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Prognostic significance of tumor budding in head and neck squamous cell carcinoma: association with clinicopathological features

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Abstract

Background Head and neck squamous cell carcinoma (HNSCC) is a significant global health concern, constituting about 4.5% of all cancer diagnoses and fatalities. Tumor budding, characterized by single cells or small clusters at the invasive tumor front, has shown promise as a prognostic marker in various carcinomas, but its role in HNSCC requires further investigation.

Materials and methods This retrospective study analyzed patients with HNSCC who underwent surgical resection from January 2023 to June 2024. Histopathological evaluation involved counting tumor buds in ten high-power fields and classifying them as low (0–4 buds), intermediate (5–9 buds), or high (≥ 10 buds). Clinicopathological parameters such as age, gender, tumor grade, stage, nodal involvement, depth of invasion (DOI), worst pattern of invasion (WPOI), lymphovascular invasion (LVI), and perineural invasion (PNI) were recorded. Statistical analyses assessed associations between tumor budding and these parameters.

Results The study included 53 patients with a mean age of 47.2 years. Tumor budding was low in 9.4%, intermediate in 62.3%, and high in 28.3% of cases. Significant associations were found between higher tumor budding and higher stage ($p = 0.01$), worst pattern of invasion ($p < 0.01$), lymphovascular invasion ($p < 0.01$) and nodal involvement ($p = 0.03$). High budding was linked to greater DOI ($p < 0.01$).

Conclusion Tumor budding is significantly associated with aggressive clinicopathological features in HNSCC, including advanced stage and nodal involvement. Incorporating tumor budding assessment into routine histopathological evaluations could enhance prognostication and guide therapeutic decisions, potentially improving patient outcomes.

Keywords Tumor budding, Head and neck, Squamous cell carcinoma

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Introduction

Head and neck cancer is a prevalent and aggressive malignancy, affecting the oral cavity, pharynx, hypopharynx, larynx, nasal cavity, and salivary glands. As the seventh most common cancer worldwide, head and neck squamous cell carcinoma (HNSCC) poses a significant health burden, with approximately 890,000 new cases and 450,000 deaths annually, accounting for about 4.5% of all cancer diagnoses and fatalities according to GLOBOCAN estimates (Sung et al. 2021). In contrast to other prevalent cancers like breast and lung cancers that have seen advancements in treatment approaches, head and neck cancers have largely relied on conventional therapies with limited development of targeted options, contributing to the poor prognosis. Identifying reliable histopathological markers that can predict aggressive behavior and guide treatment decisions is crucial in improving patient outcomes.

Tumor budding, characterized by isolated single tumor cells or small clusters of up to four cells at the invasive front of the tumor, has emerged as a promising prognostic marker in various carcinomas, including colorectal, esophageal, breast and pancreatic cancers (Zlobec et al. 2020; Huang et al. 2022; Argon et al. 2023). However, there is limited data on its role in HNSCC. The presence of tumor buds at the invasive front suggests a high degree of cellular dedifferentiation and epithelial-mesenchymal transition, processes associated with increased invasiveness and metastatic potential (Zlobec et al. 2020).

This study aims to investigate the association between tumor budding and various clinicopathological parameters in HNSCC. By examining the relationship between tumor budding and factors such as tumor grade, stage, nodal involvement, depth of invasion, lymphovascular invasion (LVI), and perineural invasion (PNI), we seek to elucidate the potential of tumor budding as a prognostic marker in HNSCC.

Materials and methods

Study design and patient selection

This retrospective cohort study included patients diagnosed with head and neck squamous cell carcinoma who underwent surgical resection between January 2023 and June 2024. Patients who received any neoadjuvant chemotherapy or radiotherapy were excluded.

Histopathological evaluation

Formalin-fixed, paraffin-embedded tissue blocks from surgical specimens were retrieved from the pathology archives. All cases included in the study were conventional keratinizing squamous cell carcinomas (SCC). There were no other subtypes known for aggressive or indolent behavior, such as basaloid SCC or verrucous carcinoma thus ensuring a homogenous cohort for evaluating tumor budding. Additionally, no HPV-related carcinomas were identified in this cohort. Hematoxylin and eosin (H&E)-stained slides were reviewed by two independent pathologists blinded to clinical outcomes. Tumor budding was assessed at the invasive front of the tumor using a standardized protocol in ten high-power fields (HPFs). Tumor budding was defined as isolated single tumor cells or small clusters of up to four cells at the invasive front. To ensure reproducibility, the calibration of the microscope was specified. The high-power field (HPF) area used for tumor budding evaluation was defined as 0.785 mm^2 (based on a 20x objective lens with a field diameter of 0.5 mm).

Based on the budding count, tumors were classified into three categories: low budding (0–4 buds), intermediate budding (5–9 buds), and high budding (≥ 10 buds). A critical addition was establishing a minimum distance from the invasive tumor front to identify a real tumor bud. Budding structures within 100 μm of the tumor front were excluded unless confirmed to be disconnected through examination of adjacent sequential sections. This approach minimizes misidentification of tumor structures potentially connected to the invasive front. In addition to tumor budding, the Worst Pattern of Invasion (WPOI) was evaluated for all cases. WPOI was categorized into low-grade (WPOI 1–4) and high-grade (WPOI 5), following ASCO/CAP guidelines (Zanoni et al. 2019). While WPOI is routinely reported for oral cavity SCC in surgical specimens, this study aimed to determine whether tumor budding provides comparable or additional prognostic value.

Clinicopathological parameters

The following clinicopathological parameters were recorded: age at diagnosis, gender, tumor grade (grade 1–3), pathological T stage, presence of lymph node metastasis, depth of invasion, lymphovascular invasion (LVI) and perineural invasion (PNI).

Statistical analysis

Statistical analyses were performed using version 25.0 (IBM, Chicago, USA). Descriptive statistics were used to summarize patient demographics and clinicopathological features. The Fisher's exact test and Chi square test were utilized to assess the association of clinicopathological variables with tumor budding. Multivariate logistic regression analysis assessed the independent association between tumor budding and tumor stage, controlling for confounding variables, including histological grade, LVI, and PNI. A p-value of less than 0.05 was considered statistically significant.

Results

Demographics and patient characteristics

The study cohort consisted of 53 patients with head and neck squamous cell carcinoma. The majority of patients (20 cases, 37.7%) were in the age group of 41–50 years, followed by 31–40 years (14 cases, 26.4%). The mean age of the patients was 47.2 ± 9.6 years. There were 41 (77.4%) and 12 (22.6%) male and female patients respectively. There were 37 (69.8%) and 16 (30.2%) cases of oral cavity and oropharyngeal carcinomas respectively.

Among these, 12 patients (22.6%) had Grade 1 tumors, 37 patients (69.8%) had Grade 2 tumors, and four patients (7.5%) had Grade 3 tumor. The distribution of tumor stages was as follows: 5 patients (9.4%) were T1, 19 patients (35.8%) were T2, 15 patients (28.3%) were T3, and 14 patients (26.4%) were T4. Nodal involvement was observed in 25 patients (47.1%). Lymphovascular and perineural invasion were observed in 14 (26.4%) and 3 (5.7%) cases respectively.

Tumor budding and clinicopathological parameters

Tumor budding was categorized into low (9.4%), intermediate (62.3%), and high (28.3%) groups [Fig. 1]. Among Grade 1 tumors, 8.3% exhibited low budding, 66.7% intermediate budding, and 25% high budding. Grade 2 tumors showed 10.8% low, 64.8% intermediate, and 24.3% high budding, while Grade 3 tumors demonstrated predominantly intermediate (25%) and high (75%) budding. Tumor site analysis revealed similar patterns, with oral cavity tumors comprising 10.8% low, 64.8% intermediate, and 24.3% high budding, and oropharyngeal tumors showing 6.2% low, 56.3% intermediate, and 37.5% high budding. Advanced tumor stages (T3/T4) were strongly associated with high budding, with 53.3% and 42.9% of cases, respectively, exhibiting this feature. Conversely, T1 tumors predominantly displayed low (60%) and intermediate (40%) budding, and T2 tumors showed 10.5% low, 84.2% intermediate, and 5.3% high budding. Nodal involvement was linked to higher budding levels, with 56% of cases with nodal involvement exhibiting high budding compared to only 3.5% in cases without nodal involvement. A similar trend was observed with the worst pattern of invasion (WPOI), where high budding was more frequent in WPOI 5 (63.2%) compared to WPOI 1–4 (8.8%). Depth of invasion (DOI) also correlated with tumor budding, with median DOI increasing from 0.4 cm in low budding to 0.8 cm in intermediate and 1.2 cm in high budding ($p = 0.04$). High budding was significantly associated with advanced tumor stage ($p < 0.01$), nodal involvement ($p < 0.01$), WPOI ($p < 0.01$), and lymphovascular invasion ($p < 0.01$), while no significant association was found with gender, tumor site, tumor grade, or perineural invasion. Multivariate analysis confirmed significant associations with tumor stage and WPOI [Tables 1 and 2].

Discussion

Tumor budding was first described by Imai et al. as "sprouting" in the 1950s. Thereafter Gabbert et al. identified the isolated tumor cells at the leading edge of the tumor in colorectal cancer (Kale and Angadi 2019). It represents an aggressive tumor phenotype characterised by loss of cell adhesion, epithelial-mesenchymal transition and local invasion (Togni et al. 2022). WPOI 1–5, defined by "tumor dispersion ≥ 1.0 mm between tumor satellites," shares conceptual similarities with tumor budding, as both parameters assess tumor invasiveness. Unlike WPOI, which is limited to surgical specimens from oral cavity SCC, tumor budding demonstrates potential as a broadly applicable parameter across all HNSCC subsites. Furthermore, tumor budding's assessment on biopsy specimens offers a distinct advantage for pre-treatment prognostication, especially in cases where surgical resection is not performed. This study found a strong correlation between high tumor budding and WPOI 1–5, suggesting tumor budding as a valuable marker for tumor invasiveness, with the potential to

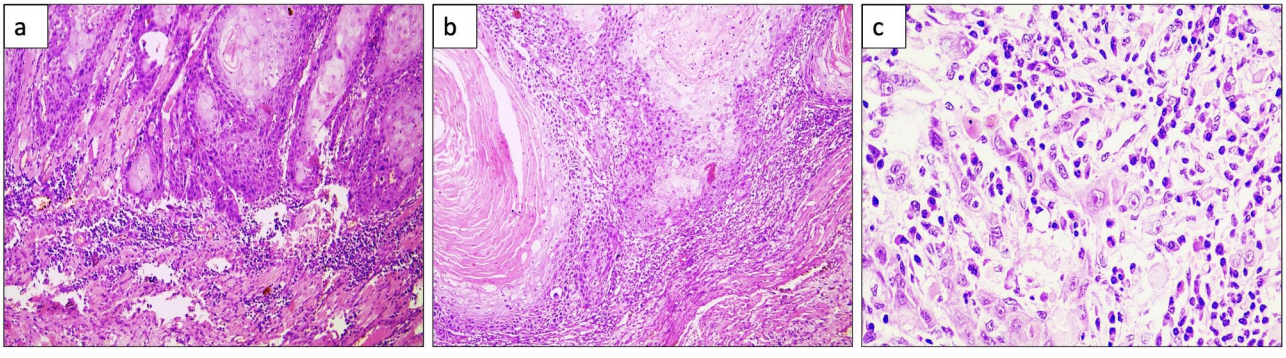

Fig. 1 Different grades of tumor budding. (**a**) A case of well differentiated squamous cell carcinoma exhibiting low budding (< 5 buds). (**b**) A case of moderately differentiated squamous cell carcinoma exhibiting intermediate budding (5–9 buds). (**c**) A case of moderately differentiated squamous cell carcinoma exhibiting high budding (> 10 buds). [Hematoxylin and eosin, 100, 200x]

Table 1 Association of clinicopathological parameters with tumor budding

Parameter	Tumor budding				
	Low [*n* = 5]	Intermediate [*n* = 33]	High [*n* = 15]	Univariate analysis (*p* value)	Multivariate analysis (*p* value)
Gender					
Male [*n* = 41]	5	26	10	0.29*	0.87
Female [*n* = 12]	0	7	5		
Tumor site					
Oral cavity [*n* = 37]	4	24	9	0.59*	0.93
Oropharynx [*n* = 16]	1	9	6		
Tumor stage					
T1-T2 [*n* = 24]	5	18	1	**< 0.01***	**0.03**
T3-T4 [*n* = 29]	0	15	14		
Nodal involvement					
N0 [*n* = 28]	5	22	1	**< 0.01***	0.05
N1-3 [*n* = 25]	0	11	14		
Grade					
G1 [*n* = 12]	1	8	3	0.31*	0.57
G2 [*n* = 37]	4	24	9		
G3 [*n* = 4]	0	1	3		
Worst pattern of invasion					
WPOI 1–4 [*n* = 34]	4	27	3	**< 0.01***	**0.02**
WPOI 5 [*n* = 19]	1	6	12		
Perineural invasion					
Present [*n* = 3]	0	1	2	0.3*	0.73
Absent [*n* = 50]	5	32	13		
Lymphovascular invasion					
Present [*n* = 14]	0	5	9	**< 0.01***	0.58
Absent [*n* = 39]	5	28	6		

*Fischer exact test

Table 2 Association of DOI (cm) with tumor budding

DOI (cm)	Low [*n* = 5]	Intermediate [*n* = 33]	High [*n* = 15]	*p* value
Mean ± SD	0.46 ± 0.26	1.02 ± 0.56	1.38 ± 0.72	**0.04**‡
Median	0.4	0.8	1.4	

‡Mann Whitney test

complement or even replace WPOI for prognostication in oral cavity SCC (Mishra et al. 2022).

The applicability of tumor budding to other HNSCC subsites further underscores its utility. In this study, we found that tumor budding can be reliably assessed in both oral cavity and oropharyngeal SCC. While oropharyngeal SCC exhibited a slightly higher proportion of high tumor budding, no statistically significant differences were observed between subsites. These findings support the role of tumor budding as a universal marker of tumor invasiveness across different HNSCC subsites.

The study exclusively included conventional keratinizing SCC. The median age of the cohort (47.2 years) is younger than typical for conventional SCC, raising concerns about potential HPV-related etiology. However, no HPV-related carcinomas were identified in this cohort.

High tumor budding was strongly associated with several aggressive clinicopathological features, including advanced tumor stage, nodal involvement, lymphovascular invasion and WPOI. Similar findings have been reported in the literature, with Bjerkli et al. and Ghosh et al. (Bjerkli et al. 2020; Ghosh and Guha 2023) identifying tumor budding as a predictor of nodal metastasis in tongue squamous cell carcinoma. Our study also confirms these associations, with high tumor budding linked to deeper tissue invasion and greater T and N stages. These results are consistent with previous studies by Niranjan et al. and Tan et al., which have also highlighted the prognostic significance of high tumor budding in relation to survival outcomes (Niranjan et al. 2023; Tan and Taskin 2023).

Almangush et al. observed that increased tumor budding correlates with poorer overall survival in tongue squamous cell carcinoma, a finding echoed in their meta-analysis, which demonstrated its association with lymph node metastasis, disease-free survival, and overall survival (Almangush et al. 2014, 2018). Silva et al. in their meta-analysis showed higher tumor budding to be associated with a lower overall and disease free survival (Silva et al. 2024). Additionally, Hong et al. highlighted the connection between tumor budding and the expression of Snail, an indicator of epithelial-mesenchymal transition, further emphasizing its prognostic relevance (Hong et al. 2018). Selvaraj et al. reported a significant correlation between tumor grade, budding activity, and cell nest sizes, where low-grade tumors exhibited low budding activity and larger cell nests, while high-grade tumors showed higher budding activity with smaller cell nests and single-cell invasions (Selvaraj et al. 2023). Mitha et al. also showed similar findings with a significant association between tumor grade and tumor budding (Mitha et al. 2024). These findings reinforce the potential of tumor budding as a valuable marker for risk stratification and prognosis in HNSCC.

The significant association between high tumor budding and advanced tumor stages (T3 and T4) highlights the role of tumor budding in promoting local invasion and deeper tissue penetration. Similarly, the correlation with nodal involvement suggests that tumor budding may facilitate lymphatic spread, contributing to the tumor's metastatic potential.

Interestingly, no significant association was found between tumor budding and lymphovascular or perineural invasion in this cohort. This finding may indicate that while tumor budding is a marker of cellular dedifferentiation and invasiveness, it operates through distinct mechanisms that may not directly involve LVI or PNI. Further

studies with larger cohorts are needed to explore these relationships in greater detail.

The lack of association with gender suggests that tumor budding is a gender-independent marker, reinforcing its potential utility across diverse patient populations. The significant difference in depth of invasion between low and intermediate to high budding groups further supports the role of tumor budding in promoting aggressive tumor behavior. A key advantage of tumor budding over WPOI is its applicability to biopsy specimens. Although tumor budding measurement in biopsy specimens is feasible, it was not done in the present study but needs to be assessed in the future. By contrast, WPOI assessment is inherently limited to surgical specimens, underscoring the broader applicability of tumor budding.

Despite its strengths, this study has certain limitations. First, the retrospective design and relatively small sample size limit the generalizability and external validation of our results. The cohort consisted primarily of conventional keratinizing SCC, and the inclusion of limited topographies and specimen types further restricts the ability to apply these findings universally across the diverse spectrum of HNSCC. While our results suggest that tumor budding is a promising prognostic marker, the lack of prospective validation and the restricted scope of specimens examined means that we cannot conclusively establish its clinical utility in routine practice at this stage. Although the associations between tumor budding and aggressive clinicopathological features are statistically significant, these findings should be viewed with caution until larger, more diverse cohorts are studied prospectively to validate the reproducibility and robustness of tumor budding as a clinical tool for prognostication.

Given the global burden of HNSCC, incorporating tumor budding assessment into routine histopathological evaluation could significantly impact clinical practice. By identifying patients with higher risk profiles, clinicians can effectively tailor treatment strategies, potentially improving survival outcomes and reducing the disease burden.

Overall, this study provides compelling evidence that tumor budding is a valuable prognostic marker in HNSCC, associated with several key indicators of poor prognosis. Incorporating tumor budding assessment into routine histopathological evaluation could enhance prognostication and guide therapeutic decision-making for patients with HNSCC.

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Author contributions
All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Sana Ahuja, Sristi Barman and Sufian Zaheer. The first draft of the manuscript was written by Sana Ahuja and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability
The data is available from the corresponding author on request.

Declarations

Ethics approval and consent to participate
The study was done in accordance with the Declaration of Helsinki and after ethical approval from the Institutional Ethics Committee.

Consent for publication
Informed consent was sought from the patient regarding participation and publication.

Competing interests
The author(s) declared no potential conflicts of interest.

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