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Prognostic role of tumor budding in breast cancer

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Abstract

Tumor budding, defined as a small number of cancer cells observed in pathology sections detached from the main tumor mass, is a common phenomenon in cancer. It is

suggested that cells in buds are in the process of actively moving away from the primary tumor in the first step of metastasis. Tumor budding has been observed in a variety of carcinomas and is best studied in colorectal cancers where it portends poor prognosis. More recently, tumor budding was found to be of prognostic significance in other cancers including breast cancer. Tumor budding in breast cancer is associated with other adverse pathologic factors, such as larger tumor size and lymphovascular invasion, but may have additional independent prognostic value. In the future, standardization of the quantification criteria for tumor budding may further aid in its adoption as a prognostic marker.

Key words: Tumor budding; Infiltration; Metastasis; Breast cancer; Prognosis; Epithelial to mesenchymal transition

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Core tip: Tumor budding, defined as scattered cells or small islands of tumor cells in the vicinity but not connected to the main tumor mass, is a common occurrence in different cancers. In breast cancer, it may portend an adverse prognosis.

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INTRODUCTION

Tumor budding is a pathologic phenomenon associated with many cancers. Although its specific definition differs from study to study, it generally consists of a small number of cells, usually up to five cells in the most commonly used definition, which have detached from the bulk of the tumor and are observed as isolated cells or small clusters of cells in histologic sections. Cancers in which tumor

budding has been observed and studied include colorectal, gastric and esophageal, lung, head and neck, and also breast cancers^[1]. Tumor buds may be observed in areas near the margins of tumors at the invasive tumor front and are called peritumoral buds, or inside the tumor mass and are thus called intratumoral buds^[2]. Identification of the tumor buds has been undertaken using plain eosin and hematoxylin sections or immunohistochemical methods. Although plain section staining is often sufficient in order to identify tumor budding, in some occasions involving significant inflammatory cell infiltration, immunohistochemical methods increase the confidence of the assessment and the inter-observer agreement. In addition to the area of the tumor where budding is observed (intratumoral versus peritumoral) as well as the method of staining used, studies have also used differing field examinations in quantifying budding. Some studies quantify budding in five high-power fields (HPF), while others count ten HPF. Some investigators use the areas of highest budding observed in order to classify cases, while others use mean counts of all fields examined. These methodological variations make comparisons across studies less straight-forward and hamper adoption of tumor budding as a more widely-used histologic phenomenon for clinical purposes such as prognostication.

PATHOPHYSIOLOGIC SIGNIFICANCE OF TUMOR BUDDING

Tumor budding is believed to represent cancer cells caught in the process of invasion^[3]. From a pathophysiologic perspective, tumor budding has been explained as a sign of cancer cell motility and as a first step in the metastatic process^[1]. The metastatic process begins with detachment of cells from the tumor bulk, infiltration through surrounding tissues into small blood vessels, and travel through the circulation to remote locations where they extravasate and may eventually establish colonies of metastatic disease. Paramount in metastasis is the process of epithelial to mesenchymal transition (EMT) and the reverse process of mesenchymal to epithelial transition (MET)^[4]. These processes, sometimes collectively referred to as epithelial mesenchymal plasticity, are part of normal embryogenesis and physiologic wound healing, and have been usurped by cancer. During EMT, detached cancer cells partially or completely lose their epithelial characteristics, detach from neighboring epithelial cells and gain mesenchymal characteristics, including expression of mesenchyme-associated proteins, to become motile. In metastatic sites, the reverse process takes place when arriving cells, helped by cues in their new microenvironment, regain epithelial properties and re-establish connections with neighboring cells^[5]. EMT/MET associated with cancer may be incomplete, and intermediate forms with partial epithelial or mesenchymal characteristics may be part of a continuous spectrum^[6,7]. In fact, cancer-associated EMT/ MET is believed to endow cells with stem cell properties, and the plasticity associated

with this stemness may help motile cells alternate along the spectrum between epithelial and mesenchymal states during their metastatic journey^[8,9]. Partial EMT may be the state of cells in tumor buds with two to five cells, where connections between them are maintained and the cells of the bud are destined to remain connected and move together through the circulation to the metastatic site. Alternatively, in some instances, buds may represent an initial step of detachment and, subsequently, individual cells may further detach from the other bud cells and move individually. Both scenarios have been observed in experimental studies^[10,11].

Tumor cells in buds of various epithelial cancers, including colorectal, pancreatic, lung and breast adenocarcinomas, lose the normal expression of membrane E-cadherin, which shows a modified cytoplasmic pattern of expression^[12]. Subsequently, the mesenchymal transcription factor ZEB1 is upregulated in the nucleus. These changes are observed in both budding cells within protrusions still connected to the main tumor mass and in cells of tumor buds already detached from the main mass^[12]. Budding cells, despite expressing the mesenchymal marker vimentin, do not completely lose cytokeratin staining, consistent with an incomplete EMT^[13]. ZEB1, along with the related transcription factor ZEB2, as well as other transcription factors such as Snail, Slug, Twist1 and FOXC2 constitute the core network of EMT^[14]. These core factors receive signals from a complement of signaling pathways and cooperate with additional transcription factors such as NF- κ B and c-Myc to influence cell fate across the epithelial-mesenchymal continuum^[5]. Interestingly, NF- κ B and Twist1 have been confirmed to be expressed in the cells of tumor buds and the surrounding stroma^[15,16]. Two additional observations, pertaining to the biologic implications of tumor budding as a first step of the metastatic process and its relationship to EMT and stemness properties, have been reported in studies done on colorectal cancer. First, cancer cells in tumor buds lose expression of the transcription factor CDX2, which is a marker of intestinal differentiation expressed in most colorectal cancers and associated with improved prognosis compared with colorectal cancers that do not express it^[17,18]. CDX2 is usually observed to be re-expressed at metastatic sites. Second, the expression of the proliferation marker Ki67 is low in tumor buds, denoting a quiescent state^[19]. These observations are consistent with the dedifferentiation of tumor cells in tumor buds and low proliferation during invasion, suggestive of their acquisition of an EMT/stemness phenotype which is reversed at the metastatic sites.

PROGNOSTIC IMPLICATIONS OF TUMOR BUDDING

The clinical significance of tumor budding has begun to be elucidated in recent years with studies associating the phenomenon with adverse clinical outcomes^[20,21]. The cancer location where tumor budding has been initially

described and remains still more extensively studied is the colon and rectum^[2]. A meta-analysis of reports of the prognostic role of tumor budding in resected stage II colorectal cancers observed worse survival outcomes in patients with tumor budding, with an odds ratio for death at five years of 6.25 (95% CI: 4.04-9.67) in patients with budding compared to those that had no tumor budding in their tumors^[22]. In rectal cancer, the presence of tumor budding in biopsies before neo-adjuvant chemo-radiation was associated with poor response to neo-adjuvant treatment^[23]. No patients among those with tumor budding had complete pathologic response rates (pCR) to neo-adjuvant treatment, whereas pCR was observed in 17% of patients without budding in their pre-treatment biopsy.

Tumor budding has also been studied in other gastrointestinal cancers. In a series of squamous esophageal cancer patients who received neoadjuvant chemotherapy with the 5-fluorouracil, cisplatin and doxorubicin regimen, tumor budding in the post-treatment surgical specimen was the most important predictive factor for overall survival (OS) and progression-free survival in multivariate analysis^[8]. Patients with high-grade budding, defined as five or more scattered cell formations (buds) in a low power field of maximal budding, had a five-year OS of 17% compared with a five-year OS of 49% in patients whose tumors had low-grade budding, defined as less than five buds in the low power field of maximal budding^[8].

In patients with gastric adenocarcinoma, high-grade tumor budding was a prognostic factor of worse OS^[24]. High-grade tumor budding was defined in this study as five or more tumor buds on average in ten HPF (400 ×), and conferred an increased risk of death with a hazard ratio of 2.26 (95% CI: 1.61-3.15) compared with patients whose tumors had low-grade budding. The prognostic value of budding for OS remained significant after adjustment for other factors in multivariate analysis. In a series of pancreatic cancer patients, tumor budding was observed in all cases where patients with high-grade budding (defined in this study as more than ten buds per HPF) had a worse OS than patients with low-grade tumor budding^[25]. Additional reports concur with a role of tumor budding as an adverse prognostic factor in pancreatic adenocarcinoma^[26,27].

Beyond gastrointestinal cancers, additional reports have shown that tumor budding is a prognostic factor in other cancers such as lung cancer and head and neck carcinomas. In an extensive study of stage I lung adenocarcinoma patients, high-grade tumor budding, defined as five or more buds in an HPF, was associated with a recurrence rate that was worse than low-grade tumor budding^[21]. This was true for all histologic subtypes investigated (acinar-predominant, papillary-predominant and solid-predominant), and for stages I A and I B. In early-stage oral squamous cell carcinomas, the presence of high-grade tumor budding of ten or more buds per HPF was associated with a worse disease-free survival (DFS) than intermediate level budding (five to less than

ten buds per HPF), and intermediate-grade budding had worse progression-free survival than low-grade budding (less than five buds per HPF)^[28]. Differences remained significant in the multivariate analysis. The study used pan-cytokeratin immunostaining to ascertain the identification of tumor buds.

TUMOR BUDDING IN BREAST CANCER

The above studies suggest that tumor budding is a phenomenon observed across cancer types and has adverse prognostic significance. Based on this evidence, studies have been undertaken to investigate whether tumor budding could be of clinical importance in breast cancer. Of note, breast cancer-associated tumor budding akin to budding observed in other cancers should not be confused with the process of tumor cells of the breast duct invading the basal membrane, which has also been referred to as "budding" by some investigators^[29]. In a study of 244 estrogen receptor (ER)-positive, human epidermal growth factor receptor 2 (HER2)-negative and 131 triple negative localized breast cancers, tumor budding was associated with worse OS in triple negative but not in ER-positive, HER2-negative patients^[30]. Interestingly, tumor budding was not predictive of DFS in either group, but it was predictive of a poorer DFS in the sub-group of ER-positive, HER2-negative patients with an intermediate Oncotype Dx score. This study examined budding in areas of maximal presence (termed H-TB) as well as the average budding in five HPF (termed A-TB), and supports the notion that H-TB is sufficient for prediction while A-TB does not add significant information^[30]. In another study that included localized breast cancers across the sub-type spectrum, higher tumor budding (> seven buds per a 200 × power field in a slide with the maximal invasive margin) was observed in about two thirds of patients, while the remaining one third displayed low tumor budding (seven or fewer buds per 200× power field in a slide with the maximal invasive margin). High tumor budding as well as tumor size, nodal status and the presence of lymphovascular invasion were independently associated with OS^[31]. Immunohistochemical studies showed that tumor bud cells had increased vimentin expression and decreased E-cadherin expression compared with the center of the tumor, suggesting that they had undergone an EMT^[13]. In addition, they were less positive for the proliferation marker Ki67 than the center of the tumor. Higher tumor budding (defined in this study as more than 20 buds at the field with the highest budding) was also independently associated with worse cancer-specific survival in a series of over 400 breast cancer patients with localized disease^[32]. With the definition used in this series, 35% of patients had high tumor budding and 65% had low tumor budding. The hazard ratio for cancer-specific survival was 2.08 (95% CI: 1.14-3.09) in patients with high tumor budding compared with patients with low tumor budding^[32]. Another series with early breast cancer patients across sub-types, but mostly consisting of luminal cancers, showed that high tumor budding was

associated with lymphatic invasion and positive lymph node disease^[33].

A series of 146 ductal carcinoma patients with operable disease was evaluated for both tumor budding, defined as less than five cells per bud, as well as for the presence of buds of five or more tumor cells not forming glands, termed "poorly differentiated clusters"^[34]. Both higher levels of tumor budding and poorly differentiated clusters were associated with a worse DFS and OS. In multivariate analysis, both phenomena remained significant, along with tumor size and nodal status. Authors of this study propose poorly differentiated clusters to be the preferred marker of prognosis, as they consider this easier to evaluate than tumor budding^[34].

Given the suggested participation of cells of tumor buds in EMT and the associated changes in protein expression, an interesting question is whether cells in the tumor buds of breast cancers maintain the same ER, progesterone receptor and HER2 profile as the main tumor mass. A study addressing this question showed that expression of hormone receptors and of HER2 is mostly concordant between the main tumor mass and tumor buds in 96.5% of tumors examined^[35]. However, another study showed that isolated tumor cells at the invasive front of ER-positive, HER2-negative luminal cancers co-expressed HER2 and aldehyde dehydrogenase, in contrast to the main tumor mass^[36]. Thus, it appears that there is heterogeneity in the stability of the profile of tumor buds. It is also possible that, at least in some cases, cells in buds, despite undergoing a partial EMT, maintain their initial hormone receptor and HER2 status. This uncertainty could be elucidated by studies examining concomitant expression of hormone receptors and the HER2 receptor, along with EMT markers at tumor buds from the same cancer specimens.

PERSPECTIVES

The association of tumor budding with the pathophysiologic correlation between metastasis and EMT is an important avenue to further explore in breast cancer clinical research. EMT is also associated with stemness characteristics, and the status of tumor bud cells across the stem cell differentiation axis would thus be interesting to define^[8,9]. Cancer stem cells are commonly quiescent, and this would correlate with the low Ki67 index shown in some cases^[19]. Further study of stem cell markers in tumor buds is warranted.

As mentioned in a previous section, tumor budding in biopsies of rectal cancer patients was predictive of response to neo-adjuvant chemoradiation^[23]. In addition, the presence of tumor budding in post-neoadjuvant chemotherapy surgical rejection specimens of esophageal carcinomas was associated with worse survival outcomes^[20]. Neoadjuvant chemotherapy is increasingly used in breast and other cancers in order to down-stage locally advanced disease prior to definitive surgical rejection of the tumor. In breast cancer, specifically, it is applied

when breast conserving surgery is desired but not initially technically possible due to the size and extent of the tumor. It is also used in node-positive disease, especially in tumors with aggressive biology, defined as triple negative or HER2-positive. These cancers tend to respond better to chemotherapy (or the combination of chemotherapy and HER2-targeting treatments in the case of HER2-positive cancers) than ER-positive cancers^[37]. Complete pCR to neoadjuvant chemotherapy range between 30% to 40% in triple negative and HER2-positive cancers, but are observed only in about 10% of hormone receptor-positive cancers^[38]. However, the majority of patients will still have residual disease after neoadjuvant chemotherapy, independent of their cancer subtype. In addition, there are no predictive markers for the response of patients to neoadjuvant treatment besides tumor subtype. Thus, in this scenario, tumor budding could be an additional predictive marker to consider in order to better predict tumor responses to treatment, should further studies confirm its predictive value.

From a therapeutic perspective, the associations of tumor budding with EMT and cancer stem cell characteristics may position tumor budding as a predictive marker for treatment with specific anti-metastatic treatments, and against stemness phenotypes that are investigated and may become clinically available in the future.

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