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## Expression of Paxillin is Correlated with Clinical Prognosis in Colorectal Cancer Patients

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**Background:** The aim of this study was to investigate the expression of Paxillin in colorectal carcinoma and its significance in clinical prognosis.

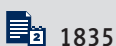
**Material/Methods:** Tissue specimens from 242 colorectal cancer patients who underwent radical resection were collected in Shaanxi Provincial People's Hospital from 2010 to 2014. The mRNA levels of Paxillin in colorectal cancer tissue and tissue adjacent to carcinoma of 62 patients were measured by quantitative real-time PCR. Immunohistochemistry staining was used to detect the expression of Paxillin in 242 samples of paraffin-embedded tissues.

**Results:** The mRNA and protein level of Paxillin in colorectal cancer tissues were significantly higher than those in the tissue adjacent to carcinoma ( $P < 0.001$  and  $P = 0.003$ , respectively). The expression of Paxillin was significantly correlated to tumor histological grade ( $P < 0.001$ ), tumor size ( $P = 0.01$ ), serum CA199 level ( $P < 0.001$ ), the clinical TNM stage ( $P < 0.001$ ), and distant metastasis ( $P < 0.001$ ). Survival analysis showed that the prognosis of the patients with high expression of Paxillin was poorer than those with low expression of Paxillin ( $P = 0.03$ ). Cox proportional hazards model with stepwise selection showed that age, Paxillin expression level, and the clinical TNM stage were independent prognostic factors influencing survival for patients ( $P = 0.01$ ,  $P = 0.004$  and  $P < 0.001$ , respectively).

**Conclusions:** Paxillin was expressed at significantly higher levels in colorectal cancer tissues and might serve as a potential prognostic indicator in patients with colorectal cancer.

**MeSH Keywords:** **Colorectal Neoplasms • Paxillin • Prognosis**

**Full-text PDF:** <http://www.medscimonit.com/abstract/index/idArt/893832>



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## Background

Colorectal cancer is one of the most common types of cancer, and is the third leading cause of cancer-related death worldwide [1]. It is estimated that at the time of diagnosis 30–40% of patients have locally advanced disease (Stage II-III) and approximately 20% have distant metastases (Stage IV) [2,3]. While substantial improvement has been achieved on the treatment of colorectal cancer in recent years, the survival rates remain low in patients, especially for those with the metastatic disease [4]. Tumor metastasis is a complicated process, and is a major cause of cancer-related death [5]. Thus, it is urgent to explore valuable molecular biomarkers to promote early diagnosis, prognosis prediction, and novel therapeutic strategies for colorectal cancer.

Paxillin is identified as a cytoplasmic protein containing tyrosine, and interacts with structural protein to regulate focal adhesion, cytoskeletal rearrangements, and cell motility [6]. It is a 68±70 kDa protein in cells transformed by the SRC oncogene and could encode cell adhesion molecule [7]. As an adapter protein, Paxillin coordinates and integrates multiple signals from the growth factors and cell surface receptors. Through these protein-protein interactions, Paxillin can modulate diverse physiological processes, such as gene expression, cell migration and invasion, cell proliferation, and tissue remodeling [8]. In some prostate cancer cell lines, early evidence showed that Paxillin might have a positive effect on androgen receptor-mediated transcription [9]. Paxillin can also combine with some oncogenic protein, including V-Src, E6, and BCR-ABL [10–12]. These oncoproteins disrupt the signal transduction of normal adhesion molecule and growth factor, which eventually affects cell growth and migration.

Paxillin has been reported to be involved in the pathogenesis of tumors. Previous studies showed that Paxillin can inhibit the proliferation and migration of lung cancer cells [13]. However, recent findings indicated that Paxillin variants played a prominent role in the mitochondrial dynamics, with direct implications for lung cancer progression [14]. It was reported that the stimulation of tyr118 phosphorylation of Paxillin in AGS cell's fibronectin was the most important factor in promoting the invasion in gastric cancer [15]. In the present study, we report that the expression level of Paxillin in colorectal cancer tissues is correlated with clinical prognosis of patients.

## Material and Methods

### Tissue samples

We enrolled 242 colorectal cancer patients in Shaanxi Provincial People's Hospital from 2010 to 2014. None of these patients

received any treatment before surgery. The tumor tissue and tissue adjacent to carcinoma were collected immediately after surgery. We divided every sample into 2 parts. One part was stored at –80°C, and the other part was fixed in 10% formaldehyde and then embedded in paraffin. This study was approved by the Shaanxi Provincial People's Hospital ethics committee. All patients signed the informed consent. The clinical stages were evaluated in accordance with the American Joint Committee on Cancer (AJCC) guidelines for colorectal cancer. The overall survival referred to the period from the date that patients underwent surgical treatment to death or the date of the last follow-up visit.

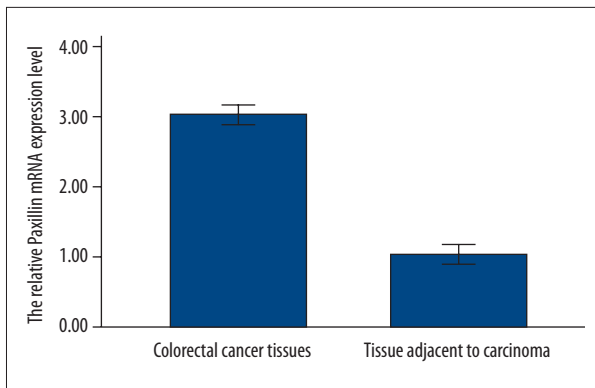
### Quantitative real-time PCR

We analyzed 62 frozen specimens using quantitative PCR. Total RNA was extracted from the tissue samples using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. First-strand cDNA was synthesized using the AMV reverse transcription system (Promega). The levels of mRNA expression were then assessed by the quantitative real-time PCR using the 7300 Real-Time PCR System (Applied Biosystems). The primers used for amplification were: Paxillin: forward 5'-ACAATCGACCCACGTTTCTC-3', reverse 5'-ATATTGTG CACCGGGATCAT-3'; glyceraldehyde 3-phosphate dehydrogenase (GAPDH): forward 5'-CATGACCAC AGTCCATGCCATCACT-3', reverse 5'-TGAGGTCCACCACCCTGTTGCTGTA-3'. Data were analyzed with the relative quantification method ( $2^{-\Delta\Delta Ct}$ ).

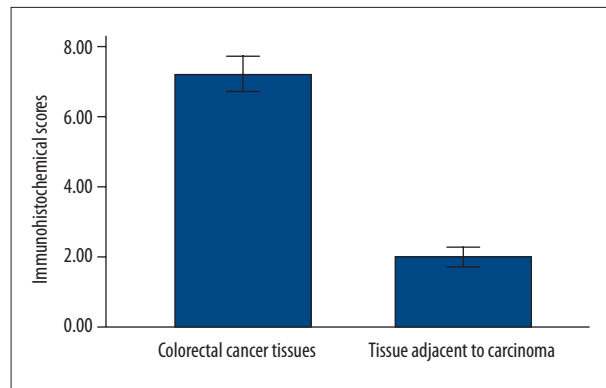
### Immunohistochemistry staining

Rabbit anti-human Paxillin antibody was purchased from Cell Signaling Technology (Beverly, MA, USA). Second antibody kits were purchased from Zhongshan Golden Bridge Bio-technology (Beijing, China). The experiment was performed strictly in accordance with the supplier's instructions. After dewaxing and hydration, endogenous peroxidase in tissue sections was neutralized with hydrogen peroxide. Then, the sections were washed with PBS buffer and incubated at room temperature with 5% goat serum for 10 minutes. After goat serum was washed, the tissue sections were incubated with rabbit anti-human Paxillin antibody (1:500) at 4°C overnight, and then washed with PBS buffer. Secondary antibody labeled with biotin was added and incubated at 37°C for 30 minutes. 3, 3'-Diaminobenzidine tetrahydrochloride (DAB) was then added as a chromogen.

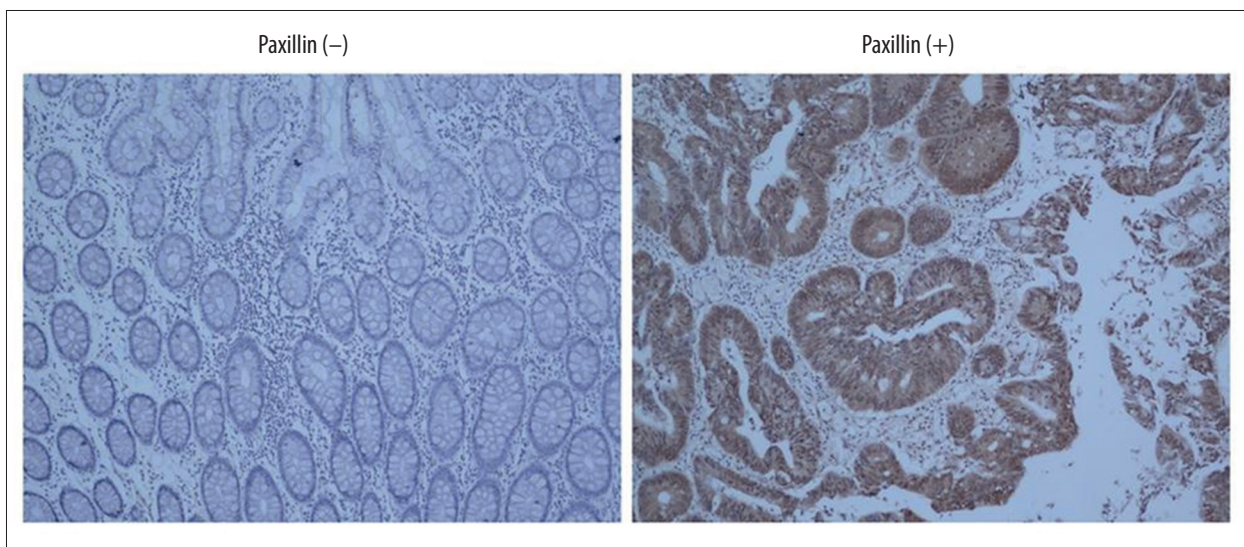
Immunohistochemical results are evaluated as follows: the proportion of cells deposited with brown granular <10%, 0 score; 11% to 25%, 1 score; 26% to 50%, 2 score; 51% to 75%, 3 score; 76% to 100%, 4 score. The staining intensity: negative, 0 score; mild positive, 1 score; moderate positive, 2 score; strong positive, 3 score. The total score equals to the proportion of positive cells' score multiplied by the staining intensity.



**Figure 1.** Bar charts of the relative Paxillin mRNA expression levels stratified by tissue sections. The charts show the mean change and 95%CI. CI=confidence interval.



**Figure 3.** Bar charts of the immunohistochemical scores by tissue sections. The charts show the mean score and 95%CI. CI=confidence interval.



**Figure 2.** The immunohistochemistry staining of Paxillin expression in colorectal cancer tissues and tissue adjacent to carcinoma.

The total scores with 6 or more were considered to be categorized as Paxillin high expression; otherwise, they were categorized as low expression of Paxillin.

### Statistical analysis

All statistical analyses were performed using SPSS13.0 software. The mRNA expression level of Paxillin between carcinoma tissue and tissue adjacent to carcinoma was analyzed by using the t test. The  $\chi^2$  test was used to explore the association between the expression level of Paxillin and the clinical pathological characteristics. The patient's survival curve was drawn according to Kaplan-Meier method and analyzed by log-rank test. Univariate and multivariate analyses for prognostic factors influencing survival in 242 patients with colorectal cancer were performed using the Cox proportional hazards model with stepwise selection.  $P < 0.05$  was considered statistically significant.

## Results

### The expression of Paxillin in colorectal cancer

The mRNA and protein level of Paxillin in colorectal cancer tissues and tissue adjacent to carcinoma were measured by quantitative real-time PCR and immunohistochemistry staining. As shown in Figure 1, Paxillin mRNA level in colorectal cancer tissues was significantly higher than that in the tissue adjacent to carcinoma ( $n=62$ ,  $P < 0.001$ ). Immunohistochemical results showed the mean scores in colorectal cancer tissues (7.21, 95% CI: 6.68–7.54) were significantly higher than that in the tissue adjacent to carcinoma (1.98, 95%CI: 1.71–2.25,  $n=242$ ,  $P=0.003$ , Figures 2, 3).

**Table 1.** The correlation between Paxillin expression and clinical pathological characteristics in 242 patients with colorectal cancer.

Clinical pathological characteristics	No. of patients with Paxillin high expression (%)	No. of patients with Paxillin low expression (%)	$\chi^2$	P value
Mean age				
≤60 years	61 (54%)	71 (55%)	0.03	0.49
>60 years	52 (46%)	58 (45%)		
Sex				
Male	76 (67%)	82 (64%)	0.36	0.32
Female	37 (33%)	47 (36%)		
Serum CEA level				
≤5 ng/ml	41 (36%)	55 (43%)	1.02	0.19
>5 ng/ml	72 (64%)	74 (57%)		
Tumor histological grade				
Height/middle	38 (34%)	109 (84%)	65.36	<0.001
Poor	75 (66%)	20 (16%)		
Tumor size				
≤5 cm	87 (77%)	81 (63%)	5.72	0.01
>5 cm	26 (23%)	48 (37%)		
Serum CA199 level				
≤35 u/ml	86 (76%)	70 (54%)	12.55	<0.001
>35 u/ml	27 (24%)	59 (46%)		
Clinical TNM stage				
I/II	28 (25%)	71 (55%)	22.82	<0.001
III/IV	85 (75%)	58 (45%)		
Distant metastasis				
With	84 (77%)	28 (21%)	67.11	<0.001
Without	29 (23%)	101 (79%)		

### The association between the Paxillin expression and clinical pathological characteristics of colorectal cancer patients

Of the total 242 patients, 30, 69, 79 and 64 patients were in clinical stage I, stage II, stage III and stage IV, respectively. As shown in Table 1, 113 patients of 242 cases (46.7%) had high expression of Paxillin, and the others (53.3%) had low expression of Paxillin. The expression level of Paxillin was significantly correlated to tumor histological grade ( $P<0.001$ ), tumor size ( $P=0.01$ ), serum CA199 levels ( $P<0.001$ ), the clinical TNM stage ( $P<0.001$ ), and distant metastasis ( $P<0.001$ ). However, the expression level of Paxillin was not related to

age, sex, or serum CEA levels ( $P=0.49$ ,  $P=0.32$  and  $P=0.19$ , respectively) (Table 1).

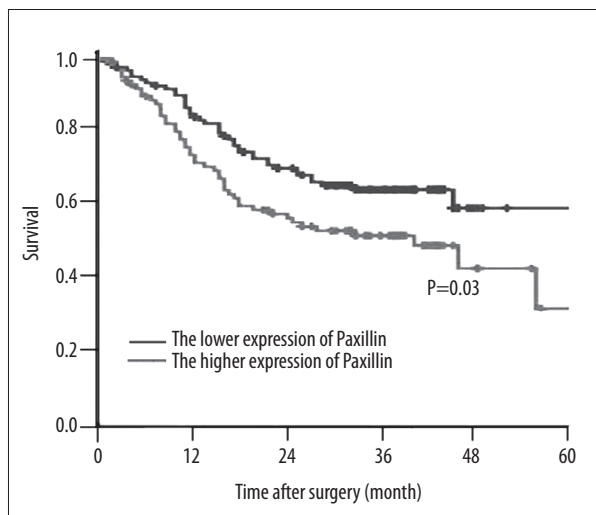
### The association between the expression of Paxillin and the survival of patients with colorectal cancer

Survival analysis showed that the prognosis of the patients with high expression of Paxillin was significantly worse than in those with Paxillin low expression ( $n=242$ ,  $P=0.03$ ). The survival rates of patients with high expression of Paxillin at 2 years, 3 years, and 5 years were 54.7%, 50.5% and 30.8%, respectively. The survival rates of patients with low expression of Paxillin at 2 years, 3 years, and 5 years were 70.5%, 62.1%,

**Table 2.** Prognostic factors influencing survival in 242 patients with colorectal cancer.

	Univariate			Multivariate		
	HR	95%CI	P value	HR	95%CI	P value
Age (>60 years)	1.06	1.02–1.11	0.01	1.06	1.02–1.08	0.01
Sex (male)	1.02	0.97–1.07	0.39			
Serum high CEA level	1.10	0.98–1.21	0.48			
High/middle grade	1.26	1.23–1.48	0.01			
Tumor size (>5 cm)	1.78	1.46–1.93	0.04			
Serum high CA199 level	1.76	0.69–4.49	0.62			
Clinical TNM III/IV stage	3.59	0.82–15.81	0.89			
With distant metastasis	2.03	1.46–3.04	0.002	1.98	1.76–2.68	0.004
Paxillin high expression	4.93	1.66–20.82	<0.001	4.21	3.96–16.81	<0.001

HR – hazard ratio; CI – confidential interval.



**Figure 4.** Patient survival compared between the patients with higher and lower expressions of Paxillin. Survival rate was calculated by the Kaplan-Meier method, and statistical significance was analyzed using the log-rank test. The black line indicates the lower expression of Paxillin and the gray line indicates the higher expression of Paxillin.

and 58.1%, respectively (Figure 4). We further examined the prognostic factors influencing survival with univariate and multivariate analyses. The univariate analysis showed that age ( $P=0.01$ ), high/middle grade ( $P=0.01$ ), tumor size (>5 cm) ( $P=0.04$ ), with distant metastasis ( $P=0.002$ ), and high Paxillin expression ( $P<0.001$ ) were significant factors. The multivariate analysis with stepwise selection showed that age ( $P=0.01$ ), distant metastasis ( $P=0.004$ ), and high Paxillin expression ( $P<0.001$ ) were significant independent factors for patients with colorectal cancer (Table 2).

## Discussion

The present study evaluated the expression level of Paxillin in the colorectal cancer tissues and its significance in clinical prognosis. Our results demonstrated that Paxillin was expressed significantly more in cancerous tissue than that in the adjacent normal tissue. Moreover, overexpression of Paxillin was correlated to tumor malignant phenotypes, such as tumor histological grade, clinical TNM stage, and distant metastasis. Additionally, the overall survival in patients with high Paxillin expression was clearly poorer than in those with low Paxillin expression. In univariate and multivariate analysis, overexpression of Paxillin was an independent prognostic factor influencing survival in patients with colorectal cancer. Our finding suggested that Paxillin might serve as a potential prognostic indicator in patients with colorectal cancer.

Many studies have characterized the expression profiles of Paxillin in tumors. It was reported that Paxillin was significantly more expressed in gastric cancer and was correlated with the malignant-phenotype tumor [16]. It was also found that Paxillin was overexpressed in premalignant areas of hyperplasia, squamous metaplasia, and goblet cell metaplasia, as well as dysplastic lesions and carcinoma in high-risk lung cancer patients [17]. The expression of Paxillin was elevated in human prostate cancer tissue microarrays [18]. Overexpression of Paxillin was relevant to the distant metastasis of salivary adenoid cystic carcinoma and the poor prognosis of hepatocellular carcinoma [19,20]. Our findings regarding Paxillin expression in colorectal carcinoma are consistent with the above results.

Cell attachment, spreading, and motility are complex processes requiring the integration of diverse signaling networks and structural assemblies [21]. The activation of the



tyrosine kinases Src and FAK is one of the earliest steps in transducing extracellular cues through integrins to the cytoskeleton [22]. FAK is activated to autophosphorylate Y397 and binds to the Src SH2 domain [23]. Then, Src phosphorylates FAK on multiple residues, which increase the kinase activity of FAK [24]. Next, several downstream binding partners for Src/FAK are targeted for phosphorylation, including Paxillin [25]. Phosphorylated Paxillin can stimulate cell motility [26]. However, to date, there are no known Paxillin inhibitors, so it remains uncertain whether inhibition of Paxillin could cause an inhibition in cell migration.

As an adaptor protein, Paxillin is involved in multiple signal transductions. It has the capacity to enhance the adhesion between tumor cells and the surrounding cells and molecules, thus promoting the migration and invasion capacities of tumor cells [27]. Numerous studies reported that the abnormal expression of Paxillin in tumor tissues was closely related with tumorigenesis and cancer metastasis [28,29]. In general, higher expression of Paxillin was accompanied with a greater likelihood of malignant behavior. The effect of Paxillin on cell proliferation was mainly regulated by tyrosine/serine

phosphorylation [30]. For example, cell migration and invasion of breast cancer is induced by the activation of breast tumor kinase (BRK) mediated by Paxillin phosphorylation [31]. In colorectal cancer, 31 and 118 sites tyrosine phosphorylation in Paxillin is an important mechanism of cell metastasis and adhesion induced by stress [32]. In this study, we found that the expression of Paxillin was markedly related with distant metastases in colorectal cancer patients. These results suggest that Paxillin may play an important role in the development and metastasis of colorectal cancer.

## Conclusions

This study demonstrates that high expression of Paxillin is significantly correlated with the poor prognosis of patients with colorectal cancer. Future studies are needed to clarify this relationship and to explore its clinical value.

## Conflict of interest statement

None declared.

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