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Original Article

Expression of Ki-67 and pAKT in colorectal cancer tissues and their clinical significance

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Abstract: Objective: Our aim was to investigate expression of Ki-67 and phosphorylated serine-threonine protein kinase (pAkt) in colorectal cancer tissues and their clinical significance, providing evidence for clinical diagnosis and treatment of colorectal cancer. Methods: A total of 80 patients pathologically diagnosed with colorectal cancer were selected. Streptavidin-Peroxidase immunohistochemical assay was used to detect positive expression rates of Ki-67 and pAKT in 80 cases of colorectal cancer tissue (CRC group), 80 cases of colorectal cancer adjacent tissue (adjacent group), and 20 cases of normal colorectal tissue (normal group). Correlation of expression of Ki-67 and pAKT in cancer tissues of CRC patients with metastasis, stage, and prognosis of colorectal cancer were analyzed. Spearman's correlation analysis was then adopted to analyze the relationship between Ki-67 expression and pAKT expression. Results: Positive expression rates of Ki-67 and pAKT in colorectal cancer group were significantly higher than those in the adjacent group and normal group (P<0.001, P<0.001, P=0.012; P<0.001, P=0.011, P=0.024). Positive expression rates of Ki-67 and pAKT in cancerous tissue of patients with lymph node metastasis were overtly higher than those in cancerous tissue of patients without lymph node metastasis (P=0.013, P=0.009). Positive expression rates of Ki-67 and pAKT in cancer tissue of patients with stage I+II cancer were clearly lower than those in cancer tissue of patients with stage III+IV cancer (P=0.003, P=0.002). There was positive correlation between expressions of Ki-67 and pAKT (r=0.520, P=0.020). Overall survival of patients with negative Ki-67 expression was statistically longer than those of patients with (+), (++) and (+++) of Ki-67 (P=0.021, P=0.022, P<0.001). In addition, overall survival of patients with negative pAKT expression showed statistically longer survival compared with those with (+), (++) and (+++) of pAKT (P=0.011, P=0.012, P=0.001). Conclusion: Abnormal expression of Ki-67 and pAKT was closely related to occurrence and development of colorectal cancer and upregulated expression of Ki-67 and pAKT was involved in the formation, development, invasion, and metastasis of tumors. They could be used as clinical markers determining the biological behavior of colorectal cancer.

Keywords: Ki-67, phosphorylated serine-threonine protein kinase, colorectal neoplasia, correlation

Introduction

Colorectal cancer (CRC) is one of the most common oncological diseases in clinical practice. It is detected in many sites including the rectum, sigmoid colon, and cecum. In recent years, its incidence has increased year by year and it has ranked third among cancers all over the world, seriously affecting patient quality of life. Moreover, most patients are already in an advanced stage when detected, losing the optimal opportunity for treatment [1]. Ki-67 is a protein in the cell nucleus and is closely related to cell proliferation. One study demonstrated that Ki-67 was a marker for cell proliferation and was closely associated with differentiation and metastasis of tumors, being widely used in marking of cell proliferation in clinical practice [2]. Serine-threonine protein kinase (AKT) is a protein kinase regulating cell proliferation and differentiation which participates in protein synthetic and anti-apoptotic processes, plays an important role in promoting cell growth and proliferation, and is not expressed in normal tissue [3]. One study indicated that phosphorylated AKT (pAKT) plays an important role in tumor angiogenesis and is involved in the metastasis and invasion of various tumors [3]. Studying gene mutation and tumor cell proliferation in colorectal cancer is of great clinical significance

Table 1. Comparison of general data between the two groups

Group	Gender		Age
	Male	Female	
Colorectal cancer group	45	35	52.85±17.04
Normal group	11	9	52.17±16.26
X^2/t	2.044		1.025
P	0.191		0.382

in correctly judging biological behaviors of colorectal cancer including occurrence, invasion, and metastasis. Previous studies have reached different conclusions on whether expression of pAKT protein can predict prognosis of patients with colorectal cancer. Therefore, pAKT protein expression cannot currently be used as a mature biological index predicting prognosis of patients with colorectal cancer [4, 5]. In this study, we observed expression of Ki-67 and pAKT in colorectal cancer tissues via immunohistochemistry and explored the correlation of expression of Ki-67 and pAKT with invasion and metastasis of colorectal cancer, aiming to investigate colorectal carcinogenesis, evaluate prognosis in early stage, and guide postoperative follow up of patients with colorectal cancer.

Materials and methods

General data

A total of 80 patients with colorectal cancer, admitted to The Second Affiliated Hospital of Xinjiang Medical University from January to December 2016, were selected as our study objects. Another 20 cases of normal colonic mucosa tissues were used as controls. All subjects signed informed consent and this study was approved by the Ethics Committee of the Second Affiliated Hospital of Xinjiang Medical University.

Inclusion criteria: Patients received no radiotherapy or chemotherapy before operation, were pathologically diagnosed with colorectal cancer with complete clinical and pathological data, and without diseases of other tissues or organs.

Exclusion criteria: Patients with other malignancies with incomplete follow up data or that were unable to cooperate with treatment due to mental disorders.

Detection methods

Streptavidin-Peroxidase immunohistochemical assay was used to determine expression of Ki-67 and pAKT in specimens of patients. Cancerous tissue specimens were collected from patients, fixed in 10.00% neutral formalin, dehydrated, embedded, sectioned, and dewaxed. Specimens were then added with mouse anti-human pAKT and mouse anti-human Ki-67 monoclonal antibodies, stained with hematoxylin, mounted, and observed under a high-powered microscope. The nuclei of stained positive cells were brown to dark brown. One thousand random stained cells were observed and the positive rate was calculated. Scoring was based on positive expression rate: 0%≤ positive rate ≤5.00%, 0 points; 5%< positive rate ≤25.00%, 1 point; 25.00%< positive rate ≤50.00%, 2 points; and positive rate >50.00%, 3 points. Scoring criteria based on staining intensity: 0 points for unstained cells, 1 point for light yellow cells, 2 points for brown cells, and 3 points for tan cells. The product of the two was defined as a combined score with 0-1 point for negative (-), 2-3 points for weakly positive (+), 4-8 points for moderately positive (++), and 9 points for strongly positive (+++).

Follow up

Patients were followed up for 5 years via phone or through outpatient after the operation. Those lost to follow up were excluded before calculating survival analysis.

Statistical analysis

SPSS 16.0 software was used for statistical analysis. Measurement data are expressed as $\bar{x} \pm$ sd. t-test was employed for comparisons between two groups. F-test was applied for comparisons among three groups. X^2 test was adopted to compare rates of Ki-67 and pAKT positive cells in different tissues. Spearman's analysis was used to analyze the relationship between Ki-67 expression and pAKT expression. α=0.05 was the test level.

Results

Comparisons of general data

In the colorectal cancer group, there were 45 males and 35 females, aged 35-77 years old with a mean age of (52.85±17.04) years old.

Table 2. Positive expression rates of Ki-67 and pAKT in three kinds of tissue

Group	Case	Positive rate	
		Ki-67	pAKT
Normal tissue	20	1 (5.00%)	1 (5.00%)
Para-carcinoma tissue	80	23 (28.75%)	19 (23.75%)
Colorectal cancer tissue	80	67 (83.75%)	71 (88.75%)
X^2		$21.273^{\Delta}/13.844^{*}/9.091^{\#}$	$19.583^{\Delta}/10.325^{*}/8.744^{\#}$
P		$<0.001^{\Delta}/<0.001^{*}/0.012^{\#}$	$<0.001^{\Delta}/0.011^{*}/0.024^{\#}$

Note: $^{\Delta}$Comparison between colorectal cancer tissue and normal tissue; *comparison between adjacent tissue and normal tissue; $^{\#}$comparison between colorectal cancer tissue and adjacent tissue. pAKT, phosphorylated serine-threonine protein kinase.

Among these, 46 patients had rectal cancer and 34 patients had colon cancer. All patients were staged according to tumor-node-metastasis (TNM) staging criteria and the results showed that there were 15 patients with stage I cancer, 23 patients with stage II cancer, 26 patients with stage III cancer, and 16 patients with stage IV cancer. There were 46 patients with lymph node metastasis and 34 patients without lymph node metastasis. According to degree of tumor differentiation, there were 45 patients with poor differentiation, 23 patients with moderate differentiation, and 12 patients with high differentiation. In our normal group, there were 11 males and 9 females, aged 33-68 years old with an average age of (52.17±16.26) years old. There were no statistically significant differences between the two groups (P=0.191, P=0.382). See **Table 1**.

Expression of Ki-67 and pAKT in different tissues: positive expression of Ki-67 and pAKT in colorectal cancer tissue, adjacent tissue, and normal tissue

pAKT was located in the cytoplasm and Ki-67 was located in the nucleus and they were brown or tan. Positive expression rates of Ki-67 and pAKT in colorectal cancer tissue (83.75%, 88.75%) were significantly higher than those in adjacent tissue (28.75%, 23.75%) and normal colorectal mucosa tissue (5.00%, 5.00%). Differences of pairwise comparisons were statistically significant (P<0.001, P<0.001, P= 0.012; P<0.001, P=0.011, P=0.024) among three groups (**Table 2** and **Figures 1**-**3**).

Correlation of Ki-67 and pAKT levels with metastasis of cancer tissue

Positive expression rates (89.13%, 97.83%) of Ki-67 and pAKT in cancer tissue of patients with lymph node metastasis were significantly higher than those (76.47%, 76.47%) in cancer tissue of patients without lymph node metastasis (P= 0.013, P=0.009). See **Table 3**.

Positive expression rates of Ki-67 and pAKT in cancer tissue of patients with different stages of cancer

Positive expression rates (68.42%, 76.32%) of Ki-67 and pAKT in stage I-II patients were evidently lower than those (97.62%, 100.00%) in stage III-IV patients (P=0.003, P=0.002). See **Table 4**.

Correlation analysis of Ki-67 and pAKT with colorectal cancer

According to Spearman's correlation analysis, Ki-67 expression and pAKT expression were positively correlated (r=0.520, P=0.020). See **Figure 4**.

Relationship of expression of Ki-67 and pAKT with prognosis of colorectal cancer patients

With the increase of Ki-67 and pAKT expression levels, median survival of patients was shortened. 5-year overall survival of patients with negative Ki-67 expression was statistically different from those of patients with (+), (++) and (+++) of Ki-67 (P=0.021, P=0.022, P< 0.001). 5-year overall survival of patients with negative pAKT expression and those of patients with (+), (++) and (+++) of pAKT were significantly different (P=0.011, P=0.012, P=0.001). See **Tables 5** and **6**, **Figure 5**.

Discussion

In recent years, more and more people have been afflicted with colorectal cancer and they tend to be younger with improvements in living standards, changes in dietary habits and components, and increased aging populations. Invasiveness and metastasis of malignant tumor tissues are the leading causes of death in cancer patients [6]. Many factors, genes, and cytokines are involved in occurrence and development of colorectal cancer and detection of

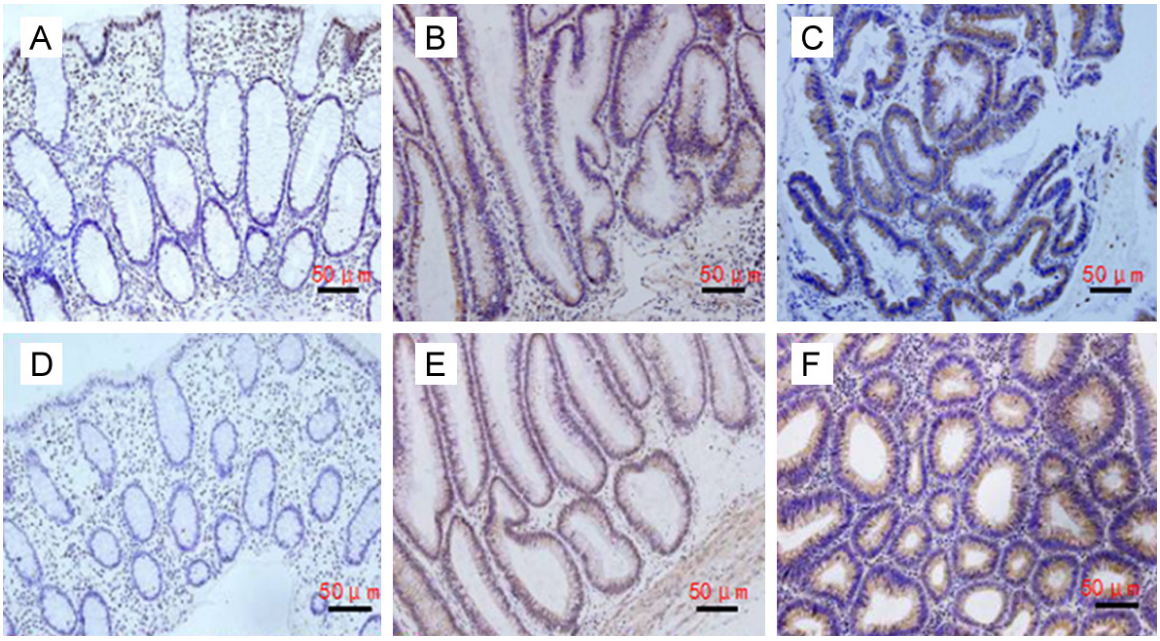

Figure 1. Expression of Ki-67 and pAKT in three different tissues (Streptavidin-Peroxidase×400). A: Normal colorectal tissue; B: Ki-67 is moderately expressed in para-cancerous tissue; C: Ki-67 is strongly expressed in colorectal cancer tissue; D: Normal colorectal tissue; E: pAKT is moderately expressed in para-cancerous tissue; F: pAKT is strongly expressed in colorectal cancer tissue. pAKT, phosphorylated serine-threonine protein kinase.

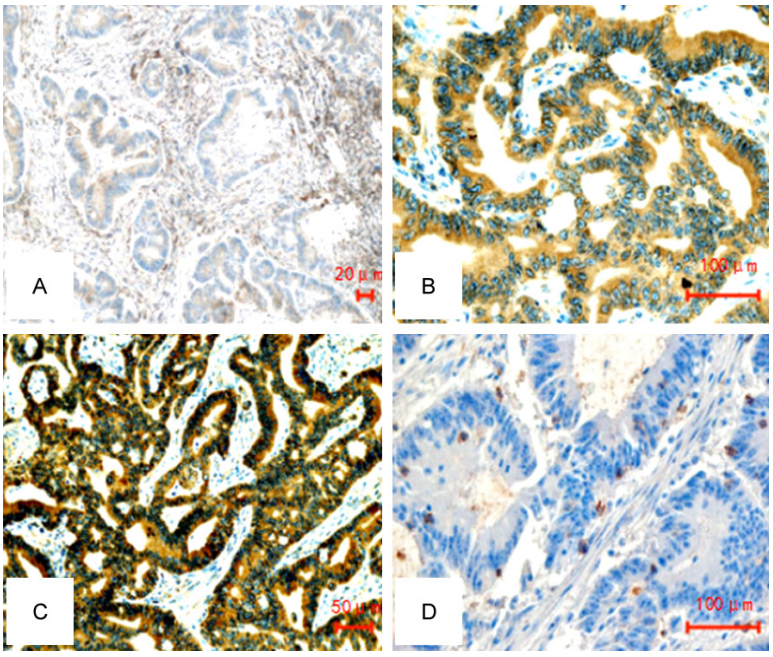

Figure 2. Expression of pAKT in different degrees. A: Weakly positive expression; B: Moderately positive expression; C: Strongly positive expression; D: Negative expression. pAKT, phosphorylated serine-threonine protein kinase.

cancer-related proteins is conducive to detecting cancer at an early stage, preventing its development, and clinically assessing colorectal cancer. Diagnosis of the development of cancer tissue at an early stage is highly significant for predicting prognosis of patients.

pAKT regulates proliferation, invasion, metastasis, and apoptosis of tumor cells. Thus, it accelerates the growth and proliferation of cells and promotes occurrence and development of tumors [7]. One study has suggested that pAKT is highly expressed in a variety of malignant tumors (including gastric carcinoma, hepatocellular carcinoma, and breast carcinoma), and plays crucial roles in occurrence and development of tumors [8]. A study found that the expression rate of pAKT in breast cancer was up to 58% [9]. Another study found that pAKT expression rate in early-stage invasive ductal carcinoma was as high as 76% [10]. An additional study also confirmed that pAKT plays crucial roles in tumor invasion and metastasis [11]. Currently,

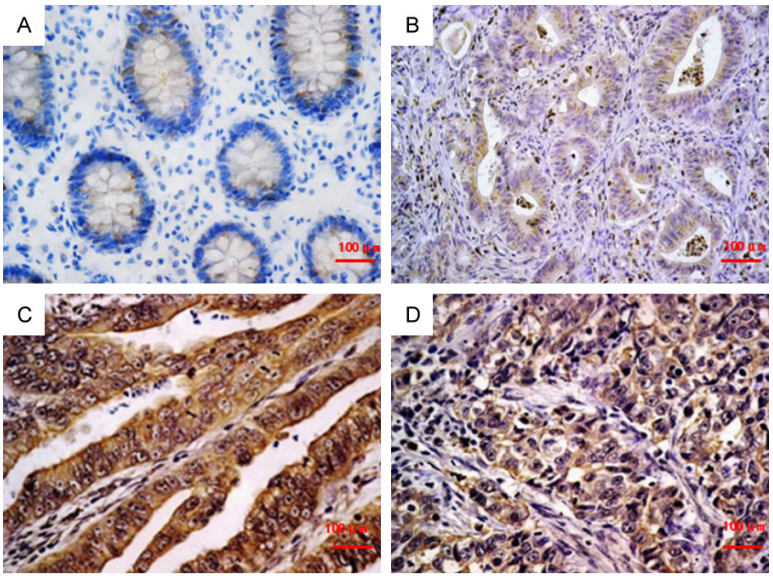

Figure 3. Expression of Ki-67 in different degrees. A: Negative expression; B: Weakly positive expression; C: Strongly positive expression; D: Moderately positive expression.

Table 3. Correlation of Ki-67 and pAKT levels with cancer tissue metastasis (n, %)

Group	Case	Ki-67		pAKT	
		+	%	+	%
Lymph node metastasis group	46	41	89.13	45	97.83
No lymph node metastasis group	34	26	76.47	26	76.47
X^2		7.484		9.953	
P		0.013		0.009	

Note: pAKT, phosphorylated serine-threonine protein kinase.

detection of pAKT in breast cancer is taken seriously. The results of this present study demonstrated that the positive expression rate of pAKT in colorectal cancer tissue was significantly higher than in adjacent tissue and normal colorectal mucosa tissue, suggesting that pAKT may be involved in differentiation of cancer cells. The positive rate of pAKT in cancer tissue of patients with lymph node metastasis was obviously higher than that in cancer tissue of patients without lymph node metastasis, indicating that pAKT is certainly associated with metastasis of colorectal cancer. The later the TNM staging in colorectal cancer patients, the higher the positive expression rate of pAKT in cancer tissue. Positive expression rate of pAKT in stage I-II cancer patients was significantly lower than that in stage III-IV patients, suggesting that cancer in later stages produces more pAKT to maintain growth of the tumor. Highly expressed pAKT was more invasive. pAKT is associated with progression of colon cancer and may be involved in carcinogenesis. It has been speculated that the mechanism of pAKT participating in occurrence and development of colon cancer may be that pAKT is involved in the proliferation of tumor cells by activating or inhibiting a large number of downstream targets such as mouse double minute 2 homolog, mammalian target of rapamycin, B-cell lymphoma 2, and glycogen synthase kinase-3, inhibiting cell apoptosis. Simultaneously, pAKT can promote immune escape of tumor cells via tumor microenvironment, avoiding being attacked. In addition, pAKT is able to promote formation of vascular growth factors and a large number of tumor cell blood vessels, promoting tumor proliferation [12]. However, the specific mechanism still further study and confirmation.

Uncontrolled proliferation of tumor cells and disordered apoptosis play important roles in occurrence and development of malignant tumors. There are many factors involved in regulation of the cell cycle and they are coordinated with each other to maintain normal proliferation of cells. Ki-67 is a protein in the cell nucleus that can reflect the degree of cell proliferation, being one of the common indicators of clinical and pathological diagnosis. Ki-67 is lowly expressed in normal tissues and highly expressed in tumor cells [13]. A study showed that Ki-67 was associated with RNA transcription, had high expression in cell division and proliferation stages, and could reflect the degree of cell division [14]. Currently, there are many studies on Ki-67 in China and foreign countries. Now, it is clear that Ki-67 has a short half-life and is difficult to be detected. Moreover, the rising trend of Ki-67 is always consistent

Table 4. Positive expression rates of Ki-67 and pAKT in cancer tissue of patients with different stages of cancer (n, %)

Group	Case	Positive rate	
		Ki-67	pAKT
Stage I-II	38	26 (68.42)	29 (76.32)
Stage III-IV	42	41 (97.62)	42 (100.00)
X^2		12.067	11.542
P		0.003	0.002

Note: pAKT, phosphorylated serine-threonine protein kinase.

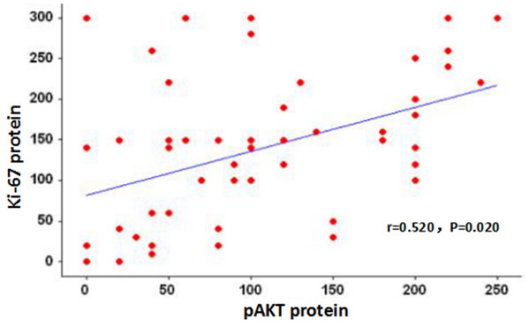

Figure 4. Correlation analysis of Ki-67 and pAKT. pAKT, phosphorylated serine-threonine protein kinase.

Table 5. Postoperative median survival and overall survival of colorectal cancer patients with different expression levels of Ki-67

Ki-67	Case	Median survival (month)	Overall survival (%)		
			<1 year	1-3 year (s)	>5 years
-	13	45.72	79.89	51.53	33.62
+	15	60.13	90.12	60.14	33.82*
++	37	30.11	71.35	41.20	19.75*
+++	15	17.23	53.84	9.70	0*

Note: Compared with Ki-67 (-), *P<0.05.

Table 6. Postoperative median survival and overall survival of colorectal cancer patients with different pAKT expression levels

pAKT	Case	Median survival (month)	Overall survival (%)		
			<1 year	1-3 year (s)	>5 years
-	9	46.14	80.31	53.57	26.33
+	25	59.94	89.34	67.11	41.26*
++	32	33.25	75.87	42.86	23.81*
+++	14	18.38	67.13	10.81	0*

Note: Compared with pAKT (-), *P<0.05. pAKT, phosphorylated serine-threonine protein kinase.

with that of tumor cell proliferation. Therefore, Ki-67 is commonly used in detection of tumor cell proliferation. One study suggested that breast cancer patients with lymph node metastasis had a strong expression of Ki-67 and a low 5-year overall survival, suggesting that Ki67 was related to metastasis and prognosis of breast cancer [15]. In addition, some scholars have confirmed that Ki-67 and COX-2 are highly expressed in breast cancer via immunohistochemistry and are considered as high risk biomarkers of breast cancer invasion, metastasis, prognosis, and recurrence [16]. The results of this study indicated that the positive expression rate of Ki-67 in colorectal cancer tissue was significantly higher than in adjacent tissue and normal colon tissue. Positive expression rate was negatively correlated with the differentiation degree of colorectal cancer, suggesting that Ki-67 plays a certain role in the differentiation process of colorectal cancer. At the same time, it was found that the later the TNM staging, the higher the positive expression rate of Ki-67 in cancer tissue would be, i.e., the positive expression rate of Ki-67 in patients with stage I-II cancer was significantly lower than that in patients with stage III-IV cancer. Positive expression rates of Ki-67 in patients with lymph node metastasis were significantly higher than in patients without lymph node metastasis. This suggests that positive expression rates of Ki-67 in colon cancer tissue have the same tendency with trends of tumor invasion depth and lymph node metastasis, are positively correlated with clinical prognosis of tumors, and are indicators of prognosis of colorectal cancer.

The process of cell carcinogenesis is very complex. It involves a variety of cytokines and genes that still remain unclear [17]. The results of this study suggest that with the increase of cancer staging and deepening of infiltration, Ki-67 and pAKT expression also get higher and higher, having guide value for diagnosis and treatment of colorectal cancer. Both Ki-67 and pAKT can be used as indicators for judging occurrence, metastasis, depth of invasion, and prognosis of colorectal cancer. Higher expression levels of Ki-67 and pAKT suggest that the invasive ability of cancerous tissue is stronger and prognosis is poorer [18, 19]. Highly expressed Ki-67

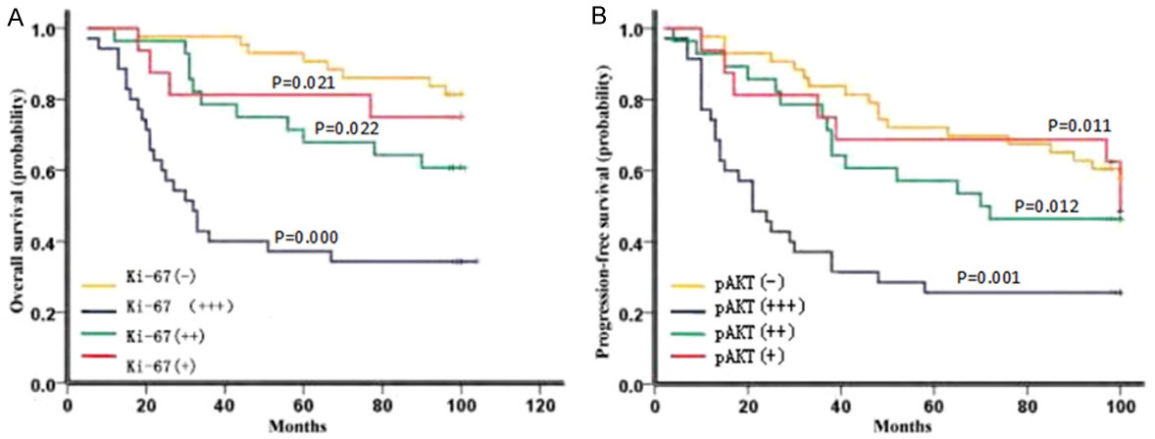

Figure 5. Correlation of Ki-67 and pAKT with prognosis of colorectal cancer patients. A: Ki-67 expression and prognosis of patients with colorectal cancer. The overall survival of patients with Ki-67 negative expression was statistically different from those of patients with (+), (++) and (+++) expression of Ki-67 (P=0.021, P=0.022, P<0.001). B: pAKT and prognosis of patients with colorectal cancer. The overall survival of patients with pAKT negative expression was statistically different from those of patients with pAKT (+), (++) and (+++) expression (P=0.011, P=0.012, P=0.001). pAkt, phosphorylated serine-threonine protein kinase.

and pAKT were positively correlated, perhaps due to the possibility that high expression of pAKT may promote the transformation of tumor cells from G1 to S phase and exacerbate proliferation and division of cells. This study suggests that pAKT and Ki-67 synergistically promote progression of colorectal cancer. pAKT can promote tumor angiogenesis and Ki-67 can promote tumor cells. They may synergistically promote tumor angiogenesis and tumor cell hyper proliferation, through interactions in tumor angiogenesis and other aspects, promoting the development of colorectal cancer. Ki-67 and pAKT levels could be used as two evaluation indexes in early diagnosis and treatment of colorectal cancer, similar to the findings of other studies [20].

In conclusion, abnormally expressed pAKT and Ki-67 are closely related to occurrence and development of colorectal cancer. In colorectal cancer tissue, pAKT expression was increased with abnormal proliferation of tumor cells. pAKT and Ki-67 were involved in the formation, development, invasion, and metastasis of tumors. Our sample size, however, was relatively small and follow up time was short. To confirm our findings, sample sizes should be increased and the relationship between the two should be further studied in the future.

Disclosure of conflict of interest

None.

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