Original Article

Talin1 Ser425 phosphorylation promotes colorectal cancer progression and metastasis

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Background: Talin1 serves as a crucial element within the multiprotein adhesion complexes that facilitate processes such as cell migration, adhesion, and integrin signaling. This study aimed to explore the underlying role of Talin1 Ser425 phosphorylation in the development of colorectal cancer (CRC).

Methods: Blank plasmids, non-phosphorylatable mutant Talin1 S425A plasmids, and phosphorylation-mimetic mutant Talin1 S425D plasmids were constructed and used for transfection of CRC cells. The expression of mRNA and protein in CRC cells or tumor tissues was assessed by The Cancer Genome Atlas (TCGA), Genotype-Tissue Expression (GTEx), and UALCAN databases, immunohistochemistry (IHC), and Western blot (WB). Cell proliferation was assessed via 5-ethynyl-2-deoxyuridine (EDU) proliferation assay and colony formation assay. Cell migration and invasion were detected by wound healing assay and transwell assay. Cell apoptosis was assessed by flow cytometry. The Kaplan-Meier Plotter was used to evaluate the prognostic value of mRNA in CRC.

Results: TLN1 was markedly downregulated in CRC tissues while the level of Talin1 Ser425 phosphorylation in CRC tissues and aggressive CRC cells was relatively higher. The S425A mutant inhibited CRC cell proliferation, migration, and invasion, whereas the S425D mutant promoted these processes. Flow cytometry assay showed that cell apoptosis was induced by S425A mutant and suppressed by S425D mutant in RKO cells. Further investigation suggested that CDK5 might be responsible for Talin1 phosphorylation.

Conclusions: Talin1 Ser425 phosphorylation is of great importance in CRC development and Talin1 is supposed to be a potential tumor marker and therapeutic target for CRC.

Keywords: Talin1 Ser425 phosphorylation; colorectal cancer (CRC); cancer development; biomarker

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Introduction

Colorectal cancer (CRC) stands as one of the most prevalent malignancies globally, ranking second among female cancer patients (9.2% of the total cases) and third among male cancer patients (10% of the total cases), respectively (1,2). Therefore, rapid diagnoses and effective therapies are essential for reducing the morbidity and mortality of CRC. Currently, surgical resection is the preferred

method for CRC treatment, supplemented by radiotherapy, chemotherapy, targeted therapy, and gene therapy (3,4). However, most CRC patients have already shown local progression or distal metastasis upon diagnosis. Although chemotherapy is a common treatment for such patients, it is often accompanied by tumor resistance, resulting in poor efficacy (5,6). It is reported that stage I CRC patients boast a remarkable 5-year survival rate of up to 90%, whereas stage III and IV CRC patients with distant metastasis have a dismal 5-year survival rate of just over 10% (7). It is urgent to explore the mechanisms of CRC occurrence and development, seek early diagnostic markers and effective therapeutic targets, and develop practical targeted drugs to enhance the prognoses of CRC patients and prolong their survival time.

The remodeling of the extracellular matrix (ECM) and alterations in the cytoskeleton play pivotal roles in tumor initiation and progression (8,9). Talins, located at the adhesion complex between cells and the ECM, serve as adaptor proteins, orchestrating focal adhesion signaling by linking integrins to the cytoskeleton (10). Among the talin isoforms, Talin1 and Talin2, encoded by TLN1 and TLN2 respectively, Talin1 predominates and exhibits ubiquitous expression across various tissues, while Talin2 is specifically expressed in the heart, muscle, testis, and brain (11,12). As a crucial constituent of multiprotein adhesion complexes regulating cell migration, adhesion, and integrin signaling, Talin1 has garnered attention in cancer research (13-16). It has been demonstrated that the level of Talin1 in the serum of colon cancer patients was significantly higher than that of healthy controls. Moreover, a notable correlation has been established between Talin1 levels and factors such as tumor grade, Tumor Node Metastasis (TNM) staging, and lymph node metastasis. These findings imply that Talin1 may contribute to the enhancement of cellular proliferation, adhesion, and angiogenesis within the context of colon cancer (17). Additionally, a separate investigation focused on the protein and mRNA expression levels of cytoskeletal components associated with focal adhesion plaques in paired samples from normal colorectal mucosa, primary colorectal adenocarcinomas, and separate lymph node metastases revealed that Talin expression in CRCs was significantly reduced when compared to normal colorectal mucosal tissue, and it exhibited a strong association with lymph node metastasis (18). Lower expression of Talin1 at both the gene and protein level was proved to be significantly associated with advanced TNM stage and worse prognosis, and the association between Talin1 and tumor aggressiveness provides new insights into the development of progression indicators for CRC patients (19). Notably, cancer-associated point mutations in Talin-1 can influence cell behavior, potentially contributing to cancer progression (20). Talin1 Ser425 phosphorylation was found to correlate with metastatic potential of cancer cells (21). However, potential roles of this phosphorylation in the development of CRC have not been assessed previously.

This study aimed to determine and analyze the phosphorylation level of Talin1 Ser425 in tumor tissue of CRC patients, and explore the effects of the activation and inactivation status of Talin1 Ser425 phosphorylation on the proliferation, migration, invasion, and apoptosis of CRC cells. This will help deepen the understanding of the impact of Talin1 function on CRC, and provide a theoretical and research basis for subsequent use of Talin1 as both a tumor marker and a potential therapeutic target. We present this article in accordance with the ARRIVE reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1283/rc).

Highlight box

Key findings

- Talin1 Ser425 phosphorylation played a vital role in colorectal cancer (CRC) cell proliferation, migration, invasion, and cell apoptosis. Talin1 Ser425 phosphorylation also enhanced the growth of CRC cells *in vivo*. CDK5 might be responsible for Talin1 phosphorylation.

What is known and what is new?

- Talin1 serves as a crucial element within the multiprotein adhesion complexes that facilitate processes such as cell migration, adhesion, and integrin signaling. Cancer related point mutations in Talin-1 can influence cell behavior, potentially contributing to cancer progression.
- This study adds a better understanding of the underlying role of Talin1 Ser425 phosphorylation in the development of CRC.

What is the implication, and what should change now?

- Talin1 Ser425 phosphorylation is of great importance in CRC development and Talin1 is supposed to be a potential tumor marker and therapeutic target for CRC.

Methods

Public data resources and tools

We used both the The Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer.gov/) and the Genotype-

Tissue Expression (GTEx) (https://www.gtexportal.org/home/) databases to investigate the levels of mRNA expression in colon adenocarcinoma (COAD) and rectum adenocarcinoma (READ) tissues compared with normal colorectal tissues. The Kaplan-Meier Plotter (http://kmplot.com/analysis/) was used to evaluate the prognostic value of mRNA in CRC. UALCAN (http://ualcan.path.uab.edu/index.html/) was employed to evaluate the mRNA and protein expression of CDK5 in normal and tumor tissues with different stages.

Human CRC samples and immunohistochemistry (IHC)

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Dongtai People's Hospital (2023-dtry-K086) and informed consent was obtained from all individual participants. A total of seventy-three pairs of clinical CRC samples along with their corresponding control tissues were acquired. The fresh samples were procured without any treatment, subsequently immersed in RNAlaterTM (Thermo Fisher Scientific, Massachusetts, USA), and stored at −80 ℃. The IHC staining was performed using the VECTASTAIN ABC Detection System. Anti-phospho-Talin1 (Ser425) antibody (bs-3719R) was purchased from Beijing Biosynthesis Biotechnology (Beijing, China). The IHC results were scored by a medical pathologist.

Cell culture and transfection

Human CRC cell lines (COLO205, RKO, SW620, and HCT116) were sourced from the National Collection of Authenticated Cell Cultures (Shanghai Institute of Cell Biology, Chinese Academy of Sciences, Shanghai, China). The cell lines were cultured in Dulbecco's Modified Eagle Medium (Gibco, Massachusetts, USA), which was supplemented with 10% fetal bovine serum (BI, 04-004-1ACS), along with 100 U/mL penicillin and 100 mg/mL streptomycin (Gibco). All cell lines were placed in a humidified incubator containing 5% CO_2 at 37 ℃. Cell transfection was performed after the cells reached 60% confluences. Blank plasmids, non-phosphorylatable mutant Talin1 S425A plasmids, and phosphorylation-mimetic mutant Talin1 S425D plasmids were constructed and used for transfection of CRC cells. After transfection for 48 h, cells were collected for function experiments.

Western blot (WB) analysis

Cells were harvested and subsequently lysed to extract the protein content. The quantification of proteins was performed using the BCA protein assay kit (Beyotime, Shanghai, China). A quantity of 20 μg of protein was loaded per lane and subjected to separation via 8–12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), followed by transfer onto nitrocellulose membranes. After the incubation of primary and secondary antibodies, WB bands were obtained via exposure. Densitometric analysis of the WB bands was performed by ImageJ software. The following primary antibodies were used: anti-Talin1 (bs-3619R, Biosynthesis Biotechnology, Beijing, China), anti-phospho-Talin1 (bs-3719R, Biosynthesis Biotechnology, Beijing, China), and anti-Actin (4970S, Cell Signaling Technology, Danvers, USA). Actin was set as the control.

EDU (5-ethynyl-2-deoxyuridine) proliferation assay and colony formation assay

The EDU proliferation assay was conducted utilizing an EDU kit (Beyotime, Shanghai, China). The cells that underwent staining were examined and captured through an inverted microscope. To assess cell viability, colony formation assays were executed. Specifically, 3,000 cells were inoculated into each well of a 6-well plate and allowed to culture for a duration of 8 to 10 days in standard growth medium. Following a fixation process in 4% paraformaldehyde at ambient temperature, the cell colonies were stained with crystal violet. Subsequently, these colonies were photographed and enumerated through visual inspection.

Wound healing assay and transwell assay

For the wound healing assay, cells were plated in 6-well plates. Once the cell monolayers achieved over 80% confluence, they were subjected to scratching using a 200 μL pipette, followed by the removal of cell debris through washing with phosphate-buffered saline (PBS). Subsequently, the cells were maintained in a medium devoid of fetal bovine serum (FBS). Photographs documenting cell migration were taken at consistent locations at 0, 12, 24, and 48 hours.

The migration and invasion assays employed transwell chambers (Corning, USA), which were either coated

or uncoated with Matrigel (BD Biosciences, USA), respectively. Approximately 10^5 cells were introduced into the upper chamber, while the lower chamber was filled with 600 µL of medium supplemented with 10% FBS. Following a 24-hour incubation period, cells adhering to the upper membrane were removed, and the cells that had invaded or migrated were fixed with methanol and subsequently stained with 0.1% crystal violet for 30 minutes. Images were captured using an inverted microscope.

Cell apoptosis analysis

To conduct the apoptosis analysis, cells were harvested using a 0.25% trypsin solution and subsequently centrifuged at 300 g for 5 minutes to eliminate the trypsin. Following this, the cells were washed twice with 1 mL of cold PBS and again centrifuged at 300 g for 5 minutes to remove the PBS. The cells were then resuspended in 100 µL of 1× binding buffer. For each sample, 5 µL of Annexin-V-FITC and 10 µL of propidium iodide were incorporated into the cell suspension, which was incubated in the dark at room temperature for 15 minutes. The stained cells were subsequently detected using the Gallios Flow Cytometer (A94303) and analyzed with FlowJo software (Version 10.8.1).

Tumor xenograft experiments

The BALB/c nude mice were purchased from Shanghai Experimental Animal Center (Shanghai, China). The nude mice were randomly divided into four groups: control group, Talin1 wild-type (WT) group, Talin1 non-phosphorylatable mutant (S425A) group, and Talin1 phosphorylation-mimetic mutant (S425D) group. To guarantee biological duplication, the mice number of each group was eight. The treated cell suspensions were subcutaneously injected into nude mice, and the tumor volume was measured every seven days. After 28 days, the mice were sacrificed and the tumors were photographed. Tumor tissue sections were stained with hematoxylin-eosin (HE) and Ki67. A protocol was prepared before the study without registration. All nude mice were maintained in specific pathogen-free condition. Animal experiments were performed under a project license (No. TOPIACUC-2023-0329) granted by the Ethics Committee of Shenzhen TopBiotech Co., Ltd., in compliance with the Guideline for the Care and Use of Laboratory Animals and the institutional laboratory animal welfare guideline.

Statistical analysis

The GraphPad Prism version 8.0 and the SPSS version 25.0 were utilized for statistical analysis. Correlations between two variables were evaluated by Spearman's rank-correlation test or Pearson correlation analysis. Overall survival (OS) rates were assessed employing the Kaplan-Meier method, with the log-rank test utilized to calculate P values. $P<0.05$ was considered statistically significant.

Results

The expression of Talin1 and Talin1 Ser425 phosphorylation in human CRC

Based on TCGA and GTEx data, TLN1 was markedly downregulated in COAD and READ tissues compared with normal colorectal tissues (*Figure 1A*). The correlation of TLN1 expression with OS of CRC patients was further explored using the Kaplan-Meier analysis. The survival analysis demonstrated that the prognosis was worse in patients with the highest quartile expression of TLN1 (*Figure 1B*). To investigate the level of Talin1 Ser425 phosphorylation in human CRC, we undertook IHC analyses in human CRC tissue microarrays containing 146 samples (73 CRC tissues and 73 adjacent normal tissues). The positive rate of Talin1 Ser425 phosphorylation in CRC tissues was 95.89% and that in adjacent normal tissues was 35.62%. The level of Talin1 Ser425 phosphorylation in CRC tissues was much higher than that in normal tissues (*Figure 1C,1D*). Meanwhile, we examined Talin1 Ser425 phosphorylation in different CRC cells. Talin1 Ser425 phosphorylation was observed relatively higher in RKO, SW620, and HCT116 which are human colorectal carcinoma cell lines known for high aggressiveness (*Figure 1E,1F*).

The effect of Talin1 Ser425 phosphorylation on cell proliferation, migration, invasion, and cell apoptosis

To determine if Talin1 Ser425 phosphorylation would affect CRC cell proliferation, migration, and invasion, we cultured CRC cells stably expressing either Talin1 WT, a non-phosphorylatable mutant (S425A), or a phosphorylation-mimetic mutant (S425D). The proliferating cells were measured using EDU proliferation assay and colony formation assay. Significantly, S425A mutant reduced both EDU incorporation and colony formation of CRC cells

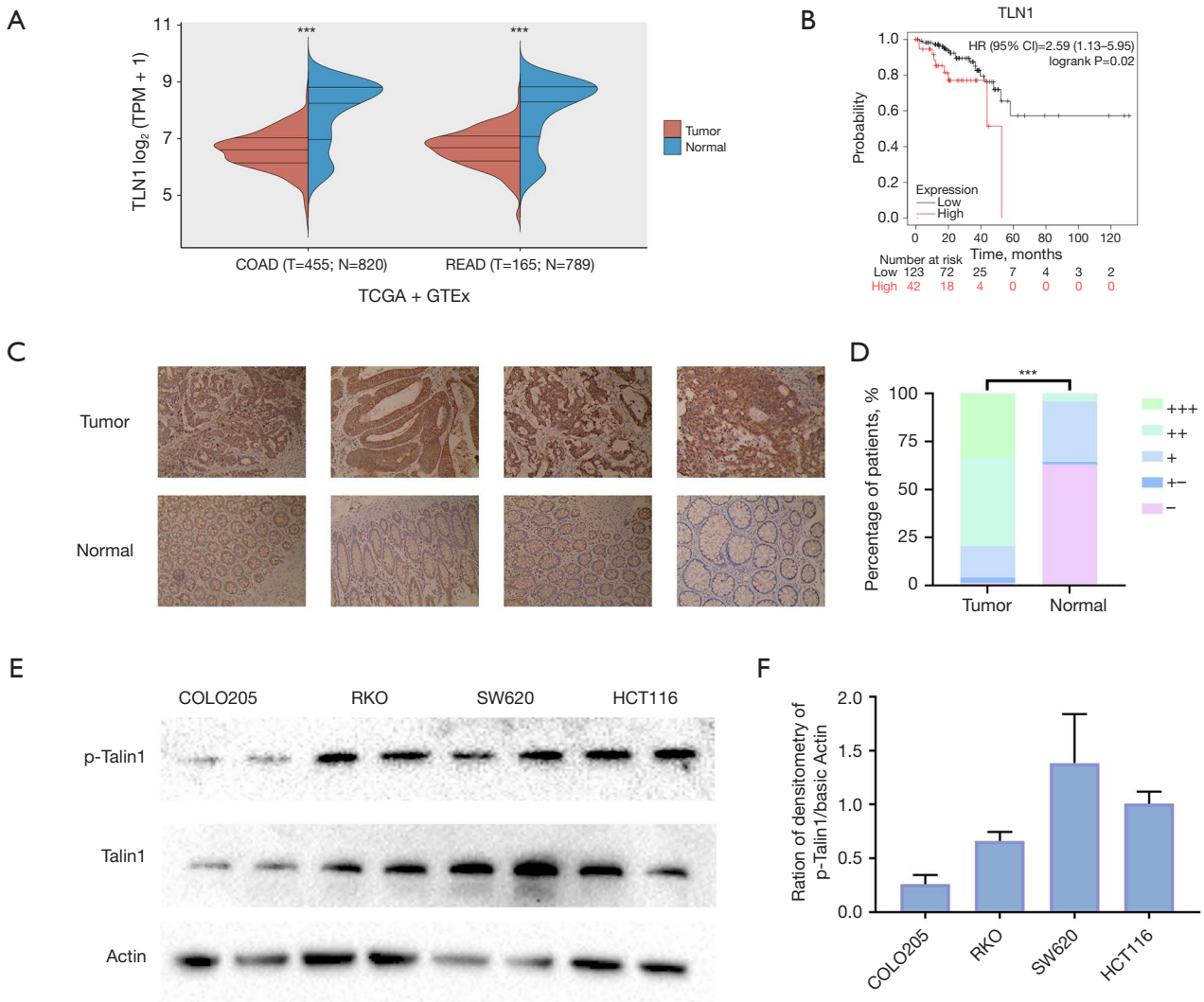

Figure 1 The expression of Talin1 and Talin1 Ser425 phosphorylation in human CRC. (A) TLN1 expression based on TCGA and GTEx databases. (B) Prognostic value of TLN1 in CRC via Kaplan-Meier Plotter. (C,D) Representative images (IHC staining, ×100 magnification) and quantitative analysis of Talin1 Ser425 phosphorylation staining in the IHC assay. (E,F) Talin1 Ser425 phosphorylation in different CRC cells was assesses by WB analysis. ***, P<0.001. TPM, transcripts per million; TCGA, The Cancer Genome Atlas; GTEx, Genotype-Tissue Expression; COAD, colon adenocarcinoma; READ, rectum adenocarcinoma; CI, confidence interval; HR, hazard ratio; CRC, colorectal cancer; IHC, immunohistochemistry; WB, Western blot.

while S425D mutant promoted (*Figure 2*). The wound healing experiment displayed that the scratch healing rates in S425A mutant cells were decreased, while those in S425D mutant cells were increased (*Figure 3*). Transwell assay demonstrated that cell migration and invasion abilities decreased in the groups with S425A mutant and increased in the groups with S425D mutant (*Figure 3*). Flow cytometry assay was used to detect cell apoptosis in RKO, SW620, and HCT116 cells among the four groups. The cell apoptotic rates were significantly increased by S425A mutant and significantly decreased by S425D mutant in RKO cells (*Figure 3*).

To further validate the tumorigenic potential of Talin1 Ser425 phosphorylation, we constructed the *in vivo* xenograft models. The control RKO cells and those transfected with blank plasmids, non-phosphorylatable mutant Talin1 S425A plasmids, and phosphorylation-mimetic mutant Talin1 S425D plasmids were injected

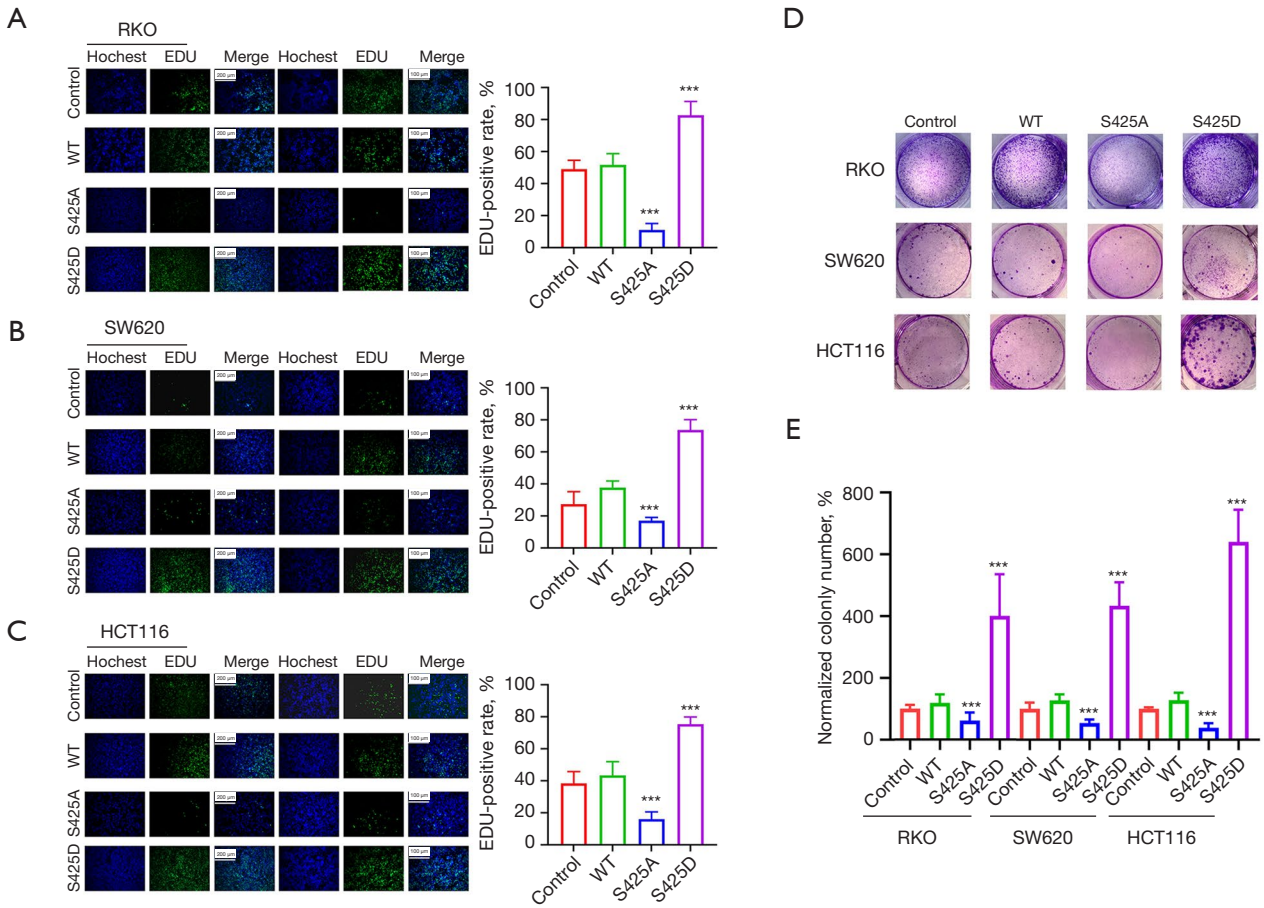

Figure 2 The effect of Talin1 Ser425 phosphorylation on cell proliferation of CRC. (A-C) Representative images (EDU staining, ×100 and ×200 magnification) and quantitative analysis of EDU proliferation assay for RKO, SW620, and HCT116 cells. (D,E) Representative images (crystal violet staining) and quantitative analysis of colony formation assay for RKO, SW620, and HCT116 cells. ***, P<0.001. EDU, 5-ethynyl-2-deoxyuridine; WT, wild-type; CRC, colorectal cancer.

into nude mice independently. Compared with the control group, we observed a significant decrease in tumor volume in S425A mutant group and a significant increase in S425D mutant group, while there was no significance in the body weight between the groups of mice (*Figure 4*). The IHC results also demonstrated that the positive rate of Ki-67 was lower much lower in the mice that were subcutaneously injected with S425A mutant cells, and much higher in the S425D mutant mice (*Figure 4*). Collectively, the phosphorylation of Talin1 Ser425 obviously enhanced the growth of CRC cells *in vivo*.

Talin1 Ser425 phosphorylation is mediated by CDK5

Next, we determined the mechanism by which Talin1 Ser425 phosphorylation is increased in CRC cells. Previous work demonstrated that Talin Ser425 is a phosphorylation site for CDK5 (22). We therefore determined whether CDK5 was responsible for Talin1 Ser425 phosphorylation in CRC cells. Based on TCGA and GTEx data, CDK5 was markedly upregulated in COAD and READ tissues compared with normal colorectal tissues (*Figure 5*). The correlation of CDK5 expression with OS of CRC patients was further explored using the Kaplan-Meier analysis. The survival analysis demonstrated that the prognosis was worse in patients with high expression of CDK5 (*Figure 5*). Furthermore, UALCAN was employed to evaluate the mRNA and protein expression of CDK5 in normal and tumor tissues with different stages. Compared with normal tissues, CRC tissues of various stages showed

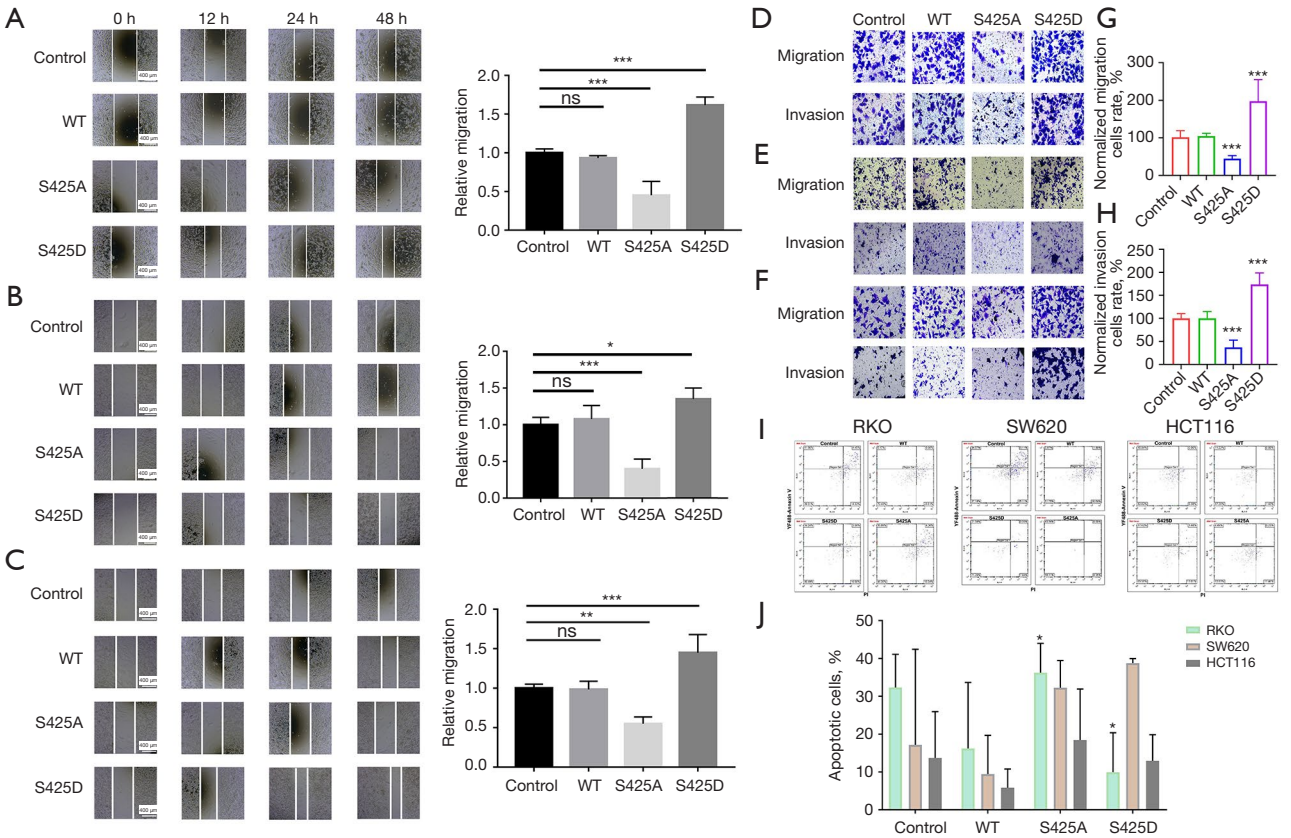

Figure 3 The effect of Talin1 Ser425 phosphorylation on cell migration, invasion, and cell apoptosis of CRC. (A-C) Representative images (×40 magnification) and quantitative analysis of wound healing assay for RKO, SW620, and HCT116 cells. (D-F) Representative images (crystal violet staining, ×100 magnification) of transwell assay for RKO, SW620, and HCT116 cells. (G,H) Quantitative analyses of migration rates and invasion rates. (I,J) Representative images of flow cytometry assay for cells and quantitative analysis of apoptotic rates. *, P<0.05; **, P<0.01; ***, P<0.001. WT, wild-type; ns, no significance; CRC, colorectal cancer.

significantly higher expression of CDK5 mRNA and protein (*Figure 5* and *Table 1*). Notably, inhibition of CDK5 by the CDK5 inhibitor, 20-223, reduced Talin1 phosphorylation in SW620 and HCT116 cells, suggesting that CDK5 might be responsible for Talin1 phosphorylation (*Figure 5*).

Discussion

Our study has revealed the expression pattern, biological role, and potential mechanism of Talin1 phosphorylation in CRC. We first identified the expression of Talin1 and Talin1 Ser425 phosphorylation in human CRC and found that TLN1 was markedly downregulated in COAD and READ tissues while the level of Talin1 Ser425 phosphorylation in CRC tissues and aggressive CRC cells was relatively higher. We then cultured CRC cells stably expressing either Talin1 WT, a non-phosphorylatable mutant (S425A), or a phosphorylation-mimetic mutant (S425D) to detect the effects of different phosphorylation status of Talin1 on the proliferation, migration, invasion, and apoptosis of CRC cells and tumor progression in CRC model mice. Our results suggest that Talin1 phosphorylation may play an important role in CRC. Moreover, we determined whether CDK5 was responsible for Talin1 Ser425 phosphorylation in CRC cells, which might partly explain the mechanism of how Talin1 phosphorylation acted in CRC.

As a major cytoskeletal protein implicated in integrin activation within cells, talin is essential for maintenance of the connection of the ECM and the cytoskeleton. It comprises a globular N-terminal head and a large flexible C-terminal rod domain (23). The talin head domain contains binding sites for β-integrin tail, FAK, and layilin,

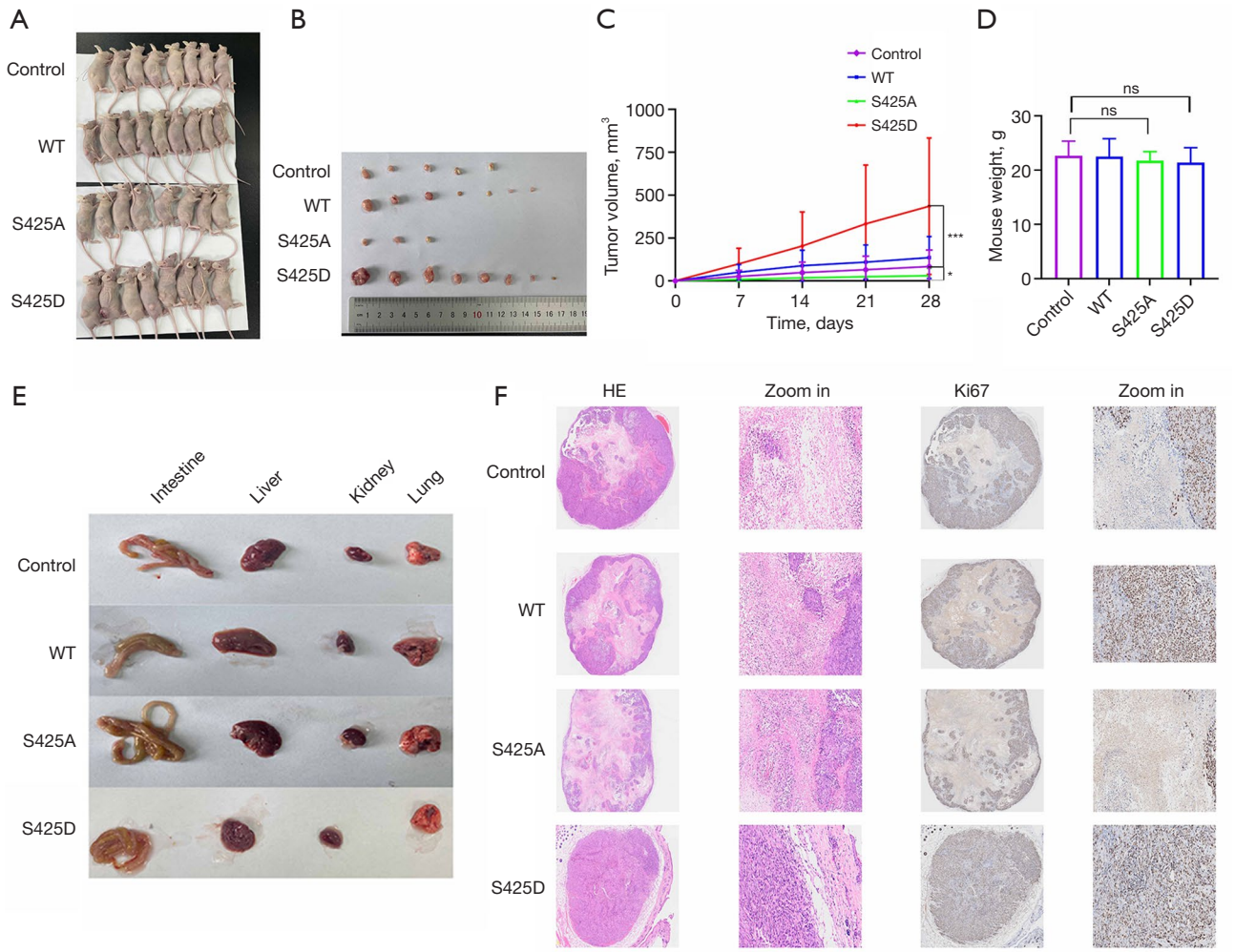

Figure 4 The effect of Talin1 Ser425 phosphorylation on the growth of CRC *in vivo*. (A) Photographs of the mice after they were sacrificed. (B) Photographs of the tumors stripped from the sacrificed mice. (C,D) Quantitative analyses of tumor volume and mouse weight. (E) Representative photographs of organs derived from the sacrificed mice. (F) Representative images (HE and IHC staining, ×20 and ×100 magnification) of HE and Ki67 staining in the IHC assay. *, P<0.05; ***, P<0.001. WT, wild-type; ns, no significance; HE, hematoxylin eosin; CRC, colorectal cancer; IHC, immunohistochemistry.

while the rod domain contains binding sites for vinculin, integrin, and actin (24-27). The specific binding of Talin1 to β-integrin tail induces structural changes in the extracellular domains of integrin, thereby augmenting their binding affinity and promoting cellular functions (28). It is worth noting that the activation of integrins facilitated by talin is crucial for integrin-mediated signaling and the initiation of subsequent survival pathways. This process ultimately contributes to the prevention of anoikis, which is a pivotal factor in the advancement of cancer towards metastatic stages (10).

The migration and invasion of tumors are dynamic processes that require the joint regulation of integrin activation mediated by talin and calpain (29). Research has shown that the talin head exhibits a stronger binding affinity for Smurf1, an E3 ubiquitin ligase that plays a critical role in cell polarity and migration, compared to the full-length talin molecule. This interaction has the potential to result in the ubiquitylation and subsequent degradation of the talin head, ultimately inhibiting integrin activation (30,31). Moreover, it has been established that the phosphorylation of talin at Ser425 influences the stability and ubiquitylation

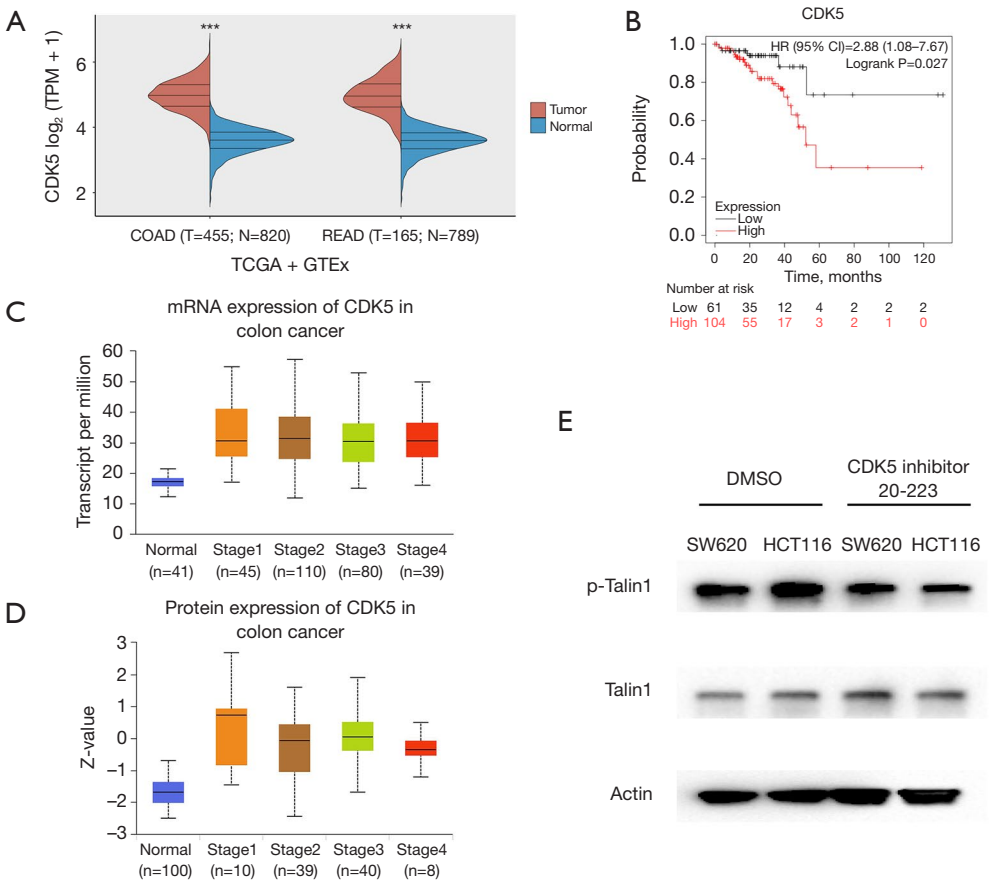

Figure 5 Talin1 Ser425 phosphorylation is mediated by CDK5 in CRC. (A) CDK5 expression based on TCGA and GTEx databases. (B) Prognostic value of CDK5 in CRC via Kaplan-Meier Plotter. (C,D) mRNA and protein expression of CDK5 in normal and tumor tissues with different stages. (E) The level of Talin1 phosphorylation in SW620 and HCT116 cells after inhibition of CDK5 by the CDK5 inhibitor, 20-223. ***, P<0.001. TPM, transcripts per million; COAD, colon adenocarcinoma; READ, rectum adenocarcinoma; TCGA, The Cancer Genome Atlas; GTEx, Genotype-Tissue Expression; HR, hazard ratio; CI, confidence interval; CRC, colorectal cancer.

of the talin protein (32). When the phosphorylation of Talin1 Ser425 site is inhibited, it can effectively reduce integrin activation and inhibit tumor cell invasion and metastasis. We found that S425A mutant reduced both EDU incorporation and colony formation of CRC cells while S425D mutant promoted. S425A mutant decreased migration and invasion rates while S425D mutant increased. Both cell function experiments and animal experiments confirmed that Talin1 Ser425 phosphorylation could promote CRC cell proliferation, migration, and invasion, and reduce cell apoptosis.

CDK5 is a unique member of the cyclin-dependent kinase family of serine/threonine kinases. Recent studies have found that CDK5 plays an important role in tumor-related biological functions, such as regulation of cell cycle and proliferation, DNA damage repair, and cell adhesion (33-35). It was demonstrated that the activity of CDK5 influences cellular processes by restraining Src activity in areas where Rho activity is essential for the contraction of stress fibers. Additionally, CDK5 facilitates the phosphorylation of the talin head, which in turn promotes the stabilization of newly formed focal adhesions (36). Jin *et al.* illustrated that the phosphorylation of talin1 by CDK5, which activates β1 integrin, represents an innovative mechanism that enhances the metastatic capabilities of prostate cancer cells. This finding indicates that both talin1 and CDK5 could serve as promising targets for the creation of new inhibitors aimed at curbing cancer metastasis (21). CDK5 was found to be highly expressed in CRC cells and tissues, and significantly associated with tumor stage and

Table 1 Comparison of the mRNA and protein expression of CDK5 in normal and tumor tissues with different stages

Groups	P value	
	mRNA	Protein
Normal *vs.* stage 1	0.001*	<0.001*
Normal *vs.* stage 2	<0.001*	<0.001*
Normal *vs.* stage 3	<0.001*	<0.001*
Normal *vs.* stage 4	0.002*	<0.001*
Stage 1 *vs.* stage 2	0.26	0.60
Stage 1 *vs.* stage 3	0.57	0.47
Stage 1 *vs.* stage 4	0.12	0.29
Stage 2 *vs.* stage 3	0.24	0.73
Stage 2 *vs.* stage 4	0.34	0.45
Stage 3 *vs.* stage 4	0.09	0.74

*, P<0.05 was considered significant.

prognosis of CRC (37,38), which corresponded to our results. As a CDK5 inhibitor, 20-223 exerts anti-tumor effects against CRC by targeting CDK 2/5 and inducing cell cycle arrest (39). CDK5 can phosphorylate the talin head at Ser425, inhibit its binding to Smurf1, and thus prevent ubiquitination and degradation of the talin head, which in turn regulates cell migration (32). Our findings showed that inhibition of CDK5 reduced Talin1 phosphorylation in CRC cells, suggesting the potential for CDK5 inhibitors in clinical therapeutic applications.

Conclusions

In summary, we demonstrated the importance of Talin1 Ser425 phosphorylation in CRC development. We further identified a potential mechanism for Talin1 Ser425 phosphorylation through CDK5 mediation in CRC. Our findings suggest that Talin1 Ser425 phosphorylation is of great significance for the diagnosis and targeted treatment of CRC.

Acknowledgments

None.

Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1283/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1283/dss

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Dongtai People's Hospital (2023-dtry-K086) and informed consent was obtained from all individual participants. Animal experiments were performed under a project license (No. TOPIACUC-2023-0329) granted by the Ethics Committee of Shenzhen TopBiotech Co., Ltd., in compliance with the Guideline for the Care and Use of Laboratory Animals and the institutional laboratory animal welfare guideline.

References

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136:E359-86.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015.

CA Cancer J Clin 2015;65:5-29.
3. Brody H. Colorectal cancer. Nature 2015;521:S1.
4. Dekker E, Tanis PJ, Vleugels JLA, et al. Colorectal cancer. Lancet 2019;394:1467-80.
5. Van der Jeught K, Xu HC, Li YJ, et al. Drug resistance and new therapies in colorectal cancer. World J Gastroenterol 2018;24:3834-48.
6. Blondy S, David V, Verdier M, et al. 5-Fluorouracil resistance mechanisms in colorectal cancer: From classical pathways to promising processes. Cancer Sci 2020;111:3142-54.
7. Brenner H, Kloor M, Pox CP. Colorectal cancer. Lancet 2014;383:1490-502.
8. Bertero T, Oldham WM, Grasset EM, et al. Tumor-Stroma Mechanics Coordinate Amino Acid Availability to Sustain Tumor Growth and Malignancy. Cell Metab 2019;29:124-140.e10.
9. Oudin MJ, Jonas O, Kosciuk T, et al. Tumor Cell-Driven Extracellular Matrix Remodeling Drives Haptotaxis during Metastatic Progression. Cancer Discov 2016;6:516-31.
10. Desiniotis A, Kyprianou N. Significance of talin in cancer progression and metastasis. Int Rev Cell Mol Biol 2011;289:117-47.
11. Malla RR, Vempati RK. Talin: A Potential Drug Target for Cancer Therapy. Curr Drug Metab 2020;21:25-32.
12. Gough RE, Goult BT. The tale of two talins - two isoforms to fine-tune integrin signalling. FEBS Lett 2018;592:2108-25.
13. Sakamoto S, McCann RO, Dhir R, et al. Talin1 promotes tumor invasion and metastasis via focal adhesion signaling and anoikis resistance. Cancer Res 2010;70:1885-95.
14. Xu YF, Ren XY, Li YQ, et al. High expression of Talin-1 is associated with poor prognosis in patients with nasopharyngeal carcinoma. BMC Cancer 2015;15:332.
15. Masi I, Ottavi F, Del Rio D, et al. The interaction of β-arrestin1 with talin1 driven by endothelin A receptor as a feature of α5β1 integrin activation in high-grade serous ovarian cancer. Cell Death Dis 2023;14:73.
16. Chen P, Lei L, Wang J, et al. Downregulation of Talin1 promotes hepatocellular carcinoma progression through activation of the ERK1/2 pathway. Cancer Sci 2017;108:1157-68.
17. Bostanci O, Kemik O, Kemik A, et al. A novel screening test for colon cancer: Talin-1. Eur Rev Med Pharmacol Sci 2014;18:2533-7.
18. Yang HJ, Chen JZ, Zhang WL, et al. Focal adhesion plaque associated cytoskeletons are involved in the invasion and metastasis of human colorectal carcinoma. Cancer Invest 2010;28:127-34.
19. Vafaei S, Saeednejad Zanjani L, Habibi Shams Z, et al. Low expression of Talin1 is associated with advanced pathological features in colorectal cancer patients. Sci Rep 2020;10:17786.
20. Azizi L, Cowell AR, Mykuliak VV, et al. Cancer associated talin point mutations disorganise cell adhesion and migration. Sci Rep 2021;11:347.
21. Jin JK, Tien PC, Cheng CJ, et al. Talin1 phosphorylation activates β1 integrins: a novel mechanism to promote prostate cancer bone metastasis. Oncogene 2015;34:1811-21.
22. Ratnikov B, Ptak C, Han J, et al. Talin phosphorylation sites mapped by mass spectrometry. J Cell Sci 2005;118:4921-3.
23. Critchley DR. Biochemical and structural properties of the integrin-associated cytoskeletal protein talin. Annu Rev Biophys 2009;38:235-54.
24. Calderwood DA, Zent R, Grant R, et al. The Talin head domain binds to integrin beta subunit cytoplasmic tails and regulates integrin activation. J Biol Chem 1999;274:28071-4.
25. Chen HC, Appeddu PA, Parsons JT, et al. Interaction of focal adhesion kinase with cytoskeletal protein talin. J Biol Chem 1995;270:16995-9.
26. Wegener KL, Basran J, Bagshaw CR, et al. Structural basis for the interaction between the cytoplasmic domain of the hyaluronate receptor layilin and the talin F3 subdomain. J Mol Biol 2008;382:112-26.
27. Critchley DR. Cytoskeletal proteins talin and vinculin in integrin-mediated adhesion. Biochem Soc Trans 2004;32:831-6.
28. Tadokoro S, Shattil SJ, Eto K, et al. Talin binding to integrin beta tails: a final common step in integrin activation. Science 2003;302:103-6.
29. Webb DJ, Parsons JT, Horwitz AF. Adhesion assembly, disassembly and turnover in migrating cells -- over and over and over again. Nat Cell Biol 2002;4:E97-100.
30. Wang HR, Zhang Y, Ozdamar B, et al. Regulation of cell polarity and protrusion formation by targeting RhoA for degradation. Science 2003;302:1775-9.
31. Sahai E, Garcia-Medina R, Pouysségur J, et al. Smurf1 regulates tumor cell plasticity and motility through degradation of RhoA leading to localized inhibition of contractility. J Cell Biol 2007;176:35-42.
32. Huang C, Rajfur Z, Yousefi N, et al. Talin phosphorylation by Cdk5 regulates Smurf1-mediated talin head ubiquitylation and cell migration. Nat Cell Biol

2009;11:624-30.
33. Pozo K, Bibb JA. The Emerging Role of Cdk5 in Cancer. Trends Cancer 2016;2:606-18.
34. Liu W, Li J, Song YS, et al. Cdk5 links with DNA damage response and cancer. Mol Cancer 2017;16:60.
35. Kawauchi T. Cell adhesion and its endocytic regulation in cell migration during neural development and cancer metastasis. Int J Mol Sci 2012;13:4564-90.
36. Tripathi BK, Zelenka PS. Cdk5: A regulator of epithelial cell adhesion and migration. Cell Adh Migr 2010;4:333-6.
37. Ruiz de Porras V, Bystrup S, Cabrero-de Las Heras S, et al. Tumor Expression of Cyclin-Dependent Kinase 5 (Cdk5) Is a Prognostic Biomarker and Predicts Outcome of Oxaliplatin-Treated Metastatic Colorectal Cancer Patients. Cancers (Basel) 2019;11:1540.
38. Zhuang K, Zhang J, Xiong M, et al. CDK5 functions as a tumor promoter in human colorectal cancer via modulating the ERK5-AP-1 axis. Cell Death Dis 2016;7:e2415.
39. Robb CM, Kour S, Contreras JI, et al. Characterization of CDK(5) inhibitor, 20-223 (aka CP668863) for colorectal cancer therapy. Oncotarget 2018;9:5216-32.

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