Cell budding

A unique and fundamental characteristic of malignant neoplastic cells is their ability to invade new tissues and metastasize. The first step in this process is the dissociation of some of these cells from the invasive front (IF) of the tumor, which is often associated with a transformation: either dedifferentiation or transdifferentiation. The invasive tumor front of oral squamous cell carcinoma (OSCC) has been an area of research interest in recent decades. Cancer cells at the IF behave aggressively compared with cancer cells in the superficial or central regions of the main tumor mass. Most importantly, cancer cells at the IF may undergo epithelial–mesenchymal transition, which is an important step in the progression of tumor metastasis. However, several researches have introduced the histopathologic representation of tumor metastasis using various terms, but the most accepted is tumor budding (TB), which has become relevant recently given TB's relationship with vascular invasion, metastasis and prognosis in oral cancer. Moreover, TB can be a new powerful prognostic marker that can be adaptable to conventional hematoxylin and eosin staining.

The term TB is not new; it was first reported almost 70 years ago in Japan. The first mention was made by Imai in 1954 as "sprouting" of tumor cells,[1] who described it as a morphological feature along the invasive margin reflecting more active tumor growth in several human cancers. In 1961, McGavran *et al.* showed that there was a significant correlation between the frequency of metastasis and the types of invasive growth patterns in squamous cell carcinoma of the larynx.[2] An important refinement of pattern of invasion, known as TB, was defined, with general agreement, as the presence of individual cancer cells or small clusters (fewer than five cancer cells) at the IF of the tumor, dissociated from the main tumor mass [Figures 1 and 2], and is considered the first step in the metastasis of a solid tumor. The term "TB" was coined by Morodomi *et al.* in 1989.[3] During the 1990s, Hase *et al.* demonstrated the association of TB with adverse clinicopathological factors such as tumor grade, tumor stage and lymphovascular and perineural invasion, as well as laying the groundwork for the evaluation of TB.[4] During the following years, little was studied until the middle 2000s, when a few studies about the morphological and molecular aspects of TB were published, expanding the knowledge about TB to other carcinomas such as colorectal, oral, breast, lung, esophagus and pancreas.

Morphologically, TB is well organized with a basal lamina that abuts the outer circumference; they have intercellular junctions and microvilli (abortive) as well as an envelope of myofibroblasts. This shows that the neoplastic cells are invading, advancing through long extensions such as dendritic extensions, called "podias" or "tubular invasion pole." However, this organized state seems to be focally disturbed: myofibroblasts may be withdrawn or absent; ultrastructurally, there is an absence of basal lamina,

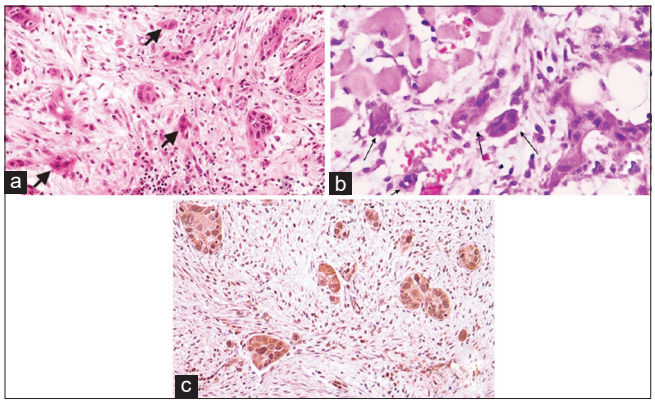

Figure 1: Tumor budding at the invasive front of oral squamous cell carcinoma. H&E section (a and b) and immunostaining for multi-cytokeratin (c) at high magnification

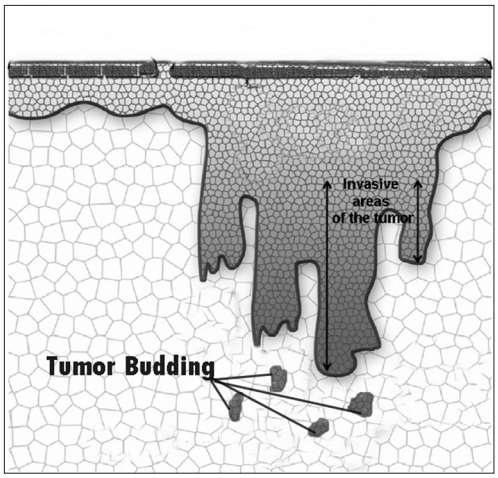

Figure 2: Schematic representation of tumor budding at the invasive front of oral squamous cell carcinoma

and the cytoplasmic junctions of the tumor cells come into direct contact with the extracellular matrix (i.e., the intercellular junctions are lost). Despite these findings, it has also been observed through experimental models and with three-dimensional reconstruction that there is indeed a true migration of individual cells from the main body of the tumor to the stroma.[5]

The importance of TB in cancer prognosis has been studied widely particularly in colorectal cancer, where it is recognized as an additional prognostic marker. In esophageal cancer, pancreatic cancer, breast cancer and lung cancer, TB has been reported as a promising prognostic marker. In OSCC, a significant correlation between high TB count, pattern of invasion and presence of occult lymph node metastasis has been observed, which has proven to be the best morphological prognostic indicator in oral cancer. Such a finding might indicate that TB is an early step en route to metastasis. As occult metastasis is the most common reason for relapse and poor prognosis in early-stage cases.[5-7]

The definite implementation of TB assessment depends on a selected, internationally accepted, scoring system. However, scoring systems of TB are different in reports on several cancers. Recently, Boxberg *et al.* described a novel grading scheme which includes cell nest size (CNS) and tumor budding activity (BA) in OSCC, analogous to SQCC-Lung developed by Weichert.[8] Budding was assessed in areas with maximal BA and was scored separately in one high-power field (HPF; ×40) displaying the highest BA and 10 HPFs. In one HPF, low BA was defined as 1–4 budding nests and high BA as ≥5 budding nests. In 10 HPFs, no budding was scored as 1, low BA and 1–14 buds were scored as 2 and high BA and ≥15 budding nests were scored as 3. Cell nests were defined as clustered tumor cells surrounded by stroma and were classified based on the size of the smallest invasive cell nest. Clusters of >15 tumor cells were classified as large cell nests (score 1), 5–15 tumor cells as intermediate cell nests (score 2), 2–4 tumor cells as small cell nests (score 3) and single-cell invasion was stated for individual tumor cells (score 4). CNS was assessed at the invasive margin and within the tumor core region. The grading score was summed for these two variables and ranged from 2 to 7. Tumors with scores 2–3 were defined as Grade 1, score 4–6 as Grade 2 and score 7 as Grade 3. Multivariate survival analysis confirmed that the prognostic impact of this grading scheme was independent of clinicopathological parameters (e.g., differentiation, age, sex and stage). This novel grading system is interesting and attractive because a combination of these two factors represents the malignant potential of a given carcinoma more reliability than each individual parameter alone.

To assess the degree of TB, immunohistochemically, pan-cytokeratin is helpful to identify epithelial cells when lymphoid infiltration obscures observation, and pan-keratin was more sensitive and easy to score than hematoxylin and eosin in OSCC. However, TB can be detected using hematoxylin and eosin-stained slide and is less expensive.[7,8]

In any case, the evaluation method for determining a TB score should be standardized because the results will be different depending on whether immunochemical staining is used to count TB.

For the assessment of TB from surgical resection specimens in OSCC, BA in preoperative biopsies has been considered. Seki *et al.* showed that TB evaluated using biopsy specimens was a good predictive factor for lymph node metastasis in squamous cell carcinoma of the tongue and floor of the mouth and was essentially unaffected by infiltrative patterns and tumor depth. TB in biopsy specimens was found to be an independent and powerful predictor of lymph node metastasis and prognosis.[9]

To conclude, TB has a prominent prognostic power for OSCC even at early stages of the disease. When considering a new prognostic marker for clinical application, the marker should also have a significant prognostic value independent from classical markers. Interestingly, for TB, most of the studies that provided multivariate analysis reported that TB has a superior prognostic value compared to other classical markers such as TNM stage and depth of invasion or WHO tumor grade. Future research on OSCC should compare the different evaluation methods with the goal of standardizing the assessment method for pathology reports. However, there are many unanswered questions about the genesis of these cells, and the underlying molecular aspects, which, once explored, will give us more information about the tumor progression in OSCC.

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Conflicts of interest

There are no conflicts of interest.

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