BMJ Open

Potential key roles of tumour budding: a representative malignant pathological feature of non-small cell lung cancer and a sensitive indicator of prognosis

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To cite: Qian L, Zhang J, Lu S, *et al*. Potential key roles of tumour budding: a representative malignant pathological feature of non-small cell lung cancer and a sensitive indicator of prognosis. *BMJ Open* 2022;**12**:e054009. doi:10.1136/bmjopen-2021-054009

► Prepublication history for this paper is available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2021-054009).

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Received 12 June 2021
Accepted 18 February 2022

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ABSTRACT

Objectives To investigate the relationship between tumour budding, clinicopathological characteristics of patients and prognosis in non-small cell lung cancer.

Study design A retrospective study was used.

Participants We selected 532 patients with non-small cell lung cancer from China, including 380 patients with adenocarcinoma and 152 with squamous cell carcinoma.

Primary and secondary outcome measures Tumour budding was visible using H&E staining as well as pancytokeratin staining. The count data and measurement data were compared using the χ^2 test and the t-test, respectively. The overall survival rate was the follow-up result. The survival curves were drawn using the Kaplan-Meier method, and the differences between groups were analysed using the log-rank method. The independent prognostic factor of patients with lung cancer was determined using a multivariate Cox proportional hazard model.

Results In patients with lung adenocarcinoma, there was a correlation between tumour budding and spread through air spaces (OR 36.698; 95% CI 13.925 to 96.715; p<0.001), and in patients with squamous cell carcinoma, tumour budding state was closely related to the peritumoural space (OR 11.667; 95% CI 4.041 to 33.683; p<0.001). On Cox regression analysis, multivariate analysis showed that tumour budding, pleural and vascular invasion, spread through air spaces, tumour size, lymph node metastasis, and tumour node metastasis stage were independent risk factors of prognosis for patients with non-small cell lung cancer.

Conclusions As an effective and simple pathological diagnostic index, it is necessary to establish an effective grading system in the clinical diagnosis of lung cancer to verify the value of tumour budding as a prognostic indicator. We hope that this analysis of Chinese patients with non-small cell lung cancer can provide useful reference material for the continued study of tumour budding.

Strengths and limitations of this study

- We selected 532 patients with non-small cell lung cancer (NSCLC) from China, including 380 patients with adenocarcinoma and 152 with squamous cell carcinoma, to explore the correlation between tumour budding, the clinicopathological characteristics of these patients and prognosis.
- Through the evaluation of tumour budding in lung cancer specimens of Chinese patients, we hope to provide reference for the establishment of tumour budding criteria in the diagnosis of lung cancer.
- Our research was limited to the tumour budding analysis of NSCLC patients in China, and the results of different ethnicities may differ.
- This study only included surgical resection specimens, no biopsy specimens.

INTRODUCTION

Lung cancer is among the most common malignant tumours in China and the world. According to global cancer data from 2020, lung cancer is the most common type of cancer (11.4% of the total) and cancer-related death (18% of total cancer deaths).[1] Early lung cancer has few clinical manifestations and is easily ignored or even missed. With the spread and infiltration of tumour cells, most patients lose the opportunity for radical surgery. In recent years, with the rapid development of medical technology, immunotherapy has become a hot spot in the treatment of lung cancer. In a meta-analysis study by Tartarone *et al*, the results showed that in pretreated patients with non-small cell lung cancer (NSCLC), three immune checkpoint inhibitors (ICIs) such as nivolumab, pembrolizumab and atezolizumab, as well as two anti-PD-1 (nivolumab and pembrolizumab) and one anti-PD-L1 (atezolizumab) can be administered. The findings support the superiority of ICIs over docetaxel in pretreated NSCLC patients, and suggest that anti-PD-1 inhibitors may have a minor advantage over anti-PD-L1 inhibitors.[2] Petrelli *et al* confirmed in their meta-analysis that there is moderate evidence that adding ICIs to chemotherapy improves

overall survival (OS) when compared with chemotherapy alone.[3] However, in a review of Zhu *et al* put forward different opinions. Their research results show that immunotherapy for patients with NSCLC after surgery or radiotherapy cannot prolong their survival time. At the same time, they noted that an interim analysis for one of these trials revealed that treated participants with stage III NSCLC had a better PFS.[4] Most current studies are combined therapies, such as dendritic cells (DCs) or DCs/cytokine-induced killer therapy in combination with chemotherapy in advanced lung cancer, according to a review by Mohsenzadegan *et al*.[5] However, these medications have only had little success in the treatment of advanced NSCLC.[5] Invasion and metastasis are among the main causes of lung cancer death and play a decisive role in lung cancer staging and management.

As a pathological phenomenon, tumour budding has been attracting increased attention. Some studies have shown that tumour budding is a factor that reflects the malignant invasion and poor prognosis of digestive tract tumours.[6] The Union for International Cancer Control (UICC) has officially recognised that tumour budding is an independent prognostic factor for colorectal cancer (CRC) patients. However, only a few studies have explored its significance in lung cancer.

In recent years, with the increasing research on cancer prognosis, some scholars have reported that the morphological characteristics of the peritumoural space are related to patient prognosis. Peritumoural spaces have been noted in breast, lung, bladder, and prostate cancers as well as other malignant tumours. Tumour cells generally spread to the corresponding lymph nodes through the lymphatic system, a phenomenon that is considered an important early event of tumour metastasis.[7 8] However, the presence of a correlation between tumour budding and the peritumoural space has been rarely reported.

In this study, we selected 532 cases of patients with NSCLC from China, including 380 cases of adenocarcinoma and 152 cases of squamous cell carcinoma, to explore the correlation between tumour budding, patients' clinicopathological characteristics and prognosis with the aim of determining a reference value for evaluating patient prognosis and clinical treatment.

MATERIAL AND METHODS

Patients' general information

We retrieved the pathological reports of patients who met the inclusion criteria from the files of the pathology system and obtained other clinical pathological information from the electronic medical record system. All 532 cases included in this study were radical surgical specimens. The data of 380 patients with primary lung adenocarcinoma and 152 patients with primary lung squamous cell carcinoma treated in the Cardiothoracic Disease Department of the Affiliated Hospital of Nantong University between June 2009 and July 2015. We excluded patients for whom follow-up information was lacking; thus, and a total of 532 patients (302 males, 230 females; 202 patients were ≤65 years old, while 328 patients were >65 years old). None of the patients received chemotherapy or radiotherapy preoperatively. The clinical and pathological information and medical records were complete for each patient.

We took the corresponding paraffin blocks of each patient from the pathological diagnosis centre and sliced them into 3 µm thick slices. Each slice was floated in 45°C warm water on a spreader to flatten the tissue, which was then picked up with a slide and baked in an oven at 65°C. Cytokeratin immunohistochemical staining (CK) and H&E staining were performed. Rabbit polyclonal anti-human pancytokeratin (CKpan) antibody was used (dilution 1:50; ab215838, Abcam, USA). The evaluations were independently performed by three experienced pathologists using a multihead microscope (Precise Instrument, Beijing, China) to reach consensus.

Patient and public involvement

The patients were followed up by telephone and outpatient service. The starting point of follow-up was the operation time for each patient, while the end point was the time of death. If the patient was still alive, we selected the last follow-up appointment as the termination point.

Histological type assessment

We observed the histopathological structure of each tissue sample under the microscope and classified the tumour tissues according to the diagnostic criteria formulated by the WHO in 2015. The tumour node metastasis (TNM) staging was based on UICC/American Joint Committee on Cancer (AJCC) eighth edition.

Evaluation of tumour budding with H&E

The slides stained with H&E were placed under a 10×20 light microscope to observe the densest portion of the budding. The areas of budding were then counted in high-power fields (HPFs).

The judgement of tumour budding refers to the standard of Ueno *et al*,[9] that is, an isolated single tumour cell or small clusters of tumour cells composed of no more than four tumour cells in the stroma at the start of the tumour invasion were considered tumour budding.

To employ a semiquantitative method to analyse tumour budding, we counted the mean number of tumour buds under 10 HPFs. The tumour budding was divided into non-budding, low budding (≤10 buds/10 HPFs) and high budding (>10 buds/10 HPFs).

Tumour cell clusters surrounded by tumour stroma were defined as tumour cell nests. Based on Moritz's research method[10] and according to the histomorphology characteristics of lung cancer, we divided the cell nests in tumour stroma into 2–4 tumour cell nests and a single invasive cancer cell in the matrix of the tumour invasion edge. We also divided tumour interstitial fibrosis into negative, very low (10% of the total tumour area), low (10%–25%), medium (25%–50%) and high (>50%).

Evaluation of tumour budding assisted with cytokeratin

The clarity of HE and pancytokeratin staining on tumour budding were compared.

It remains controversial whether H&E or cytokeratin (CK) staining should be used for budding markers. CK staining can reportedly more clearly show the bud focus covered by the significant peritumoural inflammatory reaction.[11] CK staining also aides in the observation of a large number of germinal foci mixed with stromal fibroblasts.[12] CK staining can produce three to four times more buds than H&E staining.[13] In many studies, many scholars chose CK staining for sprouting evaluations.[12 14–20] Therefore, here we used both H&E staining and pancytokeratin staining and observed the budding state of each level between methods. The budding site was more easily observed and the scope of the bud focus was clearer using pancytokeratin staining.

Statistical analysis

The data were analysed using SPSS V.26.0 software (IBM). The χ^2 test and t-test were used to compare the count data and measurement data, respectively. The follow-up result was the OS rate. The Kaplan-Meier method was used to draw the survival curves, while the log rank method was used to analyse the differences among groups. A multivariate Cox proportional hazard model was used to determine the independent prognostic factors of the lung cancer patients. The difference was statistically significant ($p<0.05$).

RESULTS

Tumour budding in NSCLC patients

In cases of lung cancer with tumour budding, the front edge was not smooth and the budding tumour cells were heteromorphic, irregularly shaped, rich in cytoplasm, often fused and eosinophilic. The nucleus was irregularly shaped and the staining was deeper than that of stromal cells. However, the tumour budding foci were sometimes easily confused with poorly differentiated stromal cells. However, compared with H&E staining, CK staining can more clearly show tumour budding spores (figure 1).

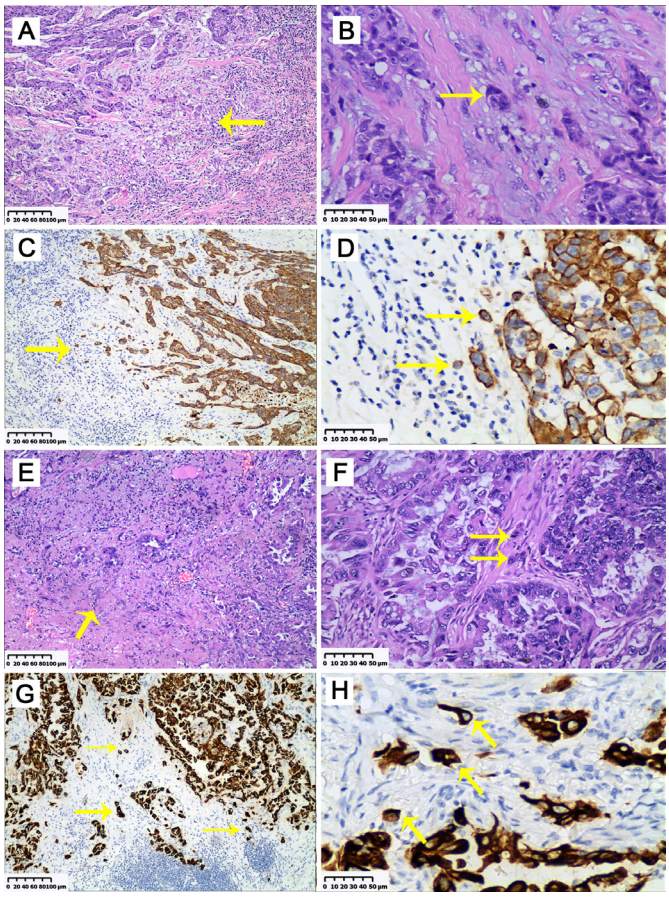

Figure 1 The tumour budding with H&E staining and immunohistochemical staining. (A–D) The budding of the tumour in lung squamous cell carcinoma. (E–H) The tumour budding in lung adenocarcinoma. A, C, E and G were ×20 magnification. B, D, F and H were ×40 magnification (bar=500 µm). The yellow arrow represents the tumour budding.

Relationship between tumour budding and clinicopathological features of patients with NSCLC

Tumour interstitial fibrosis was defined as fibrosis observed under ×100 magnification. According to the area of fibrosis, it was classified as negative, ≤10%, 10%–25%, 25%–50% and >50%. The peritumoural space, that between the tumour cells and the stroma, was the morphological manifestation of the interaction between them that clearly divided the tumour components and the stroma.[7] Shah *et al*[21] reported that the peritumoural space was very common in tumours and related to invasive cancer cell nests.

Among the 380 cases of lung adenocarcinoma, 46 showed no tumour budding and 334 showed tumour budding. Tumour budding status was closely related to the 5-year OS status of patients with lung adenocarcinoma. In addition, it was closely related to tumour histological subtype ($p<0.001$), tumour size ($p<0.001$), lymph node metastasis ($p<0.001$), vascular invasion (OR 3.693; 95% CI 1.847 to 7.383; $p<0.001$), pleural invasion (OR 13.393; 95% CI 5.512 to 32.542; $p<0.001$), spread through air spaces (STAS) (OR 36.698; 95% CI 13.925 to 96.715; $p<0.001$), tumour necrosis ($p=0.005$), tumour interstitial fibrosis ($p<0.001$) and TNM stage ($p<0.001$). However, tumour budding was not related to the patient gender (OR 1.086; 95% CI 0.583 to 2.021; $p=0.875$) or age (OR 0.959; 95% CI 0.510 to 1.804; $p=0.898$). The proportion of tumour budding in patients with vascular tumour thrombus was significantly higher than that in patients without vascular tumour thrombus. The greater the degree of lymph node metastasis, the higher the proportion of tumour budding (table 1). In the 152 patients with primary squamous cell carcinoma of the lung (table 2), tumour budding status was significantly correlated with the 5-year OS status (OR 0.098; 95% CI 0.027 to 0.350; $p<0.001$), peritumoural space (OR 11.667; 95% CI 4.041 to 33.683; $p<0.001$), vascular invasion (OR 5.426; 95% CI 1.855 to 15.865; $p<0.001$), tumour size ($p<0.001$), lymph

Table 1 The correlation of tumour budding with clinicopathological characteristics of lung adenocarcinoma patients

Characteristic	All cases	Tumour budding		χ^2	P value
		Negative	Positive		
Total	380				
Age (year)				0.016	0.898
≤65	150	18 (11.84%)	134 (88.16%)		
>65	228	28 (12.28%)	200 (87.72%)		
Gender				0.067	0.875
Male	208	26 (12.50%)	182 (87.50%)		
Female	172	20 (11.63%)	152 (88.37%)		
Histological subtype				128.953	< 0.001*
Adherent type	63	34 (53.97%)	29 (46.03%)		
Acinar type	140	1 (0.71%)	139 (99.29%)		
Papillary type	49	2 (4.08%)	47 (95.92%)		
Micropapillary type	62	7 (11.29%)	55 (88.71%)		
Solid type	66	2 (3.03%)	64 (96.97%)		
Pleural invasion				48.730	< 0.001*
Absent	151	40 (26.49%)	111 (73.51%)		
Present	229	6 (2.62%)	223 (97.38%)		
Vascular invasion				15.095	< 0.001*
Absent	179	34 (18.99%)	145 (81.01%)		
Present	201	12 (5.97%)	189 (94.03%)		
STAS				103.402	< 0.001*
Absent	102	41 (40.20%)	61 (59.80%)		
Present	278	5 (1.80%)	273 (98.20%)		
Interstitial fibrosis				141.608	< 0.001*
Negative	11	7 (63.64%)	4 (36.63%)		
≤10%	94	39 (41.49%)	55 (58.51%)		
10%–25%	99	0 (0.00%)	99 (100.00%)		
25%–50%	113	0 (0.00%)	113 (100.00%)		
> 50%	63	0 (0.00%)	63 (100.00%)		
Necrosis				10.737	0.005*
Absent	114	22 (19.30%)	92 (80.70%)		
Focal area	216	16 (7.41%)	200 (92.59%)		
A large area	50	8 (16.00%)	42 (84.00%)		
pT				115.713	< 0.001*
pT1a	18	14 (77.80%)	4 (22.22%)		
pT1b	64	6 (9.38%)	58 (90.63%)		
pT1c	65	20 (30.77%)	45 (69.23%)		
pT2a	64	4 (6.25%)	60 (93.75%)		
pT2b	84	1 (1.19%)	83 (98.81%)		
pT3	77	1 (1.3%)	76 (98.70%)		
pT4	8	0 (0.00%)	8 (100.00%)		
pN				27.761	< 0.001*
pN0	195	40 (20.51%)	155 (79.49%)		
pN1	82	1 (1.22%)	81 (98.78%)		

Continued

Table 1 Continued

Characteristic	All cases	Tumour budding		χ^2	P value
		Negative	Positive		
pN2	82	5 (6.10%)	77 (93.90%)		
pN3	21	0 (0.00%)	21 (100.00%)		
TNM stage				41.194	< 0.001*
Ia1	6	4 (66.67%)	2 (33.33%)		
Ia2	76	12 (15.79%)	64 (84.21%)		
Ia3	48	11 (22.92%)	37 (77.08%)		
Ib	41	9 (21.95%)	32 (78.05%)		
IIa	15	3 (20.00%)	12 (80.00%)		
IIb	87	2 (2.30%)	85 (97.70%)		
IIIa	76	4 (5.26%)	72 (94.74%)		
IIIb	27	1 (3.70%)	26 (96.30%)		
IIIc	3	0 (0.00%)	3 (100.00%)		
IV	1	0 (0.00%)	1 (100.00%)		
5-year survival				32.644	< 0.001*
No	183	4 (2.19%)	179 (97.81%)		
Yes	197	42 (21.32%)	155 (78.68%)		

*P < 0.05
STAS, spread through air spaces; TNM, tumour node metastasis.

node metastasis (p=0.040), STAS (OR 7.230; 95% CI 2.021 to 25.863; p=0.001), tumour necrosis (p=0.030), TNM stage (p<0.001) and tumour interstitial fibrosis (p<0.001).

Survival analysis of patients

All 532 patients were included in the survival analysis study by July 2020. The follow-up time was 3–82 months. At the end of the study, 261 patients were still alive. Among the dead patients, the proportion of high-grade budding was significantly higher than those of the low-grade budding and non-budding groups. The Kaplan-Meier method was used to analyse the postoperative survival rate, while the log rank method was used to test the intergroup differences.

In patients with lung adenocarcinoma, univariate analysis showed that tumour budding, tumour budding nucleus size, pleural and vascular invasion, STAS, histological subtype, necrosis area and TNM stage were significantly associated with 5-year survival (table 3). We then used the Cox proportional hazard regression model to analyse the statistically significant indicators of the univariate analysis. For the budding model, we took the above factors as variables, and the tumour budding (HR 1.298; 95% CI 1.033 to 1.630; p=0.025), nuclear size (HR 1.477; 95% CI 1.070 to 2.039; p=0.018), pleural invasion (HR 1.527; 95% CI 1.052 to 2.217; p=0.026), vascular invasion (HR 2.144; 95% CI 1.285 to 3.578; p=0.004), STAS (HR 2.695; 95% CI 1.597 to 4.548; p<0.001), necrosis (HR 1.328; 95% CI 1.016 to 1.734; p=0.038), histological subtype (HR 0.855; 95% CI 0.758 to 0.965; p=0.011), pT (HR 2.011; 95% CI 1.645 to 2.458; p<0.001), pN (HR 2.038; 95% CI 1.413 to 2.940; p<0.001) and TNM stage (HR 0.481; 95% CI 0.299 to 0.773; p=0.002) also showed a statistically significant correlation with the 5-year survival rate based on the Cox regression univariate analysis (figure 2).

The Kaplan-Meier survival curve showed that the higher the budding grade, the lower the 5-year OS rate (p<0.001) (figure 3). In the histological subtypes of lung adenocarcinoma, the higher the level of tumour budding, the worse the prognosis in cases with micropapillary subtypes and solid subtypes (figure 4). In the adherent subtype (p=0.356), papillary subtype (p=0.567) and acinar subtype (p=0.353), there was no statistical correlation between tumour budding degree and survival status. Compared with tumour budding cell nucleus containing fewer than three lymphocytes (small size), when the tumour budding nucleus had four or more lymphocytes (large size), the 5-year OS rate of lung adenocarcinoma patients was significantly reduced (figure 5A).

In cases of lung squamous cell carcinoma, tumour budding size, budding tumour nest, pleural and vascular invasion, STAS, tumour interstitial fibrosis area, peritumoural space, tumour size and lymph node metastasis, and TNM stage influenced patient 5-year survival rate (table 4). To eliminate the interactions between variables, multivariate Cox regression analysis was used to analyse the data. The above factors independently affected the prognosis of patients with squamous cell carcinoma (figure 2). The Kaplan-Meier survival curve showed

Table 2 The correlation of tumour budding with clinicopathological characteristics of lung squamous cell carcinoma patients

Characteristic	All cases	Tumour budding		χ^2	P value
		Negative	Positive		
Total	152				
Age (year)				3.776	0.075
≤65	52	3 (5.77%)	49 (94.23%)		
>65	100	17 (17.00%)	83 (83.00%)		
Gender				0.457	0.622
Male	94	11 (11.70%)	83 (88.30%)		
Female	58	9 (15.52%)	49 (84.48%)		
Peritumoural space				27.333	<0.001*
Absent	36	14 (38.89%)	22 (61.11%)		
Present	116	6 (5.17%)	110 (94.83%)		
Pleural invasion				1.341	0.475
Absent	132	19 (14.39%)	113 (85.61%)		
Present	20	1 (5.00%)	19 (95.00%)		
Vascular invasion				11.160	<0.001*
Absent	62	15 (24.19%)	47 (75.81%)		
Present	90	5 (5.56%)	85 (94.44%)		
STAS				11.715	0.001*
Absent	75	17 (22.67%)	58 (77.33%)		
Present	77	3 (3.90%)	74 (96.10%)		
Interstitial fibrosis				51.047	<0.001*
Negative	6	6 (100.00%)	0 (0.00%)		
≤10%	32	8 (25.00%)	24 (75.00%)		
10%–25%	49	4 (8.16%)	45 (91.84%)		
25%–50%	36	0 (0.00%)	36 (100.00%)		
> 50%	29	2 (6.90%)	27 (93.10%)		
Necrosis				6.983	0.030*
Absent	7	2 (28.57%)	5 (71.43%)		
Focal area	92	16 (17.39%)	76 (82.61%)		
A large area	53	2 (3.77%)	51 (96.23%)		
pT				31.561	<0.001*
pT1a	1	1 (100.00%)	0 (0.00%)		
pT1b	20	6 (30.00%)	14 (70.00%)		
pT1c	31	10 (32.26%)	21 (67.74%)		
pT2a	33	2 (6.06%)	31 (93.94%)		
pT2b	34	0 (0.00%)	34 (100.00%)		
pT3	22	0 (0.00%)	22 (100.00%)		
pT4	11	1 (9.09%)	10 (90.91%)		
pN				8.284	0.040*
pN0	84	17 (20.24%)	67 (79.76%)		
pN1	47	2 (4.26%)	45 (95.74%)		
pN2	19	1 (5.26%)	18 (94.74%)		
pN3	2	0 (0.00%)	2 (100.00%)		
TNM stage				32.131	<0.001*
Ia1	4	1 (25.00%)	3 (75.00%)		

Continued

Table 2 Continued

Characteristic	All cases	Tumour budding		χ^2	P value
		Negative	Positive		
Ia2	18	5 (27.78%)	13 (72.22%)		
Ia3	23	10 (43.48%)	13 (56.52%)		
Ib	16	2 (12.50%)	14 (87.50%)		
IIa	19	0 (0.00%)	19 (100.00%)		
IIb	38	1 (2.63%)	37 (97.37%)		
IIIa	25	1 (4.00%)	24 (96.00%)		
IIIb	5	0 (0.00%)	5 (100.00%)		
IIIc	3	0 (0.00%)	3 (100.00%)		
IV	1	0 (0.00%)	1 (100.00%)		
5-year survival				17.383	<0.001*
No	88	3 (3.41%)	85 (96.59%)		
Yes	64	17 (26.56%)	47 (73.44%)		

*P < 0.05
STAS, spread through air spaces; TNM, tumour node metastasis.

that the 5-year OS rate of patients with lung squamous cell carcinoma in TNM stage II was significantly higher than that of patients with high-grade tumour budding (figure 6B), while the 5-year OS rate of lung squamous cell carcinoma patients with single cell tumour budding was significantly lower (figure 5B).

DISCUSSION

Cancer is an issue of great concern worldwide, and its prognosis mainly depends on the pathological type, TNM stage, tumour differentiation degree and microvascular invasion, and patients with the same TNM stage but quite different prognoses are often seen in the clinical setting. In recent years, as a pathological phenomenon, tumour budding has attracted increasing attention. Tumour budding, also known as focal dedifferentiation is the first step in the process of a malignant tumour's invasion and metastasis. Therefore, tumour budding is considered a key step in a tumour's invasive growth process.[22] Tumour budding spores are considered cancer stem cells, which are defined as isolated single tumour cells or clusters of fewer than five tumour cells at the start of tumour invasion.[11] Some studies stated that tumour budding is not a static histological feature; rather, it involves a small focal tumour cell complex separated from the main body of the tumour that enters the surrounding tissue in a 'budding' manner, which represents a dynamic process.[23] Gabbert *et al*[22] also supported this conclusion. Shinto *et al*[14] reported that there were interconnected cytoplasmic pseudo fragments similar to pseudopodia processes between budding tumour cells, which may be related to the increase in cell invasion ability. In addition, some studies have speculated that tumour budding is a step in the progression of malignant tumours from focal lesions to systemic diseases.[24] Tumour budding is now considered of great significance in tumour invasion and metastasis.[25–28] Some studies have shown that tumour budding reflected the invasiveness and poor prognosis of digestive tract tumours.[6] The presence of tumour budding may be related to the late stage of a tumour, frequent lymphatic vascular invasion, and lymph node and distant metastasis. The UICC officially recognises tumour budding as an independent prognostic factor for CRC. It was recently used as a significant prognostic indicator for the treatment of oesophageal squamous cell carcinoma, gastro-oesophageal junction adenocarcinoma, and gastric adenocarcinoma.[29] In the current study of 380 cases of primary lung adenocarcinoma and 152 cases of primary lung squamous cell carcinoma, we found that tumour budding was closely related to the 5-year OS, tumour size, lymph node metastasis, vascular invasion, spread through air spaces (STAS), tumour necrosis, tumour interstitial fibrosis and TNM stage. This suggests that tumour budding may be an important indicator of malignant invasion and metastasis. Compared with NSCLC patients without tumour budding, those with the morphological characteristics of tumour budding have a worse 5-year OS prognosis.

The detection accuracy of abdominal B-ultrasound and abdominal CT for lymph node metastasis is reportedly 12.2%–80.0%[30] and 50%–80%, respectively.[31–34] Gulluoglu *et al*[35] evaluated 126 patients with gastric cancer and found that lymph node metastasis was the only parameter associated with tumour budding. Masaki *et al*[36] established a model formula for predicting the probability of lymph node metastasis in 76 patients with T1 stage CRC as follows: $z=0.070 \times (\text{budding count}) - 3.726$, $\text{probability}=1/1+e^{-z}$. Furthermore, the tumour budding count was included in the clinical decision-making analysis of

Table 3 The univariate analysis of 5-year survival prognostic factors in lung adenocarcinoma patients

Variable	Univariate analysis	
	P value > |z|	HR (95% CI)
Tumour budding (10 HPF)		
Low (n=141) vs high (n=193)	0.011*	1.374 (1.077 to 1.753)
Nuclear size		
Small (n=145) vs large (n=189)	0.023*	1.467 (1.054 to 2.042)
Smallest tumour cell nest		
Single cell (n=166) vs 2–4cells (n=168)	0.699	0.943 (0.702 to 1.267)
Gender		
Male (n=208) vs female (n=172)	0.252	0.835 (0.614 to 1.136)
Age(years)		
≤65 (n=150) vs > 65 (n=228)	0.050	1.362 (1.00 to 1.854)
Pleural invasion		
Absent (n=151) vs present (n=229)	0.021*	1.560 (1.071 to 2.272)
Vascular invasion		
Absent (n=179) vs present (n=201)	0.001*	2.357 (1.401 to 3.965)
STAS		
Absent (n=102) vs present (n=278)	<0.001*	2.874 (1.690 to 4.887)
Necrosis		
Absent (n=114) vs present (n=266)	0.047*	1.315 (1.004 to 1.722)
Histological subtype		
Adherent type (n=63) vs acinar type (n=140) vs papillary type (n=49) vs micropapillary type (n=62) vs solid type (n=66)	0.014*	0.858 (0.759 to 0.969)
Interstitial fibrosis		
Absent(n=11) vs present(n=369)	0.200	0.900 (0.766 to 1.057)
pT		
pT1 +pT2(n=295) vs pT3 +pT4 (n=85)	<0.001*	2.069 (1.687 to 2.538)
pN		
pN0(n=195) vs pN1 +pN2+pN3 (n=185)	<0.001*	1.974 (1.363 to 2.858)
TNM stage		
I+ II(n=273) vs III+ IV(n=107)	0.003*	0.484 (0.301 to 0.780)

*P < 0.05
HPF, high-power field; STAS, spread through air spaces; TNM, tumour node metastasis.

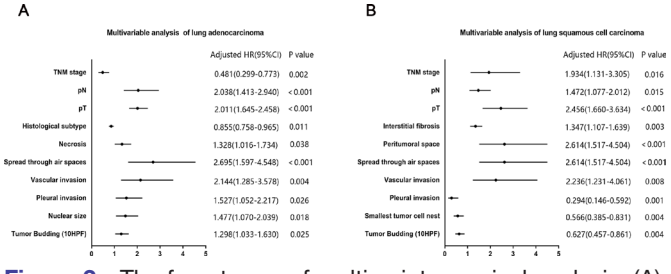

Figure 2 The forest map of multivariate survival analysis. (A) The results of multivariate analysis of lung adenocarcinoma. (B) The results of multivariate analysis of lung squamous cell carcinoma. HPF, high-power field; TNM, tumour node metastasis.

patients to determine whether patients require additional surgery after endoscopic treatment. Some studies have shown that the presence of tumour budding in biopsy specimens before CRC surgery increases the possibility of lymph node and distant metastasis. Therefore, neoadjuvant therapy and surgical treatment can be considered for these patients.[37] The Japanese Society for Cancer of the Colon and Rectum has incorporated the index of tumour budding into the guidelines for patients with pT1 disease who require further surgery.[38] In our study, 244 of 253 patients with lymph node metastasis had tumour budding. The sensitivity of budding for predicting lymph node metastasis was 96.44%, indicating that tumour budding is an effective pathological index with high sensitivity for

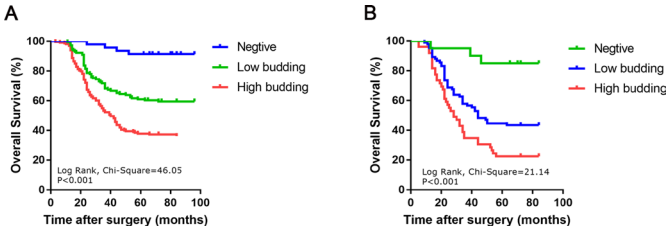

Figure 3 Kaplan-Meier analysis of the relationship between tumour budding and 5-year overall survival rate in patients with NSCLC. (A) In patients with lung adenocarcinoma, the 5-year survival rate of patients with high-grade budding group was significantly lower than that of patients without tumour budding and low-grade tumour budding. (B) In patients with lung squamous cell carcinoma, the higher the level of tumour budding, the worse the prognosis of patients was. NSCLC, non-small cell lung cancer.

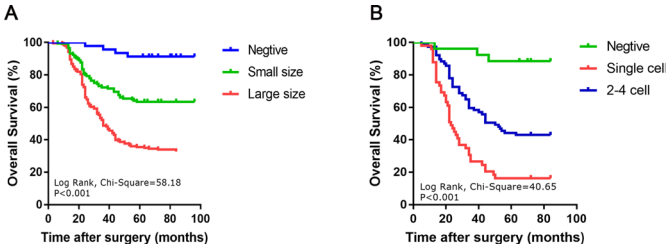

Figure 5 The relationship between the size of tumour budding nests and the nuclear size of tumour budding, as well as the 5-year survival rate of patients with NSCLC. (A) In patients with lung adenocarcinoma, the larger the nucleus of tumour budding, the lower the 5-year overall survival rate was. (B) In patients with lung squamous cell carcinoma, single cell invasion showed a worse prognosis. NSCLC, non-small cell lung cancer.

predicting lymph node metastasis. Therefore, we believe that for patients with NSCLC, we can refine the significance of tumour budding through a larger sample study to contribute to clinical decision making.

The peritumoural space is the space between the tumour cells and the stroma that divides the tumour components from the stroma and is morphological manifestation of the interaction between the tumour cells and the stromal cells. The peritumoural space is commonly seen in paraffin-embedded tissue sections fixed with formalin. The peritumoural space is one of the pathomorphological manifestations of tumour biological behaviour that is considered a prognostic factor by some scholars. Peritumoural spaces have been noted in breast, lung, bladder and prostate cancers and other malignant tumours. Tumour cells usually spread to the corresponding lymph nodes through the lymphatic system, this phenomenon is considered an important early event of tumour metastasis.[7 8] In prostate cancer, an extensive peritumural space indicates a higher tumour grade, shorter disease-free survival and poor prognosis.[39 40] At the same time, the peritumoural space in breast cancer is closely related to histological grade, lymphatic invasion, lymph node metastasis and prognosis and can be used as an important marker to judge the prognosis of breast cancer patients.[41 42] Acs *et al*[43] observed the relationship between a large peritumoural space and lymph angiogenesis, and the results confirmed a poor prognosis of patients with large peritumoural spaces, which was consistent with this hypothesis. In our study, we found that in patients with lung squamous cell carcinoma, the peritumoural space is closely related to tumour budding, which is also an independent risk factor for patient 5-year OS. A joint evaluation of the peritumoural space and tumour budding can effectively evaluate the prognosis of patients with lung squamous cell carcinoma.

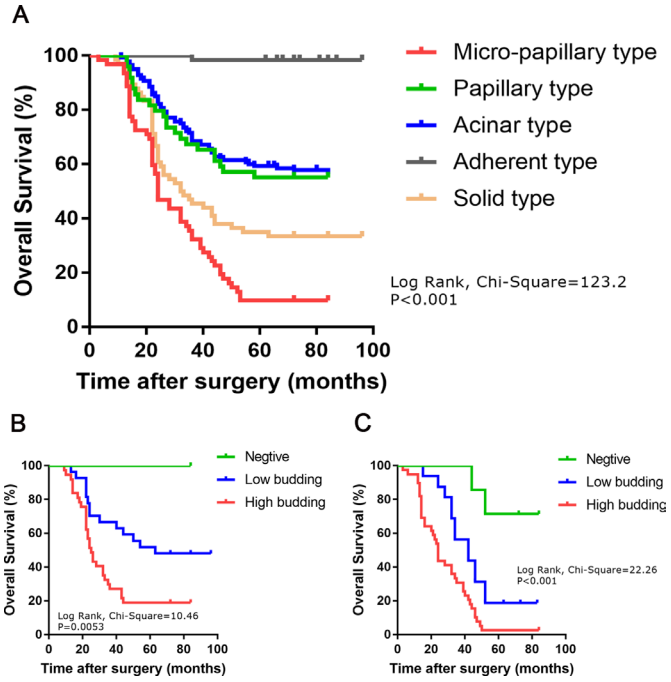

Figure 4 Kaplan-Meier analysis showed that the 5-year survival rate of patients with different histological subtypes in adenocarcinoma. (A) The survival rates of patients with different histological subtypes were different. Among them, the 5-year prognosis of patients with micropapillary subtype and solid subtype was significantly lower than that of adherent subtypes. (B) In patients with solid subtypes, the 5-year survival rate of patients with high-grade budding was significantly lower than that of patients with low-grade budding and non-budding. (C) In patients with micropapillary subtypes, the higher the grade of tumour budding, the worse the prognosis.

Lung adenocarcinoma spreads through the bronchus, known as lung metastasis, and the airways, known as airway metastasis. A small number of lung adenocarcinoma cancer cells enter the bronchial cavity, and with the respiratory movement through the bronchial discontinuous, they diffuse into other lung segments or lobes on the same or opposite side, forming new lung metastases.[44] Our study revealed that tumour budding was closely related to STAS. Tumour budding can be combined with STAS to evaluate the malignant aggressive behaviour of NSCLC.

Che *et al*[6] found that the OS rate of patients with high budding gastric adenocarcinoma was significantly lower

Table 4 The univariate analysis of 5-year survival prognostic factors in lung squamous cell carcinoma patients

	Univariate analysis	
Variable	**P value > |z|**	**HR (95% CI)**
Tumour budding (10 HPF)		
Low (n=83) vs high (n=49)	0.002*	0.589 (0.423 to 0.820)
Nuclear size		
Small (n=129) vs large (n=3)	0.159	0.390 (0.880 to 2.196)
Smallest tumour cell nest		
Single cell (n=49) vs 2–4cells (n=77)	0.002*	0.485 (0.307 to 0.769)
Gender		
Male (n=94) vs female (n=58)	0.964	1.014 (0.552 to 1.863)
Age (years)		
≤65 (n=52) vs > 65 (n=100)	0.908	0.972 (0.600 to 1.575)
Pleural invasion		
Absent (n=132) vs present (n=20)	0.001*	0.302 (0.149 to 0.613)
Vascular invasion		
Absent (n=62) vs present (n=90)	0.005*	2.397 (1.307 to 4.396)
STAS		
Absent (n=75) vs present (n=77)	0.004*	2.426 (1.327 to 4.435)
Necrosis		
Absent (n=7) vs present (n=145)	0.287	1.252 (0.828 to 1.896)
Peritumoural space		
Absent(n=36) vs present (n=116)	<0.001*	4.389 (1.920 to 10.035)
Interstitial fibrosis		
Absent(n=6) vs present (n=146)	0.009*	1.315 (1.071 to 1.614)
pT		
pT1 +pT2(n=119) vs pT3 +pT4 (n=33)	<0.001*	2.398 (1.584 to 3.629)
pN		
pN0(n=84) vs pN1 +pN2+pN3 (n=68)	0.029*	1.440 (1.038 to 1.999)
TNM stage		
I+ II(n=118)vs III+ IV(n=34)	0.016*	1.954 (1.133 to 3.372)

*P < 0.05
HPF, high-power field; STAS, spread through air spaces; TNM, tumour node metastasis .

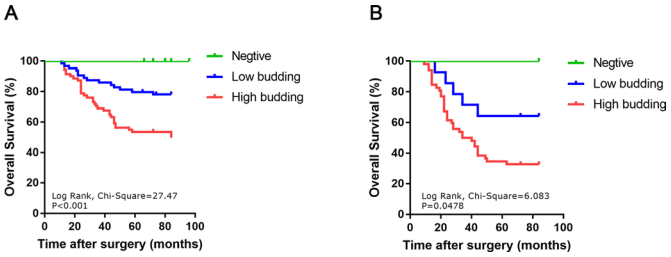

Figure 6 The relationship between tumour budding level and patients at different tumour node metastasis (TNM) stages. (A) In patients with TNM stage I lung adenocarcinoma, the higher the tumour budding level, the lower the 5-year overall survival rate. (B) In patients with TNM stage II squamous cell carcinoma, the prognosis of patients without tumour budding and low-grade tumour budding was significantly higher than that of patients with high-grade tumour budding.

than that of patients with low budding gastric adenocarcinoma. Some studies reported that the presence of tumour budding in surgical specimens of patients with gastric cancer may indicate a poor prognosis and early recurrence.[29] We also found that the 5-year OS rate of lung adenocarcinoma or squamous cell carcinoma patients with high-grade budding was significantly lower than that of patients with low-grade or no budding. However, Hass *et al*[45] emphasised that tumour budding and cancer classification based on cell differentiation were neither the same nor related. Some researchers believed that tumour budding and tumour growth pattern were independent prognostic parameters.[9] However, in our study of lung adenocarcinoma, tumour budding was closely related to histological subtype. In patients with papillary and solid subtypes of lung adenocarcinoma, the 5-year survival rate of patients with high-grade budding was significantly lower than that of patients with low-grade budding. In patients with TNM stage I, the 5-year OS rate of patients with high-grade tumour budding was lower than that of patients with low-grade or no budding (figure 6A). The results are consistent with those of Kadota *et al*.[46]

In our study, Cox regression analysis showed a significant correlation between tumour budding and 5-year OS rate. Tumour budding, pleural and vascular invasion, STAS, tumour size, lymph node metastasis and TNM stage were independent risk factors for the prognosis of NSCLC patients. In addition, tumour budding nucleus size, tumour necrosis area and histological subtype were independent prognostic factors of lung adenocarcinoma. The area of interstitial fibrosis, presence of a peritumoural space, and small tumour cell nest were independent prognostic factors in patients with squamous cell carcinoma. Therefore, we speculate that tumour budding may be a representative malignant pathological feature of NSCLC and a sensitive indicator reflective of its prognosis.

The research results of Wang *et al* suggested that tumour budding should be included in the routine histopathological report to better stratify the risk of CRC patients.[47] The AJCC and College of American Pathologists guidelines on

CRC proposed that tumour budding should be considered an optional reporting indicator and should be evaluated in all cases of stage I and II CRC. This provides us with a standardised reporting tool for tumour budding.[48] However, there is no unified scoring standard for lung cancer.

The current study had several limitations. First, our research is limited to the tumour budding analysis of NSCLC patients in China, and the results of different ethnicities may differ. For example, demographic heterogeneity in the frequency of genetic susceptibility alleles was addressed in Fathi *et al*'s review of lung cancer in the Iranian population.[49] They focused on germline and somatic gene variation, putative operable drivers of these genes, their impact on tumour immune monitoring and the drug resistance mechanism of cancer treatment in which they engage in this work. In addition, because the number of surgical specimens selected for this operation before 2015 was limited, the sample size was insufficient, which might result in sample bias. However, as an effective and simple pathological diagnosis index, it is necessary to establish an effective grading system to verify its value as a standard prognostic indicator. In addition, prospective clinical trials including multicentre samples are needed to evaluate the role of tumour budding in predicting the prognosis of lung cancer and produce reference values for the pathological diagnosis and clinical treatment of lung cancer.

CONCLUSION

To validate the utility of tumour budding as a prognostic indicator, an effective and straightforward pathological diagnostic index should be established in the clinical diagnosis of lung cancer. We selected 532 Chinese patients with NSCLC for this investigation, including 380 with adenocarcinoma and 152 with squamous cell carcinoma. Our findings reveal a link between tumour budding and STAS in patients with lung adenocarcinoma, and a connection between tumour budding and the peritumoural space in patients with squamous cell carcinoma. Multivariate analysis revealed that tumour budding, pleural and vascular invasion, STAS, tumour size, lymph node metastasis and TNM stage were independent risk variables of prognosis for NSCLC patients by Cox regression analysis. We think that this study of Chinese patients with NSCLC will be relevant for future research into tumour budding.

Contributors The authors would like to thank LQ and JZ for performing the research, YL and JS for designing the research study, JF and XH for the excellent histological sections, LQ, JF and SL for the analysis of the data. This article was written by LQ. YL is responsible for the overall content as guarantor.

Funding This work was supported by the National Natural Science Foundation of China (grant. no. 81770266), Jiangsu Post-doctoral Foundation Research Project (grant no. 2019Z142), "Transverse" scientific research project of Nantong University (grant. no. 21ZH470), and the Scientific Research Project of Nantong Municipal Health Commission (grant. no. QA2019060).

Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval This study involves human participants and was approved by ethics name ID: 2018-L068. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. The data used to support the findings of this study are available from the corresponding author upon reasonable request.

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