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Tumor Budding Density Associated with Survivin Expression in Colorectal Carcinoma

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Abstract: ***Background**: Colorectal carcinoma (CRC) is the third most common malignancy. Tumor Budding was a single or small (up to 5 cells) undifferentiated cells at the edge of tumor invasion. Cells in tumor budding show low mitotic index and apoptosis. Survivin is an apoptotic inhibitor protein. This study aims to prove the association between tumor budding density and survivin expression. **Method**: This study was an analytical cross-sectional study conducted on 43 cases of CRC. Evaluation of tumor budding density was calculated on the H-E staining using the 10 field of view, then categorized as high density when found> 100 tumor budding/10 high power field and low density when found ≤ 100 tumor budding/10 high power field. Survivin expression was assessed by immunohistochemistry (IHC) and was categorized as high if it contained> 25% of tumor cells and was low if ≤25% of tumor cells. The results of the study were analyzed by x2 test to determine differences in survivin expression in high and low density tumor budding, and the mean difference survivin expression in both groups of tumor budding with the Spearman rho test. The degree of significance is determined at p <0.05. **Results**: From 43 samples, 21 cases of high density tumor budding and 22 cases of low density tumor budding were obtained. High density tumor budding with high survivin expression were found in 18 cases (85.7%) while high density tumor budding with low survivin expression was found in 3 cases (14.3%). Low density tumor budding with high survivin expression of 6 cases (27.3%). Low density tumor budding with low survivin expression 16 cases (72.7%). There was a significant difference in survivin expression in the high and low density tumor budding group (p = 0.001), with an odd ratio of 16.00 (95% confidence interval 3.42-74.69). The Spearmans rho test showed that the mean percentage of Survivin expression in the high density CRC case group differed significantly from the mean survivin percentage in the low density CRC case group (p <0.001). **Conclusion**: Budding tumor density is related to survivin expression. CRC with high density tumor budding has 16 times higher risk showing high survivin expression compared to CRC with low density tumor budding.*

Keywords: colorectal carcinoma, tumor budding, Survivin

1. Introduction

Colorectal carcinoma is a malignancy in the colon or rectum originating from epithelial. The incidence and mortality of the CRC is quite common in developing countries. Prognosis can be predicted by risk factors, both tumor biology factors and clinicopathology.[1]

Tumor budding is the presence of single cells or small groups (up to 5) cells, which do not show glandular differentiation and assessment is carried out at the edge of tumor invasion. Tumor budding considered to be related to the process of cancer invasion and metastasis and hypothesized is a histological representation of EMT (epithelial-mesenchymal transition) which is an important change in tumor progression and spreading[2,3]. The study by Dawson et al (2013) at the CRC showed that cells in tumor budding had lower proliferation and apoptosis index than other tumor cells[4].

Survivin is an apoptotic inhibitor that plays a dual role. The first is by inhibiting apoptosis via the intrinsic pathway (mitochondrial signal) initiated by activation of Bcl-2-Associated X protein / B-Cell Lymphoma 2 (Bax / Bcl-2) which ultimately interferes with caspase 9 and extrinsic pathways through bonding with death receptors in cell surface which then inhibits caspase 8. Both of these pathways play a role in the regulation of bipolar spindle formation[5]. The uniqueness of survivin properties that are not expressed in normal tissue compared to cancer cells makes it suitable to be applied as a diagnostic factor, prognostic, and anticancer therapy[6]. Given its direct role in carcinogenesis, survivin also has a role in angiogenesis, metastasis, and chemoresistance[7].

This study is to prove the relationship between tumor budding density and survivin expression in the CRC.

2. Method

This study used a cross sectional design. The research sample was paraffin block from the material resection of non-specific colorectal adenocarcinoma which was carried out by histopathology at the Anatomical Pathology Laboratory of the Medical Faculty of Udayana University / Sanglah General Hospital Denpasar from January 30 2017 to June 30, 2018.

This tumor budding density was assessed in H-E staining. High density tumor budding when> 100 tumor budding/ 10 high power field were found using a wide scale 0.65 mm2 and low density tumor budding if ≤ 100 tumor budding was found/ 10 high power field using a wide scale of 0.65 mm[8].

Survivin expression was assessed by IHC examination. Calculations were carried out in the tumor hot spot area determined at 100x magnification. Calculation of the percentage of cells expressing survivin was carried out with 400x magnification, without taking into account the survivors' outward intensity. Tumor budding carcinoma cells are expressing survivin if malignant epithelial cells stained brown colour in the nucleus and / or cytoplasm. Survivin expression is stated to be high if the percentage is> 25% in the cytoplasm, conversely if the percentage of ≤ 25% is stated as low[9].

Data were analyzed using the SPSS Program (Statistical Package for Social Sciences) 20.0 for Windows. The x2

test was conducted to determine differences in survivin expression in high and low density tumor budding, and the mean difference in survivin expression in both groups of tumor budding with the Spearmans rho test. The degree of significance is determined at p <0.05.

3. Results

Of the 43 study samples, 23 were men and 20 were women, with an average age of 60.97 (age range 42-87 years). Case characteristics based on location, degree of tumor differentiation and depth of invasion are presented in Table.1.

Table 1: Characteristics of research subjects

Characteristic	Total (n=43)	Percentage (%)
Gender		
Men	23	53,4
women	20	46,5
Location		
Ascending colon	10	23,26
Descendent colon	20	46,51
Rectum	13	30,23
DerajatDifferensiasi		
Well differentiated	3	7
Moderate differentiated	37	86,0
Poorly differentiated	3	7
Invasion depth		
pT1	0	0
pT2	6	13,9
pT3	33	76,7
pT4	4	9,3

The relationship between tumor budding density and survivin expression was carried out by the x2 test presented in table 2.

Table 2: The x^2 test of the relationship between tumor budding density and survivin expression in colorectal carcinoma

Budding tumor	Survivin expresion		Total	P
	High	Low		
High density	18 (85,7%)	3 (14,3%)	21 (100%)	0,001
Low density	6 (27,3%)	16 (72,7%)	22 (100%)	
Total	24(55,8%)	19 (44,2%)	43 (100%)	

Statistical analysis with x2 showed tumor budding density in CRC correlated statistically with survivin expression (p = 0.001), with Odds Ratio 16.00 (95% confidence interval 3.42-74,69), mean percentage of survivin expression on the CRC with Low density tumor budding is 23.40 (SD 11.68). While the mean percentage of survivin expression in CRC with high density tumor budding was 44.52 (SD 20.42). (Table 3) The Spearmans rho test showed that the mean difference in the percentage of survivin expressions in the high density CRC case group was significantly different from the mean survivin percentage in the low density CRC case group (p <0.001).

Table 3: The mean percentage of survivin expression based on tumor budding density in colorectal carcinoma

Tumor Budding	Number of sample	Average percentage of survivin expressions	Standard Deviation
Low	22	23,40	11.68
High	21	44.52	20.42

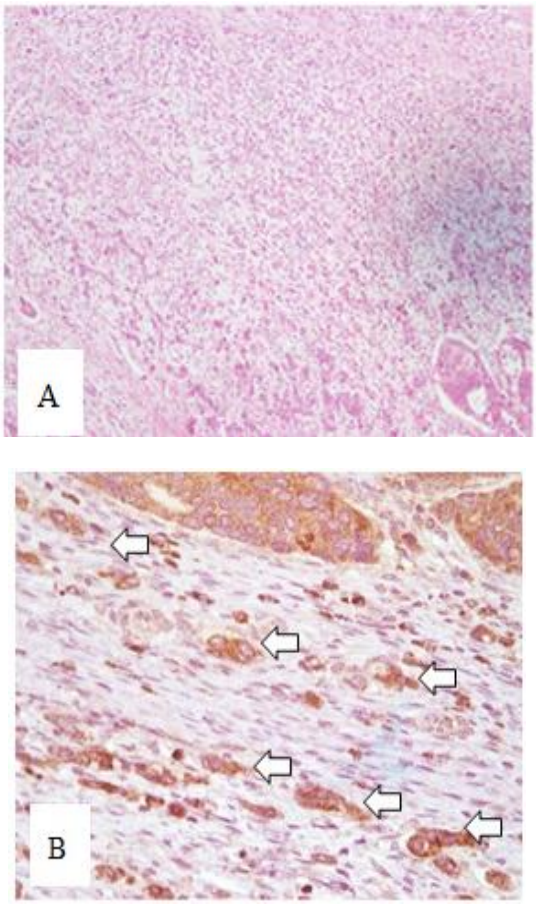

Figure 1: Colorectal carcinoma with high density tumor budding with high survivin expression A. Low magnification (x100). B. High magnification (x400)

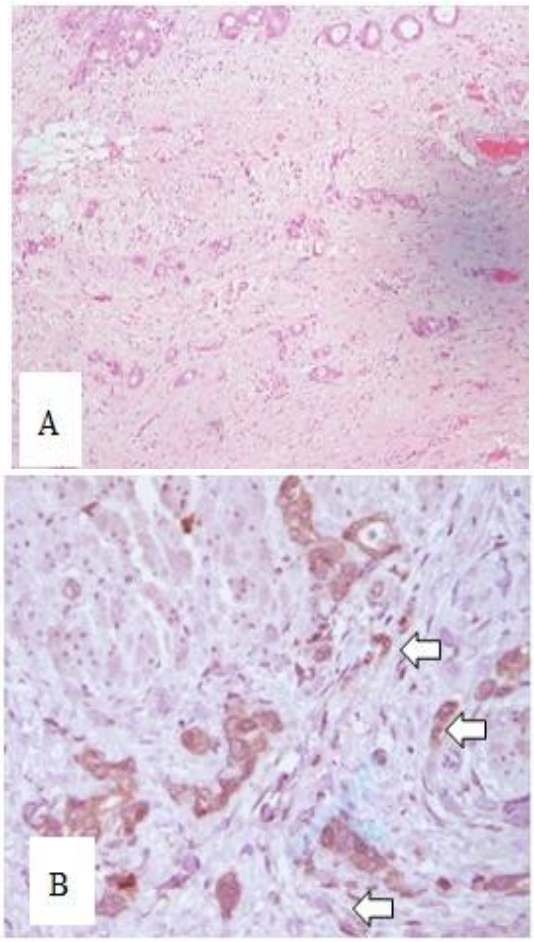

Figure 2: Colorectal carcinoma with low density tumor budding with low survivin expression. A. Low magnification (x100). B. High magnification (x400)

4. Discussion

Tumor budding is the presence of single cells or small groups (up to 5) cells, which are undifferentiated on the invasive edge of the CRC. Tumor budding considered to be related to the process of cancer invasion and metastasis and hypothesized to be a histological representation of EMT[2,3]. In addition to the CRC, tumor budding has also been studied in pancreatic, breast, neck, and lung carcinoma. At present tumor budding is known to be an additional prognosis factor in 10 CRC patients.

Budding tumors can be calculated on H-E staining, but in certain conditions it is difficult to assess because of the presence of solid inflammatory cells, reactive fibroblast stroma, or ruptured glands. In this study cases of type non-specific colorectal adenocarcinoma with solid peritumoral inflammation were excluded. Staining for tumor budding examination varies; the method of calculating tumor budding also varies. The methods used include the method of Hase, Nakamura, Ueno, one high power field method and the average method of 10 high power fields .[10,11]

High density budding tumors are significantly associated with poor prognosis and low survival rates in patients with resection of stage II CRC. Therefore tumor budding is an important additional prognosis factor that needs to be reported at the stage II CRC which allows identification of patients for adjuvant therapy[10]. Tumor budding was a marker of tumor progression associated with lymphovascular invasion, lymph node metastasis, and distant metastasis.[13]

Survivin is not expressed or expressed very weakly in normal cells. Several studies have shown survivin's strong expression in benign and preneoplastic lesions such as melanocytic naevus, colon polyps, adenoma mammae, Bowen's disease and actinic keratosis[13]. Survivin overexpression is associated with vascular invasion and KGB metastasis supported by the results of studies from Mehrotra et al[14] who found survivin has the effect of stimulating tumor cell invasion and the formation of metastases in the complex with XIAP.

Some theories reveal the possible causes of high survivin expression on cancer cells including 1) survivin expression in all cell cycles, 2) survivin's role in cell division, 3) inhibiting cell death, 4) its role in angiogenesis, 5) survivin amplification on chromosome 17q25, 6) demetilation of exon survivin, and 7) increasing signal PI3K or MAPK15 signals. The metaanalysis study in 15 KKR studies found that survivin expression was associated with a poor prognosis associated with overall survival in KKR[14]. The Lee et al (2009) study also stated that higher expression of survivin and cortactin correlated significantly with tumor stage and shorter survival[16].

The study by Dawson et al (2013) in the CRC showed that cells in tumor budding had lower proliferation and apoptotic index than other tumor cells. The low index of apoptosis in cells in tumor budding seems to occur due to survivin inhibition of apoptosis.

5. Conclusion

Based on the results of this study it can be concluded that high density tumor budding is significantly associated with high survivin expression in the CRC. Colorectal carcinoma with high density tumor budding has the risk of showing high survivors 16 times higher than low density CRC. The average percentage of survivin expression in the high density CRC case group was significantly different from the survivin mean rate in the low density CRC case group.

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