Original Article

Immunohistochemistry identifies E-cadherin, N-cadherin and focal adhesion kinase (FAK) as predictors of stage I non-small cell lung carcinoma spread through the air spaces (STAS), and the combinations as prognostic factors

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Background: Spread through air spaces (STAS) is one of the multiple modes of lung cancer dissemination, yet its molecular and clinicopathological characterization remains poorly studied. This study aimed to investigate the effect of adhesion molecule expression levels on the incidence of STAS and postoperative recurrence in stage I lung cancer patients undergoing radical resection.

Methods: E-cadherin, P-cadherin, N-cadherin, focal adhesion kinase (FAK), epithelial cell adhesion molecule (EpCAM), neural cell adhesion molecule 1 (NCAM1), vascular cell adhesion molecule 1 (VCAM1), intercellular cell adhesion molecule-1 (ICAM-1) were analyzed retrospectively using immunohistochemistry in patients undergoing radical resection for stage I non-small cell lung cancer (NSCLC). Patients were categorized into four groups based on adhesion molecule expression levels: "low/low", "high/low", "low/high", and "high/high", and the group with the lowest recurrence-free probability (RFP) was defined as high risk. Associations between those adhesion molecules' expression levels and STAS were determined by using the Chi-squared test and logistic regression model. RFP was analyzed by using the log-rank test and Cox proportional risk model.

Results: As of January 1, 2024, 12 of 60 patients undergoing radical resection for stage I lung carcinoma had a disease recurrence. All 60 patients' tissue specimens were retrospectively analyzed, and there were no significant differences between patients with STAS-positive (n=30) and STAS-negative (n=30) in baseline clinicopathologic features, except for histological growth patterns. We found that low expression of E-cadherin, high expression of N-cadherin and FAK, and males were independent predictors of higher incidence of STAS. Multivariate Cox analysis showed that tumors with low E-cadherin/high N-cadherin, low E-cadherin/high FAK, and high N-cadherin/high FAK expression were important predictors of recurrence in patients with stage I lung carcinoma. In addition, females and high N-cadherin/high FAK were associated with a high risk of recurrence in patients with STAS.

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Conclusions: E-cadherin, N-cadherin, and FAK are predictors of STAS occurrence in stage I NSCLC, and their combinations are prognostic factors. The discovery of these molecular markers provides clinicians with a reliable means that may help in the early identification of individuals with a higher risk of recurrence in lung cancer patients, targeting personalized treatment plans such as aggressive adjuvant therapy or closer follow-up.

Keywords: Spread through air spaces (STAS); non-small cell lung cancer (NSCLC); cell adhesion molecules; N-cadherin; focal adhesion kinase (FAK)

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Introduction

Spread through air spaces (STAS) is a new mode of invasion that has received widespread attention since Kadota *et al.* defined STAS in 2015 as the spread of tumor cells through the airspace into the lung parenchyma near the margins of the main tumor (1). Recent studies have increasingly validated the prognostic significance of STAS in predicting adverse clinical outcomes for patients with resected lung adenocarcinoma (2,3) and squamous cell carcinoma (4-6). Nevertheless, the correlation between STAS and the molecular profile of lung carcinoma remains underexplored.

Highlight box

Key findings

- E-cadherin, N-cadherin, and focal adhesion kinase (FAK) are predictors of Stage I non-small cell lung carcinoma spread through the air spaces (STAS), and their combinations serve as prognostic factors.

What is known and what is new?

- STAS is one of the multiple modes of lung cancer dissemination, yet its molecular and clinicopathological characterization remains poorly studied.
- In patients with stage I non-small cell lung cancer (NSCLC), our study identified significant associations between the presence of E-cadherin, N-cadherin, and FAK with the occurrence of STAS. The combined risk indices of these markers were highly correlated with an increased risk of recurrence. Additionally, high N-cadherin/FAK expression in tumors provides information about a higher risk of recurrence in STAS-positive stage I lung carcinoma.

What is the implication, and what should change now?

- Utilizing immunohistochemistry to detect E-cadherin, N-cadherin, and FAK enables the enhanced identification of patients requiring adjuvant therapy or close postoperative monitoring.

Epithelial-mesenchymal transition (EMT) is a critical physiological process in embryonic development and tissue regeneration (7). Abnormal reactivation of EMT is associated with heightened tumor cell malignancy, characterized by elevated migration and invasiveness (7-9). Many independent studies have investigated markers indicative of EMT activation, suggesting a potential association between EMT and the development of tumor STAS, alongside adverse prognostic implications (6,10,11). The defining characteristic of EMT is the disruption of epithelial cell integrity, manifested by reduced adhesion junctions and resulting in cell detachment and enhanced motility (12). Cell adhesion molecules are cell surface proteins responsible for cell-cell interactions, and a variety of adhesion molecules play a crucial role in tumor progression, encompassing growth, invasion, and metastasis (13-16). Building upon the findings of prior research on EMT-related markers, we hypothesize that the incidence of STAS may be linked to altered intercellular adhesion junctions and increased migratory capabilities.

This study was designed to assess the impact of adhesion molecule expression on the incidence of STAS and postoperative recurrence in patients with stage I lung carcinoma undergoing radical resection. We present this article in accordance with the TRIPOD reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-247/rc).

Methods

Patients

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Institutional Review Board of Nanjing

Jinling Hospital (No. DBNJ20241) and individual consent for this retrospective analysis was waived. Patients with stage I primary squamous and adenocarcinoma of the lung who underwent sublobar resection or lobectomy with systemic lymph node dissection between January 2021 and December 2021 at Jinling Hospital were enrolled in the study. Exclusion criteria encompassed: patients with pathology reports from external hospitals (due to the absence of clinical and imaging data in our records); lack of available tumor specimens; tumors exceeding 4 cm in diameter; with regional lymph node metastasis; positive surgical margins; a history of neoadjuvant therapy; and loss to follow-up. The tumor node metastasis (TNM) staging was based on the 8th edition of the American Cancer Society's TNM Staging Manual.

Histologic evaluation

Lung cancer sections stained with hematoxylin and eosin (H&E) were independently assessed by the pathologist who was blinded to the patients' clinical data. The identification of STAS was based on the presence of small tumor cell clusters or nests within the lung parenchyma, extending beyond the primary tumor margins, a feature typically noted under low-power magnification in histopathological examinations. The tumors were classified based on the 2021 World Health Organization (WHO) lung cancer classification criteria (17), utilizing pathological characteristics for classification into highly differentiated (characterized by predominant adherent growth pattern without a high-grade component or with a high-grade component constituting less than 20%), moderately differentiated (marked by predominantly vesicular or papillary growth without a high-grade component or with a high-grade component less than 20%), and poorly differentiated types (defined by the presence of a high-grade component constituting 20% or more, with the high-grade component comprising solid, micropapillary, and complex glandular structures) (18). The poorly differentiated type was defined as having a high-risk growth pattern, and the remaining highly and moderately differentiated were defined as having a low-risk growth pattern. Lymphovascular invasion was identified by the detection of at least one cluster of cells within a lymphatic vessel or vein. STAS was defined by the occurrence of micropapillary clusters, small solid tumor nests, or individual cells within the air spaces of the surrounding lung parenchyma. To differentiate from artificially isolated cells during tumor dissection, a minimum of three tumor sections were examined microscopically, with initial identification at low magnification focusing on the boundaries. The presence of STAS was confirmed with tumor cells located beyond the primary tumor margins (1,2,17).

Immunohistochemical staining

Collected samples were tissue sectioned using whole block tumors. Tumor tissue sections were dewaxed and then hydrated, followed by antigen repair using high-pressure steam. Endogenous peroxidase was quenched with endogenous peroxidase blocker (Fuzhou Maixin Biotech, Fuzhou, China) for 30 min at room temperature. Next, nonspecific proteins were blocked with 5% goat serum (BOSTER, Wuhan, China) for 30 min. Subsequently, sections were incubated with E-cadherin-specific antibody (20874-1-AP, Proteintech; dilution ratio 1:10,000), P-cadherin antibody (13773-1-AP, Proteintech; dilution ratio 1:200), N-cadherin antibody (22018-1-AP, Proteintech; dilution ratio 1: 600), focal adhesion kinase (FAK) antibody (66258-1-Ig, Proteintech; dilution ratio 1: 100), epithelial cell adhesion molecule (EpCAM) antibody (66316-1-Ig, Proteintech; dilution 1: 200), neural cell adhesion molecule 1 (NCAM1) antibody (14255-1-AP, Proteintech; dilution 1: 600), and vascular cell adhesion molecule 1 (VCAM1) antibody (66294-1-Ig, Proteintech; dilution ratio 1:600), intercellular cell adhesion molecule-1 (ICAM-1) antibody (60299-1-Ig, Proteintech; dilution ratio 1:500) was incubated at 4 ℃ overnight. Color development was performed using the diaminobenzidine (DAB) horseradish peroxidase color development kit (Fuzhou Maixin Biotech). Finally, the sections were restained with hematoxylin, dehydrated, and sealed with neutral gum.

Immunohistochemical evaluation and quantification of adhesion molecule markers

Immunohistochemical analysis was employed to assess the presence of adhesion molecules within the membrane and cytoplasm of tumor cells. The assessment involved evaluating the staining distribution and intensity across primary tumors, with five tumor areas selected for analysis under an Olympus microscope featuring an EP50 digital camera (Olympus Corp., Tokyo, Japan) and a 20× objective lens. Depending on the percentage of positively stained cells, a score of 0 (0–25% of cells were positive), 1 (26–50% of cells were positive), 2 (51–75% of cells were positive) and

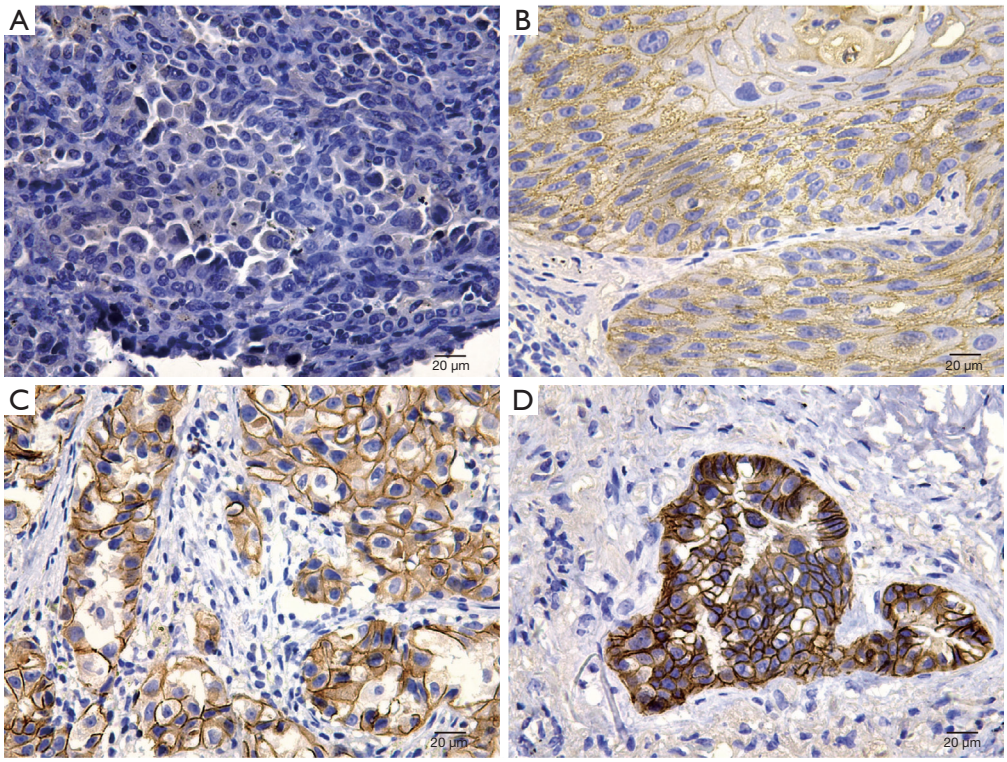

Figure 1 Evaluation of adhesion molecules expression using immunohistochemistry. (A) Score 0: negative; (B) Score 1: weakly positive; (C) Score 2: moderately positive; (D) Score 3: strongly positive.

3 (76–100% of cells were positive) was assigned. Similarly, staining intensity was classified from 0 (no expression) to 3 (strong expression) (*Figure 1*). The average expression score for each patient was derived by aggregating the scores for distribution and intensity, resulting in a composite score between 0 and 6. Expression levels were deemed high or low relative to the median score; scores above the median indicated high expression, while those below indicated low expression.

Statistical analysis

Correlations among variables were analyzed through using Chi-squared tests for categorical data and logistic regression to determine the independent correlations between STAS, clinicopathological features, and adhesion molecule expressions within a multivariate framework. Recurrence-free probability (RFP) was defined as the duration from surgical resection to the onset of disease recurrence and was calculated using the Kaplan-Meier estimator, with comparisons between groups conducted via the log-rank test. Furthermore, the impact of adhesion molecules on RFP, while adjusting for clinicopathological variables, was analyzed using Cox proportional hazards regression. Statistical tests were two-sided with a significance level set at 5%. Pearson correlation tests were utilized to explore the relationships between variables, ensuring that only variables without strong intercorrelations were included in the regression model to avoid redundancy. All statistical analyses were executed using SPSS for Windows, version 25.0 (IBM Corporation, Armonk, NY, USA).

Results

Association of STAS with clinicopathological features

Table S1 outlines the clinical and pathological features of 60 stage I lung carcinoma patients. The cohort's median age was 64 (range, 37–87) years, with males comprising 52% (31 patients) of the population. The vast majority, 92% (55 patients), were classified as pathological stage Ia. Surgical procedures consisted of lobectomy in 80% (48 patients)

Table 1 Analysis of the associations between tumor STAS and adhesion molecules

Variables	Total, n [%]	STAS, n [%]		P value
		Absent	Present	
E-cadherin expression				
Low	32 [53]	10 [33]	22 [73]	0.002
High	28 [47]	20 [67]	8 [27]	
P-cadherin expression				
Low	32 [53]	19 [63]	13 [43]	0.12
High	28 [47]	11 [37]	17 [57]	
N-cadherin expression				
Low	32 [53]	21 [70]	11 [37]	0.01
High	28 [47]	9 [30]	19 [63]	
FAK expression				
Low	30 [50]	20 [67]	10 [33]	0.01
High	30 [50]	10 [33]	20 [67]	
EpCAM expression				
Low	30 [50]	21 [70]	9 [30]	0.002
High	30 [50]	9 [30]	21 [70]	
NCAM1 expression				
Low	32 [53]	14 [47]	18 [60]	0.30
High	28 [47]	16 [53]	12 [40]	
VCAM-1 expression				
Low	30 [50]	14 [47]	16 [53]	0.61
High	30 [50]	16 [53]	14 [47]	
ICAM-1 expression				
Low	30 [50]	12 [40]	18 [60]	0.12
High	30 [50]	18 [60]	12 [40]	

STAS, spread through air spaces; FAK, focal adhesion kinase; EpCAM, epithelial cell adhesion molecule; NCAM1, neural cell adhesion molecule 1; VCAM1, vascular cell adhesion molecule 1; ICAM-1, intercellular cell adhesion molecule-1.

and limited resection (sub-lobar resection) in 20% (12 patients). During the observation period, recurrence occurred in 20% (12 patients) of the cases. The median follow-up time for patients without recurrence was 27 months (average ± standard deviation: 28±4 months). Furthermore, 50% of the cases (30 patients) exhibited STAS, which was significantly associated with histologic growth patterns (P<0.001).

Association of STAS with adhesion molecules expression

Table 1 delineates the correlations between STAS presence and adhesion molecule expression. Significant associations were noted with E-cadherin, N-cadherin, FAK, and EpCAM (P=0.002, P=0.01, P=0.01, and P=0.002, respectively). After excluding variables that are strongly correlated or have a subgroup number of zero, multivariate logistic regression, controlling for sex, age, clinical stage, and tumor size, identified sex, E-cadherin, N-cadherin, and FAK as independent predictors of STAS (P=0.03, P=0.002, P=0.02, and P=0.03) (*Table 2*). *Figure 2* shows representative images of STAS regarding E-cadherin, N-cadherin, and FAK. Univariate analysis revealed a significant association between the expression levels of E-cadherin, N-cadherin, and FAK with growth patterns and STAS, while no significant correlations were observed with sex, age, smoking status, clinical stage, surgical procedures, tumor size, lymphovascular invasion, or visceral pleural invasion (Table S2).

Associations between RFP and adhesion molecules marker expression

Table S3 presents the correlations between patient clinicopathological characteristics and RFP. Among the evaluated clinicopathological factors, clinical stage (P=0.02), growth patterns (P=0.03), and the presence of STAS (P=0.03) exhibited significant associations with recurrence rates. Patients with low E-cadherin expression (n=32; RFP, 69%) demonstrated significantly poorer RFP compared to those with high E-cadherin expression (n=28; RFP, 93%) (P=0.04). Similarly, high N-cadherin expression (n=28; RFP, 68%) correlated with significantly lower RFP than low N-cadherin expression (n=32; RFP, 91%) (P=0.03), and high FAK expression (n=30; RFP, 67%) was significantly associated with reduced RFP compared to low FAK expression (n=30; RFP, 93%) (P=0.01). No other individual adhesion molecule markers showed a significant correlation with RFP. Analysis of combinations of adhesion molecules, selected based on their significance in the univariate analysis, indicated significant prognostic implications for E-cadherin/N-cadherin, E-cadherin/FAK, and N-cadherin/FAK combinations (P=0.03, P=0.008, and P=0.004, respectively) (Table S4).

Based on the above results, paired adhesion molecules were stratified into four categories based on their combined expression levels: 'low/low', 'high/low', 'low/high', and 'high/high'. The 'low/high' (E-cadherin/FAK, E-cadherin/

Table 2 Multivariate logistic regression analysis for the factors associated with STAS in all patients included

Variables	OR	95% CI	P value
Sex (male *vs.* female)	5.686	1.234–26.197	0.03
Age (≥65 *vs.* <65 years)	1.022	0.228–4.588	0.98
Stage (Ib *vs.* Ia)	1.676	0.061–45.970	0.76
Tumor size (>2 *vs.* ≤2 cm)	2.544	0.573–11.292	0.22
E-cadherin expression (high *vs.* low)	0.078	0.016–0.382	0.002
N-cadherin expression (high *vs.* low)	6.247	1.374–28.403	0.02
FAK expression (high *vs.* low)	4.912	1.194–20.203	0.03

STAS, spread through air spaces; OR, odds ratio; CI, confidence interval; FAK, focal adhesion kinase.

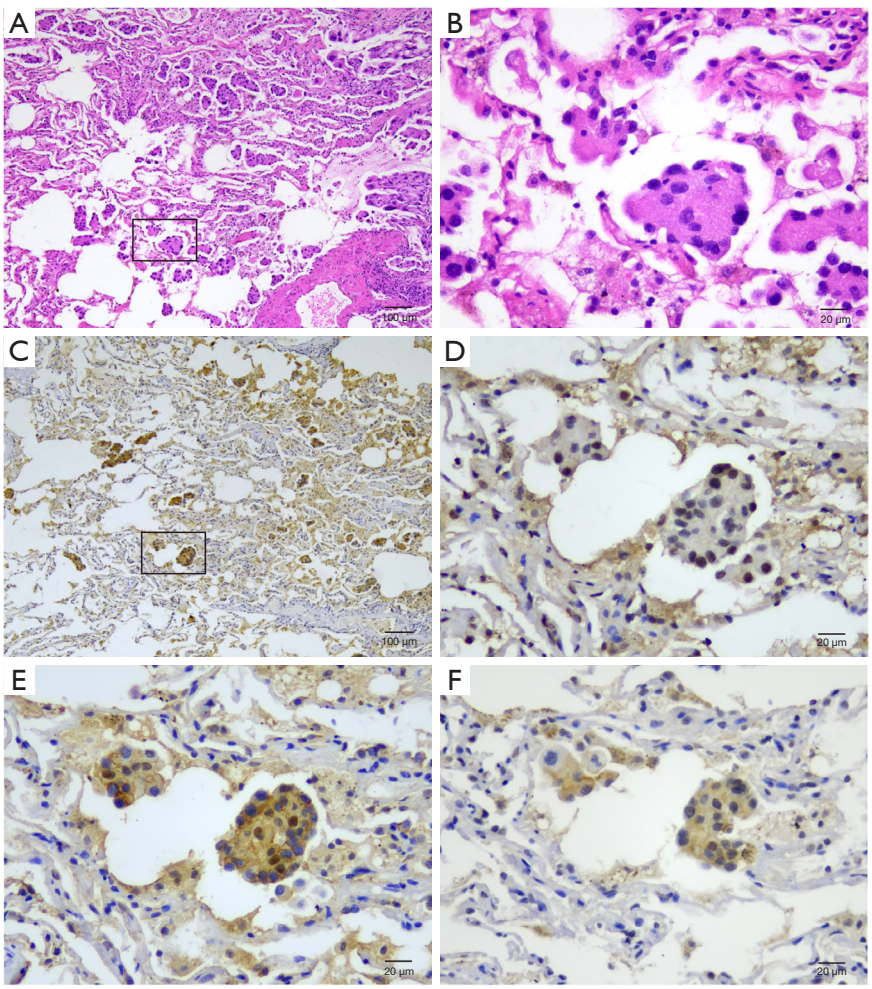

Figure 2 The localization of the primary tumor and STAS was determined using hematoxylin and eosin (H&E) staining. (A) At 40× magnification, the region external to the primary tumor is depicted, with the black square indicating the STAS area; (B) at 200× magnification, tumor cell clusters floating in air spaces are visible within the black square. Immunohistochemistry was utilized for staining adhesion molecules across the entire tissue sample: (C) immunohistochemical staining images of areas outside the tumor at 40× magnification with the black square highlighting the STAS region; representative areas of STAS at 200× magnification showing (D) low expression of E-cadherin, (E) high expression of N-cadherin, and (F) high expression of FAK. STAS, spread through air spaces; FAK, focal adhesion kinase.

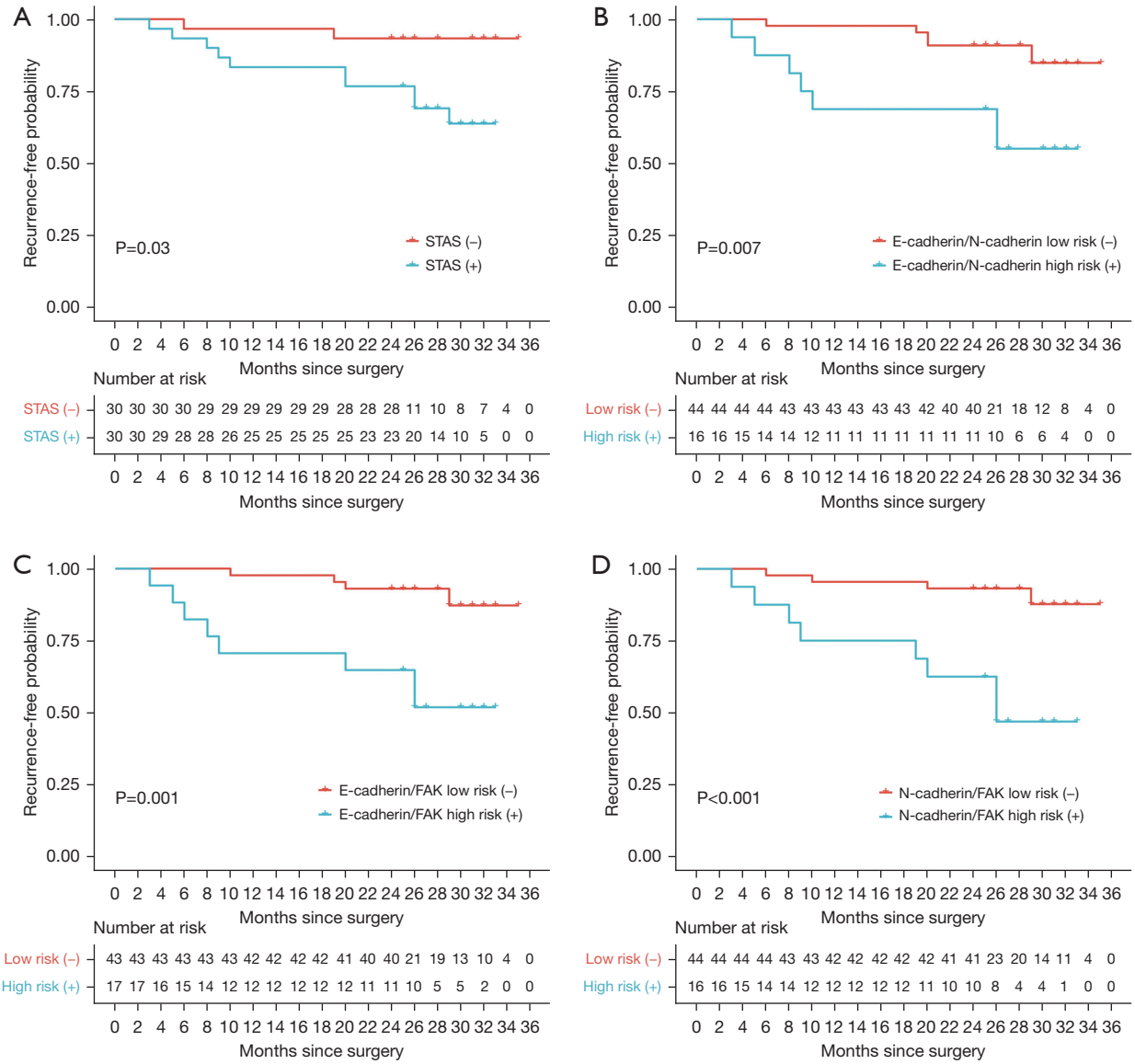

Figure 3 The association between STAS and adhesion molecule risk indices with RFP was examined. (A) The association between RFP and STAS; (B) the association of RFP with the E-cadherin/N-cadherin risk index; (C) the association between RFP and the E-cadherin/FAK risk index; (D) the association between RFP and the N-cadherin/FAK risk index were analyzed. STAS, spread through air spaces; RFP, recurrence-free probability; FAK, focal adhesion kinase.

N-cadherin) and 'high/high' (N-cadherin/FAK) category was designated as 'high-risk' due to its lowest RFP, while the other three categories were considered 'low-risk'. Patients characterized by high-risk combinations of E-cadherin/N-cadherin, E-cadherin/FAK, and N-cadherin/FAK exhibited significantly inferior RFP compared to those in the low-risk groups (P=0.007, P=0.001, and P<0.001, respectively) (*Figure 3*). The results indicated that the prognostic significance of E-cadherin/N-cadherin and E-cadherin/FAK combinations was comparable to that of the N-cadherin/FAK combination.

Multivariate survival analyses were conducted across four datasets, with redundancy minimized by retaining only one variable in cases of strong inter-variable correlations. Adjusting for sex, age, tumor size, and clinical stage, the analysis of the initial dataset revealed that STAS was

Table 3 Multivariate cox regression analysis for the factors associated with STAS in all patients included

Variables	Model 1		Model 2		Model 3		Model 4	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Sex	0.267 (0.062–1.145)	0.08	0.488 (0.140–1.700)	0.26	0.593 (0.181–1.938)	0.39	0.303 (0.077–1.192)	0.09
Age	3.724 (0.833–16.639)	0.09	3.768 (0.908–15.641)	0.07	2.251 (0.613–8.273)	0.22	3.924 (0.982–15.691)	0.053
Tumor size	0.235 (0.046–1.201)	0.08	0.385 (0.076–1.952)	0.25	0.366 (0.077–1.729)	0.20	0.462 (0.088–2.418)	0.36
Stage	4.127 (0.798–21.336)	0.09	2.770 (0.374–20.507)	0.32	3.737 (0.682–20.472)	0.13	1.360 (0.167–11.072)	0.77
STAS	7.689 (1.284–46.031)	0.03						
E-cadherin/ N-cadherin risk			4.544 (1.091–18.922)	0.04				
E-cadherin/FAK risk					4.390 (1.206–15.986)	0.03		
N-cadherin/FAK risk							10.180 (2.197–47.170)	0.003

STAS, spread through air spaces; HR, hazard ratio; CI, confidence interval; FAK, focal adhesion kinase.

independently predictive of an increased recurrence risk [hazard ratio (HR) =7.689, P=0.03]. Similarly, in the subsequent datasets, the E-cadherin/N-cadherin, E-cadherin/FAK, and N-cadherin/FAK risk indices were each found to be independently associated with elevated recurrence rates (P=0.04, P=0.03, and P=0.003, respectively) (*Table 3*).

Further examination focused on the interplay between clinicopathological features, adhesion molecule expression, and recurrence risk among STAS-positive patients. Notably, male patients with STAS exhibited significantly better RFP (n=19; RFP, 79%) compared to females (n=11; RFP, 45%) (P=0.048) (Table S5). Additionally, patients with a high N-cadherin/FAK risk index (n=13; RFP, 46%) demonstrated significantly poorer RFP than those with a low-risk index (n=17; RFP, 82%) (P=0.03) (Table S6). After adjusting for age, surgical procedures, and visceral pleural invasion, sex and the N-cadherin/FAK risk index were identified as independent predictors of RFP in STAS patients (P=0.03 and P=0.01, respectively) (Table S7).

Associations of clinicopathological features with E-cadherin/N-cadherin, E-cadherin/FAK, and N-cadherin/FAK high-risk index

Univariate analysis of the clinicopathologic characteristics of the E-cadherin/N-cadherin, E-cadherin/FAK, and N-cadherin/FAK risk indices revealed that they were significantly correlated with histological growth patterns as well as with STAS, with the N-cadherin/FAK risk index being significantly correlated with clinical staging (P=0.03). Furthermore, none of the three risk indices showed significant correlations with sex, age, smoking status, clinical stage, surgical procedures, STAS, histologic growth patterns, tumor size, lymphovascular invasion, or visceral pleural invasion (Table S8).

Discussion

This research entailed a detailed examination of various adhesion molecular markers, including E-cadherin, P-cadherin, N-cadherin, FAK, EpCAM, NCAM1, VCAM-1, and ICAM-1, in patients with stage I lung cancer who underwent surgical resection. The analysis identified low E-cadherin expression, high N-cadherin expression, and high FAK expression as independent predictors of a higher incidence of STAS. Furthermore, high-risk indices involving combinations of E-cadherin/N-cadherin, E-cadherin/FAK, and N-cadherin/FAK, significantly predicted RFP. In addition, a high risk of N-cadherin/FAK was found to be associated with the risk of recurrence in patients with STAS, after adjusting for sex, age, visceral pleural invasion, and surgical procedures.

STAS was recognized as a novel invasive pattern in the 2015 WHO classification of lung tumors (17). STAS has been identified as a detrimental prognostic indicator for recurrence and survival in non-small cell lung cancer (NSCLC) patients, a finding supported by meta-analyses and validated across large, independent cohorts internationally (2,3,17,19-21). Prior research indicates that patients exhibiting STAS and identified with high-risk factors for recurrence derive a survival advantage

from adjuvant chemotherapy (22). Thus, more precise identification of STAS patients with high-risk factors can better guide clinical treatment planning.

Cell adhesion molecules, located on the cell surface, facilitate cell-cell interactions and include cadherins, the immunoglobulin superfamily, integrins, selectins, and other non-classical adhesion molecules. These molecules play pivotal roles in mediating tumor growth, invasion, and metastasis (13,15,16).

FAK, a crucial tyrosine kinase within the integrin signaling cascade, becomes phosphorylated on tyrosine (Tyr397) following activation. This leads to the formation of a FAK/Src complex, which orchestrates the remodeling of the actin cytoskeleton via phosphorylation. Consequently, it triggers focal adhesion relaxation, exerting a substantial impact on cellular migration, cytoskeletal rearrangement, and adhesion dynamics (23-28).

Aberrant activation of EMT contributes to increased cell migration, invasiveness, and resistance to apoptosis (29). Central to this process is the epithelial cell marker E-cadherin and the mesenchymal marker N-cadherin, which play pivotal roles in EMT by facilitating the replacement of epithelial adhesion proteins (e.g., E-cadherin) with those offering greater junctional flexibility (e.g., N-cadherin), thereby promoting cell detachment and migration (12).

Previous studies in malignancies such as pancreatic cancer and colorectal cancer have demonstrated that a mesenchymal phenotype, characterized by reduced expression of epithelial markers and elevated expression of mesenchymal markers, is linked to tumor progression and adverse outcomes (5,30,31). Regarding the mechanism of STAS occurrence, Jia *et al.* found that the presence of STAS in squamous cell carcinoma and adenocarcinoma of the lung is associated with low expression of E-cadherin (32). Ikeda *et al.* found that the occurrence of STAS in NSCLC is associated with non-epithelial features, such as low expression or loss of E-cadherin (6). The study by Liu *et al.* confirmed the association between twist and slug (which act as transcription factors inducing EMT) and the occurrence of STAS (10). Yoshida *et al.* found that the tumor-associated CD68+ macrophages, CD163+ macrophages, and CD25+ lymphocytes were independently associated with the occurrence of STAS. CD163 serves as a specific marker for M2 macrophages, and the M2 polarization of tumor-associated macrophages (TAMs) promotes the EMT and invasive capacity of cancer cells (33-36). The above studies all indicate a close relationship between the occurrence of STAS and the process of EMT, which may be influenced and regulated by the immune microenvironment and upstream relevant transcription factors. Our study also confirms this viewpoint. Additionally, our study expands on the finding that high expression of N-cadherin is also an independent predictor of STAS, indicating that in lung cancer, not only the loss of epithelial markers but also the increase in mesenchymal cell markers is associated with STAS.

Moreover, previous research has shown that activation of FAK is accompanied by decreased expression of E-cadherin and increased expression of Slug and N-cadherin. Conversely, inhibition of FAK results in the opposite changes in the aforementioned three markers (37-39). Phosphorylation of FAK exacerbates adhesive disruption mediated by E-cadherin, while inhibition of E-cadherin function can further enhance Src activity by promoting FAK phosphorylation, thereby relieving E-cadherin-mediated adhesion and subsequently enhancing tumor cell motility. These findings collectively suggest active involvement of the FAK pathway in the EMT process (40). Based on our study findings, we observed that STAS is not only associated with the expression levels of E-cadherin and N-cadherin but also independently correlated with high FAK expression. This suggests that the development and progression of STAS may be related not only to the abnormal activation of the EMT process and alterations in epithelial adhesion function but also to increased cell migration and cytoskeletal reorganization mediated by the integrin-FAK pathway. FAK may serve as an upstream regulatory molecule associated with the occurrence of STAS, but caution is warranted in interpreting these results. Further in-depth research is warranted to elucidate the specific mechanisms by which FAK regulates EMT activation and promotes STAS occurrence in patients with NSCLC.

Sub-lobar resection is a recognized factor influencing poor prognosis in STAS-positive patients (1,41,42). Although our study did not observe any differences in RFP associated with surgical procedures, we still believe that the results of previous studies should be prioritized. Additionally, our study developed novel risk indices. We found that high-risk indices (comprising low E-cadherin/high N-cadherin, low E-cadherin/high FAK, and high N-cadherin/high FAK combinations) independently predict recurrence. Furthermore, the high N-cadherin/high FAK combination remains a risk factor for recurrence in NSCLC patients with STAS after adjusting for confounding factors such as surgical procedures, visceral pleural invasion, age, and sex. Moreover, we did not observe any correlation between the

risk indices, composed of E-cadherin, N-cadherin, FAK, and their combinations, and surgical procedures.

Building on previous research, we speculated that in the future, combining FAK inhibitors with monoclonal antibodies or modulators targeting relevant adhesion molecules (such as upregulators or activators of E-cadherin) may have potential applications for patients with STAS-positive NSCLC (43-45). Small molecule FAK inhibitors may exert their effects by directly or indirectly inhibiting aberrant EMT activation. Additionally, FAK inhibitors may impact the immune microenvironment, enhancing tumor sensitivity to T cells and promoting the infiltration of anti-tumor immune cells into the tumor, thereby reshaping the tumor microenvironment (46,47). However, these potential applications still face many challenges that need to be addressed. Currently, FAK inhibitors are still in the early stages of research, with limited studies on their use in early-stage NSCLC treatment. Additionally, the production of human monoclonal antibodies as therapeutic agents poses numerous challenges and is costly. Further research is needed in the future to validate the possibility of these applications.

Many studies have indicated an association between STAS and the EMT process, consistent with our findings. However, Yagi *et al.* observed E-cadherin expression in the STAS regions attached to the alveolar wall in three micropapillary (MIP) samples and suggested that tumor cells in STAS may reattach to the alveolar wall via a vascular co-selection mechanism (48). This challenges the view that STAS cells undergo EMT. We speculate that when STAS lesions detach from the primary tumor and form free tumor cell clusters, they may undergo abnormal EMT activation processes, leading to reduced cell adhesion and tumor budding, indicative of characteristic EMT activation. However, upon reattachment to the alveolar wall, STAS lesions may undergo a mesenchymal-epithelial transition (MET), promoting tumor growth, hence the expression of E-cadherin observed in the attached STAS regions (49,50). Therefore, elucidating the mechanisms underlying STAS occurrence and re-implantation, which lead to increased risk of recurrence in NSCLC patients, remains an area requiring further investigation.

This study has several limitations. Firstly, it is retrospective and covers a limited sample size from a single center, potentially introducing selection bias. Therefore, expanding the study to a multicenter environment may enhance the external validity and generalizability of the findings. Additionally, the method of assessing adhesion molecule expression using immunohistochemistry alone for patient stratification in clinical settings may be constrained by the time limitations of surgical approach selection, thus affecting a comprehensive evaluation of treatment plans. Future research could employ methods such as real-time quantitative polymerase chain reaction (PCR), ribonucleic acid (RNA) sequencing, mass spectrometry, etc., to quantitatively assess adhesion molecule expression in preoperative biopsy and postoperative tumor specimens, to ascertain the feasibility of patient risk stratification before surgery. Thirdly, our study suggests an association between the occurrence of STAS and tumor recurrence with the expression levels of relevant adhesion molecules. However, previous research has indicated that inhibiting E-cadherin function may also induce the occurrence of the EMT process. Therefore, basic and clinical research on inhibiting or activating the function of relevant adhesion molecules may be a future research direction, such as using specific antibodies for blockade or activation, to comprehensively explore the relationship between the mechanism of STAS occurrence and adhesion molecules.

Conclusions

In patients with stage I NSCLC, our study identified significant associations between the presence of E-cadherin, N-cadherin, and FAK with the occurrence of STAS. The combined high-risk indices of these markers were highly correlated with an increased risk of recurrence. Additionally, high N-cadherin/FAK expression in tumors provides information about a higher risk of recurrence in STAS-positive stage I lung carcinoma. Utilizing immunohistochemistry to detect these biomarkers enables the enhanced identification of patients requiring adjuvant therapy or close postoperative monitoring. However, the validation of these findings necessitates extended follow-up periods.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Institutional Review Board of Nanjing Jinling Hospital (No. DBNJ20241) and individual consent for this retrospective analysis was waived.

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