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Tumor budding in colorectal adenocarcinoma using cytokeratin 20 immunostaining

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Abstract

Introduction: Colorectal cancer is the third most common cancer worldwide and second most common fatal cancer. Tumor budding is an emerging prognostic indicator in cancers. Though tumor budding can be assessed using Hematoxylin and Eosin stain, sometimes peritumoral inflammation and reactive stromal fibrosis obscures the tumor buds. The present study aimed to assess tumor budding using cytokeratin 20 immunohistochemistry in colorectal cancers.

Methods: This is a cross sectional study which included 30 cases of colorectal carcinomas. cytokeratin 20 stained slides of colorectal carcinoma were assesed for tumor budding using International Tumor Budding Consensus Conference (ITBCC) 2016 consensus criteria and compared with AJCC cancer stage.

Results: Demographic analysis showed peak incidence of colorectal cancer in the age group of 55-64 years (33.3%) and male: female ratio of 1.14:1. Majority of the tumors with score 1(23.3%) showed stage I. While tumors with score 2 had similar incidence of stage I (16.6%) and II (16.6%) and lesser incidence of stage III (3.33%). Most of the tumors with score 3 tumor budding had stage III tumors (26.67%). The p value is < 0.0001, which is statistically significant.

Discussion: Tumor budding signifies the biological and molecular phenomenon of epithelial-mesenchymal transition in the tumor microenvironment. Loss of E-cadherin, alterations in transcription factors including SNAIL, ZEB, TWIST etc and switching of CMS2 to CMS4 are some of the recorded molecular profiles responsible for tumor budding.

Conclusion: This study concludes that the tumor stage increases with tumor budding and thus is a reliable marker in predicting the prognosis. This study also states that immunohistochemical study gives objective scoring of tumor buds in colorectal cancers.

Keywords: tumor budding; colorectal cancer; cytokeratin 20; prognostic marker

Introduction

Colorectal cancer contributes to a global health problem having a significant disease incidence and mortality. It is the third most common cancer worldwide and accounts to second most common fatal cancer [1]. The occurrence of colon cancer among younger adults has been increased in the recent years [2]. Increase in the modifiable risk factors has contributed to increase in sporadic colon cancers (approximately 75%) [3]. There are various modifiable risk factors having deleterious effect on the incidence of colorectal cancer, including smoking [4], obesity [5], sedentary lifestyle and physical inactivity [6] and poor dietary habits [7].

Staging of colorectal cancers using TNM system remains the gold standard for prognostification of these tumors [8]. Furthermore, some of the histopathological features have independent prognostic value in colorectal cancers including histological subtype [9], lymphovascular invasion [10], number of tumor positive lymphnodes

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collecting at least 12 lymphnodes [11], perineural invasion [12] and tumor budding [13]. Though molecular markers of prognostic significance in colorectal cancers are available [10], the histopathological factors can be considered as cost-effective and widely usable parameters in risk assessment.

Tumor budding is an emerging prognostic indicator, which is defined as single or a cluster of ≤ 4 de-differentiated tumor cells present at the invasive tumor front [14]. Tumor budding can be identified at the tumor center called as intratumoral budding, or at the invasive front which are known as peritumoral budding [15]. Tumor buds are formed due to the Epithelial-Mesenchymal transition in the tumor microenvironment [14]. International Tumor Budding Consensus Conference (ITBCC) 2016 consensus recommends the assessment of peritumoral budding at the invasive front at 20x magnification in a field that has maximum density of buds. These can be further classified as low (0-4 buds/20x), intermediate (5-9 buds/20x) and high (>9 buds/20x) [15]. This stratification of tumor budding is chiefly salient in stage I and stage II colorectal cancers [16-18]. Even though patients with TNM stage I are expected to have best prognosis, some tumors have aggressive course. Thus, patients having increased number of microscopic risk factors for tumor spread (including tumor budding) on biopsy specimen, must be considered for oncological resection [16]. Stage II colorectal cancer patients with high tumor budding have increased risk of metastasis and mortality [17]. Thus, these patients should be considered for adjuvant therapy [16].

Though tumor budding can be assessed using Hematoxylin and Eosin stain, in some cases peritumoral inflammation and reactive stromal fibrosis obscures the tumor buds [14, 20]. In addition, the results are subjective and has increased interobserver variability [19]. Thus, the present study aimed to assess the Tumor budding using Cytokeratin 20 immunohistochemistry in colorectal cancers.

Materials and methods

This is a cross sectional descriptive study done in the Department of Pathology, Mysore Medical college and research institute, Mysore during the period of 6 months from December 2019 to May 2020. Approval for conducting the study was obtained from the Institutional Ethical committee. The present study included 30 cases of colorectal carcinomas, including resected specimen of colorectal cancers. We have excluded the cases which had extensive necrosis and had undergone prior adjuvant therapy. Routine grossing and histopathological examination of the surgical specimen was done as per the standard protocol. Additional 5 micron thick sections were taken on charged slides and cytokeratin 20 immunostain was done. Cytokeratin 20 stained slides of colorectal carcinoma cases were utilised in the present study to assess tumor budding.

A single or group of less than 5 cells stained with cytokeratin 20 were counted as tumor buds. ITBCC 2016 consensus criteria was used to analyse and count the tumor budding. The slides were initially scanned under low power (10x) fields to identify the field with maximum density of buds. Subsequently, the number of buds was counted at 20x objective lens as per the criteria. Three-tiered system was used for stratification of the tumor buds into low/BD1 - 0-4 buds, intermediate/ BD2- 5-9 buds and high/BD3- >9 buds per 20x field. Results were tabulated and compared with the stage of the tumor.

Statistical analysis

All the data were entered in Microsoft Excel and analyzed using statistical software R version 4.2.2. The qualitative study variables were expressed in frequency with percentages. To find the significant difference Yates Chi-square test is used since the frequencies were ≤ 5. p value of <0.05 is considered statistically significant.

Results

The study was conducted in the Department of Pathology of our institute, in which 30 specimen of colorectal cancer have been included. Demographic results were analysed. In the present study, peak incidence of colorectal cancer was in the age group of 55-64 years (33.3%) and least was in younger age group of 35-44 years and older than 75 years (6% each). Gender wise distribution suggests that the males were slightly more affected than females with Male: female ratio of 1.14:1 (Table 1).

The H&E stained slides were studied and all the 30 cases (100%) were reported as Adenocarcinoma. Majority of the tumors were of moderately differentiated grade 2 (56.6%), followed by grade 1 (40%) and only 1/30 (3.3%) was graded as poorly differentiated adenocarcinoma. Pathological TNM staging was done according to AJCC 8th edition. Majority of the cases, 12/30 (40%) belonged to stage I followed by stage II and III (30% each) (Table 1).

The CK 20 immuno-stained slides were examined under 20x field and scored according to three scaled scoring system.

Score 0 = no staining or nonspecific staining of tumor cells.

Score 1+ = weak and incomplete staining of more than 10% of tumor cells.

Score 2+ = moderate and complete staining of more than 10% of tumor cells.

Score 3+ = strong and complete staining of more than 10% of tumor cells.

Table 1: Demographic and histomorphological data of tumor budding in colorectal cancer.

Descriptive analysis	*Adenocarcinoma (N = 30)*
Age	
35-44	2
45-54	7
55-64	10
65-74	9
75-84	2
Gender	
F	14
M	16
Tumour grade	
1	12
2	17
3	1
Stage	
I	12
II	9
III	9
CK 20 score	
1	7
2	11
3	12
Tumour budding score	
1	9
2	11
3	10

The number of tumor buds were counted using CK20 immunostaining. The tumor buds were grouped into three tiered groups as per ITBCC 2016 consensus criteria. Low tumor budding (score 1) was seen in 9/30 cases (30%) (Figure 1), 11/30 (36.6 %) were scored as intermediate (score 2) (Figure 2) which contributes to the majority of cases, and 10/30(33.3%) showed high tumor budding (Figure 3).

The tumor bud scoring was compared with the known prognostic parameter i.e, tumor staging. Majority of the tumors with score 1 (23.3%) showed stage I tumor. While tumors with score 2 had similar incidence of Stage I (16.6%) and II (16.6%) and lesser incidence of stage III (3.33%). Most of the tumors with score 3 tumor budding had stage III tumors (26.67%). The p value is < 0.0001, which is statistically significant. AJCC and TNM staging of tumors include lymphnodal metastasis also, thus these results imply that the tumor budding score increases with increase in lymph nodal metastasis and in turn results in upstaging of the tumor (Table 2).

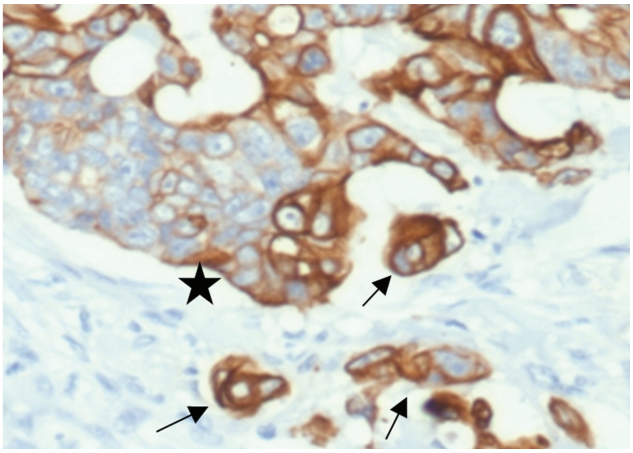

Figure 1: Low tumor budding. 20x; IHC-CK20, Shows tumor front.

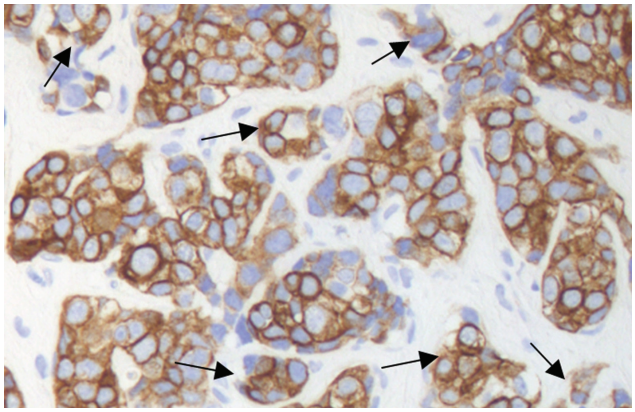

Figure 2: Intermediate tumor budding, 20x; IHC-CK20.

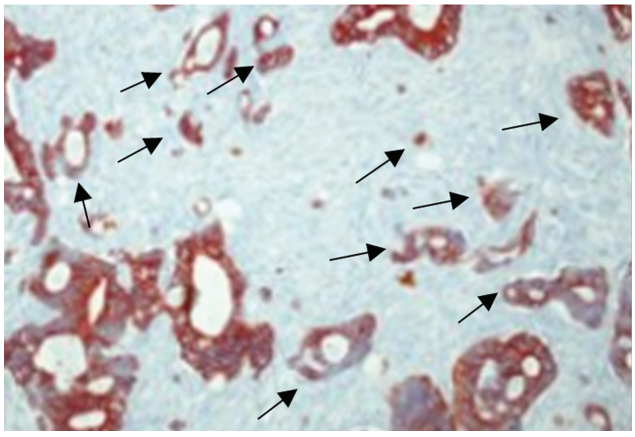

Figure 3: High tumor budding, 20x; IHC-CK20.

Table 2: Correlation between tumor budding score with the tumor stage.

Tumour budding score	*Tumor stage*			*p value*
	I (%)	*II (%)*	*III (%)*	
1	23.33	6.67	0.00	
2	16.67	16.67	3.33	<0.0001*
3	0.00	6.67	26.67	

p value < 0.05 is significant, Chi square test of significance

Discussion

Tumor budding has been a novel prognostic marker used in various organs e.g., breast [21], laryngeal squamous cell carcinoma [22] and in various other solid cancers [23]. Tumor budding signifies the biological and molecular phenomenon of epithelial-mesenchymal transition in the tumor microenvironment [23]. Many scoring systems have been proposed for the analysis of tumor budding, including Choi et al [24], Prall et al [25], Lugli et al [26], Zlobec et al [27] etc.

A review done by Lugli et al [21], catalogued various criterias to count the tumor budding, which include the following: (1) ≥10 buds in a single field (20x objective) on H&E (10/24, 42%), (2) ≥5 buds in a single field (20x objective) on H&E (3/24, 13%), (3) 10 HPF method' using IHC (≥10 HPF, 40x objective) (0/24, 0%), and (4) No cut-off used (prefer continuous scale, reporting buds/HPF) (4/24, 17%).

However, the recent ITBCC consensus criteria are been accepted and widely used in reporting of tumor budding scoring.

Tumor budding results from Epithelial-mesenchymal transition (EMT) which is a result of various molecular alterations. Loss of E-cadherin, alterations in transcription factors including SNAIL, ZEB, TWIST etc and switching of CMS2 to CMS4 are some of the recorded molecular profiles responsible for tumor budding [30].

The results of present study were compared with the studies done by other pioneers. The age incidence of CRC correlated well with Zlobec et al being more prevalent in elderly patients with a median age of 64 years. Also, they documented a significant association between high-grade tumor budding and a lack of objective response in patients with metCRC treated with anti-EGFR therapies [27].

Prall et al in their retrospective study identified that majority of patients reported with high tumor budding score had metastatic disease in future compared to those reported as low tumor budding score [25]. In a study conducted by Rieger et al., tumor budding counts showed significant relation with advanced T -stage, presence of nodal metastasis, lymphatic invasion, venous invasion, tumour grade, independent of the location of assessment. This is comparable with our study [28]. Wang et al., in their study related tumor budding with epithelial–mesenchymal transition and appears to be an independent prognostic factor [29]. Our study correlated with the study done by Kumarguru et al., where 60% of patients had high tumor budding score and 40% of patients had low tumor budding score [21].

Limitations: This study has been conducted on a small number of samples retrieved at our institution. Comparison with routine hematoxylin and eosin staining could not be done due to extensive stromal reaction and inflammationin few cases. Large sample size and multicentre studies are required to confirm these results.

Conclusion

This study concludes that the tumor stage increases with tumor budding score, and thus is a reliable marker in predicting the prognosis. This study also states that immunohistochemical evaluation gives objective scoring of tumor buds compared to routine hematoxylin and eosin staining. Tumor budding is a reliable and cost effective indicator of spread of cancer and can be a futuristic prognostic marker. We recommend the utility of this parameter in routine reporting of colorectal cancers.

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Conflicts of interest

Authors declare no conflicts of interest.

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