Intra-tumoral budding in preoperative biopsy specimens predicts lymph node and distant metastasis in patients with colorectal cancer

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Tumor budding, a histological hallmark of epithelial–mesenchymal transition in colorectal cancer, is a parameter of tumor progression and according to the International Union Against Cancer/American Joint Committee on Cancer an ‘additional’ prognostic factor. The current definition of tumor budding is reserved for the invasive tumor front of colorectal cancer (so called peri-tumoral budding), but tumor buds can also be observed in small preoperative biopsy specimens. Whereas the prognostic value of peri-tumoral budding assessed in resection specimens has found wide acceptance, the value of budding in preoperative biopsies, which normally do not encompass the invasive tumor margin and hence can be called intra-tumoral budding, has not been systematically investigated yet. Therefore, the aim of this study is to assess the predictive value of intra-tumoral budding for lymph node and distant metastasis in preoperative biopsies. Preoperative biopsy samples and consecutive resection specimens from 72 patients with pathological information on TNM stage, vascular, lymphatic and perineural invasion, and tumor border configuration were used to evaluate intra-tumoral budding and peri-tumoral budding. Both parameters were scored semiquantitatively as ‘high’ (detectable at low power magnification $\times 2.5$) and ‘low’ (occasional budding at intermediate magnification $\times 10$, difficult to find or absent). In biopsy samples high intra-tumoral budding was observed in 12/72 patients (17%) and associated with high peri-tumoral budding in the corresponding resection specimens ($P=0.008$). Additionally, there was a correlation between high intra-tumoral budding and lymph node metastasis ($P=0.034$), distant metastasis ($P=0.007$) and higher tumor grade ($P=0.025$). Peri-tumoral budding was associated with higher N stage ($P=0.004$), vascular ($P=0.046$) and lymphatic invasion ($P=0.019$) as well as with an infiltrating tumor border ($P<0.001$), reflecting the predictive power of peri-tumoral budding for tumor progression. High intra-tumoral budding in preoperative biopsy samples of colorectal cancer patients predicts high peri-tumoral budding at the invasive margin and lymph node metastasis in the corresponding resection specimens as well as distant metastasis.

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Colorectal cancer accounts for one of the most common malignancies in industrialized nations. Prognostication of newly diagnosed colorectal cancer predominantly relies on stage or anatomic extent of the disease based on the International Union Against Cancer (UICC-TNM)¹ and the American

Joint Committee on Cancer (AJCC)² staging classifications.

Tumor infiltration depth, loco-regional lymph node involvement, venous and lymphatic invasion, tumor grade, tumor budding³ and tumor border configuration⁴ are histological features associated with worse patient outcome and can only be assessed in surgical tumor resection specimens.

Tumor budding, a histological hallmark of epithelial–mesenchymal transition, was comprehensively defined in 1987 by Jass *et al*⁴ and subsequently obtained wide acceptance among surgical pathologists. Peri-tumoral budding in colorectal cancer is associated with tumor progression and has been

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retained as an adverse feature of the primary tumor according to the World Health Organisation⁵ as well as an 'additional' prognostic factor according to the UICC/AJCC.⁶ Tumor budding is defined as isolated single cancer cells or clusters composed of fewer than five undifferentiated cancer cells at the invasive tumor margin (ie, peri-tumoral budding⁷).⁸ Tumor buds are believed to have the properties of tumor stem cells,⁹ including the potential for redifferentiation both locally and at the sites of metastasis.¹⁰ As the extent of tumor budding is an adverse feature of primary tumor, it is eligible to be reported in histopathology reports. Various elaborate systems for quantification of tumor budding in colorectal cancer have been described.^{3,8,11–19} All of these tumor budding grading systems share an association of high peri-tumoral budding with a worse outcome.

In daily practice, colorectal cancer is most often diagnosed on colonoscopy biopsy specimen obtained from superficial tumor areas by forceps biopsy technique, hence usually not encompassing the invasive tumor margin. These biopsies typically suffice to confirm the clinical suspicion of malignancy. It would be helpful to have a preoperative/pre-treatment biomarker to predict the stage of disease and particularly the lymph node status from the biopsy. Only sparse data exist on the role of tumor budding in biopsy specimens.^{11,20} Morodomi *et al*¹¹ described tumor buds in preoperative biopsy specimens in rectal cancer and hypothesized that tumor buds might have a predictive value for lymph node metastasis. Nevertheless, the value of tumor budding in preoperative biopsy specimens (intra-tumoral budding), located within the main tumor body away from the invasive border, has so far not been investigated systematically.

We have previously described that tumor buds are not only observed at the invasive border of a colorectal cancer, but that they may also be encountered within the tumor bulk. We thus implemented the terms of peri-tumoral budding for traditional tumor budding at the invasive tumor front and of intra-tumoral budding defined as budding located within the main tumor body, away from the invasive border. We further found that intra-tumoral budding is associated, in both DNA mismatch repair-proficient and -deficient cancers, with histological hallmarks of aggressive tumor behavior and worse tumor outcome.⁷

The aim of the present study was therefore to investigate the predictive value of intra-tumoral budding for lymph node and distant metastasis based on preoperative biopsy samples.

Patients and methods

Patient and Specimen Characteristics

One hundred and thirty-five patients with colorectal cancer treated at the Cantonal Hospital of Aarau, Switzerland between 1990 and 2008 were entered in

the study. Patients with rectal tumors as well as patients who received neo-adjuvant therapy were excluded from the cohort. According to the clinical data files none of the patients was known to have a hereditary colorectal cancer syndrome (familial adenomatous polyposis or hereditary non-polyposis colorectal cancer). Permission to perform the study was granted by the Swiss federal health office; individual patient consent from surviving patients was obtained. For all 135 patients, histological slides of colorectal cancer resections were retrieved from the archives of the Department of Pathology, Cantonal Hospital Aarau, Switzerland. In addition, for 72 patients, the histological slides of preoperative biopsy specimens were also available. Histological work-up included TNM stage, tumor grade, presence of vascular, lymphoid, and perineural invasion. Tumor border configuration was assessed according to Jass *et al*.²¹ Patient and specimen characteristics are summarized in Table 1.

Assay Methods

Tissue samples were fixed in buffered formalin and paraffin embedded according to the standard protocols. Approximately 4- μ m tissue slices were mounted on glass slides and were stained with H&E according to the standard protocols. All colorectal cancer-containing slides were reviewed for peri-tumoral budding quantification (OG). All biopsies had three levels separated by 20–50 μ m. For resection specimen, all tumor-containing slides (average of five slides per tumor, range 2–14 slides per tumor; median 4 tissue slides) were reviewed.

Peri-tumoral budding was scored in analogy to the method proposed by Nakamura *et al*.²² Tumor budding at the invasive tumor front in the resection specimen, namely peri-tumoral budding, was designated as 'high peri-tumoral budding' when at least one focus of high tumor budding was detected at the entire invasive front at low magnification ($\times 2.5$ objective, 10 $\times$ 20 mm field of view eye piece, Zeiss Axioscope, Germany) and 'low' budding, ie, cases where tumor budding was not detected at low power magnification, with occasional budding at intermediate magnification $\times 10$, difficult to find or absent. For intra-tumoral budding in the biopsy specimens, the same criteria were applied as for peri-tumoral budding, ie, being obvious at low power and being confirmed at intermediate power magnification. Tumor budding is illustrated in Figures 1 and 2. Thirty-nine resection specimens and 14 biopsy cases were evaluated by a second pathologist (SC) to assess the inter-observer agreement of the scoring method blinded to other data.

Statistical Analysis

The associations between intra-tumoral budding or peri-tumoral budding and clinicopathological features were evaluated using the χ^2 or Fisher's

Table 1 Patients and specimen characteristics with available surgical resection (A; *n* = 135) and with matched preoperative biopsy and surgical resection (B; *n* = 72)

Feature	A (<i>n</i> = 135) Frequency, N (%)	B (<i>n</i> = 72) Frequency, N (%)
Age (median; range)	68 (30–92)	67 (30–87)
Number of lymph nodes Median, range	18 (5–52)	18 (5–50)
<i>pT</i>		
<i>pT</i> 1–2	8 (5.9)	4 (5.6)
<i>pT</i> 3–4	127 (94.1)	68 (94.4)
<i>pN</i>		
<i>pN</i> 0	57 (42.2)	31 (43.1)
<i>pN</i> 1–2	76 (56.3)	41 (56.9)
<i>pN</i> x	2 (1.5)	0
<i>pM</i>		
<i>pM</i> 0	67 (49.6)	35 (48.6)
<i>pM</i> 1	43 (31.9)	27 (37.5)
<i>pM</i> x	25 (18.5)	10 (13.9)
Tumor grade		
G1–2	92 (68.1)	52 (72.2)
G3	36 (26.7)	17 (23.6)
NA	7 (5.2)	3 (4.2)
Vascular invasion		
Absent	53 (39.3)	30 (41.7)
Present	82 (60.7)	42 (58.3)
Lymphatic invasion		
Absent	38 (28.2)	24 (33.3)
Present	97 (71.9)	48 (66.7)
Perineural invasion		
Absent	108 (80.0)	59 (81.9)
Present	27 (20.0)	13 (18.1)
Tumor border configuration		
Pushing	32 (23.7)	16 (22.2)
Infiltrating	52 (38.5)	29 (40.3)
Mixed	51 (37.8)	27 (37.5)
Intra-tumoral budding		
Low/absent	NA	60 (83.3)
High	NA	12 (16.7)
Peri-tumoral budding		
Low/absent	67 (49.6)	31 (43.1)
High	68 (51.4)	41 (56.9)

exact test where appropriate. Sensitivity, specificity, negative and positive predictive values were calculated. Adjustment for multiple testing was not performed.²³ The inter-observer reproducibility of the scoring method was assessed using the kappa statistic. *P*-values <0.05 were considered statistically significant. All statistical analyses were carried out using SAS V9.1 (The SAS Institute, Cary, NC, USA).

Results

Frequency and Inter-Observer Agreement of Intra-Tumoral Budding and Peri-Tumoral Budding

In biopsy specimens, high intra-tumoral budding was seen in 17% (12/72) whereas no or sparse tumor

budding, detectable only at high power magnification, was seen in 83% (60/72). Within the resection specimen of patients with an available preoperative biopsy, high peri-tumoral budding was seen in 57% (41/72) whereas low peri-tumoral budding was seen in 43% (31/72). A strong inter-observer concordance was detected for peri-tumoral budding scoring in resection specimen (*k* = 0.68, percent-concordance 85%) and intra-tumoral budding scoring in biopsy samples (*k* = 0.65, percent concordance 86%).

Association of Peri-Tumoral Budding, Intra-Tumoral Budding and the Histomorphological Parameters

High peri-tumoral budding in resection specimen was associated with an infiltrating tumor border configuration (*P* < 0.001), vascular (*P* = 0.046) and lymphatic invasion (*P* = 0.019), and lymph node metastasis (*P* = 0.004). There was no association with *pT* classification (*P* = 0.33) distant metastasis (*P* = 0.49) and with tumor grade (*P* = 0.78) (Table 2).

High intra-tumoral budding in biopsies was associated with lymph node (*P* = 0.034) and distant metastasis at the time of diagnosis (*P* = 0.007) and with a higher tumor grade (*P* = 0.025). There was no association with *pT* stage (*P* = 0.619), vascular (*P* = 1.0) and lymphatic invasion (*P* = 1.0), perineural invasion (*P* = 0.114) and tumor border configuration (*P* = 0.114; Table 3).

Association of Intra-Tumoral Budding (in Biopsies) for Lymph Node and Distant Metastasis

The specificity of intra-tumoral budding in biopsies for lymph node involvement and for distant metastasis was 94%, with positive predictive value for loco-regional lymph node involvement of 83% and 82%, respectively. This contrasts with lower sensitivities (24% for lymph node involvement and 33% for distant metastasis) as well as lower negative predictive value (48% for lymph node involvement and 65% for distant metastasis).

Concordance of Intra-Tumoral Budding in the Biopsy Samples and Peri-Tumoral Budding in the Corresponding Resection Specimens

The association of high intra-tumoral budding in biopsies with high peri-tumoral budding in the corresponding resection specimens was statistically highly significant (*P* = 0.008), showing a sensitivity of 27% (11/41), a specificity of 97% (30/31), a positive predictive value of 91% (11/12) and a negative predictive value of 50% (30/60; Table 4).

Figure 1 Peri-tumoral budding quantification on resection specimen with H&E staining in the top row and the extracted epithelial component from immunohistochemical staining for cytokeratin for the purpose of illustration in the bottom row. Invasive margin in analogy to Jass *et al*.⁴ (a) expanding border: tumor budding low; (b) expanding border: tumor budding high; (c) infiltrating border: tumor budding high.

Figure 2 Biopsy specimen with high intra-tumoral budding in H&E staining (top); extracted epithelial component from immunohistochemical staining for cytokeratin for the purpose of illustration (bottom).

Discussion

In this study the value of intra-tumoral budding was systematically assessed in preoperative biopsy samples of non-rectal colon cancers. On the basis of H&E-stained tissue slides from a total of 135 colorectal cancer resection specimens and 72 corresponding preoperative biopsy samples, we were able to assess intra-tumoral budding in biopsy specimens and correlate this feature with peri-tumoral budding in the resection specimens. High intra-tumoral budding in biopsies predicts lymph node and systemic metastasis. Additionally, the association of high intra-tumoral budding with high peri-tumoral budding was significant ($P=0.008$), showing a positive predictive value of 91%, confirming the strength of the assessment of intra-tumoral budding for predicting more aggressive tumor behavior in preoperative biopsy samples.

Peri-tumoral budding and intra-tumoral budding were scored in analogy to the method proposed by Nakamura *et al*.²² This method was selected for several reasons. First, in contrast to other methods such as those proposed by Ueno *et al*.^{8,24} or Wang *et al*,²⁵ the method proposed by Nakamura is not limited to the size of the tumor or choice of microscope. Second, though it is a subjective assessment of tumor budding, it still shows a significant reproducibility between different observers. Third, it avoids the requirement of counting individual tumor buds, an exercise that can be challenging particularly in small or fragmented biopsy samples.

Table 2 Association of conventional budding (PTB) in resection specimens with histomorphological features

Feature	PTB, n (%)		
	Absent/low grade	High grade	P-value
<i>pT</i> (n = 135)			
pT1	3 (4.5)	0	0.334
pT2	3 (4.5)	2 (2.9)	
pT3	44 (65.7)	47 (69.1)	
pT4	17 (25.4)	19 (27.9)	
<i>pN</i> (n = 133)			
pN0	36 (55.4)	21 (30.9)	0.004
pN1–2	29 (44.6)	47 (69.1)	
<i>pM</i> (n = 110)			
pM0	31 (64.6)	36 (58.1)	0.487
pM1	17 (35.4)	26 (41.9)	
<i>Tumor grade</i> (n = 128)			
G1–2	46 (73.0)	46 (70.8)	0.778
G3	17 (27.0)	19 (29.2)	
<i>Vascular invasion</i> (n = 135)			
Absent	32 (47.8)	21 (30.9)	0.046
Present	35 (52.2)	47 (69.1)	
<i>Lymphatic invasion</i> (n = 135)			
Absent	25 (37.3)	13 (19.1)	0.019
Present	42 (62.7)	55 (80.9)	
<i>Perineural invasion</i> (n = 135)			
Absent	58 (86.6)	50 (73.5)	0.058
Present	9 (13.4)	18 (26.5)	
<i>Tumor border configuration</i> (n = 135)			
Pushing	26 (38.8)	6 (8.8)	<0.001
Infiltrating	17 (25.4)	35 (51.5)	
Mixed	24 (35.8)	27 (39.7)	

Our findings are comparable to previous studies that used more complex and elaborate tumor budding quantification systems.^{8,11} The results of this study are also in agreement with previous work from our group using tissue microarrays, where we observed an association of intra-tumoral budding with advanced tumor stage and lymph node metastasis (even in the absence of peri-tumoral budding), vascular invasion, infiltrating tumor border configuration and shorter survival time.⁷

The identification of a histological hallmark for tumor aggressiveness in superficial tumor biopsy samples not encompassing the deep infiltration border of colorectal cancer raises a question regarding the future of tumor budding assessment in general. The survey of intra-tumoral budding may allow risk stratification for patients with worse prognosis already at the time point of the initial biopsy, and, if confirmed in prospective studies, intra-tumoral budding can be proposed as a histomorphological marker for daily practice that is applicable in preoperative biopsies and may assist the decision-making for a neo-adjuvant therapy approach.

Table 3 Association of ITB in biopsies with histomorphological features

Feature	ITB, n (%)		
	Absent/low grade	High grade	P-value
<i>pT</i> (n = 72)			
pT1&2	4 (6.7)	0	0.619
pT3	40 (66.7)	8 (66.7)	
pT4	16 (26.7)	4 (33.3)	
<i>pN</i> (n = 72)			
pN0	29 (48.3)	2 (16.7)	0.034
pN1–2	31 (51.7)	10 (83.3)	
<i>pM</i> (n = 62)			
pM0	33 (64.7)	2 (18.2)	0.007
pM1	18 (35.3)	9 (81.8)	
<i>Tumor grade</i> (n = 69)			
G1–2	46 (80.7)	6 (50.0)	0.025
G3	11 (19.3)	6 (50.0)	
<i>Vascular invasion</i> (n = 72)			
Absent	25 (41.7)	5 (41.7)	1.0
Present	35 (58.3)	7 (58.3)	
<i>Lymphatic invasion</i> (n = 72)			
Absent	20 (33.3)	4 (33.3)	1.0
Present	40 (66.7)	8 (66.7)	
<i>Perineural invasion</i> (n = 72)			
Absent	50 (83.3)	9 (75.0)	0.493
Present	10 (16.7)	3 (25.0)	
<i>Tumor border configuration</i> (n = 72)			
Pushing	15 (25.0)	1 (8.3)	0.114
Infiltrating	21 (35.0)	8 (66.7)	
Mixed	24 (40.0)	3 (25.0)	

Table 4 Accuracy of ITB for PTB

	Biopsy (ITB)		P-value
	Low/absent budding	High budding	
<i>Resection (PTB)</i>			
Low/absent budding	30 (50%)	1 (8.3%)	0.008
High budding	30 (50%)	11 (91.7%)	
Total	60 (100%)	12 (100%)	

Disclosure/conflict of interest

The authors declare no conflict of interest.

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