

The Role of Tumour Budding at the Front of Invasion and Recurrence of Rectal Carcinoma

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Abstract. *The presence of "tumour budding", i.e. single cancer cells, or a nest of poorly- differentiated cells at the front of invasion, in bowel cancer appears to be a new histopathological indicator of increased aggressiveness of colorectal carcinoma. The aim of this work was a retrospective evaluation of the front of invasion in preoperative biopsies of patients with rectal carcinoma (T1) and an analysis of the relationship between tumour budding and metastases or recurrence of the tumour at the site of resection. Patients and Methods: The study was performed based on material obtained before and during surgical treatment of 34 patients with cancer of the colon. Tissue was obtained directly following tumour resection, fixed in 10% formaldehyde and embedded in paraffin blocks using a routine method by melting with paraffin at a temperature of 56 °C. These samples were then routinely stained with haematoxylin and eosin and underwent a histopathological evaluation, with particular attention being paid to the front of invasion of the tumour. Results: Tumour budding (TB) at the front of invasion was found in 12 out of 34 patients. Statistical analysis showed a correlation between TB and the histological type of tumour, the presence of lymph node metastases, distant metastases and recurrence of the tumour in post-operative scars. Conclusion: The results suggest the usefulness of evaluating TB at the front of invasion as an additional prognostic indicator in rectal carcinoma.*

Colorectal cancer is one of the most frequent malignant tumours. The increasing incidence of this disease has stimulated research into colorectal carcinoma (1-6). The different classifications used regarding cancer of the colon, include Dukes' classification 1958 (7), Astler and Coller 1954 (8), TNM (9), AJCC (10) and Jass (11). Efforts have been made to find parameters which indicate the risk of metastases and recurrence at the site of the post- operative

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scar. There has been an increased amount of research into this subject ever since the first studies concerning tumour budding (i.e. the presence of poorly-differentiated tumour nests of up to 5 cells at the front of invasion) in bowel cancer and the association of metastases and life expectancy were published. Tumour budding would appear to be not only a parameter of the invasiveness of tumours, but, according to the study by Ueno *et al.* (6), may be an additional early indicator of metastatic potential.

The study aimed to evaluate the association between tumour budding and blood vessel and lymph vessel invasion (vascular invasion) at the front of invasion in pre-operative biopsy specimens of rectal cancers and metastases and loco-regional recurrence.

Patients and Methods

Collection of samples. The samples were obtained from 34 patients with T1 rectal carcinoma, curatively resected at the Department of Surgery, Sniadecki Hospital of Białystok, Poland, between 1999 and 2003. Patients diagnosed with familial polyposis, following other malignant disease, or with inflammatory changes were not included into the study.

Sections of formalin - fixed and paraffin - embedded specimens were stained with hematoxylin-eosin before examination. Particular attention was paid to the front of invasion in pre-operative samples, where the presence or absence of tumour budding (TB) was assessed. The criteria used for determining were those described by Morodomi *et al.* (12). Two groups were used for classification of the tumour budding phenomenon. The first, where no bud was observed, TB(-) and the second, where at least one bud was found, TB(+) at the front of invasion in the examined slice. The front of invasion determined as a budding field was measured in a visual 500 µm x 2500 µm square at 4 locations in each slide. Figure 1 illustrates the presence of tumour budding at the front of invasion.

Clinical information. The group of patients were clinically monitored for a 36 - month period. The patients underwent follow-up examinations every 6 months, including abdominal ultrasound examination, CT and colonoscopy. Local recurrence around the post - operative scar was confirmed histopathologically. Lymph node metastasis was confirmed in post -operative material, and distal (hepatic) metastases were confirmed by ultrasound - guided fine - needle aspiration cytology.

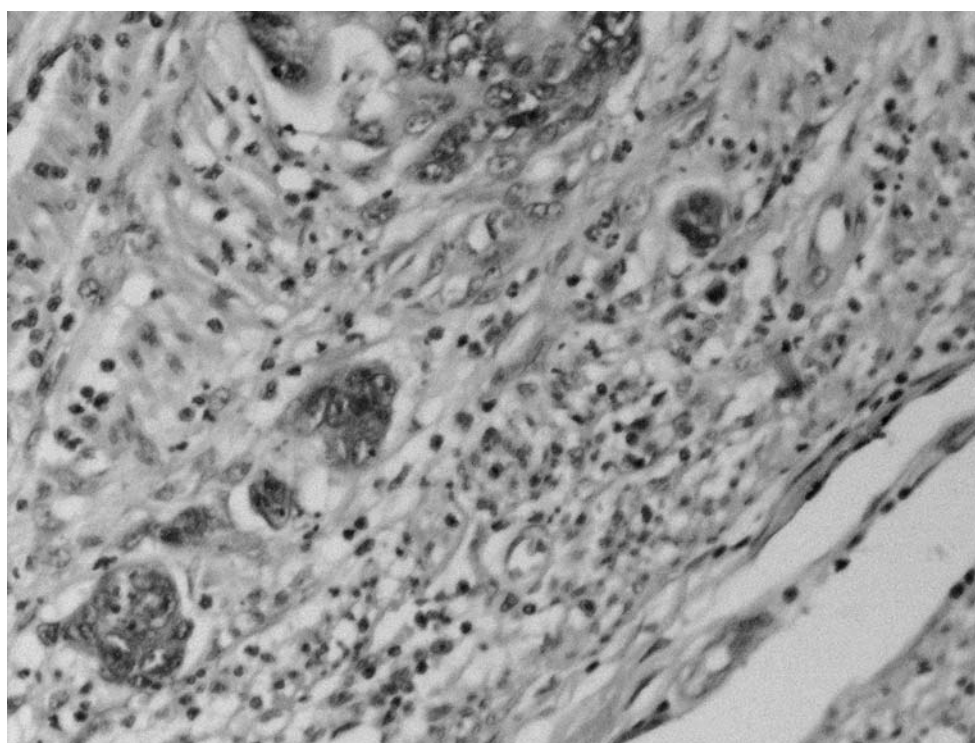


Figure 1. Representative photographs of tumour budding at the invasive front. Original magnification x 200.

Table I. Correlation between tumour budding and histological type.

Parameter	n	Tumour budding		All
		Absent	Present	
G1	20	18(90.0%)	2(10.0%)	58.8%
G2	12	3(25.0%)	9(75.0%)	35.3%
G3	2	1(50.0%)	1 (50.0%)	5.9%

$p=0.0008$

Table II. Correlation between tumour budding and vascular invasion.

Parameter	n	Tumour budding		All
		Absent	Present	
With vascular invasion	14	3(21.4%)	11(78.6%)	41.2%
Without vascular invasion	20	19(95.0%)	1(5.0%)	58.8%

$p=0.00001$

Statistical analysis. The associations between tumour budding and clinico-pathological parameters were examined using the χ^2 test. Fisher's exact test was used for statistical analysis. The values for $p<0.05$ were considered significant.

Results

Tables I-V show the correlation between tumour budding and clinico-pathological parameters. Tumour budding was found in 12 out of 34 tumours. The presence of tumour budding at the front of invasion of rectal cancer strongly correlated with the histological type, where in 9 out of 12 (75%) cases of G2, tumour budding was observed ($p<0.01$). Tumour budding was also found in cases with the presence of vascular invasion, 11/12 (91.7%), $p<0.01$, lymph node metastasis 11/12 (91.7%), $p<0.01$ and distant metastases 12/12(100%), $p<0.01$. Furthermore, in all cases with local recurrences, tumour budding was observed at the front of invasion 3/3(100%), $p<0.05$.

Discussion

A recent widely studied parameter, possibly indicating an increased aggressiveness of a given tumour, along with a decreased 5-year survival in patients with colorectal carcinoma is tumour budding at the front of invasion. In the studies which have been published, tumour budding at the front of invasion has been associated with a strong tendency for metastases in colorectal carcinoma. Morodomi *et al.* (12) were the first to present results based on the analysis of

Table III. Correlation between tumour budding and lymph node metastasis.

Parameter	n	Tumour budding		All
		Absent	Present	
With lymph node metastasis	16	5(31.3%)	11(68.7%)	47.1%
Without lymph node metastasis	18	17(94.4%)	1(5.6%)	52.9%

$p=0.0002$

Table IV. Correlation between tumour budding and distant metastasis.

Parameter	n	Tumour budding		All
		Absent	Present	
With distant metastasis	14	2(14.3%)	12(85.7%)	41.2%
Without distant metastasis	20	20(100%)	0(0%)	58.8%

$p=0.000001$

Table V. Correlation between tumour budding and local recurrences.

Parameter	n	Tumour budding		All
		Absent	Present	
With local recurrences	3	0(0%)	3(100%)	8.8%
Without local recurrences	31	22(70.9%)	9(29.1%)	91.2%

$p=0.036$

pre-operative biopsies, where the intensity of tumour budding at the front of invasion was assessed. The results of studies suggest that the intensity of tumour budding strongly correlates with the presence of lymph node metastases. Furthermore, other studies have shown moderately-differentiated tumours to have a higher intensity of budding than others (6). Our study similarly showed moderately-differentiated tumours to have the greatest budding intensity. Some studies, such as that of Okuyama *et al.* (13), have examined the relationship between the presence of tumour budding and vascular invasion in pT1 and pT2 colorectal cancers. This author described a group of 101 patients with well - differentiated colorectal cancers and concluded that the evaluation of the front of invasion (vascular invasion and tumour budding) in histopathological samples should be a routine part of the pathomorphological diagnosis, since these two parameters are indicators of lymph node metastasis. The findings of the present study imply a strong association between the presence of tumour budding in pre-operative material and the appearance of proximal and distal metastases. Moreover, vascular invasion

was strongly associated with the presence of tumour budding. Ueno *et al.* (6) examined 638 patients with rectal cancer and concluded that tumour budding would be a good index to estimate the aggressiveness of rectal cancer. Other authors have noted the usefulness of evaluating tumour budding in association with the presence of metastases and life expectancy (14). Tanaka *et al.* (15) described that the appearance of tumour budding in Stage II, T3 colon cancer indicated a high risk of tumour budding recurrence after curative surgery. In the present study, we found tumour budding to be present in all three cases where there was local recurrence. From the results of the studies above, it would appear that the presence of tumour budding in pre-operative samples provides significant information for the pathologist, gastroenterologist and surgeon. This is why we similarly propose that the routine histopathological evaluation of biopsies should provide information relating to the presence of tumour budding at the front of invasion. Although the group studied was relatively small, it needs to be stressed that the observations and follow-up evaluations were performed meticulously through a 36-month period, and that the statistical significance achieved allows the above conclusions to be drawn. A multi-centred study should be performed to confirm this criterion as being a prognostic factor.

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