal. 2022; 135: 584–590.

[5] Hong Z, Tang P, Liu B, Ran C, Yuan C, Zhang Y, *et al*. Ferroptosis-related Genes for Overall Survival Prediction in Patients with Colorectal Cancer can be Inhibited by Gallic acid. International Journal of Biological Sciences. 2021; 17: 942–956.

[6] Güllü N, Kobelt D, Brim H, Rahman S, Timm L, Smith J, *et al*. Saffron Crudes and Compounds Restrict MACC1-Dependent Cell Proliferation and Migration of Colorectal Cancer Cells. Cells. 2020; 9: 1829.

[7] Kotera E, Tasaka M, Shikanai T. A pentatricopeptide repeat protein is essential for RNA editing in chloroplasts. Nature. 2005; 433: 326–330.

[8] Sugita M. An Overview of Pentatricopeptide Repeat (PPR) Proteins in the Moss *Physcomitrium patens* and Their Role in Organellar Gene Expression. Plants (Basel, Switzerland). 2022; 11: 2279.

[9] Köhler F, Müller-Rischart AK, Conradt B, Rolland SG. The loss of LRPPRC function induces the mitochondrial unfolded protein response. Aging. 2015; 7: 701–717.

[10] Sterky FH, Ruzzenente B, Gustafsson CM, Samuelsson T, Larsson NG. LRPPRC is a mitochondrial matrix protein that is conserved in metazoans. Biochemical and Biophysical Research Communications. 2010; 398: 759–764.

[11] Rolland SG, Motori E, Memar N, Hench J, Frank S, Winklhofer KF, *et al*. Impaired complex IV activity in response to loss of LRPPRC function can be compensated by mitochondrial hyperfusion. Proceedings of the National Academy of Sciences of the United States of America. 2013; 110: E2967–E2976.

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

[12] Zong WX, Rabinowitz JD, White E. Mitochondria and Cancer. Molecular Cell. 2016; 61: 667–676.

[13] Jiang X, Li X, Huang H, Jiang F, Lin Z, He H, *et al*. Elevated levels of mitochondrion-associated autophagy inhibitor LRPPRC are associated with poor prognosis in patients with prostate cancer. Cancer. 2014; 120: 1228–1236.

[14] Gao W, Xua J, Wang F, Zhang L, Peng R, Zhu Y, *et al*. Mitochondrial Proteomics Approach Reveals Voltage-Dependent Anion Channel 1 (VDAC1) as a Potential Biomarker of Gastric Cancer. Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology. 2015; 37: 2339–2354.

[15] Wei WS, Wang N, Deng MH, Dong P, Liu JY, Xiang Z, *et al*. LRPPRC regulates redox homeostasis via the circANKHD1/FOXM1 axis to enhance bladder urothelial carcinoma tumorigenesis. Redox Biology. 2021; 48: 102201.

[16] Wang L, Luo J, Li Y, Lu Y, Zhang Y, Tian B, *et al*. Mitochondrial-Associated Protein LRPPRC is Related With Poor Prognosis Potentially and Exerts as an Oncogene *Via* Maintaining Mitochondrial Function in Pancreatic Cancer. Frontiers in Genetics. 2022; 12: 817672.

[17] Tian T, Ikeda JI, Wang Y, Mamat S, Luo W, Aozasa K, *et al*. Role of leucine-rich pentatricopeptide repeat motif-containing protein (LRPPRC) for anti-apoptosis and tumourigenesis in cancers. European Journal of Cancer (Oxford, England: 1990). 2012; 48: 2462–2473.

[18] Yang Y, Yuan H, Zhao L, Guo S, Hu S, Tian M, *et al*. Targeting the miR-34a/LRPPRC/MDR1 axis collapse the chemoresistance in P53 inactive colorectal cancer. Cell Death and Differentiation. 2022; 29: 2177–2189.

[19] Jiang T, Xing L, Zhao L, Ye Z, Yu D, Lin S. Comprehensive analysis of m6A related gene mutation characteristics and prognosis in colorectal cancer. BMC Medical Genomics. 2023; 16: 105.

[20] Liang L, Huang J. Oxymatrine inhibits epithelial-mesenchymal transition through regulation of NF-κB signaling in colorectal cancer cells. Oncology Reports. 2016; 36: 1333–1338.

[21] Liang L, Sun W, Wei X, Wang L, Ruan H, Zhang J, *et al*. Oxymatrine suppresses colorectal cancer progression by inhibiting NLRP3 inflammasome activation through mitophagy induction *in vitro* and *in vivo*. Phytotherapy Research: PTR. 2023; 37: 3342–3362.

[22] Hu Y, Cui J, Jin L, Su Y, Zhang X. LRPPRC contributes to the cisplatin resistance of lung cancer cells by regulating *MDR1* expression. Oncology Reports. 2021; 45: 4.

[23] Fahrmann JF, Grapov D, Phinney BS, Stroble C, DeFelice BC, Rom W, *et al*. Proteomic profiling of lung adenocarcinoma indicates heightened DNA repair, antioxidant mechanisms and identifies LASP1 as a potential negative predictor of survival. Clinical Proteomics. 2016; 13: 31.

[24] Michaud M, Barakat S, Magnard S, Rigal D, Baggetto LG. Leucine-rich protein 130 contributes to apoptosis resistance of human hepatocarcinoma cells. International Journal of Oncology. 2011; 38: 169–178.

[25] Liu JY, Chen YJ, Feng HH, Chen ZL, Wang YL, Yang JE, *et al*. LncRNA SNHG17 interacts with LRPPRC to stabilize c-Myc protein and promote G1/S transition and cell proliferation. Cell Death & Disease. 2021; 12: 970.

[26] Wang H, Tang A, Cui Y, Gong H, Li H. LRPPRC facilitates tumor progression and immune evasion through upregulation of m^6A modification of PD-L1 mRNA in hepatocellular carcinoma. Frontiers in Immunology. 2023; 14: 1144774.

[27] Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nature Reviews. Cancer. 2002; 2: 442–454.

[28] Arias AM. Epithelial mesenchymal interactions in cancer and development. Cell. 2001; 105: 425–431.

[29] Sabbah M, Emami S, Redeuilh G, Julien S, Prévost G, Zimber A, *et al*. Molecular signature and therapeutic perspective of the epithelial-to-mesenchymal transitions in epithelial cancers. Drug Resistance Updates: Reviews and Commentaries in Antimicrobial and Anticancer Chemotherapy. 2008; 11: 123–151.

[30] Yang J, Weinberg RA. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. Developmental Cell. 2008; 14: 818–829.