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Original Research Paper

The Role of Tumor Budding as a Prognostic Factor in Colorectal Adenocarcinoma

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ABSTRACT

Background: Colorectal cancer is among the most common malignancies globally. Tumor budding has been proposed as a potential prognostic indicator in colorectal adenocarcinoma. **Objective:** This study aimed to analyze the relationship between tumor budding and histopathological grade, lymphovascular invasion, and lymph node metastasis in colorectal adenocarcinoma, and to evaluate its potential as a prognostic factor. **Methods:** A cross-sectional analysis was conducted on 93 colorectal adenocarcinoma samples. Tumor budding was assessed histologically, and its association with histopathological grade, lymphovascular invasion, and lymph node metastasis was evaluated using Chi-square and Mann-Whitney tests. **Results:** Of the 93 cases, tumor budding showed a statistically significant association with lymphovascular invasion ($p = 0.003$). However, no significant relationship was found between tumor budding and histopathological grade ($p = 0.685$) or lymph node metastasis ($p = 0.092$). A strong correlation was noted between lymphovascular invasion and lymph node metastasis ($p < 0.001$). **Conclusion:** Tumor budding is significantly associated with lymphovascular invasion in colorectal adenocarcinoma and may serve as a useful prognostic marker. However, its association with histopathological grade and lymph node metastasis was not statistically significant in this study.

Introduction

Colorectal cancer is one of the most common cancers worldwide[1]. According to Globocan data in 2022, colorectal cancer ranks third in terms of incidence and second in terms of mortality[1,2]. Both the incidence and mortality rates of colorectal cancer continue to rise each year[1]. This increase is caused by various factors, including lifestyle, obesity, alcohol consumption, environmental exposure, physical activity, and others[2–4].

The prognosis of colorectal cancer patients is influenced by several factors, including medical history, history of chronic inflammatory bowel disease, tumor location, histopathological grading, the degree of tumor-infiltrating lymphocytes (TILs), tumor budding, lymphovascular invasion, and metastatic status (either lymph node metastasis or distant organ metastasis) [5–7]. The most common type of colorectal cancer is colorectal adenocarcinoma[8,9]. According to the 5th edition of the WHO classification in 2019, histopathological grading of colorectal adenocarcinoma is based on gland formation, where it is categorized as low grade if gland formation is more than 50%, and high grade if gland formation is less than 50%[9].

One of the prognostic factors of colorectal adenocarcinoma that is currently widely studied is the presence of tumor budding[10,11]. Tumor budding is defined as a single cell or a small cluster of cells located at the invasive front of the tumor[11–13]. A standardized reporting system for tumor budding has been

developed by the International Tumor Budding Consensus Conference (ITBCC) 2017, which classifies tumor budding into three grades: low grade (0–4 buds/0.785 mm²), intermediate grade (5–9 buds/0.785 mm²), and high grade (≥10 buds/0.785 mm²)[10,12,14.] Tumor budding is associated with epithelial-mesenchymal transition (EMT) [12]. EMT is a complex process characterized by morphological and functional changes of epithelial cells into mesenchymal cells, marked by the loss of cell-cell adhesion (indicated by decreased expression of E-cadherin, occludin, claudin, and cytokeratin)[14,15]. And increased expression of N-cadherin, vimentin, fibronectin, and smooth muscle actin. Additionally, there is a shift in cell polarity from apico-basolateral to anterior-posterior[12]. Other features of EMT include cytoskeletal remodeling, the formation of spindle-shaped cells and podia, and degradation of the extracellular matrix by matrix metalloproteinases (MMP7 and MMP9) [12,15]. This entire process enhances tumor cell motility and facilitates tumor cell migration, invasion, and metastasis to both regional lymph nodes and distant organs[12,15].

This study aims to analyze the relationship between tumor budding and histopathological grading, lymphovascular invasion, and lymph node metastasis in colorectal adenocarcinoma.

Materials and Methods

Research Design

This study employed an analytical observational approach with a cross-sectional design to examine the association between tumor budding and several clinicopathological parameters in colorectal adenocarcinoma. Specifically, it aimed to investigate the relationship between tumor budding and histopathological grading, lymphovascular invasion, and lymph node metastasis. Data were collected from histopathological examination reports and tissue slide analyses of colorectal adenocarcinoma cases. Tumor budding was assessed based on standardized criteria, while grading, lymphovascular invasion, and lymph node involvement were determined by pathologists. This study provides insight into the potential role of tumor budding as a prognostic marker in colorectal cancer progression.

Sample

A total of 93 samples were examined, collected from January 2021 to May 2024. Samples were obtained from the Anatomical Pathology Laboratory at Dr. Wahidin Sudirohusodo Central General Hospital, Hasanuddin University Teaching Hospital, and the Diagnostic Center for Pathology in Makassar.

Inclusion Criteria

Samples included paraffin-embedded tissue blocks of colon and/or rectal tumors containing lymph node tissue, with or without lymphovascular invasion, and with or without lymph node metastasis. These samples were previously sent to the Anatomical Pathology Laboratory of Dr. Wahidin Sudirohusodo Central General Hospital, Hasanuddin University Hospital, and the Diagnostic Center for Pathology Makassar.

Data Collection Techniques

Histopathological examination was performed on all tissue samples to assess tumor budding, histopathological grade, presence of lymphovascular invasion, and lymph node metastasis.

Data Analysis Techniques

Statistical analysis was conducted using SPSS software version 27. Data were presented univariately in the form of frequency tables and clinicopathological characteristic distributions. Bivariate analysis was used to examine the relationship between tumor budding and histopathological grading, lymphovascular invasion, and lymph node metastasis, using

Chi-square and Mann-Whitney tests. A p-value of less than 0.05 was considered statistically significant.

Ethical Consideration

This study utilized archived tissue samples and data obtained from pathology laboratories with no direct patient interaction. Therefore, it did not involve new interventions or personal data collection from patients. All procedures complied with ethical standards regarding the use of existing clinical specimens for research purposes, and approval was obtained from the relevant institutional review boards or ethics committees as required.

Results

Characteristics of Colorectal Adenocarcinoma Samples

Table 1 shows the distribution of colorectal adenocarcinoma samples based on age, gender, tumor location, histopathological grading, TILs grading, depth of invasion (pT), tumor budding, lymphovascular invasion, and lymph node metastasis. Based on Table 1, colorectal adenocarcinoma cases were more commonly found in patients over 50 years old, totaling 66 samples (71.0%). Regarding gender, colorectal adenocarcinoma was more frequently found in males with 52 samples (55.9%) compared to females, who had 41 samples (44.1%).

The prevalence of low-grade colorectal adenocarcinoma (80.6%) was higher than high-grade colorectal adenocarcinoma (19.4%). Based on TILs grading, intermediate-grade TILs were found in 50.5% of cases, followed by low-grade TILs (32.3%) and high-grade TILs (17.2%). Low-grade tumor budding was found in 9 samples (9.7%), intermediate-grade in 31 samples (33.3%), and high-grade tumor budding in 53 samples (57.0%). Regarding depth of invasion, the most common was pT2 (54.84%), followed by pT3 (41.93%) and pT1 (3.23%). For lymphovascular invasion, 28 samples (30.1%) showed lymphovascular invasion, while 65 samples (69.1%) did not. The group with lymph node metastasis consisted of 32 samples (34.4%), and the group without lymph node metastasis consisted of 61 samples (65.6%).

Table 1. Characteristics of Colorectal Adenocarcinoma Samples

Characteristic	n (%)
Age (Years)	
< 50	27 (29.0)
≥ 50	66 (71.0)
Gender	
Male	52 (55.9)
Female	41 (44.1)
Tumor Location	
Proximal	24 (25.8)
Distal	35 (37.6)
Rectum	27 (29.0)
Rectosigmoid	7 (7.5)
Histopathological Grading	
Low	75 (80.6)
High	18 (19.4)
Tumor Infiltrating Lymphocytes (TILs)	
Low	30 (32.3)
Intermediate	47 (50.5)
High	16 (17.2)
Tumor Budding	
Low	9 (9.7)
Intermediate	31 (33.3)
High	53 (57.0)
Lymphovascular Invasion	
Positive	28 (30.1)
Negative	65 (69.1)
Lymph Node Metastasis	
Positive	32 (34.4)
Negative	61 (65.6)
Depth of Invasion (pT)	
pTis	0 (0)
pT1	3 (3.23)
pT2	51 (54.84)
pT3	39 (41.93)
pT4	0 (0)
Total	93

Tumor budding can be identified histologically at the invasive front of the tumor and is classified based on the number of budding foci observed in a defined microscopic field. This classification is crucial for assessing the aggressiveness of colorectal adenocarcinoma. The following figure illustrates the different grades of tumor budding observed in hematoxylin and eosin (H&E) stained sections.

We can see an image of tumor budding in Figure 1 below.:

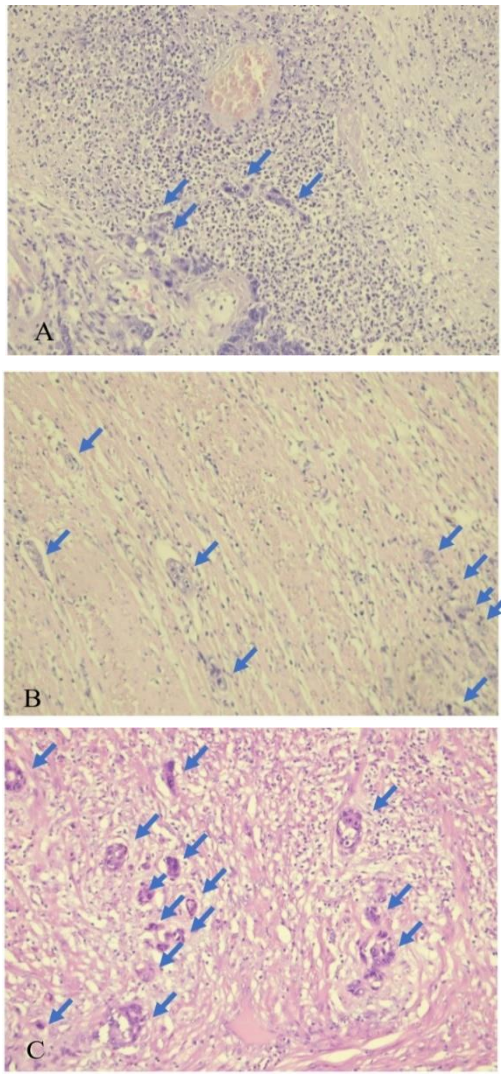

Figure 1. A. Low grade tumor budding, B. Tumor budding intermediate grade; C. High grade budding tumor

Relationship between Tumor Budding and Histopathological Grading, Lymphovascular Invasion, and Lymph Node Metastasis in Colorectal Adenocarcinoma

Based on the Mann-Whitney test (Table 2), there is no statistically significant difference in tumor budding scores between low-grade and high-grade histopathological groups (p = 0.685). However, there is a significant difference in tumor budding scores between groups with and without lymphovascular invasion (p = 0.003), with higher tumor budding scores in the lymphovascular invasion-positive group. For lymph node metastasis, there is no statistically significant difference in tumor budding scores between groups with and without metastasis (p = 0.092).

Table 2. Relationship Between Tumor Budding and Histopathological Grading, Lymphovascular Invasion, and Lymph Node Metastasis in Colorectal Adenocarcinoma

Variable	Category	n	Tumor Budding Score (Mean ± SD)	p-value
Histopathological Grading	Low Grade	75	9.83 ± 4.22	0.685
	High Grade	18	10.06 ± 3.49	
Lymphovascular Invasion	Negative	65	9.06 ± 3.89	0.003*
	Positive	28	11.75 ± 3.91	
Lymph Node Metastasis	Negative	61	9.26 ± 3.84	0.092
	Positive	32	11.03 ± 4.30	
Total		93		

**Mann-Whitney test; *p < 0.05 indicates statistical significance*

Table 3. Relationship Between Lymphovascular Invasion and Lymph Node Metastasis in Colorectal Adenocarcinoma

Lymphovascular Invasion	Lymph Node Metastasis Negative	Lymph Node Metastasis Positive	Total	p-value
Negative	52 (80.0%)	13 (20.0%)	65	<0.001*
Positive	9 (32.1%)	19 (67.9%)	28	
Total	61	32	93	

**Chi-square test; *p < 0.05 indicates statistical significance*

The Chi-square test (Table 3) shows a significant association between lymphovascular invasion and lymph node metastasis (p < 0.001). Patients with lymphovascular invasion are more likely to have lymph node metastasis compared to those without lymphovascular invasion.

Discussion

Cancer is a condition characterized by progressive and uncontrolled cell growth[16]. Colorectal cancer is the most common type of gastrointestinal cancer and contributes significantly to cancer-related mortality

worldwide[1]. The high mortality rate is due to a high rate of recurrence, metastasis, and resistance to previous treatments[17,18]. Several factors have been studied and reported to influence the prognosis of patients with colorectal adenocarcinoma, one of which is tumor budding.[11,12].

In this study, we analyzed the relationship between tumor budding and histopathological grading, lymphovascular invasion, and lymph node metastasis in colorectal adenocarcinoma. Based on histopathological grading, our results showed no significant difference in tumor budding scores between low-grade and high-grade colorectal adenocarcinomas ($p = 0.685$). We also analyzed the relationship between tumor budding and lymphovascular invasion. Our findings revealed a significant difference in tumor budding scores between the sample group with lymphovascular invasion and the group without lymphovascular invasion ($p = 0.003$). These results are consistent with the study conducted by Ozer et al. (2019) [19].

The epithelial-mesenchymal transition (EMT) process[12] is a mechanism that facilitates tumor cell dissemination and invasion, both into surrounding tissues and into blood or lymphatic vessels, ultimately leading to metastasis to regional lymph nodes or distant organs. EMT begins with the reduction of intercellular adhesion among tumor cells. The loss of these cell-to-cell adhesions allows tumor cells to detach from the primary tumor mass. This is followed by morphological changes where tumor cells become more spindle-shaped, accompanied by cytoskeletal remodeling and the formation of lamellipodia, which enhances their motility[15]. Additionally, tumor cells undergoing EMT release matrix metalloproteinase enzymes, which degrade the extracellular matrix and create pathways for tumor cell migration[12,15,20]. Ultimately, the EMT process—histologically marked by tumor budding—facilitates tumor cells in invading lymphovascular structures and eventually metastasizing.

We also analyzed the relationship between tumor budding and lymph node metastasis. Our results showed no significant difference in tumor budding scores between the sample group with lymph node metastasis and the group without lymph node metastasis ($p = 0.092$). Furthermore, we analyzed the relationship between lymphovascular invasion and lymph node metastasis. Our findings demonstrated a significant association between lymphovascular invasion and lymph node metastasis ($p < 0.001$). These results indicate that tumor budding is associated with lymphovascular invasion, and lymphovascular invasion is associated with lymph node metastasis in colorectal adenocarcinoma.

Conclusion

There is a significant relationship between tumor budding scores and lymphovascular invasion, as well as a significant association between lymphovascular invasion and lymph node metastasis. These findings suggest that tumor budding can be an important prognostic factor in colorectal adenocarcinoma.

For future research, it is recommended to use tumor budding markers such as Twist, Snail, and ZEB through immunohistochemical analysis to assess tumor budding in colorectal adenocarcinoma.

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