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# Prognostic Value of Selected Histologic Features for Lung Squamous Cell Carcinoma

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## Abstract

The recent histologic subtyping of lung adenocarcinoma has demonstrated the prognostic values of histologic patterns in this malignancy. However, the histological features of lung squamous cell carcinoma (SCC) are much less established. This short review discusses several promising histological prognostic markers for SCC, including tumor budding, tumor cell nesting, and the spreading of tumors through air spaces. Wherever appropriate, the biological significance of these morphological features was also discussed. The investigators consider that histological prognostic markers are highly valuable in understanding the cancer biology of SCC, and in guiding clinical treatment. However, larger clinical cohorts are needed to better establish the prognostic values of the aforementioned histological markers. The application of modern technologies, including machine-learning, would make the histological analysis accurate and reproducible.

### Keywords

Lung squamous cell carcinoma; Tumor budding; Tumor cell nests; Tumor spread through air spaces (STAS)

## Introduction

Lung cancer is the most common form of cancer, and is the leading cause of cancer-related deaths. Lung squamous cell carcinoma (LSCC) is the second most common type of non-small cell lung carcinoma, accounting for a quarter to a third of all lung cancers. At present, the tumor-node-metastasis (TNM) staging manual is the most widely used prognostic system for non-small cell lung cancers.[1] The TNM staging manual emphasizes a series of



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Author contributions

Analysis and interpretation of data, manuscript writing, and critical revision (JF); Analysis and interpretation of data, manuscript writing, and critical revision (SMD); Study design, analysis and interpretation of data, manuscript writing, critical revision, and administration (HW). All authors made a significant contribution to the study, and approved the final manuscript.

Conflict of interest

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general tumor histologic parameters, including tumor size, vascular invasion, surrounding tissue invasion, and lymph node metastasis, with proven prognostic values.[2] However, the recent histologic subtyping of lung adenocarcinoma has shown value in identifying prognostically significant histologic patterns, in addition to treatment decisions.[3] Thus, lung adenocarcinomas with solid and micropapillary growth patterns hold the worse prognosis, when compared to carcinomas with lepidic growth patterns.[4,5] According to the 2015 World Health Organization classification of lung cancers, LSCCs can be further classified into keratinizing, nonkeratinizing and basaloid subtypes. However, the prognostic relevance of these subtypes remains largely unknown.[6] Over the past six years, the prognostic value of distinct histopathological patterns has been highlighted for LSCC. The present mini review briefly summarizes the present literature on several promising prognostic histologic markers based on cohorts with reasonable clinical case numbers (Fig. 1).

## Tumor budding: Refined counting approach and “hotspot”

Tumor buds are defined as clusters of four or less tumor cells that infiltrate the adjacent parenchyma. Tumor budding emerged as a prognostic marker for colorectal cancer several decades ago,[7] and its prognostic value has since been established in other cancers, such as oral squamous cell carcinoma, cervical squamous cell carcinoma, and pancreatic cancer.[8] Numerous studies have focused on utilizing various approaches to count tumor buds, including different staining methods, the number of evaluated high-power fields, and the categorical *vs*. continuous values. Ultimately, the International Tumor Budding Consensus Conference (ITBCC) in 2016 provided a consensus on how to evaluate the tumor budding of colorectal cancer.[9] According to the ITBCC, the assessment of buds should be based on the H&E staining, and all available tumor slides should be scanned at medium power (×10). Subsequently, slide(s) with the highest number of buddings at the invasive front (“hotspot”) should be selected, and the tumor buddings in one “hotspot” should be counted at ×20 magnification (within an area of 0.785 $mm^2$). Budding is assessed using a three-tier system (0–4 buds: low budding, BD1; 5–9 buds: intermediate budding, BD2; ≥10 buds: high budding, BD3), and the absolute quantification of the total number of buds is recorded. It is noteworthy that regions with extensive peritumoral inflammation or tumor tissue fragments should be excluded from the count. In a recent study, Neppl *et al*. adopted the ITBCC scoring approach, and evaluated their well-characterized cohort of 354 resected primary LSCC tumors.[10] Their results confirmed that tumor budding is an independent prognostic factor for shorter overall, disease-specific and progression-free survival (OS, DSS and PFS, respectively). The ITBCC has also been shown to have a significant correlation between tumor budding and pleural invasion, larger tumor size, higher pT-category, pN-category, UICC/AJCC-stage and resection status, but not with distant metastasis or conventional tumor grading. ITBCC tumor budding categories can serve as independent prognostic factors for OS, DSS and FFS, while other factors include tumor size, tumor stage, age and gender. This report, together with earlier studies, suggest that tumor budding is a promising prognostic marker for LSCC.[11,12]

To underline the mechanisms through which tumor buddings act in cancer, previous studies have suggested that this may involve the biological processes surrounding the epithelial-mesenchymal transition, and result in the increased migration and invasion of cancer

cells. The epithelial mesenchymal transition is characterized by heightened mesenchymal differentiation (e.g. vimentin) and reduced epithelial differentiation (e.g. E-cadherin).[13] In one study of LSCC, tumor budding was found to be associated with E-cadherin downregulation and the presence of vimentin expression in LSCC cells.[1] This finding was confirmed by an immunohistochemical study, which revealed that budding cells exhibit a reduced expression of particular cellular adhesion molecules (E-cadherin and catenin) and increased levels of laminin-5γ2, when compared to cancer cells that form solid nests. On the other hand, there was no observable difference in the expression of lineage markers (p63 and podoplanin) between budding cells and cancer cells in the nests.[14] Tumor budding, together with single cell invasion, are invasive phenotypes associated with poor prognosis in LSCCs and SCCs from several other locations.[15–17] One study of LSCC revealed that tumor budding is associated with lymph node metastasis, lymphovascular invasion and scirrhous stroma.[18] In oral SCC, tumor budding is an independent predictor of regional metastasis, lymphovascular invasion and perineural invasion.[19]

## Tumor spread through air spaces: Beyond lung adenocarcinoma

Tumor spread through air spaces (STAS) is a newly recognized pattern of tumor invasion in lung adenocarcinoma, and is characterized by the clustering of tumor cell nests beyond the outer border of the main tumor, and within air spaces throughout the lung parenchyma.[20,21] STAS is typically observable in low-power fields, and is confirmed when tumor cell clusters are found to be present outside of the tumor's borders, even if it is only observed within the first alveolar layer from the tumor edge. In order to avoid misinterpretation with the detached cells, which are attributable to tumor dissection, tumor cells are considered as STAS only when detached small clusters of tumor cells are identified within the air spaces in a continuous manner. Furthermore, its distribution should be in line with the shape of the tumor's circumferential edge(s). Haphazard-shaped fragments of tumor nests should be considered as artifacts when these have sharp, jagged, or irregular contours. In a Japanese cohort with LSCC, the multivariable regression analysis revealed that STAS is independently associated with worse relapse-free survival.[22] Furthermore, patients with STAS had a higher risk of locoregional and distant recurrence, when compared to patients without STAS. In addition, a study on 445 resected primary stage I–III LSCC tumors revealed that STAS is associated with tumor recurrence and cancer-specific survival, but not with OS.[23] However, these observations were not confirmed in a recent study that observed a cohort of 354 LSCC patients. Mechanistically, it has been suggested that STAS-associated air spaces spread with a low expression of E-cadherin in ROS1-rearranged lung cancers, and consequently, reduce disease-free survival (DFS).[24]

## Tumor cell nests: Nest location and nest cell number

Tumor cell nests are clusters of tumor cells that are surrounded by tumor stroma. The smallest invasive tumor nest can be further classified into subtypes, and characterized, as follows: large nests (composed of >15 tumor cells), intermediate nests (composed of 5–15 tumor cells), small nests (composed of 2–4 tumor cells), or single-cell invasion. In most studies, the size of the tumor nests are evaluated at both the invasive front and center of the tumor, respectively, in which the smallest nest size is recorded. In one study with a

cohort of 541 LSCC patients, 17.1% of cases had single-cell invasion, 30.9% of cases had tumor cell nests that comprised of <5 cells, 21% of cases had tumor cell nests, in which the smallest nest comprised of 5–15 cells, and 31% of cases had tumor cell nests that only comprised of large tumor cell nests (>15 cells).[12] The tumor cell nest size at the invasive front typically and strongly correlates with the tumor cell nest size in the central tumor area. In addition, the presence of smaller cell nests at the tumor invasive front is linked to worse prognosis, larger tumor size, and more advanced tumor stage, but these may not be correlated with nodal metastases, remote metastases, or tumor grades. It is noteworthy that two of the four cell nest size increments, which are single cell invasion and small tumor nests, also represent tumor budding. Therefore, the evaluation of tumor cell nest size in daily pathological practice may be redundant, according to some studies.[10]

Characteristically, tumor cell nests that contain tumor buds are best regarded as 2D histological biomarkers for tumor fragments, and are a measure of tumor invasiveness. LSCC presents varying degrees of keratinization and/or formation of intercellular bridges between cancer cells. As a result of this tight intercellular connection, collective cell migration is more prominent in LSCC, which subsequently leads to a network of epithelial branches. These can be observed by 3D imaging, and exhibits high variability in the sizes of tumor cells when observed by 2D histology. In an image-based computational quantification study of LSCC, it was revealed that tumor fragmentation is linked to increased number of blood vessels, mediastinal and perineural tumor invasion, and worse survival rate.[24] Similarly, the RNA-seq and LC-MS/MS data analyses revealed the upregulation of extracellular matrix (ECM) remodeling processes, focal cell-adhesion, and the characteristic increase in cell motility. The association of tissue fragments with two known ECM proteins, periostin and versican, was also confirmed.

## Other potential morphological predictors of LSCC

One report suggested that patients with keratinizing LSCC have shortened OS, when compared to patients with nonkeratinizing or basaloid squamous cell carcinomas.[25] One multivariate analysis study confirmed this link. However, the association between keratinization and DFS was not evident.[12]

Regarding the stromal content, moderate-to-high levels of stromal content (*vs.* low stromal content) was linked to worse OS, but not to DFS, in LSCC patients,[12,26] and this was also associated with nodal metastasis.

Other potential morphological predicators of LSCC include nuclear size, the Ki-67 proliferative index, lymphoplasmacytic reaction in the stroma, and the ratio of stromal plasma cells.[2,27–30] However, these markers have not been extensively studied in large cohorts, and the clinical implications require further evaluation.

In summary, emerging prognostic markers for LSCC are being evaluated in large clinical/pathological cohorts. Similar to lung adenocarcinoma markers, these markers can guide the clinical management of LSCC patients, and accelerate the development of future targeted therapies for LSCC.

## Future direction

The investigators hypothesize that emerging histological features of LSCC can enhance the prognostic value of the TNM staging manual alone. The application of modern technologies, including machine-learning, can make the histological analysis accurate and reproducible.

## Conclusions

The present review highlights the prognostic potential of emerging histological features of LSCC, including tumor budding, tumor cell nesting, and the spreading of tumors through air spaces. The investigators consider that a large scale clinical study would be able to solidify the value of these features in clinical practice.

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## Abbreviations:

| | |
|---|---|
| **SCC** | squamous cell carcinoma |
| **STAS** | Tumor spread through air spaces. |

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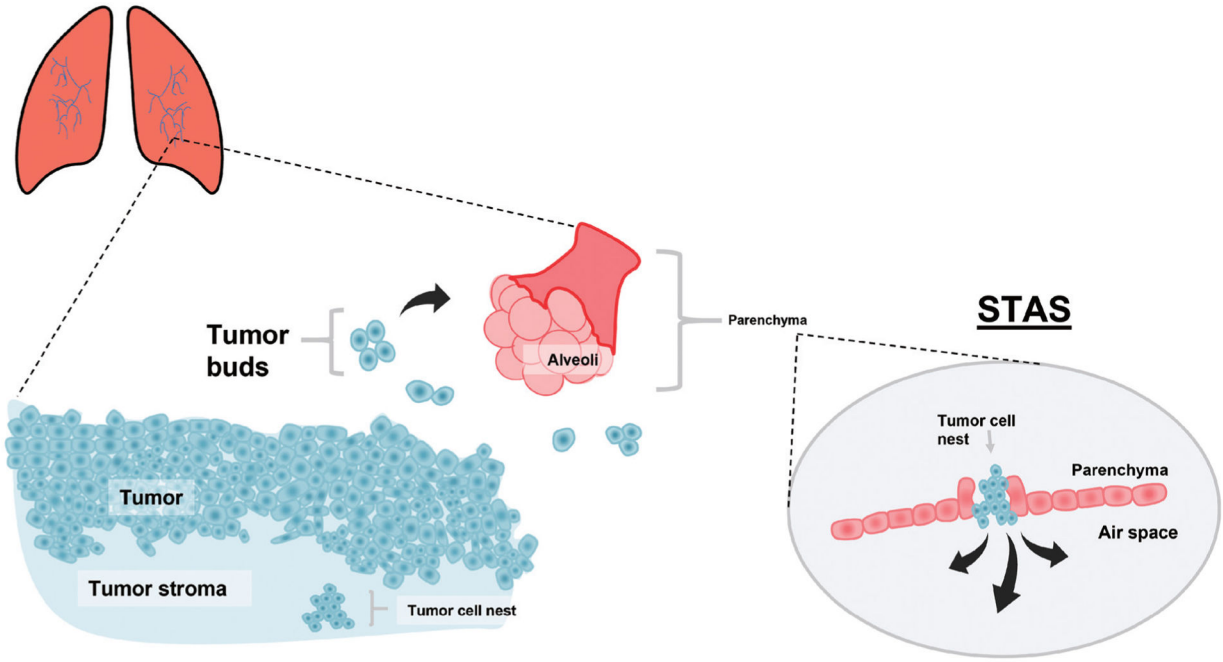


**Fig. 1.**

The relationship among tumors, tumor budding, tumor spread through air spaces, and tumor cell nests.