Clinical and Experimental Gastroenterology Dovepress

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Correlation Between Tumor Budding and Survivin Expression in Colorectal Cancer

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Aim: Correlation of Survivin expression levels in tumor tissues and degree of tumor outgrowth with colorectal cancer characteristics.

Methods: The pathological tissues of 90 cases of colorectal cancer were observed by HE staining, and the tumor budding was judged by Ueno standard, and the expression of Survivin was detected by immunohistochemistry (IHC) technique (EnVision method), so as to analyze the correlation between tumor budding, the expression level of Survivin and the degree of tumor budding, and the correlation between the tumor budding and the patients' clinical characteristics.

Results: The expression level of Survivin was significantly correlated with TNM stage, lymph node metastasis and distant metastasis in patients with colorectal cancer; tumor outgrowth was significantly correlated with TNM stage, lymph node metastasis and distant metastasis in patients with colorectal cancer ($P < 0.05$); the expression level of Survivin was significantly correlated with the degree of tumor budding in patients with colorectal cancer ($P < 0.05$).

Conclusion: In this paper, we tested the relationship between Survivin and tumor budding in colon cancer, and analyzed its relationship with clinicopathological features, with a view to providing a reference for the mechanism related to colorectal cancer.

Keywords: colorectal cancer, Survivin, tumor budding

Introduction

Colorectal cancer has the 3rd and 2nd highest incidence and mortality rates of malignant tumors China.[1] With the improvement of people's material living standard, the incidence rate of colorectal cancer has been increasing year by year and showing a trend of youthfulness.[2] Tumor budding is a valid indicator for assessing the aggressive biological behavior of colorectal cancer and a poor prognosis for colorectal cancer because. Literature reports that tumor budding is closely associated with colorectal cancer histological grading, nerve invasion, lymphovascular infiltration, and tumor staging.[3] Survivin is the most apoptosis-inhibiting protein among the apoptosis-inhibiting protein family discovered so far, and is highly expressed in a variety of tumor tissues, which can inhibit apoptosis and promote tumor cell invasion and metastasis.[4] At present, the relationship between Survivin and tumor outgrowth in colorectal cancer tissues has not been reported. In this paper, we tested the relationship between Survivin and tumor outgrowth and analyzed its relationship with clinicopathological features, with a view to providing a reference for the mechanism related to colorectal cancer.

Methods

Colorectal Cancer Samples

Ninety specimens of colorectal adenocarcinoma, 42 males and 58 females, surgically resected in Ya'an People's Hospital, Sichuan Province, China, from January 1, 2022, to January 1, 2023, were collected; The age of the patients ranged from 25 to 86 years, with a mean age of 60 years.

Received: 5 June 2024
Accepted: 4 October 2024
Published: 14 October 2024

Interpretation of Tumor Budding

Correlation sections of 90 colorectal adenocarcinomas were performed for tumor budding interpretation, and hotspot areas were selected at 200x to assess scattered single tumor cells or small focal clusters of cells in the forefront mesenchyme of tumor infiltration (≤4), which were categorized as low (BD1), medium (BD2), and high (BD3), corresponding to 0–4, 5–9, and ≥10 buds (ie, 0.785 mm^2 area).[5] In this experiment, BD1 and BD2 were defined as low level and BD3 as high level.

Immunohistochemistry

Paraffin-embedded histologic specimens were cut into 4 μm thick sections. Then, sections were routinely dewaxed and rehydrated in xylol and graded alcohol. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in a phosphate-buffered solution for 15 minutes and nonspecific binding was blocked with 2% bovine serum for 20 minutes. The slides were incubated with 1:200 diluted primary antibody against human CKpan and Survivin (Fujian Maixin Biotechnology Inc.) for 18 hours at 4°C in 2% bovine serum albumin in phosphate-buffered solution. The horseradish peroxidase–conjugated goat anti-mouse IgG secondary antibody was added and incubated for 1 hour at 37°C. The immune reaction was developed with 3,3′-diaminobenzidine-tetrahydrochloride-dihydrate. Slides were washed with distilled water, counterstained with hematoxylin, dehydrated, and mounted. All sections were observed and analyzed under a light microscope. The scoring of immunostaining was evaluated based on staining intensity and percentages of 3 randomly positive stained areas by 2 pathologists in a double-blinded manner. To score the expression of Survivin, we used a three-level scale: 1=weakly positive; 2=moderately positive; 3=strongly positive, ranging from: 1: 0–30%; 2: 30–60%; 3: 60–100%.

Statistical Analyses

SPSS 22.0 statistical software (SPSS Inc.) was used to analyze all data. The Student *t* test was employed to compare the differences between the 2 groups. Analysis of the relationship between Survivin and tumor budding and clinicopathological indicators using chi-square tests, and clinical features. A P-value <0.05 was considered statistically significant.

Results

Survivin expression level in colorectal cancer tissues and its correlation with clinical features. IHC revealed that Survivin was mainly expressed in the cytoplasm. Among them, Survivin expression level was relatively low in 27 cases and higher in 63 cases. The expression of Survivin was correlated with lymph node metastasis (P=0.000), distant metastasis (P=0.005), and TNM stage of colorectal cancer (P=0.030) ($P < 0.05$), but not with the patient's age and sex ($P > 0.05$) (Table 1).

Tumor budding in patients with colorectal cancer and its correlation with clinicopathological features. Among 90 colorectal cancer patients, 26 cases had lower tumor budding ratings (see Figure 1) and 64 cases had higher ratings. Statistical analysis showed that tumor budding rating was significantly correlated with lymph node metastasis (P=0.004), distant metastasis (P=0.010), and TNM stage (P=0.020) in patients with clinical and colorectal cancer ($P < 0.05$), and was not related to gender and age (Table 1).

Tumor budding is significantly associated with Survivin expression in colorectal cancer patients. Analysis of the correlation between Survivin protein expression and tumor budding revealed that the number of tumor outgrowth was higher in cancer tissues with higher levels of Survivin protein expression (P= 0.033) (Table 2).

Discussion

Colorectal cancer is one of the most important malignant tumors affecting the health of our population, with about 376,300 new cases and 190,000 deaths per year, ranking the 5th among malignant tumors.[6] The survival prognosis of colorectal cancer patients is poor, the 5-year survival rate of early-stage patients is about 77.4–93.8%, and the

Table 1 Correlation of Survivin Expression and Degree of Tumor Budding with Patient Characteristics in 90 Colorectal Cancer Tissues

Clinicopathological Parameters	Number of Samples (n)	Survivin Score		*P* value	Tumor Budding Number Rating		*P* value
		Low (0~9) (n=39)	High (≥10) (n= 51)		Low (0~9)	High (≥10)	
Sex							
Male	42	17	25	0.609	26	16	0.055
Female	48	22	26		20	28	
Age (years)							
≥50	57	21	36	0.102	24	33	0.557
<50	33	18	15		16	17	
Lymph node metastasis				0.000			0.004
Yes	35	7	28		9	26	
No	55	32	23		31	24	
Distant metastasis				0.005			
Yes	38	10	28		13	25	0.010
No	52	29	23		32	20	
TNM stage							
I + II	53	28	25	0.030	36	17	0.020
III + IV	37	11	26		16	21	

5-year survival rate of late-stage patients is relatively low, about 7–14%.[7] Therefore, the discovery of key clinical and molecular pathological features that affect the survival and prognosis of patients is clinically important for the development of new clinical intervention programs.

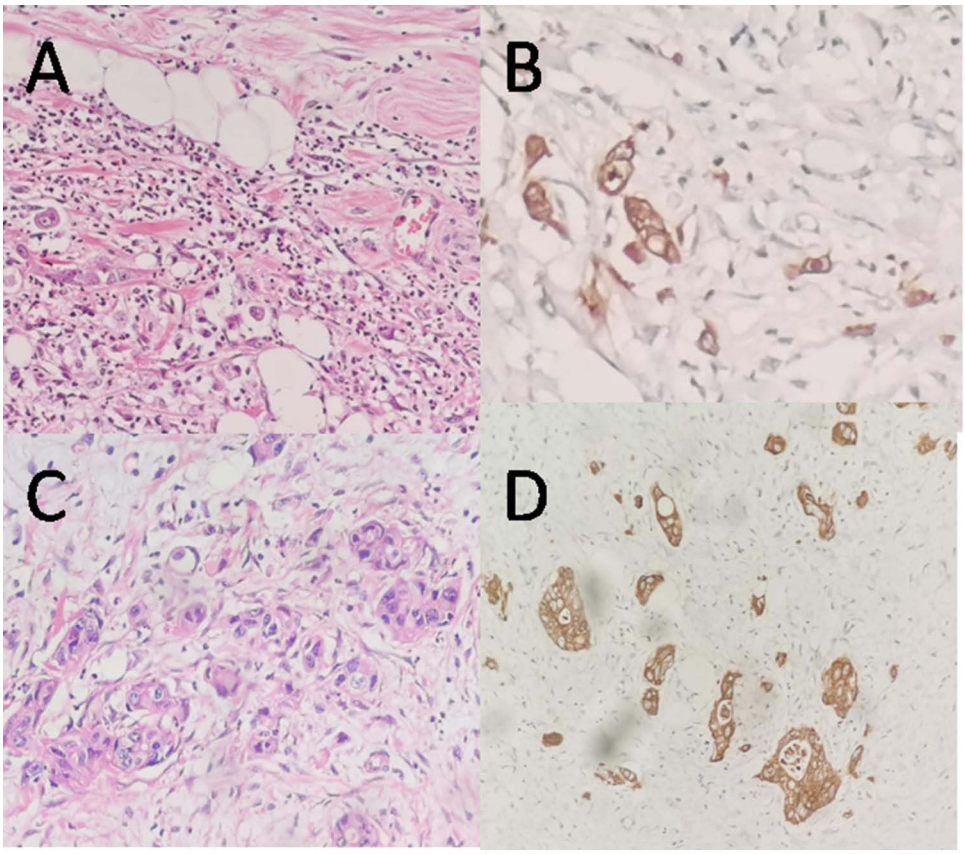

Figure 1 HE and immunohistochemical images of tumor outgrowth. (**A**) Low-grade tumor budding. (**B**) Low-grade tumor outgrowth CKpan immunohistochemistry. (**C**) High-grade tumor budding. (**D**) High-grade tumor budding CKpan immunohistochemistry.

Table 2 Correlation of Survivin with Tumor Budding (P=0.033, P < 0.05)

Grading	Tumor Budding Number Rating		Value
	Low (0~9)	High (≥10)	
Survivin gradin glow (1–2 score)	12	15	χ^2=4.543
High (3 score)	14	49	*P*= 0.033

Tumor budding is considered an independent risk factor for tumor aggressiveness and prognosis, and can occur in various parts of the body, such as the rectum, colon, stomach, esophagus, larynx, tongue, nasopharynx, gallbladder, pancreas, lungs, breast glands, and endometrium.[8] Tumor budding is scattered, undifferentiated single tumor cells or small foci of four cells at the leading edge of the tumor infiltrate.[5] Tumor outgrowth is not a static histological feature; it represents a dynamic process of progression of an aggressive tumor with infiltrative and metastatic potential, implying not only a simple detachment of tumor cells, but also an important step in the progression of malignant tumors from focal to systemic disease. There have been a number of reports on the relationship between tumor budding and clinicopathological indicators of colorectal cancer. Okuyama et al[9] studied 83 cases of highly and moderately differentiated adenocarcinomas of the rectum in PT3 and found that the incidence of tumor budding was significantly higher in rectal cancers accompanied by lymph node metastasis and lymphovascular invasion than in those without lymph node metastasis, and that local recurrence and hepatic metastases were also more frequent than in those with tumor budding. Ueno et al[10] studied tumor budding in 638 cases of rectal cancer, and the results showed that tumor budding was closely associated with pathological features reflecting the aggressive behavior of the tumor, such as tumor differentiation, extra-plasma membrane spread, lymph node involvement, lymphocyte infiltration, and extra-plasma membrane vein invasion. Our study of 90 cases of colorectal cancer found that the number of tumor budding was significantly increased in pathological specimens with lymph node metastasis, distant metastasis, and TNM stage III + IV, which is consistent with what has been reported in the literature, suggesting that tumor budding is able to reflect the invasive behavior of tumors, and that the greater the number of tumor budding, the more invasive the invasive behavior.

Survivin is overexpressed in most tumors and promotes tumor proliferation and metastasis. Survivin acts as a nodal protein and interacts with a variety of signals involved in mitosis, apoptosis, functionally integrated proliferation, cell death, and stability of the intracellular environment.[11] It has been found that Survivin is associated with the metastasis of malignant tumors such as gastric, breast, colon and cervical cancers and cervical cancer.[12,13] A study by Dawson et al[14] showed that, in addition to Ki-67 and caspase-3 positivity in tumor outgrowth cells being associated with an increased risk of death in patients, changes in various proteins associated with apoptosis were found in tumor outgrowth cells. It has now been found[15] that epithelial cells isolated from the surrounding extracellular matrix undergo a physiological form of programmed cell death known as loss-of-nest apoptosis. Therefore, budding, which are individual cells, must develop mechanisms to resist loss-of-nest apoptosis if they are to survive the process of migrating to the vasculature and generating metastasis. Survivin, an apoptosis inhibitory protein, inhibits apoptosis, and it is possible to resist loss-of-nest apoptosis mechanisms, and survivin proteins also promote cellular proliferation, which then has the potential to promote tumor outgrowth generation. Our study of 90 colorectal cancer patients found that Survivin expression levels increased in tissues with high ratings for the number of tumor outgrowths, suggesting that Survivin may play a promotional role in tumor outgrowth. It was also found that Survivin has the potential to promote local metastasis, distant metastasis and invasion of colorectal cancer through tumor budding.

Conclusions

We conducted a preliminary analysis of the clinical characteristics of colorectal cancer patients as well as on the Survivin expression and tumor outgrowth degree of tumor tissues. It was found that Survivin expression level was significantly correlated with colorectal cancer germination and clinical characteristics and TNM stage, and Survivin expression level was significantly

correlated with the degree of tumor budding. This suggests that Survivin may regulate colorectal cancer cell budding, thus promoting colorectal malignant progression and poor prognosis. In the future, the molecular mechanism of Survivin in regulating tumor outgrowth should be further investigated and analyzed, so as to provide a target site for treatment. The experiment also has some limitations, such as a small sample size, and only a superficial study, not an in-depth study, is currently being conducted.

Ethical Approval

The studies involving human participants were reviewed and approved by the Institutional Ethical Committee of the Ya'an People's Hospital (ethical review number: YARY: NO. 2022-003). The patients/participants provided their written informed consent to participate in this study. The present study fulfils the requirements of the Declaration of Helsinki.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare no conflicts of interest.

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