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# High expression of LIMD2 predicts a poor prognosis and promotes migration of colon cancer cells

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## Abstract

**Background** LIM domain-containing protein 2 (LIMD2) is known to promote metastasis in several cancers. However, its role and underlying mechanisms in colon cancer remain unclear. This study focused on investigating the prognostic value, functional impact, and molecular mechanisms of LIMD2 in colon cancer.

**Methods** LIMD2 expression in colon cancer tissues and matched non-cancerous tissues was detected using RT-qPCR and immunohistochemistry. The chi-square test was used to assess the association between LIMD2 expression and clinicopathological characteristics. Kaplan–Meier survival analysis and Cox proportional hazards models were applied to evaluate the prognostic value of LIMD2. The functional role of LIMD2 in colon cancer cell migration was examined through Transwell and wound healing assays. Additionally, RNA sequencing (RNA-seq), co-immunoprecipitation (CoIP), silver staining, and mass spectrometry were performed to uncover the role of LIMD2 in colon cancer cell migration.

**Results** LIMD2 expression was significantly increased in colon cancer samples and cell lines. High LIMD2 expression was positively correlated with lymph node metastasis and TNM stage. Patients with elevated LIMD2 expression exhibited poorer overall survival (OS). Gain-of-function assays demonstrated that LIMD2 accelerated colon cancer cell migration. RNA-seq analysis revealed that LIMD2 regulates ECM-receptor interactions and focal adhesion pathways. Furthermore, CoIP and mass spectrometry identified cofilin1 as a LIMD2-interacting protein.

**Conclusions** Our findings indicate that LIMD2 serves as a novel prognostic biomarker and potential target for colon cancer treatment.

**Keywords** LIMD2, Colon cancer, Migration, Prognosis

## 1 Introduction

Colon cancer is a common malignancy with a high rate of morbidity and mortality [1]. Metastasis remains the leading reason of mortality in patients with colon cancer [2], yet effective clinical strategies for preventing metastasis are still lacking. Therefore, to

further elucidate the molecular mechanisms of colon cancer metastasis is important for improving patient prognosis. The ability of cancer cells to migrate is a key driver of metastasis. Several metastasis-associated pathways, such as the ECM-receptor interaction and focal adhesion pathways, play important roles in regulating cytoskeletal dynamics, adhesion and metastasis of cancer cell [3]. These pathways are often abnormally activated in tumors, leading to cancer metastasis.

LIM domain containing 2 (LIMD2) belongs to the LIM domain protein family, which is known to regulate multiple aspects of tumor progression, including gene expression, cytoskeletal remodeling, cell adhesion, motility, and signal transduction [4, 5]. For instance, LIMK1 and LIMK2 have been shown to enhance colorectal cancer metastasis and contribute to chemoresistance by regulating the cofilin pathway [6]. Additionally, LIM-HD and LIM-only proteins function as transcription factors or components of transcriptional complexes in the nucleus, thereby influencing gene expression and tumorigenesis [7–9].

LIMD2 contains one LIM domain. Janete M. et al. [10] first reported that LIMD2 mRNA expression was higher in metastatic lesions of papillary thyroid carcinoma than in primary tumors and normal tissues, suggesting a potential role for LIMD2 in metastasis. Subsequently, a Brazilian research team confirmed through immunohistochemical staining that LIMD2 protein expression was higher in lymph node metastases of papillary thyroid carcinoma than that in primary tumors [11]. Further studies demonstrated that LIMD2 knockdown in thyroid cancer cell lines reduced cancer cell invasion and migration [12]. Beyond thyroid cancer, emerging evidence indicates that LIMD2 is upregulated in various malignancies, including non-small cell lung cancer [13, 14], renal clear cell carcinoma [15], esophageal cancer [16], and ovarian cancer [17]. Additionally, LIMD2 overexpression was positively associated with poor prognosis and increased metastasis. These studies demonstrate that LIMD2 plays a key role in cancer cell motility and metastatic progression. However, its function and underlying mechanisms in colon cancer remain unknown.

In the current research, we aim to explore the correlation between LIMD2 expression levels and the prognosis of patients with colon cancer, as well as its association with clinicopathological characteristics. Additionally, we examine the function of LIMD2 in promoting colon cancer cell migration. Moreover, we identify the involvement of the ECM-receptor interaction and focal adhesion pathways, along with cofilin1 signaling, in LIMD2-driven cancer cell migration.

## 2 Materials and methods

### 2.1 Cell culture

The human colonic mucosal epithelial cell line FHC and the human colon cancer cell lines SW480, RKO, SW620, HCT116 were purchased from the American Type Culture Collection (ATCC, USA). DMEM Medium (Gibco, USA) supplemented with 20% FBS (Gibco, USA) was used to culture FHC cells. Cancer cell lines were maintained in the recommended medium with 10% FBS.

### 2.2 Colon cancer samples

The colon cancer tissue microarray (HColA180Su17) containing 96 cases of colon cancer was purchased from Shanghai Outdo Biotech Company (Shanghai, China). The

Ethics Committee of Shanghai Outdo Biotech Company approved the use of microarray for research purposes. Fresh colon cancer tissues and paraffin-embedded specimens were obtained from Jiangsu Province People's Hospital Suqian Hospital (Suqian, China). All patients signed the informed consent, and the Ethics Committee of Jiangsu Province People's Hospital Suqian Hospital (Suqian, China) approved the use of clinical samples for research purposes.

### 2.3 Immunohistochemistry (IHC)

IHC staining and quantitative H-score were performed as described in our previous study [18]. Briefly, the H-score was calculated as follows: H-score = (% of strong × 3) + (% of moderate × 2) + (% of weak × 1). The resulting score is between 0 and 300. The primary antibody was anti-LIMD2 (1:200, 15471-1-AP, Proteintech, USA).

### 2.4 Construction of LIMD2 overexpression vector

The LIMD2 and LIMD2-Flag overexpression vectors were constructed by Ruibiotech (Guangzhou, China). Briefly, the coding sequences of LIMD2 were generated and cloned into the pcDNA3.1 or pcDNA3.1-Flag plasmids. Lipofectamine 3000 (Invitrogen, USA) was used to conduct transfection.

### 2.5 RNA isolation and reverse transcription (RT)

RNA isolation and RT were carried out as described in previous study [18]. RNA was isolated using RNAiso Plus reagent (#9109, Takara, Japan). RT was performed using PrimeScript™ RT reagent Kit with gDNA Eraser (RR047A, Takara, Japan).

### 2.6 Quantitative reverse transcription polymerase chain reaction (RT-qPCR)

RT-qPCR was conducted in the ABI 7500/7500 Fast Real-Time PCR System or LightCycler-480 according to our previous study [18, 19]. SybrGreen qPCR Mastermix (DBI2043, DBI Bioscience, Germany) was used in RT-qPCR. The relative expression levels of mRNA were calculated using the $2^{-\Delta\Delta Ct}$ method. GAPDH was the internal reference. Ruibiotech (Guangzhou, China) generated all primers used in this research, and their sequences are listed in Supplementary Table S1.

### 2.7 Western blot

Cells were lysed on ice using lysis buffer (FD008, FDbio, China) for 30 min. The lysate was centrifuged at 12,000 rpm for 15 min at 4 ℃. Proteins were quantified, separated by 12% SDS-PAGE, and transferred onto a PVDF membrane (Millipore, USA). The incubation of PVDF membrane was performed at 4 ℃ overnight with primary antibodies: anti-LIMD2 (1:1000, 15471-1-AP, Proteintech, USA), anti-cofilin1 (1:1000, 10960-1-AP, Proteintech, USA), anti-p-cofilin1 (Ser3) (1:1000, 310036, ZENBIO, China), and anti-β-tubulin (1:2000, 10094-1-AP, Proteintech, USA). The next day, the PVDF membrane was incubated with the second antibodies HRP-conjugated goat anti-mouse IgG (1:5000, SA00001-1, Proteintech, USA) and goat anti-rabbit IgG (1:5000, SA00001-2, Proteintech, USA) for 1 h at room temperature. An enhanced chemiluminescence [20] detection Kit (FD8020, FDbio, China) was used to visualize proteins. The original blots are provided in the Supplementary information file.

### 2.8 Transwell migration assay

Briefly, cells were transfected with either LIMD2 overexpression vector or the control vector. After 24 h, cells were trypsinized, resuspended in serum-free medium, counted, and added to the Transwell insert. After 48 h, migrated cells were fixed, washed and stained. Pictures of migrated cells were captured under a microscope and analyzed for quantification.

### 2.9 Wound healing assay

Colon cancer cells were transfected with the LIMD2 overexpression plasmid. After 48 h, the confluence of cells reached 90%. A linear scratch was generated by a 10-μl plastic pipette tip to simulate a wound. Then, cells were washed by PBS, and serum-free medium was added to the plates. Images of the wounds were captured under a microscope at 200× magnification at 0, 24, and 48 h.

### 2.10 RNA-seq

RNA sequencing (RNA-seq) and bioinformatics analysis were conducted by The Beijing Genomics Institute (BGI, China). The raw RNA-seq data have been uploaded in the NCBI Sequence Read Archive (SRA) database under accession number PRJNA1181289 (https://www.ncbi.nlm.nih.gov/sra/PRJNA1181289).

### 2.11 Co-immunoprecipitation (CoIP) and immunofuorescence (IF)

These experiments were performed according to the methods used in our previous study [18]. Briefly, HCT116 and SW620 cells were transfected with the LIMD2-Flag overexpression plasmid, and proteins were extracted 48 h later using lysis buffer (FD011, FDbio, China). The lysate was then incubated with an anti-Flag antibody (YM3001, Immunoway, USA) or control IgG (AC011, ABclonal, China) at 4 ℃ for 1 h. The mixture was subsequently incubated overnight at 4 ℃ with 20 μL of A/G agarose beads (SC-2003, SANTA Cruz Biotechnology, USA). The proteins were eluted and subjected to electrophoretic separation, silver staining (#P00175, Fast Silver Stain Kit, Beyotime, China), mass spectrometry (Wininnovate Bio, Shenzhen, China), or western blot analysis. Anti-cofilin1 (1:200, 10960-1-AP, Proteintech, USA) and dyLight 488 labeled goat anti-rabbit IgG (1:200, #RS23220, immunoway, USA) were used in IF assay.

### 2.12 Statistical analysis

GraphPad Prism 9.0 (La Jolla, CA, USA) and SPSS23.0 software (IBM, USA) were used to perform statistical analysis. Comparison between the two groups was carried out using a two-tailed Student's t-test. LIMD2 expression levels in colon cancer tissues and matched non-cancerous tissues were compared using the paired t-test. The correlation between LIMD2 expression and clinicopathological parameters was evaluated using a Chi-square ($\chi 2$) test. The overall survival rates of patients with low and high LIMD2 expression were analyzed using the Kaplan–Meier method and the Log-rank test. The prognostic value of LIMD2 expression was assessed using univariate and multivariate Cox proportional hazards models. A P-value $< 0.05$ was considered statistically significant.

## 3 Results

### 3.1 LIMD2 is upregulated in colon cancer tissues and colon cancer cell lines

To evaluate LIMD2 expression levels in colon cancer, we analyzed LIMD2 protein expression in ten paired colon cancer samples and matched non-cancerous normal tissues using IHC staining. IHC analysis revealed that LIMD2 expression was upregulated in colon cancer samples compared to matched non-cancerous samples (Fig. 1A). Furthermore, LIMD2 mRNA levels were measured in ten paired colon cancer tissues and matched adjacent non-cancerous tissues using RT-qPCR. The results revealed that LIMD2 mRNA expression was higher in colon cancer samples than in matched non-cancerous normal tissues (Fig. 1B), which was consistent with the IHC staining findings. Additionally, LIMD2 expression was analyzed in colon cancer cell lines and the normal colonic mucosal epithelial cell line FHC using RT-qPCR. The results indicated that LIMD2 expression was significantly higher in the colon cancer cell lines SW480, SW620, RKO, and HCT116 than in FHC. Moreover, LIMD2 expression was higher in the metastasis-derived cell line SW620 than the primary tumor-derived cell line SW480 (Fig. 1C). These findings confirm that LIMD2 is upregulated in both colon cancer tissues and colon cancer cell lines.

### 3.2 LIMD2 is correlated with lymphatic metastasis and TNM stage in patients with colon cancer

To assess the association between LIMD2 expression and the clinicopathological parameters of patients with colon cancer, we determined LIMD2 expression using a tissue microarray containing 96 available cases of colon cancer. IHC staining revealed that LIMD2 expression varied, exhibiting strong, moderate, or weak staining in colon cancer

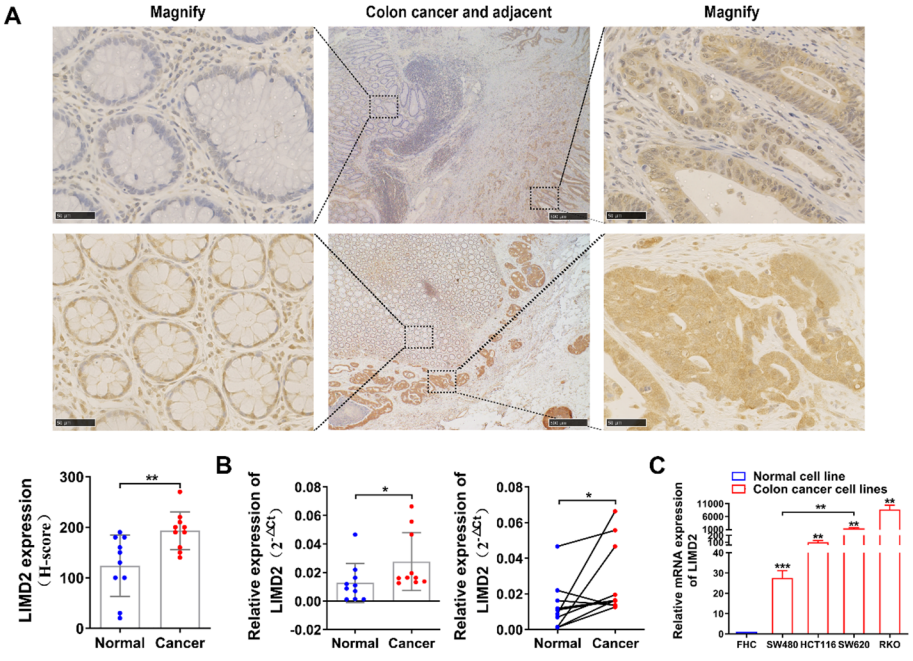


**Fig. 1** LIMD2 is upregulated in colon cancer tissues and cell lines. (A) LIMD2 expression was higher in colon cancer samples ($n=10$) compared to adjacent non-cancerous tissues ($n=10$). Scale: 50 μm and 500 μm. (B) LIMD2 mRNA expression was higher in the colon cancer tissues ($n=10$) than in the matched non-cancerous normal tissues ($n=10$). (C) RT-qPCR showed LIMD2 expression was higher in the colon cancer cell lines SW480, SW620, RKO, and HCT116 than in the normal colonic mucosal epithelial cell line FHC

tissues (Fig. 2A). Based on staining strength and area, the H-score of IHC staining was calculated, and patients were stratified into a high LIMD2 expression group ($n = 48$) and a low LIMD2 expression group ($n = 48$), using the median H-score as the cutoff value (Fig. 2B). Statistical analysis demonstrated that higher LIMD2 expression was positively correlated with both lymphatic metastasis and TNM stage (Fig. 2C and D). However, no significant association was observed between LIMD2 expression and age, tumor size, or tumor differentiation (Table 1).

### 3.3 High LIMD2 expression in colon cancer is associated with poor prognosis

To evaluate the prognostic value of LIMD2, we performed Kaplan–Meier survival analysis and Cox proportional hazards modeling using both univariate and multivariate approaches basing on the LIMD2 protein expression data used in Fig. 2. Kaplan–Meier survival analysis revealed that patients with lower LIMD2 expression had significantly higher overall survival (OS) rates compared to those with higher LIMD2 expression (Fig. 3A). Furthermore, univariate Cox proportional hazards analysis demonstrated that lower LIMD2 expression was associated with a 70% reduction in the risk of death compared to patients with higher LIMD2 expression (Fig. 3B). Multivariate Cox regression analysis verified this association, showing that patients with lower LIMD2 expression had a reduced risk of death (Fig. 3B). Consistent with our findings, the cox proportional hazards model confirmed that the higher LIMD2 expression increased the risk of death based on a colon cancer cohort (CPTAC-2) of TCGA (Supplementary Fig. 1). These

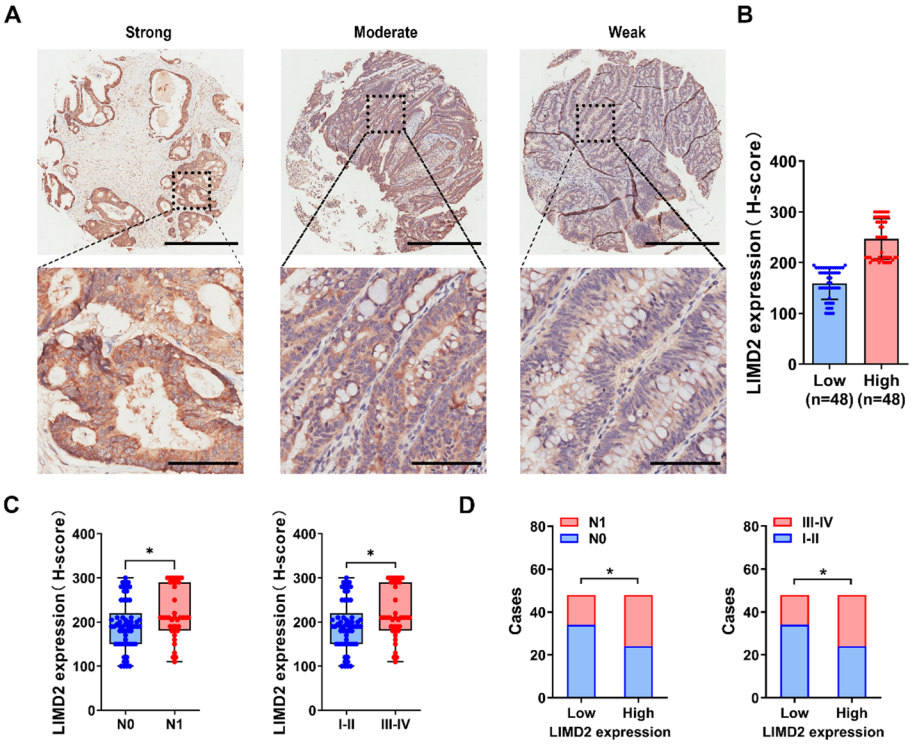


**Fig. 2** Correlation between LIMD2 expression and lymphatic metastasis in colon cancers. (A) Representative IHC images show strong, moderate, and weak staining of LIMD2 expression in a colon cancer tissue microarray ($n = 96$). (B) Colon cancers were stratified into a high LIMD2 expression group and a low LIMD2 expression group based on the median H-score of LIMD2 expression. (C) and (D) LIMD2 expression was positively correlated with lymphatic metastasis and TNM stage in patients with colon cancer

**Table 1** Correlation between LIMD2 expression and clinicopathologic characteristics in colon cancer

| Characteristics | LIMD2 expression ($n=96$) [a] | | P value |
|---|---|---|---|
| | Low ($n=48$) | High ($n=48$) | |
| Age (years) [b] | | | |
| < 69 | 26 | 21 | 0.3073 |
| >= 69 | 22 | 27 | |
| Diameter (cm) [c] | | | |
| < 5 | 19 | 21 | 0.6149 |
| >= 5 | 29 | 26 | |
| Differentiation | | | |
| Well | 10 | 10 | 0.3878 |
| Moderate | 32 | 27 | |
| Poor | 6 | 11 | |
| Lymphatic metastasis | | | |
| No | 34 | 24 | 0.0369 |
| Yes | 14 | 24 | |
| TNM classification | | | |
| I-II | 34 | 24 | 0.0369 |
| III-IV | 14 | 24 | |

a) Patients were divided according to the median expression of LIMD2

b) The age used for group was the median of patients

c) Tumor size was grouped according to median

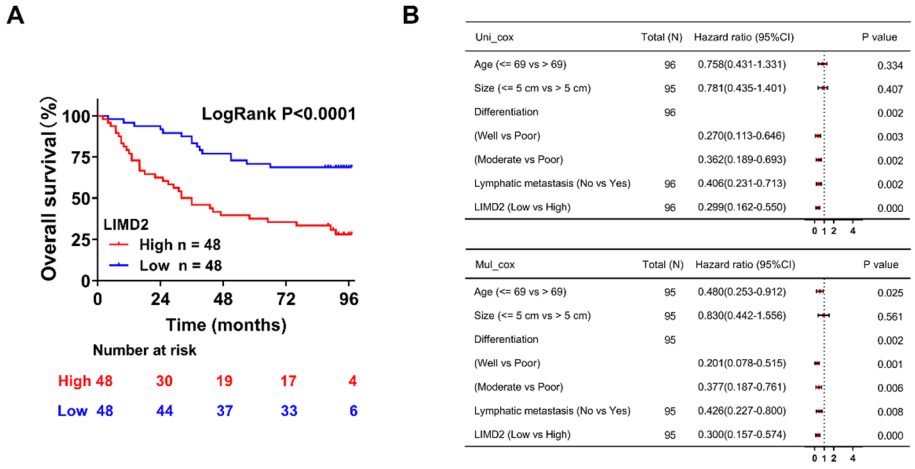


**Fig. 3** High expression of LIMD2 in colon cancer predicts a poor prognosis in patients. (A) Kaplan-Meier survival analysis demonstrated that LIMD2 expression was negatively correlated with the overall survival rate in patients with colon cancer. (B) Forest plots from the univariate and multivariate Cox proportional hazards model showed the prognostic value of LIMD2

findings strongly indicate that LIMD2 expression is associated with poor prognosis in patients with colon cancer.

### 3.4 LIMD2 promotes migration of colon cancer cells

The above findings revealed that LIMD2 is associated with metastasis in patients with colon cancer and is upregulated in highly metastatic cells, suggesting that LIMD2 may possess the ability to promote tumor cell migration. To test this hypothesis, we conducted a gain-of-function assay. First, LIMD2 mRNA and protein levels were successfully upregulated in SW620, HCT116, and RKO cells following transfection with a

LIMD2 overexpression vector (Fig. 4A, B). Transwell migration assays demonstrated that LIMD2 overexpression increased the number of migrated cells (Fig. 4C). Consistent with these findings, wound healing assays revealed that elevated LIMD2 expression enhanced the migratory capacity of colon cancer cells (Fig. 4D). Additionally, the migration ability of primary tumor-derived SW480 cells was also enhanced following LIMD2 overexpression (Supplementary Fig. 2). These results collectively demonstrate that LIMD2 promotes the migration of colon cancer cells.

### 3.5 LIMD2 regulates the ECM-receptor interaction and focal adhesion pathways

To investigate the molecular pathways through which LIMD2 influences colon cancer cell migration, we performed RNA sequencing (RNA-seq) and KEGG analysis using HCT116 cells overexpressing LIMD2. RNA-seq identified 815 differentially expressed genes (DEGs), including 553 upregulated and 262 downregulated genes (Fig. 5A). KEGG pathway analysis revealed that these DEGs were enriched in the ECM-receptor interaction, focal adhesion, and PI3K/Akt signaling pathways (Fig. 5B). Among these pathways,

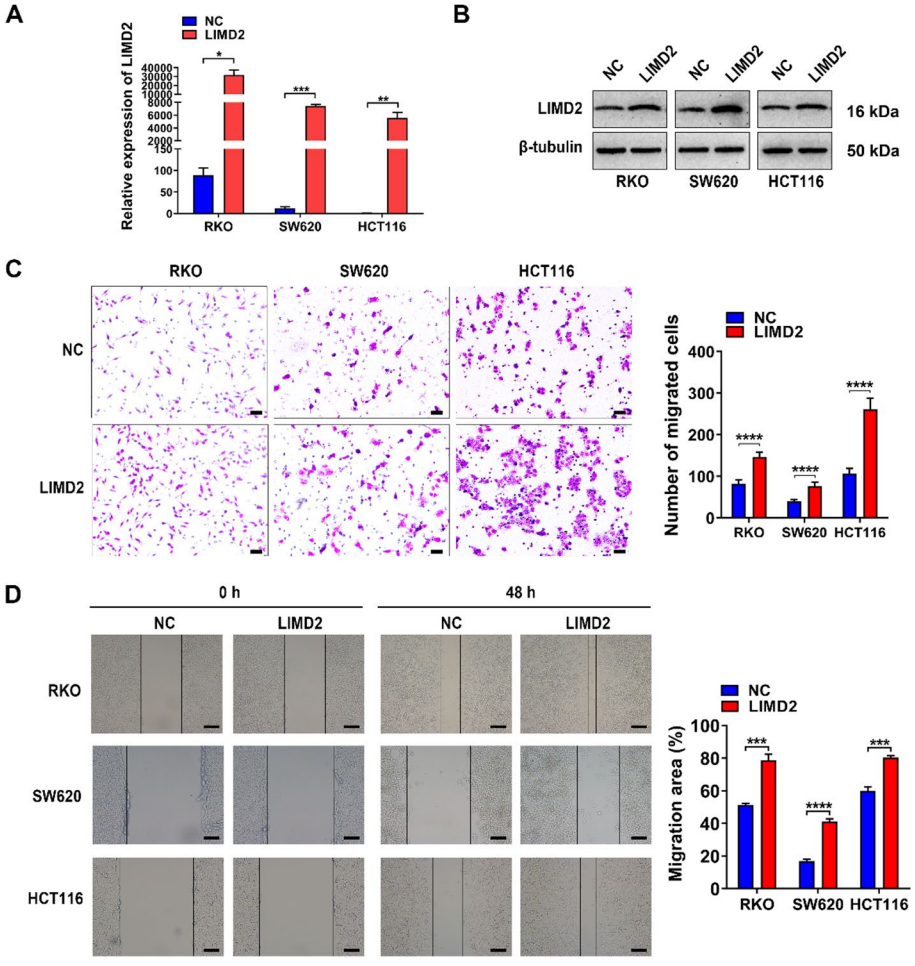


**Fig. 4** LIMD2 promotes the migration of colon cancer cells. (A) mRNA and (B) Protein levels of LIMD2 were upregulated in colon cancer cells either transfected with the LIMD2 overexpression vector (LIMD2) compared to the control cells transfected with pcDNA3.1 (NC). (C) Transwell migration assay revealed that LIMD2 overexpression resulted in more migrated cells than those in the control group. Scale: 50 μm. (D) Wound healing assay indicated that the upregulated LIMD2 promoted the migration of colon cancer cells. Scale: 100 μm. All experiments were repeated three times

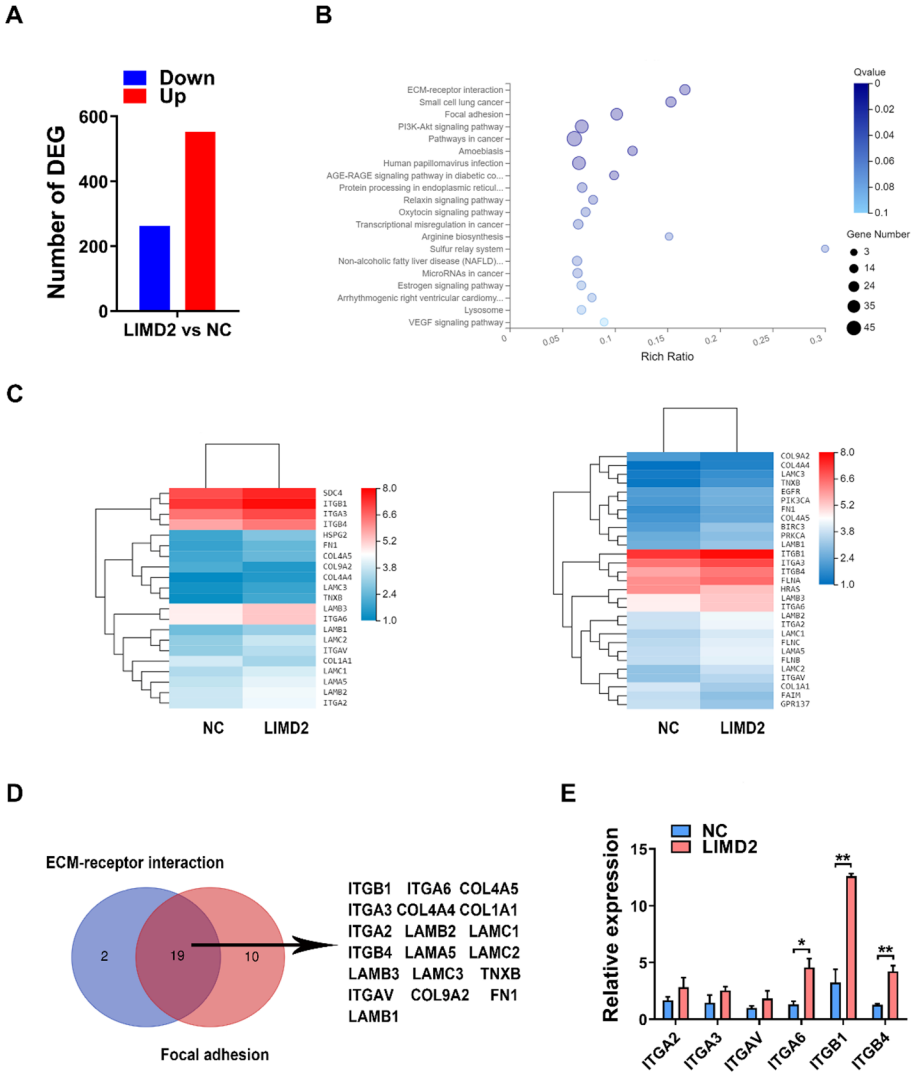


**Fig. 5** LIMD2 regulates the ECM-receptor interaction and focal adhesion pathways. (A) Number of upregulated and downregulated differentially expressed genes. (B) Bubble diagram showing the top 20 pathways enriched by the differentially expressed genes. (C) Heatmaps showing the differentially enriched genes in the ECM-receptor interaction pathway (left) and the focal adhesion pathway (right). (D) Nineteen genes were enriched in both the ECM-receptor interaction and focal adhesion pathways. (E) Six overlapping integrin genes were identified in HCT116 cells with LIMD2 overexpression using the RT-qPCR method

21 genes were specifically enriched in the ECM-receptor interaction pathway, and 29 genes were enriched in the focal adhesion pathway (Fig. 5C). Additionally, 19 genes overlapped between these two pathways, including six integrin genes (Fig. 5D). Given the critical role of integrins in cancer metastasis, we further examined the expression of these six integrin genes in HCT116 cells using the RT-qPCR method. Results demonstrated that all six integrins were upregulated following LIMD2 overexpression, with three genes—ITGB1, ITGB4, and ITGA6—showing statistically significant differences (Fig. 5E).

### 3.6 LIMD2 interacts with cofilin1

To identify proteins that interact with LIMD2, we performed CoIP assays. HCT116 cells were transfected with a LIMD2-Flag plasmid, and subsequent CoIP followed by silver

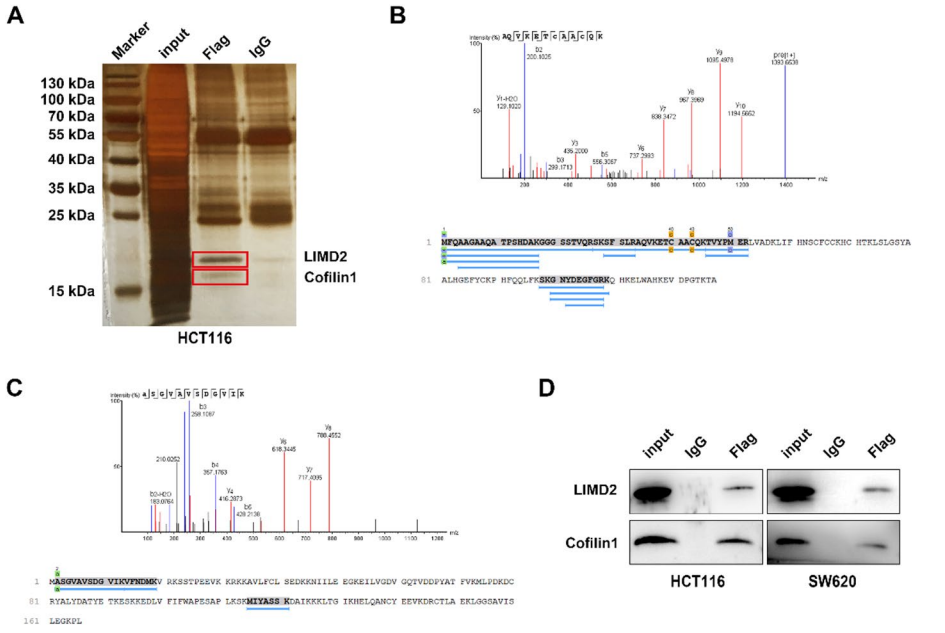


**Fig. 6** LIMD2 interacts with cofilin1. (A) CoIP and silver staining showed that the differential bands were pulled down by the anti-Flag antibody. (B) Mass spectrometry identified peptide fragments of LIMD2. (C) Mass spectrometry identified peptide fragments belonging to cofilin1. (D) Interaction between LIMD2 and cofilin1 was verified using CoIP, followed by western blotting

staining revealed two differential bands pulled down by the anti-Flag antibody (Fig. 6A). Mass spectrometry analysis identified the upper band as LIMD2 (Fig. 6B, Supplementary Table S2), while the lower band was confirmed to be cofilin1 (Fig. 6C, Supplementary Table S3). Additionally, CoIP, followed by western blotting, validated the interaction between LIMD2 and cofilin1 (Fig. 6D). Using western blotting, we found that LIMD2 overexpression didn't affect the expression of cofilin1 and p-cofilin1. However, immunofluorescence indicated that the upregulation of LIMD2 in HCT116 cells promoted cofilin1 transferred into nucleus (Supplementary Figure S3).

## 4 Discussion

In this study, we found that LIMD2 was upregulated in both colon cancer tissues and cell lines. Furthermore, higher LIMD2 expression in colon cancer tissues was positively correlated with shorter patient survival, suggesting that LIMD2 may serve as a promising prognostic biomarker. To the best of our knowledge, this is the first study to investigate the relationship between LIMD2 expression and colon cancer. Consistent with our findings, LIMD2 overexpression has been reported in several malignancies, including non-small cell lung cancer (NSCLC), esophageal cancer, renal clear-cell carcinoma, and ovarian cancer [13–17].

Additionally, gain-of-function analyses demonstrated that LIMD2 overexpression significantly enhanced the migratory capacity of colon cancer cells. Similarly, LIMD2 overexpression has been shown to promote proliferation, invasion, and epithelial-mesenchymal transition (EMT) in renal carcinoma cells [15]. In ovarian cancer, LIMD2 knockdown suppresses cancer cell proliferation and migration, as well as reduces tumor growth in xenograft models [21]. Moreover, downregulation of LIMD2 has been shown to reduce proliferation, migration, and invasion in NSCLC cells [14]. These findings

collectively indicate that LIMD2 functions as an oncogene, driving tumor progression, and may represent a potential therapeutic target for cancer treatment.

While the oncogenic role of LIMD2 has been explored in certain malignancies, its precise molecular mechanisms in cancer progression remain largely unknown. In this study, we found that LIMD2 expression in colon cancer cells led to significant enrichment of DEGs in the ECM-receptor interaction and focal adhesion signaling pathways. Consistent with our findings, LIMD2 regulates the ECM-receptor interaction and focal adhesion signaling pathways to promote proliferation and invasion in ovarian cancer [21]. Integrins, key mediators of ECM-receptor interaction and focal adhesion pathways, facilitate bidirectional intracellular and extracellular signal transduction, thereby activating the FAK and PI3K/AKT signaling pathways and promoting cytoskeletal remodeling. Numerous studies have demonstrated that integrins play important roles in tumor growth, invasion, metastasis, and drug resistance [22–24]. Through RNA-seq and verification, we found that LIMD2 upregulates the expression of ITGB1, ITGB4, and ITGA6. These integrins have been reported to enhance metastatic potential in various tumors. For instance, ITGA6 promotes metastasis in breast cancer, pancreatic cancer, and hepatocellular carcinoma through activation of the PI3K/MAPK pathway or by forming the integrin α6β4 complex [25–27]. Additionally, high ITGB1 expression has been identified as a biomarker of poor prognosis in pancreatic [28] and colorectal cancers [29], while ITGB1 inhibition suppresses tumor migration in colorectal [30] and breast cancers [31]. Moreover, ITGB4 has been implicated as a key driver of metastasis in various tumors [32]. It is upregulated in colon cancer and contributes to cancer cell invasion and metastasis [33, 34]. Taken together, these findings suggest that these integrins may be integral to LIMD2-mediated colon cancer cell migration.

In this study, we also identified cofilin1 as an interacting protein of LIMD2 in colon cancer. Cofilin1 is a key regulator of the actin cytoskeleton, facilitating actin filament reorganization to promote pseudopodia formation and tumor cell motility. Several studies have demonstrated that cofilin1 is upregulated in various cancers [35, 36], and enhances tumor cell invasion and migration by regulating cytoskeletal rearrangement and EMT [37, 38]. In addition, we found that LIMD2 does not alter the phosphorylation of cofilin1 at Serine 3 but does induce its translocation to the nucleus by an unknown mechanism, which might be a possible explanation for the function of LIMD2 on cells migration. Although our findings establish a correlation between LIMD2 and metastasis-related pathways, as well as its interaction with cofilin1, further studies are required to elucidate the molecular mechanisms underlying the regulatory role of LIMD2 in integrin expression and cofilin1 function.

In conclusion, we determined that LIMD2 overexpression is associated with poor prognosis in colon cancer tissues. Furthermore, we demonstrated that LIMD2 promotes colon cancer cell migration and proposed that ECM-receptor interaction, focal adhesion pathways, and cofilin1 may contribute to its oncogenic role. These findings suggest that LIMD2 may serve as a potential prognostic marker and therapeutic target in colon cancer.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s12672-025-03424-z.

Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

Supplementary Material 4. Supplementary Figure 1. The cox proportional hazards model revealed that the high LIMD2 expression increased the risk of death based on a colon cancer cohort (CPTAC-2) of TCGA.

Supplementary Material 5. Supplementary Figure 2. LIMD2 overexpression promoted the migration of SW480 cells. (A) LIMD2 was upregulated in SW480 cells after being transfected with the LIMD2 overexpression vector; (B) The wound healing assay and (C) Transwell assay indicated that increased LIMD2 expression promoted colon cancer migration. Scale: 100 μm and 50 μm.

Supplementary Material 6. Supplementary Figure 3. LIMD2 overexpression promoted cofilin1 transferred into nucleus. (A) Western blot results showed that LIMD2 didn't affect the expression of cofilin1 and p-cofilin1; (B) Immunofluorescence indicated that LIMD2 overexpression in HCT116 cells promoted cofilin1 transferred into nucleus.

Supplementary Material 7.

**Acknowledgements**
Not applicable.

**Author contributions**
LXM，WXY designed the study. LXM, WJJ, LGD, LDD carried out the experiments. CFJ and WY collected the samples. CYJ, ZJT and XWJ interpreted part of data. LXM wrote the manuscript. All authors reviewed the manuscript and approved the submitted version.

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**Data availability**
The RNA-seq raw data have been deposited in the NCBI Sequence Read Archive (SRA) database (https://www.ncbi.nlm.nih.gov/sra/PRJNA1181289).

## Declarations

**Ethics approval and consent to participate**
The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards and was approved by the Ethics Committee of Jiangsu Provincial People's Hospital Suqian Hospital (Suqian, China) and the Ethics Committee of Shanghai Outdo Biotech Company (Shanghai, China).

**Informed consent**
All patients signed the informed consent.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

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**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 Cancer J Clin. 2021. https://doi.org/10.3322/caac.21660.
2. Valastyan S, Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. Cell. 2011;2147. https://doi.org/10.1016/j.cell.2011.09.024.
3. Chen J, Wu F, Shi Y, Yang D, Xu M, Lai Y, et al. Identification of key candidate genes involved in melanoma metastasis. Mol Med Rep. 2019;20(2):903–14. https://doi.org/10.3892/mmr.2019.10314.
4. Dawid IB, Breen JJ, Toyama R. LIM domains: multiple roles as adapters and functional modifiers in protein interactions. *Trends Genet* (1998) 14 (4): 156–62. https://doi.org/10.1016/s0168-9525(98)01424-3
5. Rétaux S, Bachy I. A short history of LIM domains (1993–2002): from protein interaction to degradation. Mol Neurobiol. 2002;26(2–3):269–81. https://doi.org/10.1385/mn:26:2-3:269.
6. Aggelou H, Chadla P, Nikou S, Karteri S, Maroulis I, Kalofonos HP, et al. LIMK/cofilin pathway and slingshot are implicated in human colorectal cancer progression and chemoresistance. Virchows Arch. 2018;472(5):727–37. https://doi.org/10.1007/s00428-018-2298-0.

7. Wang X, He C, Hu X. LIM homeobox transcription factors, a novel subfamily which plays an important role in cancer (review). Oncol Rep. 2014;31(5):1975–85. https://doi.org/10.3892/or.2014.3112.
8. Matthews JM, Lester K, Joseph S, Curtis DJ. LIM-domain-only proteins in cancer. Nat Rev Cancer. 2013;13(2):111–22. https://doi.org/10.1038/nrc3418.
9. Sang M, Ma L, Sang M, Zhou X, Gao W, Geng C. LIM-domain-only proteins: multifunctional nuclear transcription coregulators that interacts with diverse proteins. Mol Biol Rep. 2014;41(2):1067–73. https://doi.org/10.1007/s11033-013-2952-1.
10. Cerutti JM, Oler G, Michaluart P Jr., Delcelo R, Beaty RM, Shoemaker J, et al. Molecular profiling of matched samples identifies biomarkers of papillary thyroid carcinoma lymph node metastasis. Cancer Res. 2007;67(16):7885–92. https://doi.org/10.1158/0008-5472.can-06-4771.
11. Pinheiro Dos Santos MJC, Bastos AU, da Costa VR, Delcelo R, Lindsey SC, Colozza-Gama GA, et al. LIMD2 is overexpressed in BRAF V600E-Positive papillary thyroid carcinomas and matched lymph node metastases. Endocr Pathol. 2018;29(3):222–30. https://doi.org/10.1007/s12022-018-9526-7.
12. Araldi RP, de Melo TC, Levy D, de Souza DM, Maurício B, Colozza-Gama GA, et al. LIMD2 regulates key steps of metastasis cascade in papillary thyroid cancer cells via MAPK crosstalk. Cells. 2020;119. https://doi.org/10.3390/cells9112522.
13. Wang F, Li Z, Xu L, Li Y, Li Y, Zhang X, et al. LIMD2 targeted by miR–34a promotes the proliferation and invasion of non–small cell lung cancer cells. Mol Med Rep. 2018;18(5):4760–66. https://doi.org/10.3892/mmr.2018.9464.
14. Zhang F, Qin S, Xiao X, Tan Y, Hao P, Xu Y. Overexpression of LIMD2 promotes the progression of non-small cell lung cancer. Oncol Lett. 2019;18(2):2073–81. https://doi.org/10.3892/ol.2019.10473.
15. Zhong QY, Xie YW, Chen ZL, Chen YQ, Liu YX, Xie WL, et al. LIMD2 promotes tumor proliferation, invasion, and epithelial-mesenchymal transition in clear cell renal cell carcinoma. Neoplasma. 2022;69(4):832–40. https://doi.org/10.4149/neo_2022_211130N1701.
16. Chen Y, Huang X, Zhu K, Li C, Peng H, Chen L, et al. LIMD2 is a prognostic and predictive marker in patients with esophageal cancer based on a CeRNA network analysis. Front Genet. 2021;12:774432. https://doi.org/10.3389/fgene.2021.774432.
17. Hu H, Wang Y, Dong Y, Wang L, Chen Y, Zhou Y, et al. Knockdown of LIMD2 inhibits the progression of ovarian carcinoma through ERK1/2 pathway. Mol Biol Rep. 2023;50(11):8985–93. https://doi.org/10.1007/s11033-023-08733-6.
18. Li X, Wang J, Lin W, Yuan Q, Lu Y, Wang H, et al. circEXOC6B interacting with RRAGB, an mTORC1 activator, inhibits the progression of colorectal cancer by antagonizing the HIF1A-RRAGB-mTORC1 positive feedback loop. Mol Cancer. 2022;21(1):135. https://doi.org/10.1186/s12943-022-01600-1.
19. Li X, Wang J, Zhang C, Lin C, Zhang J, Zhang W, et al. Circular RNA circITGA7 inhibits colorectal cancer growth and metastasis by modulating the Ras pathway and upregulating transcription of its host gene ITGA7. J Pathol. 2018;246(2):166–79. https://doi.org/10.1002/path.5125.
20. Giuliano S, Dufies M, Ndiaye PD, Viotti J, Borchiellini D, Parola J, et al. Resistance to lysosomotropic drugs used to treat kidney and breast cancers involves autophagy and inflammation and converges in inducing CXCL5. Theranostics. 2019;9(4):1181–99. https://doi.org/10.7150/thno.29093.
21. Chen L, Qian J, You Q, Ma J. LIM domain-containing 2 (LIMD2) promotes the progress of ovarian cancer via the focal adhesion signaling pathway. Bioengineered. 2021;12(2):10089–100. https://doi.org/10.1080/21655979.2021.2000732.
22. Campbell ID, Humphries MJ. Integrin structure, activation, and interactions. Cold Spring Harb Perspect Biol. 2011;3(3). https://doi.org/10.1101/cshperspect.a004994.
23. Cooper J, Giancotti FG. Integrin signaling in cancer: mechanotransduction, stemness, epithelial plasticity, and therapeutic resistance. Cancer Cell. 2019;35(3):347–67. https://doi.org/10.1016/j.ccell.2019.01.007.
24. Hamidi H, Ivaska J. Every step of the way: integrins in cancer progression and metastasis. Nat Rev Cancer. 2018;18(9):533–48. https://doi.org/10.1038/s41568-018-0038-z.
25. Brooks DL, Schwab LP, Krutilina R, Parke DN, Sethuraman A, Hoogewijs D, et al. ITGA6 is directly regulated by hypoxia-inducible factors and enriches for cancer stem cell activity and invasion in metastatic breast cancer models. Mol Cancer. 2016;15:26. https://doi.org/10.1186/s12943-016-0510-x.
26. Wu Y, Tan X, Liu P, Yang Y, Huang Y, Liu X, et al. ITGA6 and RPSA synergistically promote pancreatic cancer invasion and metastasis via PI3K and MAPK signaling pathways. Exp Cell Res. 2019;379(1):30–47. https://doi.org/10.1016/j.yexcr.2019.03.022.
27. Zheng G, Bouamar H, Cserhati M, Zeballos CR, Mehta I, Zare H, et al. Integrin alpha 6 is upregulated and drives hepatocellular carcinoma progression through integrin α6β4 complex. Int J Cancer. 2022;151(6):930–43. https://doi.org/10.1002/ijc.34146.
28. Iwatate Y, Yokota H, Hoshino I, Ishige F, Kuwayama N, Itami M, et al. Transcriptomic analysis reveals high ITGB1 expression as a predictor for poor prognosis of pancreatic cancer. PLoS ONE. 2022;17(6):e0268630. https://doi.org/10.1371/journal.pone.0268630.
29. Liu QZ, Gao XH, Chang WJ, Gong HF, Fu CG, Zhang W, et al. Expression of ITGB1 predicts prognosis in colorectal cancer: a large prospective study based on tissue microarray. Int J Clin Exp Pathol. 2015;8(10):12802–10.
30. Wang X, Li T. Ropivacaine inhibits the proliferation and migration of colorectal cancer cells through ITGB1. Bioengineered. 2021;12(1):44–53. https://doi.org/10.1080/21655979.2020.1857120.
31. Ren L, Mo W, Wang L, Wang X. Matrine suppresses breast cancer metastasis by targeting ITGB1 and inhibiting epithelial-to-mesenchymal transition. Exp Ther Med. 2020;19(1):367–74. https://doi.org/10.3892/etm.2019.8207.
32. Huang G, Zhou M, Lu D, Li J, Tang Q, Xiong C, et al. The mechanism of ITGB4 in tumor migration and invasion. Front Oncol. 2024;14:1421902. https://doi.org/10.3389/fonc.2024.1421902.
33. Li M, Jiang X, Wang G, Zhai C, Liu Y, Li H, et al. ITGB4 is a novel prognostic factor in colon cancer. J Cancer. 2019;10(21):5223–33. https://doi.org/10.7150/jca.29269.
34. Zhu X, Jiang X, Zhang Q, Huang H, Shi X, Hou D, et al. TCN1 deficiency inhibits the malignancy of colorectal cancer cells by regulating the ITGB4 pathway. Gut Liver. 2023;17(3):412–29. https://doi.org/10.5009/gnl210494.
35. Werle SD, Schwab JD, Tatura M, Kirchhoff S, Szekely R, Diels R, et al. Unraveling the molecular Tumor-Promoting regulation of Cofilin-1 in pancreatic cancer. Cancers (Basel). 2021;413. https://doi.org/10.3390/cancers13040725.
36. Howard J, Goh CY, Gorzel KW, Higgins M, McCann A. The potential role of cofilin-1 in promoting triple negative breast cancer (TNBC) metastasis via the extracellular vesicles (EVs). Transl Oncol. 2022;15(1):101247. https://doi.org/10.1016/j.tranon.2021.101247.

37. Sousa-Squiavinato ACM, Rocha MR, Barcellos-de-Souza P, de Souza WF, Morgado-Diaz JA. Cofilin-1 signaling mediates epithelial-mesenchymal transition by promoting actin cytoskeleton reorganization and cell-cell adhesion regulation in colorectal cancer cells. Biochim Biophys Acta Mol Cell Res. 2019;1866(3):418–29. https://doi.org/10.1016/j.bbamcr.2018.10.003.
38. Chen L, Cai J, Huang Y, Tan X, Guo Q, Lin X, et al. Identification of cofilin-1 as a novel mediator for the metastatic potentials and chemoresistance of the prostate cancer cells. Eur J Pharmacol. 2020;880:173100. https://doi.org/10.1016/j.ejphar.2020.173100.

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