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The Correlation of Tumour Budding and Epithelial-Mesenchymal Transition Associated with The Prognosis of Oral Squamous Carcinoma- A Systematic Review

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ABSTRACT

Oral squamous cell carcinoma (OSCC) is a prevalent and aggressive cancer that arises from the epithelial cells found in the oral cavity. Despite advances in diagnosis and treatment, its prognosis remains poor due to its high potential for local invasion and metastasis. Prognostic outcomes are influenced by factors such as tumor size and stage, lymph node involvement, patient demographics, lifestyle habits, histological grade, tumor margins, perineural and lymphovascular invasion, tumor budding, and molecular markers like E-cadherin and p16. Among these, tumor budding, characterized by solitary individual cells or small clusters at the invasive edge, serves as a significant predictor of lymph node metastasis and signifies aggressive tumor behavior. It has already been acknowledged as an important prognostic factor in various cancers, such as colorectal, esophageal, and lung cancers. Tumor budding is believed to represent the epithelial-mesenchymal transition (EMT), a biological process in which epithelial cells gain mesenchymal characteristics, allowing them to separate, migrate, and invade adjacent tissues. During EMT, cells lose polarity and adhesion molecules such as E-cadherin while gaining mesenchymal markers like vimentin and fibronectin. This transformation enhances tumor aggressiveness and is linked to increased mortality. Tumor buds frequently exhibit features associated with epithelial-mesenchymal transition (EMT), such as the activation of the WNT/β-catenin signaling pathway and the downregulation of E-cadherin. While these observations indicate a significant link between tumor budding and EMT, the precise nature of their relationship in oral squamous cell carcinoma (OSCC) has yet to be completely clarified.

Keywords: *E-cadherin, EMT (Epithelial-Mesenchymal Transition) , Lymph node metastasis, Prognosis, Tumor budding*

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1. INTRODUCTION

Oral squamous cell carcinoma (OSCC) is a common and aggressive type of cancer that arises from the epithelial cells in the mouth. Even with improvements in diagnostic methods and treatment options, the outlook for OSCC continues to be fairly grim because of its tendency for local invasion and spread to other parts of the body. The prognosis of oral squamous cell carcinoma (OSSC) is influenced by a variety of clinical, pathological, and molecular factors, including tumor size and stage, lymph node involvement, patient age and health, lifestyle factors such as tobacco and alcohol use, histological grade, tumor margins, perineural and lymphovascular invasion, tumor budding, specific biomarkers (e.g., E-cadherin, p16), and molecular subtypes.[1]

One reliable indicator of the existence of lymph node metastases is Tumor Budding. Isolated single cells or tiny cell clusters (up to four cells) scattered throughout the stroma ahead of the invasive tumor front (ITF) are indicative of tumor budding. Cellular discohesion and aggressive invasion are two malignant characteristics that are indicated by this phenomenon. Tumor buds are thought to be a sign of aggressive cancer behavior.[2] It has previously been determined that tumor budding is a useful prognostic indicator for patients with colorectal cancer. In more recent times, it has also been found to be a significant prognostic factor for patients with ampullary adenocarcinoma, lung cancer, and esophageal malignancies.[3-4]

It is thought that invasion, metastasis, and disease-related mortality depend on the Epithelial Mesenchymal Transition (EMT), in which cancer cells change from epithelial to mesenchymal characteristics. A migratory phenotype develops as a result of the loss of cell-cell adhesions during EMT, including desmosomes, tight junctions, and adherence junctions.[5] Epithelial cells decrease the expression of proteins like E-cadherin, keratin, and occludin, along with the loss of polarity features, while simultaneously up-regulating the expression of vimentin, N-cadherin, α-smooth muscle actin (αSMA), and fibronectin. Due to its malignant characteristics, tumor budding might serve as the histomorphologic indication of cells having undergone epithelial-mesenchymal transition (EMT).[6] The discovery that tumor budding phenotypes show constitutive activation of the WNT signaling pathway, with its downstream molecules, β-catenins, being essential components of EMT, is evidence in favor of this theory. Moreover, transcriptional repressors of E-cadherin are expressed more frequently in tumor budding cells. The exact link between tumor budding and EMT in oral squamous cell carcinoma (OSCC) is still unknown, despite recent studies indicating that tumor budding cells have undergone EMT and histologically indicate an EMT process.[7]

The epithelial-mesenchymal transition (EMT) is a vital process in tumor progression and metastasis, intricately linked with tumor budding (TB). This review offers an in-depth analysis of the current understanding of tumor budding (TB) and epithelial-mesenchymal transition (EMT), examining their interconnection and distinct contributions to the tumor microenvironment.

2. METHODOLOGY

Search Strategy

- Literature search was according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses criteria (PRISMA). Detailed automated literature search was conducted on *PubMed* (English) and *Google scholar* until May 2024. Initial pool of articles was searched for references leading to additional papers missed in automated searches. Search keywords used were 'Tumor budding, Epithelial mesenchymal transition and Oral squamous cell carcinoma'. PRISMA flowchart for present review is shown in Fig. 1.
- For Google scholar search keywords should be present in the title.

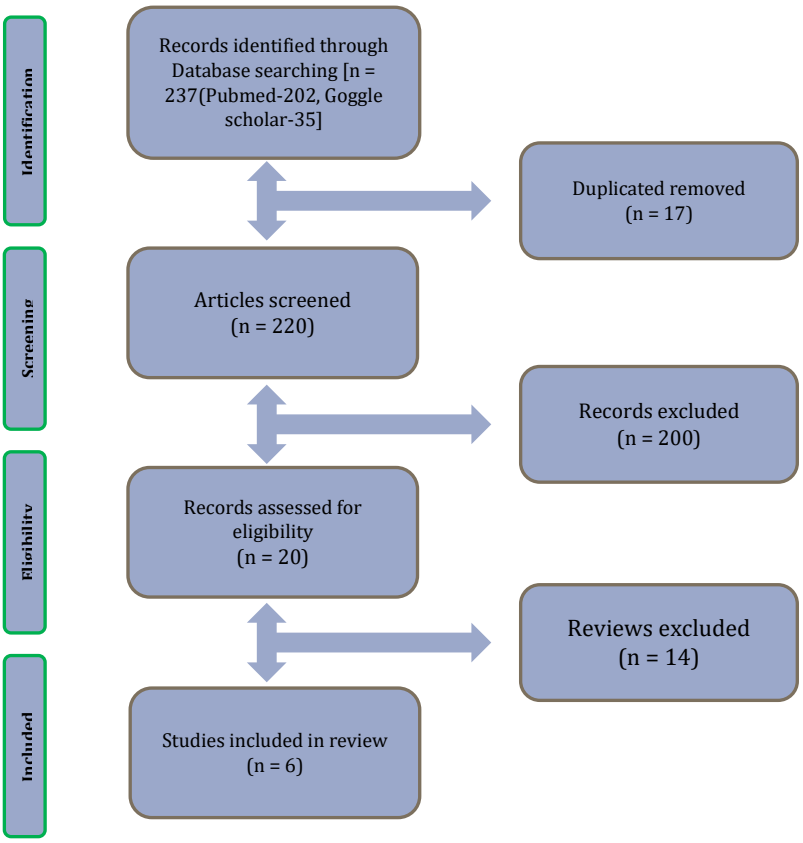
INCLUSION CRITERIA

- All original studies that examined possible correlation of Tumor Budding and Epithelial Mesenchymal Transition in Oral Squamous Cell Carcinoma.

EXCLUSION CRITERIA

- Duplicate, repetitive and irrelevant studies.
  - Studies including carcinomas other than oral squamous cell carcinoma
  - Case reports and review articles.
  - Articles in language other than English.

PRISMA flow diagram for literature search performed in this study (Fig. 1)



Preferred Reporting Items for Systematic Reviews and Meta-Analyses criteria (PRISMA)

3. TUMOR BUDDING

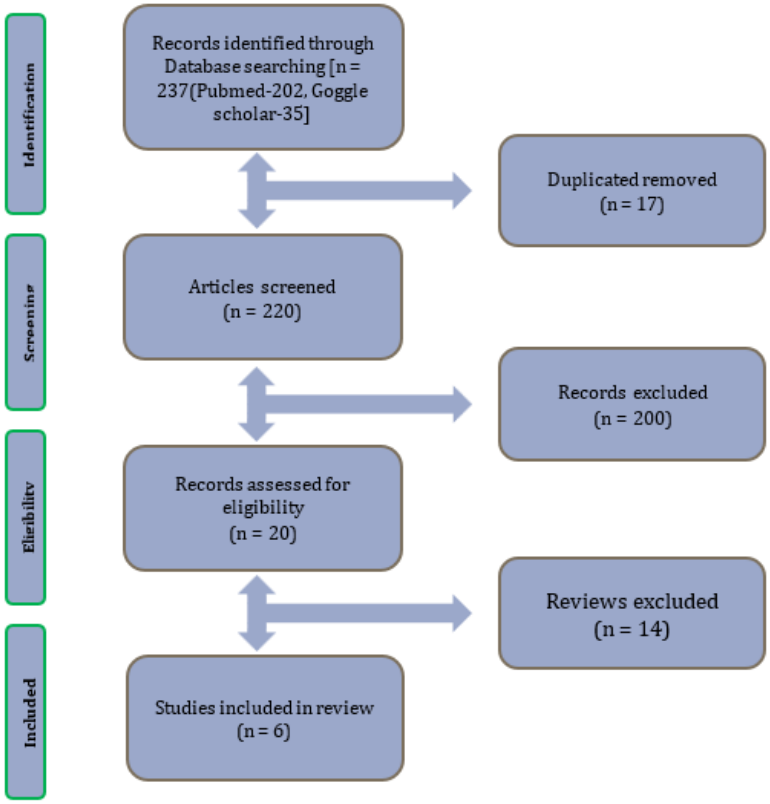
Tumor budding is a histopathological feature characterized by a dispersed invasive pattern, in which individual tumor epithelial cells or small clusters of up to five cells are observed, predominantly at the invasive front, infiltrating variably into the surrounding stromal tissue.[8] It serves as a predictor for lymph node metastasis, distant metastatic disease, local recurrence, and poorer overall and disease-free survival outcomes.[9] Angadi et al. were the first to recognize tumor budding as an independent prognostic marker for predicting lymph node metastasis in oral squamous cell carcinoma (OSCC).[10] Subsequent studies have also identified tumor budding as a significant prognostic indicator in early-stage OSCC. Quantification of tumor buds is typically performed within a microscopic field measuring 0.785 mm², corresponding to a field diameter of 0.5 mm under 20x objective magnification. The grading of tumor budding is then determined based on the number of buds present within this specified area. The grading system is as follows [2]

- **Low Budding (Bd1):** 0-4 buds per 0.785 mm², indicating a lower risk of aggressive tumor behavior and better prognosis.
- **Intermediate Budding (Bd2):** 5-9 buds per 0.785 mm², suggesting a moderate risk and intermediate prognosis.
- **High Budding (Bd3):** 10 or more buds per 0.785 mm², associated with a higher risk of aggressive behavior, increased likelihood of metastasis, and poorer prognosis.[2]

4. EPITHELIAL MESENCHYMAL TRANSITION

- An epithelial-mesenchymal transition (EMT) is a biologic process that allows a polarized epithelial cell, which normally interacts with basement membrane via its basal surface, to undergo multiple biochemical changes that enable it to assume a mesenchymal cell phenotype, which includes enhanced migratory capacity, invasiveness, elevated resistance to apoptosis, and greatly increased production of ECM components.[11]
- The early stages of primary epithelial malignancies are characterized by pronounced epithelial cell proliferation and increased angiogenesis. In contrast, the progression to later stages is marked by the acquisition of invasive capabilities, particularly the ability to breach the basement membrane, ultimately facilitating metastatic spread. Emerging evidence suggests that the activation of the epithelial-mesenchymal transition (EMT) program plays a pivotal role in enabling epithelial cancer cells to acquire malignant and metastatic phenotypes.[12]

PRISMA flow diagram for literature search performed in this study (Fig. 1)



Preferred Reporting Items for Systematic Reviews and Meta-Analyses criteria

5. DISCUSSION

Our review showed that high-grade tumor budding was significantly correlated with poor prognosis. To the best of our knowledge, this is the first comprehensive systematic review to evaluate the correlation between TB expression and epithelial mesenchymal transition in oral squamous cell carcinoma prognosis based on all available data pooled.

The infiltration of tumor cells into lymphovascular spaces through the endothelial cell layer is a critical step in the metastatic process and is recognized as a reliable prognostic marker in carcinomas, often indicating a poorer prognosis. Cases with high tumor budding intensity show a greater incidence of lymphovascular invasion, which consequently increases the likelihood of lymph node metastasis. **Almangush et al** found high-risk tumor budding (≥5/×20 field), depth of invasion ≥4 mm, and high-risk POI are associated with poor prognosis in early-stage oral tongue SCC.[13]

Tumor cells within budding foci exhibit distinct morphological alterations, notably de-differentiation and a reduction in intercellular adhesion. These cells often assume a spindle-shaped, fibroblast-like appearance, characteristic of epithelial-to-mesenchymal transition (EMT), a process that enhances cellular motility and confers increased aggressiveness and invasive capacity. Such phenotypic changes are closely linked to poor clinical outcomes and reduced patient survival. At the molecular level, these transitions are typified by a downregulation of E-cadherin, a key component of adherens junctions, and a concurrent upregulation of Vimentin, a widely expressed mesenchymal intermediate filament. The diminished expression of E-cadherin at the invasive tumor front (ITF) and in budding cells is consistently associated with elevated Vimentin levels, reinforcing the EMT-driven invasive phenotype which was proved by a study conducted by **Attramadal et al.** and **Yadav et al.**[6,14]

Similarly, **Wang et al.** investigated tumor budding in tongue squamous cell carcinoma (TSCC) and found that it was associated with the dysregulated expression of E-cadherin and vimentin, two key markers of EMT. Their study highlighted tumor budding as a prevalent histopathological feature in TSCC and concluded that it serves as an independent prognostic factor. This finding underscores the potential of tumor budding as a reliable marker for predicting clinical outcomes, further emphasizing its importance in cancer prognosis and therapeutic decision-making.[2]

Key EMT markers, Snail and Twist, show a significant correlation with tumor budding, further linking it to cancer progression. Tumor budding is significantly correlated with lymph node metastasis and decreased overall survival, underscoring its prognostic significance. Hong K et al. reported that the expression of the transcription factors Snail and

Twist was associated with lymph node involvement and adverse survival outcomes. Moreover, tumor budding exhibited a strong association with Snail expression and demonstrated a tendency toward elevated Twist expression, further emphasizing its role in tumor progression and metastasis. Immunohistochemical analysis of OSCC tissues provided concrete evidence of the interplay between tumor budding and EMT. As key EMT regulators, Snail and Twist suggest that tumor budding histologically represents the EMT process, with Snail playing a more prominent role in OSCC cell budding.[7]

Malic enzyme 1 (ME1) enhances the Warburg effect and promotes epithelial-mesenchymal transition (EMT) in oral squamous cell carcinoma (OSCC). Tumor budding at the invasive front is linked to cancer malignancy and shows a significant correlation with ME1 expression, which strengthens as cancer progresses. In OSCC cells, ME1 knockdown reduced lactate secretion, increased extracellular pH, and suppressed the EMT phenotype, whereas inhibition of oxidative phosphorylation increased lactate secretion, lowered pH, and promoted EMT. Hypoxia-induced ME1 overexpression, along with HIF1α activation, further enhanced EMT and tumor budding by increasing matrix metalloproteinases, reducing mitochondrial membrane potential, and shifting metabolism from oxidative phosphorylation to glycolysis. **Nakashima et al** suggested that tumor budding and ME1 expression serve as markers of OSCC malignancy, with ME1 as a potential therapeutic target.[15]

To summarize the key points, Epithelial-to-mesenchymal transition, is usually marked by the loss of E-cadherin, the gain of N-cadherin, and increased levels of Vimentin. An analysis of six studies revealed that tumor budding in oral squamous cell carcinoma (OSCC) is associated with reduced expression of epithelial markers such as E-cadherin and matrix metallopeptidase 9 (MMP9). Conversely, there is an increased expression of mesenchymal markers, including Vimentin and matrix metallopeptidase 2 (MMP2), in tumor budding cells. Notably, Vimentin was localized in the cytoplasm of OSCC cells at the invasive front. Given the critical roles of the transcription factors Snail and Twist in regulating epithelial-to-mesenchymal transition (EMT), these findings suggest that tumor budding may represent a morphological manifestation of EMT, with Snail potentially playing a more dominant role than Twist in driving this process. To summarize all the findings of the articles studied we have curated **Table 1, 2 and 3**.

Author and Year	Cases(N)	Tumor Site	TNM Staging	Tumor buds	Marker	Results
CECILIE GJØVAAG ATTRAMADAL (2015)	N=62 22 Female 40 Male	Gingiva: 13 Tongue: 17 Floor of mouth: 25 Other sites: 7	T1-29 T2-33	High budding tumors: 30 (10 recurrences) Low budding tumors: 28 (4 recurrences)	• Vimentin • E-cadherin • N-cadherin • Nuclear β-catenin	• Vimentin was up-regulated in tumor buds, and observed in 26 out of the 30 high-budding tumors. • Membranous E-cadherin was generally lost in tumor buds. Only two out of 30 high-budding tumors showed membranous E-cadherin immunoreactivity. • N-Cadherin was expressed in 17% of the tumors, mostly in the front. • Nuclear β-catenin expression in buds was observed in 77% of the high-budding tumors.
KUSUM YADAV (2023)	N= 200 37 Female 163 Male	Buccal Mucosa:100 Tongue: 76 Oropharynx: 24		High budding:100 Low budding: 54	• Vimentin • E-cadherin	• Upregulation of Vimentin in tumor buds in 65/154 cases (42.2%). Out of these 57/65 (87.6%) showed high-budding intensity. • Membranous E-cadherin was generally reduced in tumor buds. Only 24/62 (38.7%) high-budding tumors showed preservation of membranous E-cadherin expression

Table 1

Author and Year	Cases(N)	Tumor Site	TNM Staging	Tumor buds	Marker	Results
Cheng Wang (2011)	230 patients with Tongue SCC	Tongue		High- intensity tumor budding: 111 cases (48.36%) Low-intensity budding: 119 cases (51.74%) Of the 119 low-intensity budding cases, no tumor bud was observed in 65 cases.	• Vimentin • E-cadherin	• Intensive reduction in membranous E-cadherin expression was observed in the Invasive tumor front and tumor budding, when compared to that in the center/superficial tumor parts (64% vs 90%, $P<0.05$). • Increased expression of Vimentin was detected in the ITF and tumor budding (40% vs 76%, $P<0.05$) • High-intensity tumor budding is associated with reduced E-cadherin expression ($P<0.001$) and enhanced Vimentin expression ($P<0.001$)
Kyoung-Ok Hong (2018)	Fifty-six specimens (Male 48 Female 8)		T1: 7 N0: 19 T2: 27 N1:16 T3: 3 N2: 21 T4:19 M0: 54 M1: 2	Tumor budding was found in 33.9% (19/56) OSCC specimens. Tumor budding was positively associated with lymph node metastasis (P = .001)	• Snail • Twist	• Of the 19 tumor budding-positive cases, positive expression of Snail and Twist was detected in 14 (73.7%) and 9 (47.4%) cases, respectively. • Positive tumor budding was associated with positive expression of Snail (P=.003) and showed a tendency toward higher expression of Twist (P = .08)

Table 2

Author and Year	Cases(N)	Tumor Site	TNM Staging	Tumor buds	Marker	Results
DH Jensen (2015)	199 formalin-fixed, paraffin-embedded (FFPE) primary tumour tissues (Male-132 Female-67)	Tongue: 105 Floor of the mouth: 94	T1-T2: 169 T3-T4: 30 N0: 137 N+: 62	Below median: 99 Above median: 100	• ZEB1 and PRRX1 • E-cadherin • Vimentin	• Expression of ZEB1 and PRRX1, was increased in the tumour buds. • E-cadherin was expressed significantly less in budding tumour cells, and that a modest increase in vimentin was also present in budding tumour cells.
Chie Nakashima (2020)	96 OSCC cases (Male: 82 Female: 14)	Gingiva: 26 Tongue: 35 Pharynx: 17 Floor: 18	T1: 13 T2: 37 T3: 39 T4: 7 N0: 57 N1: 28 N2: 11		• Malic enzyme 1	• ME1 expression was low in cases where budding was not seen at the tumor front, whereas it was higher in cases where budding was observed at the tumor front. • Malic enzyme 1 and tumor budding a significant correlation was found between the two ($p = 0.005$)

Table 3

6. CONCLUSION

- In summary, tumor budding (TB) is commonly observed in oral cancers and has shown a strong correlation with traditional prognostic markers such as tumor grade, lymph node metastasis, lymphovascular invasion (LVI), and patterns of invasion.
- Furthermore, tumor budding shows a strong correlation with the expression of key epithelial-mesenchymal

transition (EMT) regulators, suggesting that it is not only a marker of poor prognosis in oral squamous cell carcinoma (OSCC) but also a histopathological indicator of EMT activity within these tumors.

- Tumor buds undergoing epithelial-to-mesenchymal transformation are believed to enhance the tumor's invasive and metastatic potential, thereby contributing to a significantly poorer clinical outcome.

Our findings demonstrate that high-grade tumor budding is associated with unfavorable prognosis in patients with OSCC. Incorporating tumor budding assessment into routine pathological evaluation may offer valuable prognostic insight and inform treatment planning.

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