

# TUMOR BUDDING IN BREAST CARCINOMA- AS A POTENTIAL NOVEL PROGNOSTIC MARKER

## Abstract

Tumor budding (TB) is a pathological phenomenon observed in various solid malignancies. It has gained significant attention as a promising prognostic biomarker in solid malignancies like colorectal carcinoma. TB has also been investigated as a prognostic marker in other malignancies, such as gastric, esophageal, pancreatic, and urinary bladder cancers. In these cancers, TB has shown promise as a novel prognostic marker that can provide independent prognostic information, irrespective of tumor grade and stage. However, it's important to note that the role of tumor budding in breast cancer is still not confirmed. While TB has been extensively studied in various malignancies, its significance in breast cancer prognosis and management has not been firmly established. Further research is needed to determine the potential role of TB as a prognostic marker in breast cancer. Overall, tumor budding has emerged as an important feature in the assessment of CRC and other solid malignancies. Its inclusion in grading systems and prognostic classifications highlights its relevance in predicting tumor behavior and patient outcomes. Ongoing research may shed light on the potential role of tumor budding in breast cancer and further refine its clinical utility in various malignancies.

**Keywords:** Tumor budding, cancer, Pathophysiology

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## I. INTRODUCTION

Breast cancer is indeed a significant global health concern and is responsible for a substantial number of cancer-related deaths. It is the most commonly diagnosed cancer and ranks fifth in terms of cancer-related deaths worldwide, with approximately 2.3 million new cases and 685,000 deaths reported in the GLOBOCAN 2020 data. Breast cancer is a complex and heterogeneous disease which means it can manifest in various forms with different biological behaviors and clinicopathological features. This heterogeneity leads to significant variations in prognosis among patients. The molecular and histological subtypes of breast cancer further contribute to this diversity. Understanding these subtypes is essential for tailoring treatment approaches and predicting outcomes accurately. Metastatic potential, which refers to the ability of cancer cells to spread from the primary tumor to other parts of the body, plays a crucial role in determining the aggressiveness of breast cancer and its prognosis. Despite the favorable overall survival rates for breast cancer, the recurrence rate remains relatively high, with more than 40% of patients experiencing recurrence within fifteen years after initial treatment.

To improve the management and prognosis of breast cancer, it is crucial to identify and assess various prognostic markers. These markers can provide valuable information about the behavior of the tumor, allowing healthcare professionals to make informed decisions regarding treatment strategies. Additionally, standardizing these markers across different settings and populations is essential for consistency in evaluating prognosis and selecting appropriate therapeutic approaches. In summary, breast cancer is a prevalent and significant cause of cancer-related deaths globally. Its complex nature, characterized by molecular and histological subtypes, makes it challenging to predict outcomes accurately. Understanding the prognostic markers associated with breast cancer and establishing standardized approaches for their assessment are essential for better management, prognosis, and targeted therapeutic interventions.

## II. TUMOR BUDDING

The concept of TB was first introduced by Morodomi in 1989, where it was defined as a collection of isolated malignant cells without a distinct structure. The term "tumor budding" was used because the tumor cells appeared to bud out from the larger malignant mass. In the Japanese classification, TB was defined as an isolated single cell or a cluster of fewer than five cells at the invasive front of the tumor. This definition focused on the location of the buds within the tumor. The International Tumor Budding Consensus Conference (ITBCC) later established a uniform and evidence-based scoring system specifically for colorectal cancer (CRC) TB. According to this system, a tumor bud was defined as a single cell or a cluster of up to four tumor cells.

Tumor budding (TB) is a pathological phenomenon observed in various solid malignancies and was initially described in colorectal cancer (CRC). It refers to the presence of isolated single cells or small clusters of less than five malignant cells at the invasive front of the tumor. TB has been extensively studied in several types of solid cancers, including head and neck, lung, gastric, esophageal, and colorectal cancers. There are two types of tumor buds based on their location within the tumor: peritumoral buds and intratumoral buds.

Peritumoral buds are observed near the tumor margins at the invasive tumor front, while intratumoral buds are found within the tumor mass itself.

TB has gained significant attention as a promising prognostic biomarker in solid malignancies. Numerous studies have investigated the association between TB and clinical outcomes, such as tumor aggressiveness, metastasis, and patient survival. High levels of tumor budding have been correlated with worse prognosis, including increased tumor invasiveness, lymph node involvement, distant metastasis, and reduced overall survival rates. The assessment of tumor budding requires careful evaluation of histological sections by pathologists, as it involves identifying and quantifying the presence of tumor buds. However, the standardization of scoring systems and criteria for tumor budding assessment is an ongoing challenge, as different studies may employ varied methods and thresholds.

The ITBCC scoring system utilizes a three-tier grading system based on the number of tumor buds observed in a defined area at the invasive front of the tumor. The evaluation is typically performed in a field of 0.785 mm<sup>2</sup>. The scores for TB are as follows: 0-4 indicate low budding (Bd1), 5-9 indicate intermediate budding (Bd2), and 10 or more indicate high budding (Bd3). This standardized scoring system helps in assessing and categorizing the degree of tumor budding in CRC. It provides valuable information about the tumor's aggressiveness and has been associated with various clinical outcomes, including prognosis and response to treatment. It's important to note that the scoring system may vary slightly for different cancer types and further research is ongoing to establish standardized protocols for tumor budding assessment in other malignancies. Overall, the development of a consensus-based scoring system for tumor budding allows for consistent evaluation and facilitates the incorporation of TB as a prognostic marker in clinical practice.

Tumor budding was included as a major criterion in the 2019 World Health Organization (WHO) classification of colorectal cancer (CRC). The recognition of TB as an important feature in CRC grading highlights its significance in assessing tumor behavior and prognosis. Furthermore, the importance of TB in CRC prognosis was underscored by its inclusion as an additional prognostic factor in the TNM classification of CRC in 2017 and the 2019 WHO classification. This recognition indicates that TB can provide valuable prognostic information beyond traditional staging systems. In patients with pT1 CRC, TB has been identified as an independent prognosticator of lymph node metastasis. This finding emphasizes the role of TB in predicting the potential for tumor spread and can aid in treatment decision-making for early-stage CRC.

Beyond CRC, TB has also been investigated as a prognostic marker in other malignancies, such as gastric, esophageal, pancreatic, and urinary bladder cancers. In these cancers, TB has shown promise as a novel prognostic marker that can provide independent prognostic information, irrespective of tumor grade and stage. However, it's important to note that the role of tumor budding in breast cancer is still not confirmed. While TB has been extensively studied in various malignancies, its significance in breast cancer prognosis and management has not been firmly established. Further research is needed to determine the potential role of TB as a prognostic marker in breast cancer. Overall, tumor budding has emerged as an important feature in the assessment of CRC and other solid malignancies. Its inclusion in grading systems and prognostic classifications highlights its relevance in predicting tumor behavior and patient outcomes. Ongoing research may shed light on the

potential role of tumor budding in breast cancer and further refine its clinical utility in various malignancies.

### **III. PATHOPHYSIOLOGY OF TUMOR BUDDING**

Tumor budding is considered an indicator of the motility of malignant cells and is believed to be an initial step in the metastatic process. It involves the detachment of tumor cells from the primary tumor mass, infiltration into surrounding tissues, intravasation into blood vessels, dissemination through the bloodstream, extravasation at distant sites, and the establishment of metastatic deposits. Epithelial-mesenchymal transition (EMT) is a crucial process in metastasis, where epithelial cells acquire mesenchymal characteristics, allowing them to become more invasive and migratory. Conversely, mesenchymal-epithelial transition (MET) refers to the reversal of this process. Together, EMT and MET are components of epithelial-mesenchymal plasticity and are observed as normal phenomena in embryogenesis and wound healing. Tumor buds are believed to exhibit properties of cancer stem cells, which include migration and the ability to redifferentiate into various cell types.

The expression of specific markers, such as CD44 and ALDH1A1, has been observed in tumor buds, further supporting their cancer stem cell properties. These markers are associated with cell migration, invasion, and resistance to therapy. The presence of tumor buds in the tumor microenvironment can promote pro-tumor development by creating an immunosuppressive milieu that inhibits the anti-tumor immune response. High levels of TB have been linked to a low CD8+ T lymphocyte index, which is associated with a poor prognosis. Overall, tumor budding plays a crucial role in the metastatic cascade and is associated with epithelial-mesenchymal plasticity, cancer stem cell properties, and modulation of the immune response. Understanding these processes can provide insights into the aggressive behavior of tumors and guide the development of targeted therapies aimed at inhibiting metastasis and improving patient outcomes.

The association between tumor budding (TB) and EMT is closely linked to the tumor microenvironment, which is characterized by factors such as hypoxia, acidity, inflammation, and immunosuppression. The presence of these conditions in the tumor microenvironment can promote EMT and enhance the invasive and metastatic potential of cancer cells. The immune cells present in the tumor microenvironment play a significant role in the EMT process. Cytokines and chemokines secreted by immune cells can interact with cancer cells and induce cell plasticity, release immunosuppressive substances, and establish an immunosuppressive microenvironment. These interactions contribute to the promotion of invasion and metastasis.

Furthermore, tumor buds are known to overexpress stem cell markers such as ALDH1, CD44, and LGR5. These stem cell markers equip tumor buds with the ability to self-renew, contributing to their survival and growth at both primary and metastatic sites. The interdependency between tumor buds and the tumor microenvironment in the metastatic cascade highlights the complex interactions and dynamics that drive cancer progression. Understanding these relationships can provide insights into the mechanisms of tumor invasion and metastasis and may lead to the development of targeted therapies aimed at disrupting these processes and improving patient outcomes.

#### **IV. DETECTION OF TUMOR BUD**

Tumor buds can be routinely detected on hematoxylin and eosin (H&E) stained sections. However, in cases where there is ambiguity, immunohistochemical staining, such as pan-cytokeratin, can be used to confirm the presence of tumor buds. These staining techniques help to distinguish tumor buds from other structures. Studies have revealed that tumor buds exhibit lower proliferation activity compared to the bulk of the tumor. This suggests that the dissociation of tumor cells into buds is not influenced by tumor size, differentiation, or proliferation activity. Additionally, altered expression of E-cadherin and vimentin has been observed in relation to TB. High-grade TB is associated with diminished E-cadherin expression, while unusual vimentin expression is found in cases of low-grade TB.

It is important to be aware of potential mimickers of tumor buds on H & E stained sections, which can include inflammatory cells, multinucleated giant cells, fibroblasts, endothelial cells, smooth muscle cells, and artifacts. These structures can resemble tumor buds, emphasizing the need for careful evaluation and, if necessary, additional staining or immunohistochemistry to confirm the presence of true tumor buds. One of the challenges in reporting TB lies in the lack of uniformity in nomenclature and methods used to identify and describe TB. There are variations in the method of counting tumor buds, the power of the objective used, and the area of the field employed to express TB. This leads to qualitative heterogeneity in reporting TB across different studies.

To address these issues, there is a need for standardized training and protocols for counting and reporting tumor buds. Pathologists should be trained to recognize TB mimickers and differentiate them from true tumor buds. By standardizing the method of counting and reporting TB, inter-observer variability can be reduced, allowing for more accurate and consistent identification of TB in various malignancies. Overall, efforts towards standardization and training in identifying and reporting TB are necessary to improve the consistency and reliability of TB assessment, facilitating its integration as a prognostic marker in clinical practice.

#### **V. TUMOR BUDDING IN BREAST CANCER**

TB is believed to be a manifestation of the epithelial-mesenchymal transition (EMT) process, which endows tumor cells with increased invasiveness and the potential for metastasis. The presence of TB suggests a more aggressive tumor phenotype and an increased likelihood of metastasis, leading to poorer overall survival. In breast cancer, although the information about the role of TB is limited compared to other solid malignancies, studies have started to emerge on this topic. Some studies have indicated that high TB in breast cancer is associated with adverse clinicopathological features, such as higher tumor grade, larger tumor size, lymph node involvement, and lympho-vascular invasion. These findings suggest that TB may serve as a prognostic indicator in breast cancer as well.

Metastasis is a major cause of death in a significant proportion of breast cancer patients. Inhibiting the tumor cells involved in the early metastatic cascade, including those present in tumor buds, could have significant clinical value. Targeting these cells and preventing or delaying their ability to invade and metastasize could potentially improve patient outcomes. Further research is needed to better understand the role of TB in breast

cancer and its potential as a prognostic marker and therapeutic target. By unraveling the mechanisms underlying TB formation and its relationship with metastasis, we can advance our knowledge and develop strategies to intervene in this process, ultimately improving outcomes for breast cancer patients.

Several studies have shown that high-grade tumor budding in breast cancer is significantly associated with ER-positive breast cancers. Estrogen has been implicated in promoting EMT in breast cancer cell lines with stem cell properties, leading to the disruption of tight junctions and enhanced cell motility. This suggests that ER-positive tumors with high-grade TB may undergo a high degree of EMT and potentially have a greater potential for metastasis. If further studies confirm this association, it opens up the possibility of utilizing anti-estrogen therapies to decrease the degree of TB in ER-positive breast cancers. Targeting estrogen signaling pathways could potentially inhibit EMT and reduce the invasive behavior of tumor cells.

Additionally, high-grade TB has been found to predict worse disease-free survival in HER2-positive, luminal A, and triple-negative breast carcinomas. This highlights the potential prognostic value of TB in various subtypes of breast cancer, independent of other pathological features and the tumor microenvironment. Overall, the presence of high-grade TB in breast carcinoma has been associated with adverse clinicopathological features and worse survival outcomes. Further studies are needed to validate and expand upon these findings, which could potentially lead to the incorporation of TB assessment into routine clinical practice for breast cancer patients. By identifying patients with high-grade TB, appropriate treatment strategies and interventions can be implemented to improve patient outcomes.

The variation in describing and scoring TB across different research papers can lead to inconsistencies and challenges in interpreting and comparing study results. In the context of breast carcinoma, TB has been less frequently examined compared to other solid tumors. As a result, the available studies on TB in breast cancer need further validation, and there is a need to establish a universally accepted parameter for scoring and defining cutoff criteria. By developing a standardized protocol for TB assessment, it can be included as an additional prognostic biomarker in pathology reports, providing valuable information for clinicians.

Establishing an internationally acceptable and uniform pathological criteria to identify and quantify TB would enhance accuracy and repeatability across different laboratories and institutions. This standardization would contribute to the consistency and reliability of TB assessment, facilitating its integration into routine clinical practice. The strong association between TB and lymphatic invasion and lymph node involvement in breast cancer suggests that TB assessment could be valuable in predicting prognosis for breast cancer patients. By including TB assessment in pathology reports, clinicians can have additional prognostic information to guide treatment decisions and improve patient outcomes. In summary, the establishment of a standardized protocol for TB assessment in solid malignancies, including breast carcinoma, is crucial. This would enable consistent and reliable evaluation of TB, allowing for its inclusion as an additional prognostic biomarker in pathology reports. Standardization would enhance the accuracy and repeatability of TB assessment and provide valuable prognostic information for clinicians.

## **VI. AUTOMATION IN TUMOR BUDDING**

The newer gene sequencing methods, such as Oncotype DX, Mammaprint, and Endoprint, are highly expensive and practically difficult to be implemented in underdeveloped and developing countries. In this context, tumor budding (TB) has the potential to serve as a cost-effective and easily reproducible prognostic biomarker in breast carcinoma. However, the lack of standardization in reporting TB in breast cancer remains a challenge for its widespread implementation. To address this issue, automated tissue microarray systems and automated tumor budding evaluation tools have been proposed. These automated methods can aid in the detection and quantification of tumor buds per image, providing objective and reproducible results.

Implementing automated systems in TB assessment can help overcome the subjectivity and inter-observer variability associated with manual quantification. These tools can potentially improve the accuracy and efficiency of TB evaluation, making it easier to incorporate TB assessment as part of routine pathology practice. Automation has shown its efficiency and effectiveness in various fields, and its application to TB evaluation holds promise for the future. By combining automated systems with standardized criteria for TB assessment, we can enhance the reliability and consistency of TB reporting, facilitating its integration as a prognostic biomarker in breast carcinoma. Further research and validation studies are needed to establish the efficacy and feasibility of automated TB evaluation tools. If successful, these advancements can significantly contribute to the wider implementation of TB assessment in breast cancer and improve prognostic evaluation in resource-constrained settings.

## **VII. CONCLUSION**

The strong association between high tumor budding (TB) and lympho-vascular invasion, lymph node metastasis, primary tumor staging and overall prognosis in breast cancer. TB has demonstrated its potential as a valuable prognostic factor in assessing the aggressiveness and metastatic potential of invasive breast cancer. To effectively utilize TB as a prognostic parameter in breast cancer, it is important to establish standardized criteria for evaluating and reporting tumor buds. Incorporating TB assessment as an additional parameter in the reporting protocol for breast cancer would provide essential information for clinicians in determining prognosis and making treatment decisions.

Standardizing the evaluation of TB would enhance the consistency and reliability of its assessment across different laboratories and institutions. This would also facilitate the comparison of findings among studies, allowing for a more comprehensive understanding of the clinical implications of TB in breast cancer. By establishing TB as a prerequisite yardstick in evaluating invasive breast cancer, healthcare professionals would have an additional tool to assess tumor behavior and make more informed decisions regarding treatment strategies. Incorporating standardized TB evaluation into the reporting protocol would provide valuable prognostic information and contribute to improving patient management and outcomes in breast cancer. Further research and collaboration among pathologists and researchers are needed to develop and implement standardized criteria for TB assessment in breast cancer. This effort would help establish TB as an essential component of breast cancer pathology

reports, enabling a more comprehensive evaluation of the disease and facilitating personalized treatment approaches.

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