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Budding invasive margin and prognosis in colorectal cancer – no direct association with β-catenin expression

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ABSTRACT

Cancer cell budding at the invasive margin has been associated with poor prognosis in rectal cancer. β-Catenin is an adhesion protein involved in the nuclear Wnt/β-catenin pathway, and mesenchymal transition of colorectal cancer cells. Hence, we investigated the relationship between cancer cell budding at the invasive margin, β-catenin expression, and 5-year-survival in colorectal cancer. Four hundred and sixty six colorectal cancer specimens were analysed for budding margin, and 108 specimens from the same set for β-catenin by immunohistochemistry. A budding margin was present in 24.0% of the cases and predicted a poor 5-year-survival (15.4%, $P < 0.00001$). Nuclear β-catenin expression increased from the central area towards the invasive margin ($P < 0.001$), but did not predict budding. Budding margin is an independent factor associated with poor prognosis in colorectal cancer, and could be utilised in diagnostic pathology. Nuclear β-catenin was often found at the invasive margin, but is unlikely to be the sole cause of budding.

1. Introduction

Colorectal cancer is the second most common malignancy in the Western World and one of the leading causes of cancer deaths. After surgical resection, neoadjuvant radiotherapy or combined adjuvant chemo- and radiotherapy are utilised for patients with local lymph node metastases, especially in rectal cancer.[1] Patients without lymph node metastases usually do not receive adjuvant therapy although a considerable proportion of these patients eventually die of cancer. Hence, better prognostic methods are needed to determine which patients are likely to benefit from adjuvant therapy.

Invasion and metastasis are the hallmarks of malignant tumours. Cellular de-differentiation and dissemination leading to invasive growth is characterised by disordered cell–cell interactions and cell adhesion. In colorectal cancer, mesenchymal transition of cancer cells at the invasive margin enables them to detach from the main bulk of the tumour and migrate beyond the outer margin.[2] Disintegration of cell adhesion molecules, especially β-catenin, has been implicated in this process.

Inactivation of the adenomatous polyposis coli (*APC*) suppressor gene is the earliest frequent event in colorectal adenoma–carcinoma sequence.[3] *APC* mutations are associated with an accumulation of intracellular β-catenin, which under normal circumstances is part of the cell membrane-bound adherens complex. This accumulation leads to loss of control of normal β-catenin signalling,[4] causing activation of the Wnt signalling pathway and oncogenic β-catenin actions in the nucleus.[5–7]

The nature of the invasive edge in colorectal cancer has an influence on survival.[8] Tumour budding – initially termed sprouting by Imai[9] – is defined as the presence of isolated cells

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or small cell clusters scattered in the stroma at the invasive margin of the tumour.[10] It has been associated with poor prognosis in colorectal carcinoma.[10,11]

The aim of this study was to investigate the possible association of tumour budding and β-catenin expression, as shown by immunohistochemistry, and to determine its prognostic significance.

2. Materials and methods

A total of 466 patients who underwent radical surgical operation for colorectal cancer between the years 1986 and 1996 were included in this study. Complete follow-up data could be obtained in 386 patients who were included in the assessment of survival statistics. The patients were followed up for 60 months or until their death (mean 41 months). Medical histories and clinical details were reviewed from the case records and the outcome of the patients from the cancer registry files (Finnish Cancer Registry). All the studies were approved by the Ethical Committee of Oulu University Hospital.

Selected samples from surgical specimens were fixed in 10% buffered formalin solution, embedded in paraffin and 5 μm sections were stained with Haematoxylin and Eosin. In each case, all slides containing cancer tissue were evaluated for the presence or absence of a budding margin. Best representative samples were selected from a random set of 108 cases (23.1%), and were used in immunohistochemical analysis against β-catenin. A random subset of 53 cases (11.4% of all cases, and 49% of cases included in β-catenin immunohistochemical analysis) were analysed using pan-cytokeratin antibody MNF116.

2.1. Immunohistochemistry

Sections (5 μm-thick) were cut from the tissue blocks and were hydrated overnight at 37 °C. After the samples were deparaffinised, rehydrated and rinsed with PBS; antigen retrieval for β-catenin was carried out using 0.01 M Tris–EDTA in a microwave oven at 850 W for 2 min, followed by 10 min at 150 W. For MNF 116, antigen retrieval was achieved by 0.1% w/v trypsin digestion for 15 min at 37 °C. After antigen retrieval, sections were cooled for 20 min and rinsed with PBS. Dako EnVision™ blocking solution (Dako, Copenhagen, Denmark) was used to block endogenous activity. After a 10 min rinse in PBS, slides were incubated in a primary monoclonal IgG antibody for β-catenin (Clone 14; Transduction Laboratories, Lexington, KY, USA) at a dilution of 1:2000 overnight at 4 °C. Incubation of the monoclonal wide spectrum anticytokeratin antibody MNF116 (Dako, Copenhagen, Denmark) was carried out at a dilution of 1:100 for 1 h at room temperature. Sections were then rinsed with PBS for 10 min and incubated with prediluted 1:200 goat anti-mouse secondary antibody (Dako, Copenhagen, Denmark) for 30 min at room temperature. Bound antibodies were detected with the EnVision™ system (Dako Inc., Copenhagen, Denmark). Diaminobenzidine (DAB) was used as the chromogen, and sections were counterstained with haematoxylin and then mounted. Negative controls (by omitting either primary or secondary antibody) were used to exclude the possibility of non-specific staining.

2.2. Budding

Budding was considered to be present when narrow strands or clusters of cancer cells of one to three cells in width were observed extending beyond the tumour margin and where this finding appeared to be unrelated to glandular disruption associated with inflammatory cell infiltration.[10] In a random subset of 53 cases, the presence of cancer cell clusters was separately assessed by immunohistochemistry using the pan-cytokeratin antibody MNF116.

2.3. β-Catenin scoring

Slides were first interpreted by one of the investigators (T.T.H.) blinded to the clinical data, and confirmed by second observer (M.J.M.). In problem cases, the final grading of β-catenin intensity was set up after discussion. The intensity of membranous, cytoplasmic and nuclear staining was graded semi-quantitatively into four categories: negative (0), weakly positive (1), moderately positive (2) and strongly positive (3). Nuclear reaction was considered positive if diffuse or punctuate brown staining existed within nuclei. Normal epithelium and adenoma components were analysed separately from cancer if they were present in a specimen. For normal and adenomatous epithelium, β-catenin positivity was analysed separately from the basal zone (basal half of the crypts and basal part of an adenoma) and from the top zone (upper half of the crypts and superficial epithelium or superficial part of an adenoma). In cancer, the central part and invasive margin were analysed separately, and budding cells were analysed separately if they were present.

2.4. Assessment of intra- and interobserver variation

A total of 25 randomly selected cases were used to assess the intra- and interobserver variation in the analysis of budding margin. Four observers, two pathologists (M.J.M., H.J.T.) and two residents (J.M.M., J.P.N.) made the observations independently and without knowing the clinical and pathological information. Training of the observers was performed on a separate set of cases (N = 25) not belonging to the original series of 466 colorectal cancers.

2.5. Statistical analysis

Computer-assisted statistical analysis software was used (SPSS, version 12.0, SPSS, IL, USA) for statistical analysis. Pearson's χ^2 test was used unless otherwise stated. Fisher's exact test was used for small numbers. For the evaluation of survival statistics, Kaplan–Meier and Cox regression models were used. A P-value of less than 0.05 was considered to be statistical significant.

3. Results

The clinical and pathological characteristics of the cancers are presented in Table 1.

3.1. Budding margin and Dukes' stage

The contour of the invasive margin of the cancer in the bowel wall was more frequently sharply infiltrative in the more advanced cancers. This was observed in 35.7% (35/98) of Dukes' A cancers, in 70.7% (133/188) of Dukes' B cancers, in 76.1%; (83/109) of Dukes' C cancers and in 90.1% (64/71) of Dukes' D cancers ($P < 0.0001$, χ^2).

The occurrence of budding also increased with the Dukes' stage, but was less prevalent (Fig. 1A and B). In Dukes' stage A, budding was observed in 9.2% ($N = 9/98$) of the cases. In Dukes' stages B, C and D, budding was observed in 19.1% ($N = 36/188$), 30.3% ($N = 33/109$) and 47.9% ($N = 34/71$) of the cases, respectively ($P < 0.0001$).

3.2. β-Catenin expression

In the normal epithelium, β-catenin was localised to the cell membranes, and nuclear expression was absent in most cases (Table 2). In tumours, membranous, cytoplasmic and nuclear expression of β-catenin was present in the majority of cells. In the central regions of cancers (Table 3), β-catenin membranous staining was observed in all cases (108/108; 100%), and nuclear staining in 82.4% (89/108) of the cases; 27 (25%) being strongly positive. In the invasive margin, cytoplasmic expression of β-catenin was present in all cases, and nuclear β-catenin was frequent (102/108; 94.4%; Fig. 1C and D), 51.9% (56/108) of the cases being strongly positive (Table 3). The intensity of the nuclear β-catenin immunoreactivity increased from the central area (25% strongly positive; 82.4% positive) towards the invasive margin (51.9% strongly positive; 94.4% positive; $P < 0.001$; Wilcoxon signed rank test). In Dukes' stage A cancers, β-catenin nuclear expression in the central part of the tumour was slightly weaker than at the invasive margin, but this difference was not statistically significant ($P = 0.056$, Wilcoxon). In Dukes' stage B cancers, nuclear β-catenin expression was clearly higher at the invasive margin of tumour than in the central part ($P = 0.0001$; Wilcoxon), and the trend was similar for stages C and D ($P = 0.058$ and $P = 0.005$, respectively). To find out whether the difference between Dukes' stages A ($N = 21$) and B ($N = 45$) was just due to smaller numbers of Dukes' A cases, we used a subset of randomly selected Dukes' stage B cases (subset $N = 21$) equivalent to the number of Dukes A cases, and also in this subset of Dukes' stage B cases the increase of nuclear β-catenin at the tumour margin was clearly evident ($P = 0.002$, Wilcoxon).

Table 1 – Dukes' stage, grade of differentiation, location, mucin production, contour of the tumour and budding margin

	N	%
Dukes' Stage	466	100.0
A	98	21.0
B	188	40.3
C	109	23.4
D	71	15.2
WHO grade	466	100.0
Grade 1	106	22.7
Grade 2	276	59.2
Grade 3	84	18.0
Location	466	100.0
Proximal colon	147	31.6
Distal colon	133	28.5
Rectum	186	39.9
Mucinous cancer	466	100.0
No	417	89.5
Yes	49	10.5
Contour of the tumour	466	100.0
Sharply invasive	151	32.4
Pushing border	315	67.6
Budding margin	466	100.0
No	354	76.0
Yes	112	24.0

3.3. Relationship between β-catenin expression and the budding margin

The presence or intensity of β-catenin cytoplasmic or nuclear expression did not show any significant differences with the occurrence of budding ($P = 0.507$, 1.000, 0.076 and 0.352, Fisher's exact test). In five cases only, β-catenin nuclear staining was negative at the invasive margin. Budding was frequent in cases where nuclear β-catenin was moderately or strongly positive (Table 4), but cases with budding did not differ significantly from the cases without a budding margin ($P = 1.0$, Fisher's exact test; Fig. 1C and D). In both the central part and the invasive margin, we did not demonstrate any relationship between nuclear β-catenin expression and grade ($P = 0.706$ and $P = 0.166$, Fisher's exact test) or mucin production of the tumour ($P = 0.855$ and $P = 0.785$). High intensity (moderate to strong) nuclear β-catenin expression in both the central part and at the invasive margin was more frequent in rectal carcinoma (37.5% and 89.6%) than in colon carcinoma (15.5% and 61.7%; $P = 0.002$ and $P = 0.003$, Fisher's exact test), but when the rectal and colon carcinomas were compared within a same grade of differentiation, such difference was not observed (data not shown).

3.4. Budding margin and pan-cytokeratin staining

The presence of a budding margin was confirmed with pan-cytokeratin MNF116 staining in 53 cases (Fig. 1E and F). Budding was observed in H&E stained slides in 15/53 cases (28.3%), and in 45.1% of the cases by MNF116. There was a clear association with the findings of H&E stained slides and pan-cytokeratin immunohistochemistry. 86.1% (13/15) of cases with a budding margin had an irregular budding contour on MNF116 staining, and in 72.2% of the cases with a non-budding margin, the margin in MNF116 staining had pushing border contour and was non-budding ($P = 0.039$, McNemar). In 18.9% (10/53) of the cases, MNF116 staining showed some budding cells not detected by H&E stained slides, but the use of MNF116 staining did not improve survival statistics (data not shown).

3.5. β-Catenin, budding, recurrences and survival

The relationship between cumulative 5-year-survival statistics and the degree of budding and nuclear β-catenin

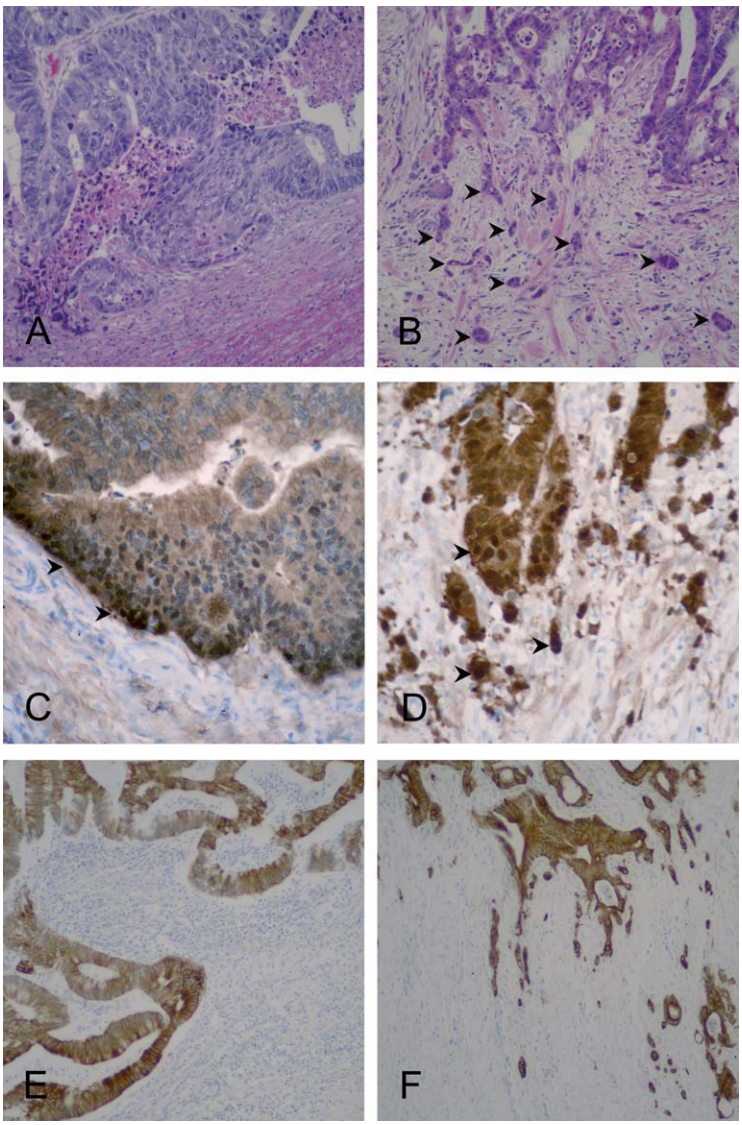

Fig. 1 – Examples of a non-budding (A,C,F) and a budding (B,D,F) margin. (A,B) In the absence of budding (A), the contour of the invasive margin is smooth, whereas budding cells (B) form isolated small clusters (arrowheads). Haematoxylin & Eosin. (C,D) β-catenin nuclear expression is seen at the invasive margin (arrowheads) in both non-budding (C) and budding margin (D). β-catenin. (E,F) Pan-cytokeratin MNF116 immunohistochemistry highlights the absence (E) and presence of budding (F). Pan-cytokeratin MNF116.

Table 2 – β-Catenin expression in normal colorectal epithelium

Localisation of immunoreaction	N	Normal epithelium; basal part								Normal epithelium; superficial part							
		Negative		Weak		Moderate		Strong		Negative		Mild		Moderate		Strong	
		N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Membranous	105	–	–	–	–	9	8.6	96	91.4	–	–	–	–	13	12.4	92	87.6
Cytoplasmic	105	6	5.7	88	83.8	11	10.5	–	–	8	7.6	89	84.8	8	7.6	–	–
Nuclear	105	85	81.0	1	1.0	5	4.8	14	13.3	96	91.4	1	1.0	–	–	8	7.6

expression levels at the central part of the tumour and the tumour margin were studied. To simplify the analysis, negative and weak positive reactions for β-catenin were grouped together (low β-catenin nuclear expression), and similarly, moderate and strong positive reactions were grouped together (high β-catenin nuclear expression). β-Catenin expression at the tumour margin did not show any significant influence on 5-year-survival. When the β-catenin nuclear expression was analysed from the central part of the cancer, 5-year-survival was 64.9% for the cases

Table 3 – β-Catenin expression in tumours

Localisation of immunoreaction	N	Central area of cancer								Invasive margin of cancer							
		Negative		Mild		Moderate		Strong		Negative		Mild		Moderate		Strong	
		N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Membranous	108	–	–	10	9.3	42	38.9	56	51.9	–	–	3	2.8	22	20.4	83	76.9
Cytoplasmic	108	3	2.8	78	72.2	25	23.1	2	1.9	–	–	27	25.0	72	66.7	9	8.3
Nuclear	108	19	17.6	9	8.3	53	49.1	27	25.0	6	5.6	7	6.5	39	36.1	56	51.9

Table 4 – β-Catenin nuclear expression intensity in relation to the budding margin

Nuclear β-catenin	No budding margin		Budding margin		Total N
	N	%	N	%	N
Negative	5	6.6	2	6.3	7
Weakly positive	3	3.9	4	12.5	7
Moderately positive	26	34.2	13	40.6	39
Strongly positive	42	55.3	13	40.6	55
Total	76	100.0	32	100.0	108

$P = 0.301$ (Fisher's exact test).

with low β-catenin expression, and 50.1% for the cases with high nuclear β-catenin expression ($P = 0.964$; log-rank). When the β-catenin nuclear expression at the invasive margin was analysed, 5-year-survival was 36.5% for the cases with low β-catenin expression, and 56.1% for the cases with high nuclear β-catenin expression ($P = 0.706$; log-rank).

Tumour budding was a strong indicator of poor prognosis. In the presence of tumour budding, 5-year-survival was only 15.4%, whereas 63.5% of the patients without budding survived regardless of the stage ($P < 0.00001$, log-rank; Fig. 2). The results were similar in both colon cancer (15.0% vs. 70.3%; $P < 0.00001$; log-rank) and rectal cancer (16.2 vs. 54.1%; $P < 0.00005$; log-rank). In localised disease, recurrences were more frequent in the presence of budding (Dukes' stage A: 66.7% vs. 15.6%; $P = 0.002$, Fisher's exact test; Dukes' stage B: 55.2% vs. 23.6%, $P = 0.001$, Fisher's exact test). In Dukes' stage A, 5-year-survival in the presence of tumour budding was only 29.2%, whereas 82.2% of the patients without budding survived ($P = 0.009$, log-rank). In Dukes' stage B, 5-year-survival in the presence of tumour budding was 29.7%, compared to 72.3% in patients without budding ($P < 0.00001$, log-rank). In Dukes' stage C, the difference was not significant (Dukes C: 17.6% vs. 41.7%; $P = 0.096$, log-rank), but in Dukes' stage D, no patients with budding survived to 5 years whereas 27.6% of cases without budding survived ($P = 0.0002$, log-rank). The sharply invasive contour of the tumour was also an indicator of worse survival, with 5-year-survival rate of 41.5%, whereas 73.1% of the patients with pushing border contour survived regardless of the stage ($P < 0.00005$, log-rank).

In Cox-regression analysis (including Dukes' stage, WHO histological grade, gender, budding, contour of the tumour, localisation and the size of the tumour and mucin production); Dukes' stage was the most significant factor in survival if Dukes stage D patients, (i.e., those with palliative operation)

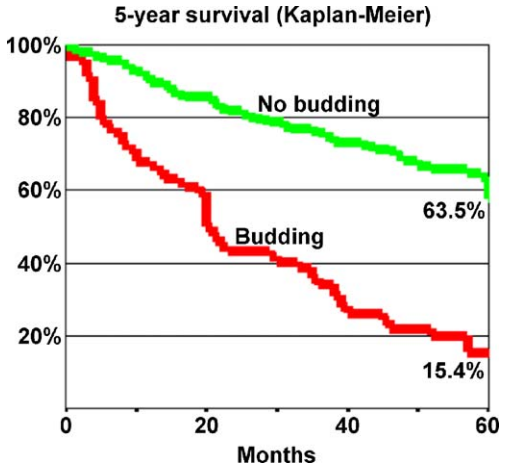

Fig. 2 – The relationship between budding and 5-year-survival in colorectal cancer patients.

were included ($P < 0.0005$, Cox-regression analysis, LR). Tumour budding was also an independent prognostic factor ($P < 0.0005$, Cox-regression analysis, LR, Table 5). When Dukes' stage D patients were removed from the analysis, tumour budding was a better predictor of poor prognosis than Dukes' stage (Table 6). In both analyses, WHO histological grade, size, mucin production and the localisation of the tumour (proximal colon/distal colon/rectum) did not show independent prognostic significance (Tables 5 and 6).

3.6. *Cox-regression analysis in the colon and rectum*

When colon and rectal cancers were evaluated separately, tumour budding showed an independent prognostic

Table 5 – Cox-regression analysis for the assessment of the independent prognostic significance of budding margin, Dukes' stage, gender, mucin production, size, contour and location of the tumour for Dukes' stage A–C cancers in terms of 5-year-survival (Cox stepwise regression analysis)

		P	Risk ratio	95.0% Confidence interval	
				Lower	Upper
Step 1	Budding	<0.0005	3.141	2.065	4.777
Step 2	Budding	<0.0005	2.386	1.539	3.697
	Dukes'stage	<0.0005			
	A vs. B	0.121	1.602	0.883	2.909
	A vs. C	<0.0005	3.181	1.735	5.830
Step 3	Budding	<0.0005	2.593	1.672	4.020
	Location	0.003			
	Proximal vs. distal colon	0.135	1.579	0.867	2.876
	Proximal colon vs. rectum	0.001	2.420	1.437	4.076
	Dukes 'stage	0.000			
	A vs. B	0.041	1.871	1.025	3.416
	A vs. C	<0.0005	3.549	1.937	6.505

Table 6 – Cox-regression analysis for the assessment of independent prognostic significance of budding in Dukes' stage A to D cancers in relation to Dukes' stage, gender, mucin production, size, contour and location of the tumour, in terms of 5-year-survival (Cox stepwise regression analysis)

		P	Risk ratio	95.0% Confidence interval	
				Lower	Upper
Step 1	Dukes 'stage	<0.0005			
	A vs. B	0.090	1.673	0.922	3.034
	A vs. C	<0.0005	3.905	2.170	7.024
	A vs. D	<0.0005	14.384	7.886	26.236
Step 2	Budding	<0.0005	2.824	1.978	4.032
	Dukes 'stage	<0.0005			
	A vs. B	0.138	1.570	0.865	2.850
	A vs. C	<0.0005	2.914	1.600	5.307
	A vs. D	<0.0005	11.923	6.454	22.025
Step 3	Budding	<0.0005	2.970	2.072	4.258
	Location	0.004			
	Proximal vs. distal colon	0.023	1.727	1.080	2.762
	Proximal colon vs. rectum	0.001	1.978	1.319	2.966
	Dukes 'stage	<0.0005			
	A vs. B	0.067	1.754	0.961	3.198
	A vs. C	<0.0005	3.183	1.743	5.811
	A vs. D	<0.0005	15.628	8.267	29.543
Step 4	Budding	<0.0005	2.656	1.832	3.849
	Contour	0.024	1.680	1.070	2.636
	Location	0.005			
	Proximal vs. distal colon	0.027	1.706	1.063	2.739
	Proximal colon vs. rectum	0.001	1.948	1.293	2.934
	Dukes 'stage	<0.0005			
	A vs. B	0.169	1.535	0.833	2.827
	A vs. C	0.001	2.902	1.583	5.322
	A vs. D	<0.0005	13.505	7.057	25.847

significance, with a risk ratio of 3.7 ($P < 0.0005$, 95% CI: 2.253–5.980) for colon cancer, and 1.8× risk ratio ($P = 0.031$, 95% CI: 1.056–3.224) for rectal cancer. When Dukes' stage D patients were removed from the analysis, tumour budding was still an independent prognostic factor in both colon cancer (risk ratio 4.198, $P < 0.0005$, 95% CI: 2.281–7.726) and rectal cancer (risk ratio 2.098, $P = 0.016$, 95% CI: 1.149–3.831).

3.7. *Intra- and interobserver variation*

Estimations of the presence of budding margin in 25 cases were well reproducible. The interobserver variation between all observers showed good agreement (mean $\kappa = 0.764$; range 0.635–0.905; $P < 0.0001$, *t*-test), and was equal to intraobserver (M.J.M.) variation ($\kappa = 0.764$, $P = 0.001$). Calculated mean κ for

all observations was $\kappa = 0.707$ ($P < 0.00001$, t-test), demonstrating good agreement between all observations.

4. Discussion

The aim of this study was to discover possible relationships between β-catenin expression and tumour budding at the invasive margin, and their influence on survival in colorectal cancer. A budding margin was infrequently seen in Dukes' stage A cases, but was more common in more advanced stages. A budding margin was a very strong indicator of poor prognosis in this study. In the presence of a budding margin, recurrences were fairly common and the 5-year-survival was only 15.4%, whereas 63.5% of the patients without a budding margin survived, regardless of stage. We found that the intensity of nuclear immunoreactivity for β-catenin was increased at the invasive margin in advanced Dukes' stage tumours. The presence or intensity of β-catenin expression did not show any significant association with the occurrence of the budding margin.

The major roles of β-catenin are in cell adhesion and in the mediation of the Wnt signal transduction pathway.[4–7,12,13] Translational stabilisation of β-catenin and its passage into the nucleus are required for the oncogenic function of β-catenin in colorectal cancer.[14] Nuclear accumulation of β-catenin is associated with mesenchymal transition of colorectal cancer cells, (i.e., dedifferentiated mesenchyme-like transformation of the cells) at the invasive margin, and membranous and cytoplasmic β-catenin expression is more common in the central areas of colorectal cancers.[6,15]

Loss or decrease of membranous β-catenin expression is related to decreased cell–cell adhesion and is frequently observed in colorectal cancer.[7] Increased nuclear β-catenin expression at the invasive margin has been shown to predict poor survival in rectal cancer and it has been correlated to more advanced Dukes' stage, tumour recurrence and MMP-7 expression.[16] Our results support this finding, as we found that nuclear β-catenin expression is increased in advanced cancers but not in Dukes' stage A cancers. 8.5% (9/106) of the well-differentiated and 11.6% (32/276) of moderately differentiated carcinomas in our study had a budding margin and concurrent lymph nodes or distant metastases, and in these tumours the degree of differentiation was similar to the primary tumour. In general, colorectal cancer metastases usually resemble their primary tumours. As a budding margin was associated with more frequent recurrences and poor survival in this study, budding cells are the most likely cells to be responsible for the metastases. In such case, the morphology of budding cells has to revert back to a more differentiated phenotype after implantation of the cancer cells at the metastatic site. Tumour budding is therefore likely to be a reversible phenomenon. Brabletz and colleagues speculated that regaining epithelial characteristics may be necessary for tumour cell proliferation at metastatic sites; because dedifferentiation of disseminating tumour cells appears to include an arrest in proliferative activity.[17]

In this study, membranous, cytoplasmic and nuclear β-catenin expression were more intense at the invasive margin than in the central part of the cancers. Cytoplasmic β-catenin has been shown to accumulate in the cytoplasm of tumour cells in spite of nuclear expression.[7] Activated integrin-linked kinase (ILK) has been shown to induce translocation of β-catenin from the plasma membrane to the nucleus, as well as induce the formation of the LEF-1/β-catenin complex without an increase of cytosolic β-catenin.[18]

The presence of a budding margin has been shown to be a relatively reproducible feature and an independent factor for poor prognosis in rectal cancer,[10] and it has also been associated with venous invasion, lymph node metastases and distant metastases.[19–22] We were able to show that budding is also an important prognostic feature in colon cancer. In this study, 5-year-survival in colon cancer was only 15.0% in the presence of a budding margin, whereas 70.3% of the patients without a budding margin survived. The presence of a budding margin was easily identified from routine sections, and a similar conclusion was made in an earlier report.[23] We also tested the use of pan-cytokeratin antibody MNF116 as an aid for the detection of budding cells.[22] Use of pan-cytokeratin antibody improved detection of isolated cells, but did not improve the prognostic value of budding in the present study. Pan-cytokeratin immunohistochemistry specifically detects epithelial cells, but other important histopathological parameters, such as inflammation-related disruption of cancer cell islands at the invasive margin, are more difficult to interpret from the Haematoxylin-counterstained slides. Isolated cancer cells at the invasive margin that were related to tumour-destructive inflammation, were not considered to represent budding in our study, as they likely are a result of tumour disruption caused by a host immune reaction at the invasive margin.[23]

Brabletz and colleagues hypothesised that β-catenin could be implicated in the development of tumour budding.[15,17] We could not find any significant association between budding and β-catenin expression. Moderate or strong nuclear β-catenin expression was observed in 81.2% of cases with a budding margin and in 89.5% of the cases without. An obvious explanation of this similarity is the high frequency (93.6%) of nuclear β-catenin expression at the invasive margin of all the tumours. As a budding margin was much less frequently seen (24.0%), it would have been very unlikely that any statistically significant connections between budding and β-catenin expression would have been observed. Our results suggest that nuclear accumulation of β-catenin at the invasive margin is clearly a requirement for invasion, as it was almost invariably present in the advanced cancers. It is likely that there are factors other than nuclear β-catenin, which are more directly related to tumour budding and poor prognosis. Jung and colleagues postulated that the expression of p 16 and the disruption of the Wnt pathway might serve as joint prerequisites for tumour budding.[24]

In conclusion, the presence of tumour budding is a pertinent indicator of poor prognosis in colorectal cancer. The assessment of the budding margin can be reliably performed in routine sections and it can offer useful prognostic information for clinicians. Whether patients with localised disease and a positive budding margin benefit from adjuvant therapies requires further study. β-catenin cytoplasmic and nuclear translocation is likely to be a background event in tumour budding, but as it was similarly observed in cases without

budding, it is likely that altered β-catenin expression is not the only explaining factor in tumour budding.

Conflict of interest statement

None declared.

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