A clinicopathological study of Tumor budding in invasive breast cancer

Reshma K V, Hemalatha J, Preeta Naik, Sowmya T.S, Purushotham Ramaiah
Department of Pathology, Hassan Institute of Medical Science, Hassan, Karnataka, India

Abstract

Background: Tumor budding is defined as a small cluster of tumor cells located at the invasive edge of tumor and was assumed to be linked with epithelial-mesenchymal transition (EMT), which is an early event in metastasis.

Objective: This study aimed to evaluate clinicopathologic significance of tumor budding in carcinoma breast and to correlate with other clinicopathological prognostic parameters of breast cancer.

Method: It was a retrospective study consisting of 60 resected specimens of primary breast carcinoma. The number of foci (tumor budding) was counted in Haematoxylin & Eosin slides under 20x magnification and were categorised into low and high tumor budding. Subsequently tumor budding association with other clinicopathological parameters were studied. Chai square test was performed to find the association between tumor budding and each of the clinicopathologic variables and p value of less than 0.05 was considered statistically significant

Result: Cases were categorized as low (0-10 buds) and high (≥10 buds) tumor budding groups based on the count of foci. High tumor budding shares significant positive association with lymph nodal status, necrosis and adipocyte infiltration and does not show association with tumor grade, tumor size, lymphovascular invasion, perineural invasion, calcification, type of carcinoma and insitu component.

Conclusion: In conclusion, tumor budding in carcinoma breast is associated with undesirable pathologic factors, such as positive nodal status, adipocyte infiltration and necrosis. In the future, standardized quantification criteria for tumor budding may further aid in its implementation as a prognostic marker.

Keywords: Tumor budding, breast cancer, lymph nodal status.

Introduction

Tumor budding is a pathologic phenomenon associated with various malignancies. It is made up of a few cells, typically five or six, that have separated from the main tumor bulk. Tumor budding has been observed and researched in a number of malignancies, including head and neck, lung, gastric and esophageal, colorectal, and breast cancers[1]. Peritumoral buds, which are found close to the margins of invasive tumors, and intratumoral buds, which are found inside the tumor mass, are two locations where tumor buds can be found[2]. High tumor bud counts are linked to lymphovascular invasion (LVI) and/or lymph node metastases in breast cancer (BC) and other malignancies[3]. Furthermore, in BC, a shorter overall and cancer-specific survival is associated with greater tumor bud counts[4]. Due to the correlation between lymphovascular invasion and tumor budding, it was hypothesized that tumorbuds undergo epithelial-mesenchymal transition (EMT), which plays a role in the early stages of the metastatic process[5]. It is commonly known that tumor cells going through epithelial-mesenchymal transition (EMT) are more invasive and likely to metastasize, which can lower cancer patients' overall survival rates[6].

Minimizing tumor cells involved in the early metastatic process would be of immense clinical value since metastatic disease remains the leading cause of deaths with around 30% of BC patients developing metastasis[7]. Therefore, an improved understanding of the metastatic process is required to develop novel targeted treatments for extremely aggressive and invasive cancer. It would be beneficial to ascertain whether and how tumor buds can be specifically targeted if they are implicated in the first stages of the metastatic process.

Address for Correspondence:

Dr. Hemalatha J
Department of Pathology,
Hassan Institute of Medical Science, Hassan, Karnataka, India.
Email: drhemalathaj@gmail.com

From a pathophysiologic perspective, tumor budding has been interpreted as a marker of cancer cell motility and as the initial stage of metastasis[1], leading one to believe that the tumor is made up of cancer cells that have invaded. The first steps in the metastatic process are the detachment of cells from the bulk of the tumor, their infiltration into small blood vessels through adjacent tissues, and their route through the circulation to distant sites where they spread and may eventually form colonies of metastatic disease.

The processes of epithelial to mesenchymal transition (EMT) and mesenchymal to epithelial transition (MET) in reverse are crucial to metastasis[4]. Together, these processes—known as epithelial mesenchymal plasticity—are essential to physiologic wound healing and normal embryogenesis, but cancer has usurped them. During epithelial-mesenchymal transition (EMT), detached cancer cells either totally or partially lose their epithelial properties. They then separate from neighbouring epithelial cells and express acquire mesenchymal properties, such as the production of mesenchyme-associated proteins, to become motile. The reverse process takes place in metastatic sites when newly arrived cells regain epithelial characteristics and re-establish connections with neighbouring cells, helped by cues in their new microenvironment[5].

Over the last few years, numerous publications have shown the importance of tumor budding as an independent predictor of lymph node positivity, local and distant relapse, lymphatic invasion, and poor prognosis among patients with Colorectal carcinoma of all pathological stages. However, the definite implementation of tumor budding into clinical practice is currently limited by the lack of an internationally standardized scoring system. Whether tumor budding can be considered a histomorphologic feature in Breast cancer remains to be elucidated. Therefore, a study on the clinical and prognostic role of TB in Breast cancer is needed for its prognostic value in appropriate management of patients with Breast cancer. Few studies have been conducted on significance of tumor budding in breast carcinoma. However, the present study has included many clinicopathological parameters like adipocyte infiltration and leukocyte infiltration, which were not evaluated in any of the previous studies. The current study has correlated tumor budding with maximum number of clinicopathological parameters which give an extensive detail than previous studies conducted on tumor budding in breast carcinoma. The aim of the current study is to document the number of tumor buds at the invasive front of breast cancer and dividing them into low and high grade and to correlate these tumor buds with other clinicopathological parameters like age, histological type, tumor size, grade (modified Bloom–Richardson), presence of necrosis, in situ component, calcification, leukocyte infiltration, type of margins, lymphovascular invasion, perineural invasion, adipocyte infiltration and lymph node status

Materials and Methods

The present study being a retrospective study was conducted in the Department of Pathology, Hassan institute of Medical sciences (HIMS). Haematoxylin & Eosin stained slides of all mastectomy cases during the study period of 2 years were retrieved. All surgically resected mastectomy cases were included in the study. Small biopsies and lumpectomy cases were not included. Slides with tumor and adjacent breast tissue were used for counting tumor budding. Tumor budding was counted at the invasive margin using X20 objective. Cases were separated into two groups according to tumor budding density per X20 field as low grade (<10) and high grade (≥ 10). This tumor budding was correlated with clinicopathological characteristics such as age, histological type, tumor size, grade (modified Bloom–Richardson), presence of necrosis, in situ component, calcification, leukocyte infiltration, type of margins, lymphovascular invasion, perineural invasion, adipocyte infiltration and lymph node status. Data was entered in MS Excel sheet and was compiled, tabulated, interpreted, and analyzed. Association between tumor budding and each variable were analysed by chi square test. The socio-demographic variables were represented using frequencies and percentages. The data analysis of association between the tumor budding and histological parameters and available clinical details were done by Chi-square test. All statistical calculations were done through statistical software STATA version 14.1

Results

In the present study, 60 cases of invasive breast carcinoma were studied. The lesions were seen in females in the range of 36–77 years. Clustering of cases was seen in the fifth and sixth decades. Invasive breast carcinomas are more commonly involved in left breast [27 cases (54%)] than right breast [23 cases (46%)]. The most common site of involvement was upper outer quadrant [29 cases (48.3%), followed by lower outer quadrant [13 cases (21.7%)], upper inner quadrant [11 cases (18.4%)], central quadrant [4 cases (6.7%)] and lower inner quadrant [3 cases (5%)].

Table 1: Distribution of clinico-pathological parameters of study sample.

Feature	Observation	Frequency
Age	<45 years	6 (10%)
	>45 years	54 (90%)
Histological type	Invasive ductal carcinoma	58 (96.6%)
	Medullary carcinoma	1 (1.7%)
	Mucinous carcinoma	1 (1.7%)
Tumor size	<2cm	3 (5%)
	2-5 cm	50 (83.3%)
	>5cm	7 (11.7%)
Histological grade	Grade 1	18 (30%)
	Grade 2	37 (61.7%)
	Grade 3	5 (8.3%)
Necrosis	Present	28 (46.7%)
	Absent	32 (53.3%)
Insitu component	Present	49 (81.7%)
	Absent	11 (18.3%)
Calcification	Present	12 (20%)
	Absent	48 (80%)
Leucocyte infiltration	Present	52 (86.7%)
	Absent	8 (13.3%)
Type of margins	Infiltrating	59 (98.3%)
	Pushing borders	1 (1.7%)
Lymphovascular invasion	Present	46 (76.7%)
	Absent	14 (23.3%)
Perineural invasion	Present	4 (6.7%)
	Absent	56 (93.3%)
Adipocyte infiltration	Present	31 (51.7%)
	Absent	29 (48.3%)
Lymph node status	Lymph nodes involved by tumor	38 (63.3%)
	Lymph nodes not involved by tumor	22 (36.7%)

Invasive carcinoma of no special type (Ductal carcinoma NST) was the most common histological type constituting 58 cases (96.6%), followed by invasive medullary carcinoma [1 case (1.7%)], and invasive mucinous carcinoma [1 case (1.7%)].

Majority of the lesions were of size 2-5 cm (50 cases, 83.3%) and most of them belonged to histological grade 2 (37 cases, 61.66%), followed by grade 1(18 cases, 30%). Necrosis was noted in 28 cases (28 cases, 46.66%), and calcification was seen in 12 cases (20%).

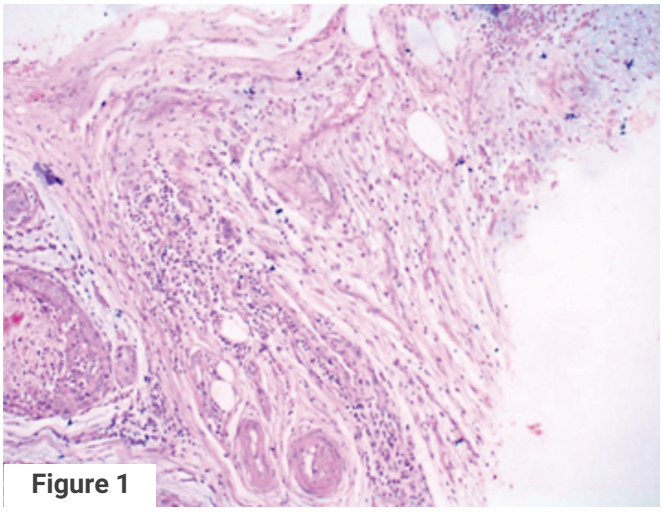
Ductal carcinoma *in situ* (DCIS) component was noted in 49 cases (81%). 46 cases (76.6%) showed lymphovascular invasion while only 4 cases (6.66%) showed perineural invasion. Adipocyte infiltration by tumor cells were noted in 31 cases (51.66%). Lymphnodes were involved by tumor cells in 38 cases (63.66%).

Tumor budding was evaluated in all the 60 cases. Tumor buds ranged in number from 4–20 tumor buds/ HPF. Tumor budding was classified into high tumor budding (Tumor buds >10/ HPF), and low tumor budding (Tumor buds ≤10/HPF). High tumor budding was seen in 26 cases (43.33%), and low tumor budding was seen in 34 cases (56.67%). Number of tumor budding observed in all categories were evaluated and tabulated. P values were calculated and significant association between each categorical variable and tumor budding were evaluated [Table 1].

Table 2: Summary of significant results and tumor budding correlation

Feature	Observation	Tumor budding		p value
		Low (<10) 34	High (>/=10) 26	
Age	<45 years	4 (50%)	2 (50%)	0.53
	>45 years	30 (55.5%)	24(44.5%)	
Histological type	Invasive ductal carcinoma	32 (55.2%)	26 (44.8%)	0.053
	Medullary carcinoma	1 (100%)	0	
	Mucinous carcinoma	1 (100%)	0	
Tumor size	<2cm	3 (100%)	0	0.088
	2-5 cm	30(60%)	20 (40%)	
	>5cm	1 (14.3%)	6 (85.7%)	
Histological grade	Grade 1	9 (50%)	9 (50%)	0.287
	Grade 2	22 (59.4%)	15 (40.6%)	
	Grade 3	3 (60%)	2 (40%)	
Necrosis	Present	11 (39.3%)	17 (60.7%)	0.012
	Absent	23 (71.9%)	9 (28.1%)	

Insitu component	Present	29 (59.2%)	20 (40.2%)	0.327
	Absent	5 (45.4%)	6 (54.6%)	
Calcification	Present	8 (66.7%)	4 (33.3%)	0.654
	Absent	26 (54.2%)	22 (45.8%)	
Leukocyte infiltration	Present	28 (53.8%)	24 (46.2%)	0.349
	Absent	6 (75%)	2 (25%)	
Type of margins	Infiltrating	33 (55.9%)	26 (44.1%)	0.698
	Pushing borders	1 (100%)	0 (0%)	
Lymphovascular invasion	Present	21 (45.6%)	25 (54.4%)	0.142
	Absent	13 (92.8%)	1 (7.2%)	
Perineural invasion	Present	2 (50%)	2 (50%)	0.284
	Absent	32 (57.1%)	24 (42.9%)	
Adipocyte infiltration	Present	13 (41.9%)	18 (58.1%)	0.002
	Absent	21 (72.4%)	8 (27.6%)	
Lymph node status	Lymph nodes involved by tumor	14 (36.8%)	24 (63.2%)	0.038
	Lymph nodes not involved by tumor	20 (90.9%)	2 (9.1%)	



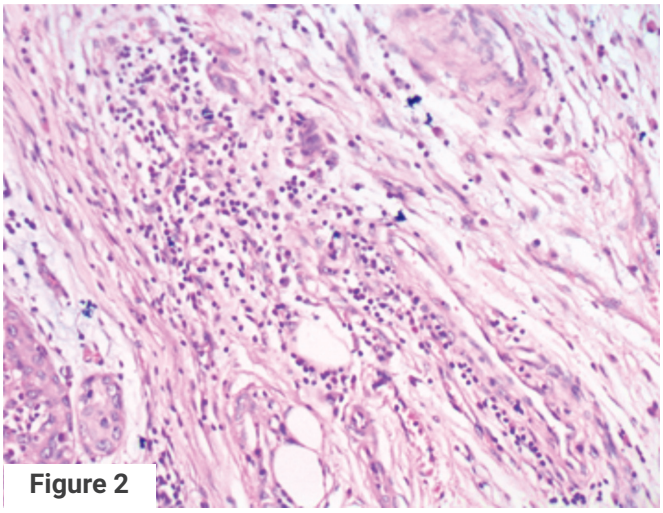


Figure 1: Low tumor budding at the invasive front, H&E stained section in 20X (Figure 1A) and 40x (Figure 1B)

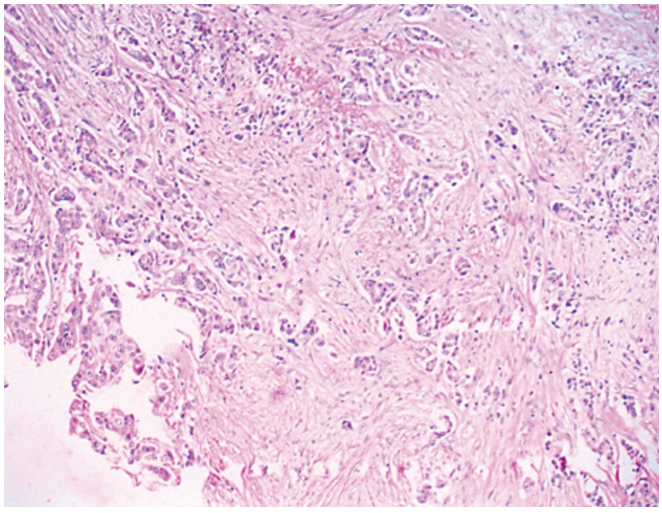


Figure 2: High tumor budding in invasive breast carcinoma, H&E stained section, 20x

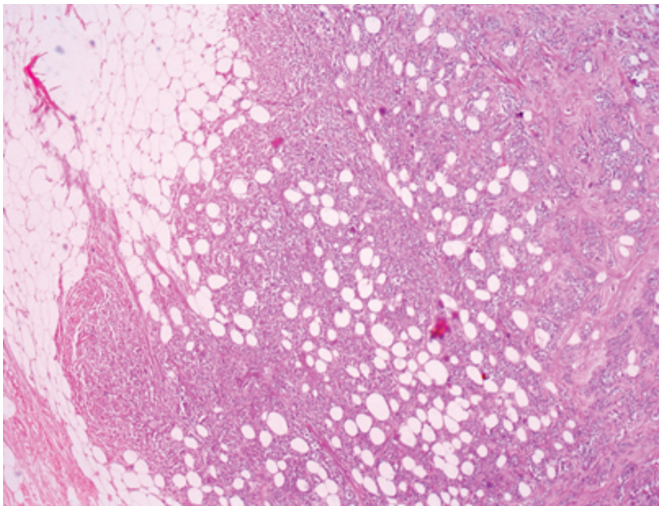


Figure 3: Adipocyte infiltration by tumor cells in breast carcinoma, H&E stained section, 20x

The association of high tumor budding with lymph node metastasis (LN METS), necrosis (NEC), and adipocyte infiltration was highly significant. Whereas, the association of high tumor budding with parameters like age, histological type, tumor size, histological grade, Insitu component, calcification, leukocyte infiltration, type of margin, lymphovascular invasion and perineural invasion was statistically not significant [Table 2].

Discussion

It is becoming more widely acknowledged that tumor budding is a significant negative prognostic factor[3]. Prognostic significance of tumor budding has mainly been examined in the field of colorectal cancer and several solid organs[5,6]. Breast cancer represents extremely diverse lesions with a wide range of morphological types[7,8] . The purpose of the current study is to assess the role of tumor budding in invasive breast cancer.

Sriwidyani NP et al.[9] reported case clustering in the fifth decade, which is comparable to the current study. The majority of the research only looked at invasive breast cancer—NOS. On the other hand, different histological forms of invasive breast cancer were included in this study [Table 1]. According to Man YG, the invasion mechanism of all breast cancer subtypes is probably the same: tumor cell budding from a focally disrupted tumor capsule. Double immunostained sections were used by the author to illustrate the results[7].

To count the tumor buds, Salhia et al.[8] and Sriwidyani NP et al.[9] employed X40 (high power) objective. Liang F et al.[5], Gujam FJA et al.[6], and the current investigation, on the other hand, used the X20 objective. In 20X objective on H and E stained sections, some authors have reported having trouble distinguishing tumor buds from their mimics [fibroblasts or inflammatory cells]. To get around this practical issue, Liang F et al.[5] employed IHC-stained slides.

Tumor buds were counted in five different fields by Salhia et al.[8], Gujam FJA et al.[6], Sriwidyani NP et al.[9], and Liang F et al.[5]. Different cut-off levels have been employed in different investigations. This is due to the fact that several researchers counted tumor buds using different techniques.

Tumor buds were counted on sections stained with H and E by Gujam FJA et al.[6] and the current study. However tumor buds were counted on IHC-stained sections by Salhia et al.[8] and Sriwidyani NP et al.[9]. Both of these were used by Liang F et al.[5]. It might be suggested that tumor buds should be regularly counted in sections stained with H and E, and that IHC should only be used in specific situations when it would be challenging to differentiate between tumor buds and their mimics; and the number of tumor buds is borderline, or close to the cut-off range, since a high number of tumor buds indicates a strong correlation with a number of histological criteria.[10,11]. IHC might be best avoided in situations where the initial H and E stained sections clearly demonstrate high tumor budding. IHC should therefore be used selectively[12,13].

It is evident that many researchers have evaluated the tumor buds using various techniques. As a result, there was a difference in the cut-off value, the number of fields counted, the objective's power for counting, the stain used to evaluate the tumor buds, and the range of tumor buds. To achieve uniformity in assessment, it is necessary to standardize the criteria used to evaluate the tumor buds.

Several studies found a substantial correlation between increased tumor budding and lymph node metastasis and lymphovascular invasion.[14,15] In the current study, there was no significant correlation between tumor budding and lymphovascular invasion, but there was a strong correlation with necrosis, adipocyte infiltration, and lymph node metastases. The initial stage of invasion and metastasis was thought to be TB at the invasive front[16]. This explains why tumor budding was linked in this study to lymph node metastasis as well as necrosis and adipocyte infiltration.

Studies by Liang F et al.[5] and Sriwidyani NP et al.[9] indicated a significant correlation between high tumor budding and primary tumor stage, but the current study found no such correlation. Both the current investigation and research by Salhia et al.[8] found a strong correlation between high tumor budding and regional lymph node staging. Both the current study and others by Gujam FJA et al.[6] and Liang F et al.[5] found no significant correlation between age group distribution and high tumor budding. The current study and those by Gujam FJA et al.[6], Liang F et al.[5], and others did not find a significant correlation between high tumor budding and overall histologic grade. On the other hand, it was significant in Sriwidyani NP's study.[9]

None of the studies have tried to find any association between adipocyte infiltration and high tumor budding in the literature. In the present study we have found a significant association between high tumor budding and adipocyte infiltration by the tumor cells in breast carcinoma, which add on the existing data on tumor budding in breast carcinoma. Adipocytes are a major component of breast tissue, and cancer-associated adipocytes (CAAs) are a key part of the tumor microenvironment. CAAs promote breast cancer progression by secreting cytokines and adipokines

that remodel the extracellular matrix and change the tumor immune microenvironment. CAAs also interact with breast cancer cells and immune cells.

Kumarguru et al[17] found most of the patients with grade 3 tumor budding had both lymph node metastasis and necrosis. Necrosis is a type of cell death brought on by external factors such as hypoxia. It frequently occurs in conjunction with aggressive, quickly spreading cancers. In the present study also we have found a significant association between tumor budding and necrosis which is a marker of poor prognosis in breast cancer.

From a biological perspective, the epithelial–mesenchymal transition is intimately associated with tumor budding[3,10]. The term "epithelial-mesenchymal transition" (EMT) describes the process by which epithelial cells transform into mesenchymal cells. Cancer stem cell behaviour, induced pluripotency, and embryonic stem cell differentiation are all regulated by EMT and mesenchymal-epithelial transition (MET). EMT causes epithelial cells to remodel their cytoskeleton, lose their junctions and apical-basal polarity, and modify how their genes are expressed. This promotes individual cell motility and permits the emergence of an invasive phenotype.[18,19]

Key transcription factors, including Snail homolog 1 (SNAIL), Zinc-finger E-box binding (ZEB), and basic helix-loop-helix transcription factors, mediate the change in cell differentiation and behaviour. Their functions are finely regulated at the transcriptional, translational, and post translational levels. An important mechanism of cancer invasion and metastasis is tumor budding.[20] Transforming growth factor family signalling is a major factor in EMT.

Conclusion

In summary, we found that tumor buds were linked to a poor prognosis for BC. More standardisation is needed to identify tumor buds, frame scoring parameters, and establish cut-off criteria based on sensitivity and specificity before tumor budding may be included in the histopathological report. According to the data, tumor budding may increase the likelihood of metastasis and is linked to an aggressive tumor phenotype. The results of the current study demonstrated the potential benefit of tumor budding in BC for improving prognosis and call for more research.

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References

1. *Malvia S, Bagadi SA, Dubey US, Saxena S. Epidemiology of breast cancer in Indian women: Breast cancer epidemiology. Asia-Pac J Clin Oncol 2017;13:289-95.*
2. *Mohapatra M, Sathyanarayana S. Evaluation of clinico: Patholoical findings of breast carcinoma in a general hospital in Southern India. Indian J Cancer 2013;50:297-301.*
3. *Prall F. Tumor budding in colorectal carcinoma. Histopathology 2007;50:151-62.*
4. *Lakhani SR, Ellis IO, Schnitt SJ, Tan PH, van de Vijver MJ. WHO Classification of Tumors of the Breast. Lyon, France: IARC Press; 2007. p. 8-11.*
5. *Liang F, Cao W, Wang Y, Li L, Zhang G, Wang Z. The prognostic value of tumor budding in invasive breast cancer. Pathol Res Pract 2013;209:269-75.*
6. *Gujam FJA, McMillan DC, Mohammed ZMA, Edwards J, Going JJ. The relationship between tumor budding, the tumor microenvironment and survival in patients with invasive ductal breast cancer. Br J Cancer 2015;113:1066-74.*
7. *Man Y. Tumor cell budding from focally disrupted capsules: A common pathway for all breast cancer subtype derived invasion? J Cancer 2010;1:32-7.*
8. *Salhia B, Trippel M, Pfaltz K, Cihoric N, Grogg A, Ladrach C, et al. High tumor budding stratifies breast cancer with metastatic properties. Breast Cancer Res Treat 2015;150:363-71.*
9. *Sriwidyani NP, Alith-Artha G, Mantik-Astawa N, Manuaba IBTW. Tumor budding in breast carcinoma: Relation to E-Cadherin, MMP-9 expression, and metastatic risk. Bali Med J 2016;5:497-501.*
10. *Mitrovic B, Schaeffer DF, Riddell RH, Kirsch R. Tumor budding in colorectal carcinoma: Time to take notice. Mod Pathol 2012;25:1315-25.*
11. *Laedrach C, Salhia B, Cihoric N, Zlobec I, Tapia C. Immunophenotypic profile of tumor buds in breast cancer. Pathol Pract. 2018; 214(1):25-9.*
12. *Centeno I, Sohns AP, Flury M, Galván JA, Zahnd S, Koelzer VH et al. DNA profiling of tumor buds in colorectal cancer indicates that they have the same mutation profile as the tumor from which they derive. Virchows Arch. 2017; 470(3):341-6.*
13. *Dawson H, Lugli A. Molecular and pathogenetic aspects of tumor budding in colorectal cancer. Front Med. 2015; 2:11.*
14. *Karamitopoulou E, Zlobec I, Born D, Kondi-Pafiti A, Lykoudis P, Mellou A et al. Tumour budding is a strong and independent prognostic factor in pancreatic cancer. Eur J Cancer. 2013; 49(5):1032-9.*
15. *Lugli A, Kirsch R, Ajioka Y, Bosman F, Cathomas G, Dawson H et al. Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016. Mod Pathol. 2017; 30(9):1299.*
16. *Mehlen P, Puisieux A. Metastasis: a question of life or death. Nat Rev cancer. 2006; 6(6):449.*
17. *Kumarguru BN, Ramaswamy AS, Shaik S, Karri A, Srinivas VS, Prashant BM. Tumor budding in invasive breast cancer-An indispensable budding touchstone. Indian Journal of Pathology and Microbiology. 2020 Feb 1;63(Suppl 1):S117-22.*
18. *Heerboth S, Housman G, Leary M, Longacre M, Byler S, Lapinska K et al. EMT and tumor metastasis. Clin Transl Med. 2015; 4(1):6.*
19. *Grigore A, Jolly M, Jia D, Farach-Carson M, Levine H. Tumor budding: the name is EMT. Partial EMT. J Clin Med. 2016; 5(5):51.*
20. *Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol 2014;15:178-96.*

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