

Tumor Budding in Colorectal Carcinomas

Kolorektal Karsinomlarda Tümör Tomurcuklanması

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ABSTRACT

Objective: In colorectal carcinomas, tumor budding has been defined as the presence of isolated single tumor cells or small cell clusters in the stroma at the invasive tumor margin. In this study, the relationship between tumor budding density at the invasive tumor margin and pathological parameters is investigated.

Material and Method: Haematoxylin and eosin stained slides of 73 cases with colorectal carcinoma were retrospectively evaluated for the presence and intensity of tumor budding by 2 observers. After the specimens were assessed, the highest density of tumor budding area was counted in a microscopic field of x200. Cases were separated into 2 groups according to tumor budding density as low grade (<10) and high grade (≥10). The relationship of these groups with depth of tumor invasion, histological grade, vascular invasion and lymph node involvement was investigated.

Results: Of the 73 colorectal carcinoma cases, 33 (45.2%) had low and 40 (54.8%) had high grade tumor budding density, respectively. There was a statistically significant relationship between high grade tumor budding density and histological grade (p=0.042), lymph node involvement (p=0.0001) and vascular invasion (p=0.0034).

Conclusion: High grade tumor budding density is associated with aggressive phenotypical features in colorectal carcinoma.

Key Words: Colorectal neoplasms, Neoplasm invasion, Lymph node, Metastasis

ÖZ

Amaç: Kolorektal karsinomlarda tümör tomurcuklanması, invaziv tümör komşuluğundaki stromada bulunan izole tümör hücreleri ya da küçük tümöral hücre kümeleri olarak tanımlanmaktadır. Bu çalışmada kolorektal karsinomlarda invaziv tümör komşuluğundaki stromada bulunan tümör tomurcuklanması yoğunluğunun patolojik özelliklerle ilişkisi araştırılmıştır.

Gereç ve Yöntem: Retrospektif olarak, kolorektal karsinomlu 73 olguya ait hematoksilen-eozin boyalı preparatlar tümör tomurcuklanması varlığı ve yoğunluğunun tespit edilmesi amacıyla 2 gözlemci tarafından değerlendirilmiştir. Preparatlar tekrar değerlendirildikten sonra tümör tomurcuklanmaları, en yoğun oldukları alan tespit edilerek x200 büyütme alanında sayılmıştır. Olgular, tümör tomurcuklanması yoğunluğu 10'dan az olanlar düşük dereceli, 10 ve üzeri olanlar yüksek dereceli olmak üzere 2 gruba ayrılmıştır. Bu grupların tümörün invazyon derinliği, histolojik derecesi, lenf nodu tutulumu ve vasküler invazyon gibi patolojik özelliklerle ilişkisi araştırılmıştır.

Bulgular: Kolorektal karsinomlu 73 olgunun 33'ünde (%45,2) düşük, 40'ında (%54,8) yüksek dereceli tümör tomurcuklanması yoğunluğu tespit edilmiştir. Yüksek dereceli tümör tomurcuklanması yoğunluğu ile histolojik derece (p=0.042), lenf nodu tutulumu (p=0,0001) ve vasküler invazyon (p=0,0034) arasında istatistiksel olarak anlamlı ilişki saptanmıştır.

Sonuç: Yüksek dereceli tümör tomurcuklanması yoğunluğu kolorektal karsinomlarda agresif fenotipik özelliklerle ilişkilidir.

Anahtar Sözcükler: Kolorektal tümörler, Neoplazi invazyonu, Lenf nodları, Metastaz

INTRODUCTION

Tumor budding is a peculiar feature that is observed in colorectal adenocarcinomas. In colorectal carcinomas, 'tumor budding' has been defined as the presence of isolated single neoplastic cells or small cell clusters of up to four cells in the stroma at the invasive tumor margin (1-8). It was first reported by Imai (9). Although he used the term 'sprouting' instead of 'budding' he was the one who showed

that tumors with this feature have more aggressive features (9). Morodomi et al. used the term 'tumor budding' first in 1989 (10).

Since Dukes' original classification, staging systems, which consider parameters; lymph-node involvement of the tumor and depth of mural invasion, are in use to predict the long-term survival following surgical resection in colorectal cancers (3). Dukes' staging system comprises

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two parameters: tumor penetration depth and lymph node involvement (11). The TNM classification of International Union Against Cancer (UICC), the most widely used standardized pathological staging system, considers depth of invasion, lymph node involvement and distant metastasis as parameters (12). Efforts have been made to improve the prognostic prediction of these systems because of the observation of different clinical outcome between the patients with same Dukes' or TNM stage, which is usually observed in colorectal adenocarcinomas. This ambiguity may be explained by the fact that these standardized pathological staging systems do not reflect biological behavior of carcinoma exactly, which may correlate with tumor aggressiveness and risk of recurrence.

There are reports which determines the association of tumor budding at the invasive tumor margin and tumor behavior (3-8). Dedifferentiation and dissociation of cancer cells has been reported to be the first event of invasion and metastasis in experimental studies. Tumor budding is the morphologic expression of this event (6). It has also been shown that it is an independent and a reproducible prognostic factor (1). In addition, tumor buds can be appreciated in conventional haematoxylin and eosin stained (H&E) slides by careful observation (3).

Correlation between tumor budding and lymph node metastasis has already been shown by several studies in the past few years (2-4). Today it is widely accepted that tumor budding is an important predictor for recurrence and poor prognosis in advanced colorectal cancers. Because tumor budding is associated with prognosis, Association of Directors of Anatomic and Surgical Pathology (ADASP) recommended that budding should be included in staging systems and it should be scored as present or absent (8). In this study the relationship between the intensity of tumor budding at the invasive tumor margin and pathological parameters is investigated.

MATERIAL and METHODS

Our study comprised 73 patients who were diagnosed as colorectal adenocarcinoma and underwent surgery in Süleyman Demirel University School of Medicine between 2003 and 2007. The primary tumor was located in the colon in 50 (68.5%) cases and in the rectum in 23 (31.5%) cases. Tumors were resected with right hemicolectomy in 12 cases, with transvers hemicolectomy in 11 cases, with left hemicolectomy in 15 cases, with sigmoidectomy in 12 cases and with low anterior resection in 23 cases. None of the operative specimens had positive surgical margins. In macroscopic examination 4 to 10 samples were obtained from each tumor specimen. The pathological staging of all cases was performed according to the 6th edition of UICC/TNM classification system (13).

Retrospectively, paraffin embedded and H&E stained slides of 73 surgically resected colorectal carcinoma cases were reevaluated simultaneously by 2 observers to assess the presence and intensity of tumor budding in the invasive tumor margin. For these tumors, morphological features of known prognostic importance such as tumor diameter, depth of invasion, histological type, histological grade, extramural vascular invasion and lymph node involvement were recorded from the pathology reports. None of the patients had familial polyposis coli, hereditary nonpolyposis colorectal carcinoma, multiple colorectal carcinoma or inflammatory bowel disease.

From each resected specimen all the slides were reviewed. After the specimens were assessed, one slide containing the highest density of tumor budding was selected for evaluation by using a Nikon Optiphot-2 microscope. For the detection of the area that contained the highest tumor budding density, at least 10 areas were assessed in each resection specimen. Tumor buddings were counted in the area of highest tumor budding density within a microscopic field of x200 in this slide. An isolated single cancer cell and/or a cluster composed of up to 4 neoplastic cells were defined as budding foci (Figure 1). After counting the highest density of tumor budding in selected slide of each case, the tumor budding density was classified into 2 groups as low grade (less than 10 tumor buds) and high grade (10 or more tumor buds) as Shinto et al., Ueno et al., and Choi et al. did in their studies to investigate the correlation of tumor budding density with depth of tumor invasion, histological grade, vascular invasion and lymph node involvement (Figure 2A,B) (5-7).

The chi-square test and Fisher's exact test were used to determine the relationship between categorized variables. A p-value of <0.05 was considered statistically significant.

RESULTS

Of the 73 patients, 41 (56.2%) were male and 32 (43.8%) were female. The mean age was 63.7 years (range 34-93 years). In these 73 cases, the histological grade of the tumors were well differentiated in 37 (50.7%) cases, moderately differentiated in 28 (38.4%) cases and poorly differentiated in 8 (10.9%) cases. There was lymph node involvement in 41 (56.2%) cases and no lymph node involvement in 32 (43.8%) cases. Vascular invasion was present in 27 (37%) cases, 46 (63%) cases were without vascular invasion. Of the 73 patients, 64 (87.7%) tumors were pT3, 7 (9.6%) tumors were pT2, 1 (1.35%) tumor was pT1 and 1 (1.35%) tumor was pTis. Clinicopathological characteristics of 73 patients are presented in Table I.

In 33 (45.2%) cases tumor budding density was lower than 10 and in 40 (54.8%) cases there were 10 or more tumor

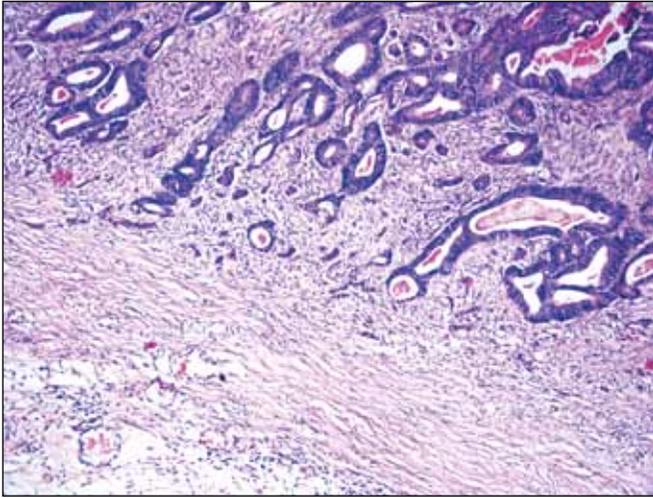


Figure 1: Tumor buddings at the invasive margin of a colorectal adenocarcinoma (H&E, x100).

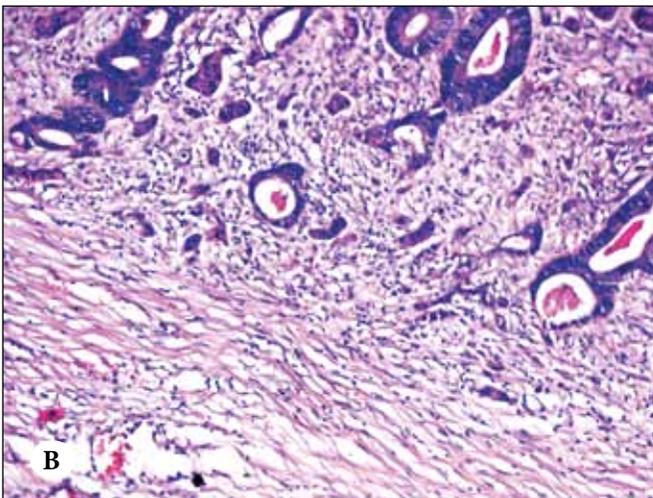
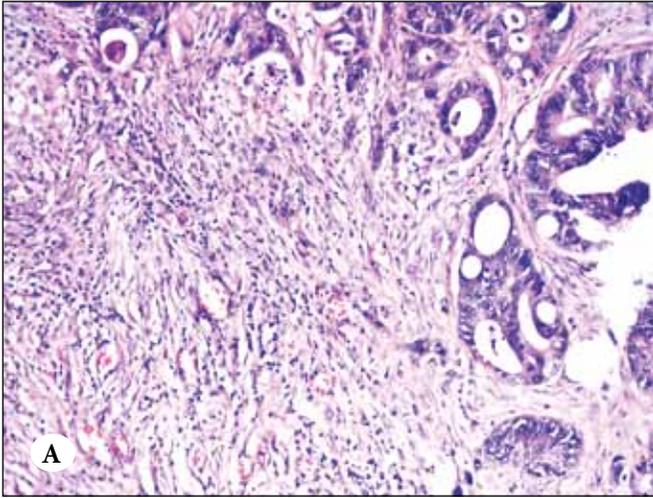


Figure 2: (A) Low grade (H&E, x200), (B) and high grade tumor budding density (H&E, x200).

Table I: Clinicopathologic characteristics of 73 colorectal carcinoma patients

Variable	Number of patients (N=73)
Mean age	63.7
Sex	
Male	41 (56.2%)
Female	32 (43.8%)
Histological grade	
Well differentiated	37 (50.7%)
Moderately differentiated	28 (38.4%)
Poorly differentiated	8 (10.9%)
Depth of tumor invasion	
pTis	1 (1.35%)
pT1	1 (1.35%)
pT2	7 (9.6%)
pT3	64 (87.7%)
Lymph node involvement	
pN0 (0)	32 (43.8%)
pN1 (1-3)	22 (30.2%)
pN2 (≥ 4)	19 (26%)
Vascular invasion	
Absent	46 (63%)
Present	27 (37%)

buddings. In the cases whose tumor budding density was <10 , histological grade was well differentiated in 21 (63.6%) cases, moderately differentiated in 10 (30.3%) cases and poorly differentiated in 2 (6.1%) cases. In the cases that tumor budding density was ≥ 10 , histological grade was well differentiated in 16 (40%) cases, moderately differentiated in 18 (45%) cases and poorly differentiated in 6 (15%) cases.

Among the 33 cases with low grade tumor budding density there was no lymph node involvement in 23 (69.7%) cases, while 10 (30.3%) cases were with lymph node involvement. Of 10 cases, 1 to 3 lymph nodes were involved in 5 (15.15%) and 4 or more lymph nodes were involved in the remaining 5 (15.15%). Among the 40 cases with high grade tumor budding density there was not lymph node involvement in 9 (22.5%) cases and there was lymph node involvement in 31 (77.5%) cases. Among these 31 cases 1 to 3 lymph nodes were involved in 17 (42.5%) cases and 4 or more lymph nodes involved in 14 (35%) cases.

There was a statistically significant relationship between high grade tumor budding density and lymph node metastasis ($p=0.0001$). In addition, tumors with 1 to 3 (pN1) ($p=0.001$) and 4 or more (pN2) ($p=0.003$) lymph node metastasis had significantly higher tumor budding

density than nonmetastatic group (pN0). However, there was no statistically significant difference between pN1 and pN2 tumors for tumor budding density.

In the group with a tumor budding density of <10, 27 (81.8%) cases were with vascular invasion, while 6 (18.2%) cases had no vascular invasion. In the high grade tumor budding density group (≥ 10), 19 (47.5%) cases were without vascular invasion and 21 (52.5%) cases had vascular invasion.

In low grade tumor budding density group 29 (87.9%) tumors were pT3, 2 (6.1%) tumors were pT2, 1 (3%) tumor was pT1 and 1 (3%) tumor was pTis according to their depth of invasion. In the high grade tumor budding density group 35 (87.5%) tumors were pT3 and 5 (12.5%) tumors were pT2.

There was a statistically significant association between high grade tumor budding density and histological grade ($p=0.042$) and vascular invasion ($p=0.0034$). However, there was no statistically significant relationship between the depth of tumor invasion and tumor budding density ($p=0.4475$). The association of clinicopathological parameters and tumor budding density is presented in Table II.

DISCUSSION

Lymph node metastasis is one of the most important prognostic factors in colorectal carcinomas (14). The depth of submucosal invasion, tumor grade at the deepest

invasive portion and existence of lymphovascular invasion are predictive markers for lymph node metastasis of colorectal carcinoma (15). It has been reported that tumor budding that represents dedifferentiated histology at the invasive margin is useful in predicting lymph node metastasis or hematogenous metastasis (16). In this regard tumor budding has been shown to reflect the biological aggressiveness of colorectal cancers (15,17). It seems to be the first histological event of invasion, metastasis and poor prognosis. It is a histological feature that appears at invasive front of the lesion rather than at the predominant tumor site. As the biologically most relevant area with the malignant potential of the tumor is the area of invasive front in colorectal carcinoma, tumor budding might reflect the biological aggressiveness of the tumor itself (15,17). The results of our study provides evidence about the prognostic value of tumor budding.

According to our results, high grade tumor budding density is significantly associated with higher histological grade, vascular invasion and presence of lymph node metastasis in colorectal carcinomas. Our results are concordant with the results of Morodomi et al., Goldstein et al., and Okuyama et al., who reported that tumor budding is associated with pathological parameters including venous invasion and lymph node metastasis in colorectal carcinomas (10, 18, 19). These findings support the idea that tumor buds originate from the cells which have higher malignant potential. Although there was a significant relationship with lymph

Table II: Association of clinicopathological parameters and tumor budding density

Parameters	Tumor budding density		
	<10 tumor buds (N=33)	≥ 10 tumor buds (N=40)	p value
Histological grade			
Well (N=37)	21 (63.6%)	16 (40%)	0.042*
Moderate (N=28)	10 (30.3%)	18 (45%)	
Poor (N=8)	2 (6.1%)	6 (15%)	
Vascular invasion			
Absent (N=46)	27 (81.8%)	19 (47.5%)	0.0034*
Present (N=27)	6 (18.2%)	21 (52.5%)	
Lymph node metastasis			
Absent (N=32)	23 (69.7%)	9 (22.5%)	0.0001*
Present (N=41)	10 (30.3%)	31 (77.5%)	
Depth of tumor invasion			
pTis (N=1)	1 (3%)	0 (0%)	0.4475
pT1 (N=1)	1 (3%)	0 (0%)	
pT2 (N=7)	2 (6.1%)	5 (12.5%)	
pT3 (N=64)	29 (87.9%)	35 (87.5%)	

*: Statistically significant ($p < 0.05$)

node involvement and high grade tumor budding density there was no association between the number of involved lymph nodes and tumor budding density according to our results. In addition to this, our results revealed that depth of tumor invasion is not associated with tumor budding density in contrast to the study of Ueno et al. (20).

The importance of tumor budding and the mechanism of its occurrence is also stated by a group of molecular studies (16,21). The loss of both glandular differentiation and cell cohesion that gives rise to dissociated elements probably is a crucial event in the development of invasion and metastatic properties in colorectal carcinomas (21). This would be consistent with the important and independent adverse prognostic significance of tumor budding. In addition tumor budding was found to be correlated with less favourable outcome (7,22).

In the present study, histological grade, lymph node involvement and vascular invasion were significantly associated with high grade tumor budding density, but in some studies a significant correlation was found between budding and lymph node involvement even when budding regarded only as positive or negative (15). Most of the studies in the literature were performed to investigate the prognostic importance of tumor budding considering them only as absent or present. All the tumors included in our study had at least 2 tumor buddings at the invasive margin. Because of this feature we could not investigate the importance of buddings considering them as absent or present. Our study is performed to investigate the intensity of tumor budding and its relationship with aggressive histopathological features. We separated the cases into two groups as high grade and low grade tumor budding density. Tumors with less than 10 tumor buds considered as low grade group and tumors with 10 or more tumor buds considered as high grade group just as Shinto et al., Ueno et al., and Choi et al. did in their studies (5-7). The intensity of tumor budding was also investigated by grouping the cases as mild (<5), moderate (5-9) and marked (≥ 10) (23). We also used this classification in our study too, but our results were not statistically significant.

Although there are reports which advise to use immunohistochemistry to detect tumor budding, in our study we evaluated the tumor buds in conventional H&E stained slides (2,24). The minimum requirements for an ideal prognostic parameter are, first, that the grading should be reproducible and widely available with quality control, and second, that the grading has substantial predictive value beyond recognized prognostic systems (23). Tumor budding can be identified and graded easily with the use of light microscopy which makes it simple and applicable in routine pathological settings.

In conclusion the results of our study reveal that high grade tumor budding density is associated with aggressive phenotypical features in colorectal carcinoma and it can be used as a practical and reliable parameter to identify higher malignancy potential. Although we admit that, the number of patients analyzed in the present study is not large enough to draw a definite conclusion, we recommend the examination of budding in the routine pathological diagnosis of colorectal carcinomas at least as present or absent. We suggest tumor budding as a risk factor for an adverse outcome in invasive colorectal carcinoma. Evaluating tumor budding can be helpful in improving the staging systems and treatment approach and it can be an additional pathological parameter which helps determining tumor behavior.

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