Predictive Value of Tumor Budding and Tumor-Infiltrating Lymphocytes for Lymph Node Metastasis in Esophageal Squamous Cell Carcinoma (postprint)

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Abstract

Background The status of lymph node metastasis in esophageal cancer affects patients' surgical strategies and prognosis, necessitating the identification of risk factors that can predict lymph node metastasis in esophageal cancer to assist clinical diagnosis and treatment. Objective To investigate the value of tumor budding (TB) and tumor-infiltrating lymphocytes (TILs) in predicting lymph node metastasis in patients with esophageal squamous cell carcinoma. Methods A total of 124 patients with esophageal squamous cell carcinoma who underwent radical esophagectomy and had paraffin-embedded specimens preserved at Ningbo University Affiliated People' s Hospital from January 2013 to March 2022 were selected as study subjects. Light microscopy and CK5/6 immunohistochemical staining were used to evaluate TB count and TILs density in cancer tissues. Multivariate Logistic regression analysis was employed to explore influencing factors of lymph node metastasis in esophageal squamous cell carcinoma patients, and receiver operating characteristic (ROC) curve analysis was utilized to investigate the predictive value of TB and TILs for lymph node metastasis in these patients. Results Multivariate Logistic regression analysis revealed that TB (OR=20.078, P<0.001), TILs (OR=0.218, P=0.008), and lymphovascular invasion (OR=28.609, P<0.001) were influencing factors for lymph node metastasis in esophageal squamous cell carcinoma patients. The area under the ROC curve (AUC) for TB in predicting lymph node metastasis was 0.835 [95%CI (0.763, 0.907)], and the AUC for TILs was 0.656 [95%CI (0.558, 0.753)]. Conclusion TB and TILs are influencing factors for lymph node metastasis in esophageal squamous cell carcinoma patients, and TB grade and TILs density demonstrate good diagnostic efficacy for predicting lymph node metastasis in these patients.

Full Text

Predictive Value of Tumor Budding and Tumor-infiltrating Lymphocytes on Lymph Node Metastasis of Esophageal Squamous Cell Carcinoma

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Abstract

Background The surgical management and prognosis of esophageal squamous cell carcinoma (ESCC) patients are significantly influenced by lymph node metastasis status. Identifying reliable risk factors to predict lymph node metastasis is essential for optimizing clinical treatment strategies.

Objective To investigate the predictive value of tumor budding (TB) and tumor-infiltrating lymphocytes (TILs) for lymph node metastasis in ESCC patients.

Methods A total of 124 ESCC patients who underwent radical esophagectomy with retained paraffin-embedded specimens at the Affiliated People' s Hospital of Ningbo University between January 2013 and March 2022 were enrolled. Light microscopy and CK5/6 immunohistochemical staining were employed to evaluate TB counts and TILs density in cancer tissues. Multivariate logistic regression analysis was used to identify factors influencing lymph node metastasis, and receiver operating characteristic (ROC) curve analysis was performed to assess the predictive value of TB and TILs for lymph node metastasis in ESCC patients.

Results Multivariate logistic regression analysis revealed that TB (OR=20.078, P<0.001), TILs (OR=0.218, P=0.008), and intravascular tumor thrombus (OR=28.609, P<0.001) were independent influencing factors for lymph node metastasis in ESCC patients. The area under the ROC curve (AUC) for TB in predicting lymph node metastasis was 0.835 [95%CI (0.763, 0.907)], while the AUC for TILs was 0.656 [95%CI (0.558, 0.753)].

Conclusions TB and TILs are significant influencing factors for lymph node metastasis in ESCC patients. Both TB grade and TILs density demonstrate good diagnostic efficacy in predicting lymph node metastasis.

Keywords Esophageal squamous cell carcinoma; Tumor budding; Tumor-infiltrating lymphocytes; Lymph node metastasis; Esophageal neoplasms;

Logistic models; Forecasting

Introduction

Esophageal cancer represents a major malignant tumor threatening human health, with China being a high-incidence country that accounts for 53.7% of new cases and 55.3% of deaths worldwide [1-2]. The status and extent of lymph node metastasis directly determine the N stage and subsequent treatment strategies for esophageal cancer patients, serving as a critical prognostic factor [3-4]. However, the submucosal lymphatic network of the esophagus is richly distributed with complex and extensive drainage pathways involving more than 20 lymph node stations in the cervical, thoracic, and abdominal regions [5]. Lymph node metastasis can occur once early tumors invade beyond the mucosal layer, characterized by regional, bidirectional, continuous, and skip metastasis patterns [6]. Therefore, identifying risk factors for lymph node metastasis and developing predictive methods are crucial for surgical approach selection, lymph node dissection extent, postoperative adjuvant therapy, and prognostic evaluation, particularly for cases with insufficient lymph node harvest that preclude accurate N staging.

Esophageal squamous cell carcinoma (ESCC) is the predominant histological subtype in China, whereas esophageal adenocarcinoma is more common in Western countries [7]. Despite rapid advances in molecular pathology and immunotherapy, histomorphology remains the cornerstone of pathological diagnosis. Tumor budding (TB) is a histopathological phenomenon observed across multiple malignant tumors that reflects strong invasive and metastatic potential. Numerous studies have validated its prognostic value [8-10] and demonstrated its correlation with lymph node metastasis in early-stage colorectal, esophageal, and gastric cancers [11-13]. Tumor-infiltrating lymphocytes (TILs) are lymphocyte populations within the tumor microenvironment that exert anti-tumor effects through immune responses [14]. Evidence supports the use of TILs for predicting immunotherapy efficacy and prognosis in malignant tumors [15-17], with studies suggesting they serve as predictive factors for lymph node metastasis in early gastric cancer [18]. This study primarily investigates the correlation between TB, TILs, and clinicopathological features of ESCC, and evaluates their predictive value for lymph node metastasis to provide histopathological evidence for clinical treatment decisions.

Methods

Study Subjects

We retrospectively enrolled 124 ESCC patients who underwent radical esophagectomy with retained paraffin-embedded specimens at the Affiliated People' s Hospital of Ningbo University between January 2013 and March

2022. Patient ages ranged from 46 to 82 years, with a median age of 62 years. Inclusion criteria were: (1) pathologically confirmed ESCC; (2) complete clinicopathological data including patient sex, age, tumor size, differentiation degree, invasion depth, lymph node metastasis status, and intravascular tumor thrombus; (3) no neoadjuvant therapy before surgery. Exclusion criteria were: (1) patients with concurrent primary tumors at other sites; (2) patients with prior major thoracic or abdominal surgery. This study was approved by the Ethics Committee of the Affiliated People' s Hospital of Ningbo University (approval number: (2021) Research Ethics Review No. 055).

Immunohistochemical Staining

Tissue sections of approximately 4 m thickness were prepared, deparaffinized with xylene, rehydrated through graded ethanol series (100%, 95%, 75%), and subjected to high-temperature antigen retrieval for 5 minutes in citrate buffer (pH 6.0). Immunohistochemical staining was performed using an automated immunostainer (LEICA BOND MAX, Germany) following the manufacturer' s protocol.

TB Assessment

TB represents the histopathological manifestation of epithelial-mesenchymal transition (EMT) observed under microscopy [19]. As standardized TB evaluation methods for esophageal cancer are currently lacking, we adopted the colorectal cancer TB assessment method recommended by the 2016 International Tumor Budding Consensus Conference (ITBCC) [20]. The most densely populated TB area ("hotspot") was selected for counting ($\times 20 field, corresponding to 0.785 mm\hat{}\{2\}$). $Scattered undifferentiated tumor cell clusters within the stroma at t$ $grade (0-4 buds), intermediate-grade (5-9 buds), or high-grade$ ($10 buds). In this study, TB counts were determined by CK5/6 immunohistochemical staining, and low-grade and intermediate-grade TB were combined as low-intermediate grade TB for further comparative analysis with high-grade TB.

TILs Assessment

We employed the breast cancer TILs evaluation criteria recommended by the International TILs Working Group in 2014 [21] for morphological assessment of TILs in esophageal cancer. TILs density was defined as the percentage of total mononuclear lymphocyte area relative to total stromal area within the invasive tumor boundary, evaluated comprehensively across the tumor region rather than focusing solely on hotspot areas, and generally rounded to the nearest 5% or 10%. In this study, two pathologists jointly evaluated TILs on HE-stained sections; TILs <30% were classified as low-density, while TILs $ $30% were classified as high-density [22].

Staging

Pathological TNM (pTNM) staging was performed according to the 8th edition esophageal cancer staging system jointly published by the American Joint Committee on Cancer and the International Union Against Cancer [23].

Statistical Analysis

All data were analyzed using SPSS 27.0 statistical software. Normally distributed continuous variables were expressed as ($\bar{x}\pm s$) and compared between groups using independent samples t-test. Categorical data were expressed as percentages and compared using chi-square test or Fisher' s exact test. Multivariate logistic regression analysis was performed to investigate influencing factors for lymph node metastasis in ESCC patients, and ROC curve analysis was used to evaluate the predictive value of TB and TILs for lymph node metastasis. $P<0.05$ was considered statistically significant.

Results

TB Count and Grading in ESCC

HE staining revealed single isolated cancer cells or small cancer cell clusters within the fibrous stroma at the invasive tumor front. CK5/6 immunohistochemical staining showed strong positive expression in all 124 esophageal cancer cases, with membranous staining used as a reference for TB grading [Figure 1: see original paper].

Comparison of Clinicopathological Parameters Between ESCC Patients With and Without Lymph Node Metastasis

Among the 124 patients, 60 (48.3%) had lymph node metastasis and 64 (51.6%) did not. Statistically significant differences in lymph node metastasis rates were observed among patients with different tumor sizes, differentiation degrees, invasion depths, intravascular tumor thrombus status, TB grades, and TILs densities ($P<0.05$). Patients were further categorized into four groups based on combined TB grade and TILs density: low-intermediate grade TB + high-density TILs, high-grade TB + high-density TILs, low-intermediate grade TB + low-density TILs, and high-grade TB + low-density TILs [Figure 2: see original paper]. Significant differences in lymph node metastasis rates were found among these combined groups ($P<0.05$). Lymph node metastasis showed no association with patient sex or age ($P>0.05$) .

Multivariate Logistic Regression Analysis of Lymph Node Metastasis and Clinicopathological Features

Using lymph node metastasis status as the dependent variable (assignment: 0=no, 1=yes) and tumor size (0=$ $2.5 cm, 1=>2.5 cm), differentiation degree (0=high-intermediate, 1=low), invasion depth (0=pT1, 1=pT2+pT3), TB

grade (0=low-intermediate, 1=high), intravascular tumor thrombus (0=absent, 1=present), and TILs density (0=low, 1=high) as independent variables, multivariate logistic regression analysis revealed that TB (OR=20.078), intravascular tumor thrombus (OR=28.609), and TILs (OR=0.218) were independent influencing factors for lymph node metastasis in ESCC patients (P<0.05) .

Predictive Value of TB and TILs for Lymph Node Metastasis in ESCC

ROC curve analysis demonstrated that TB predicted lymph node metastasis with an AUC of 0.835 [95%CI (0.763, 0.907), P<0.001], sensitivity of 70%, and specificity of 85.9%. TILs predicted lymph node metastasis with an AUC of 0.656 [95%CI (0.558, 0.753), P<0.001], sensitivity of 70%, and specificity of 60.94%. These results indicate that both TB grade and TILs density have good diagnostic efficacy for predicting lymph node metastasis in ESCC patients [Figure 3: see original paper].

Discussion

TB was initially studied extensively in colorectal cancer. A retrospective multicenter study of 806 pT1 colorectal cancers reported that among 203 patients with invasion depth <1,000 m, only 4 (2%) had lymph node metastasis, which was lower than the rate in those with invasion depth >1,000 m. The proportion of high-grade TB cases with lymph node metastasis (25%) was significantly higher than that of low-grade TB cases (7%) (P<0.001). Submucosal invasion depth $ $1,000 m and high-grade TB were identified as independent risk factors for lymph node metastasis in pT1 colorectal cancer [24]. In advanced colorectal cancer, studies have confirmed that high-grade TB is an important histological feature indicating lymph node metastasis [25]. A systematic review of 12 studies comprising 1,652 patients demonstrated that high-grade TB was associated with shorter 5-year survival in pT2 colorectal cancer patients [26]. Given the significant value of TB in invasion, metastasis, and prognosis of colorectal cancer, TB has been incorporated into routine pathological reporting for colorectal cancer, though not yet for ESCC.

Our study revealed a lymph node metastasis rate of 68.3% in high-grade TB cases, significantly higher than the 31.7% rate in low-intermediate grade TB cases. Fuchinoue et al. [13] analyzed 50 superficial ESCC patients and confirmed that TB grade correlated with lymph node metastasis in superficial ESCC (P<0.001), consistent with our findings that high-grade TB cases have higher lymph node metastasis rates. Clinically, TB grading should be included in pathology reports for both early endoscopic submucosal dissection (ESD) specimens and radical esophagectomy specimens. The presence of high-grade TB suggests the need for more aggressive treatment, such as additional surgery or chemoradiotherapy for early ESD cases, and postoperative chemoradiotherapy or extended lymph node dissection for radical resection cases. Particularly for patients with insufficient lymph node harvest that precludes accurate N staging, TB grade can serve as a reference indicator for postoperative adjuvant therapy.

TILs have been studied in various malignant tumors including melanoma, colon cancer, breast cancer, and bladder cancer, with evidence supporting their correlation with prognosis [27-30]. Regarding TILs and lymph node metastasis, studies have reported that TILs correlate with lymph node metastasis in early gastric cancer, with low-density TILs being an independent predictive factor [18]. Our study demonstrates that TILs correlate with lymph node metastasis in esophageal cancer, and multivariate analysis confirms that TB, TILs, and intravascular tumor thrombus are influencing factors for lymph node metastasis. Previous research has indicated that low-grade TB combined with high-density TILs correlates with longer survival in colorectal cancer [31]. In our study, patients were stratified into four groups: low-intermediate grade TB + high-density TILs, high-grade TB + high-density TILs, low-intermediate grade TB + low-density TILs, and high-grade TB + low-density TILs. The low-intermediate grade TB + high-density TILs group showed lower lymph node metastasis rates, while the high-grade TB + low-density TILs group showed higher rates. ROC curve analysis further confirmed the good diagnostic efficacy of combined TB grade and TILs density for predicting lymph node metastasis. These findings have important clinical implications for guiding subsequent treatment and improving prognosis.

This study has several limitations. It is a single-center retrospective study with a limited sample size. Due to substantial loss to follow-up, we were unable to analyze the impact of TB grade and TILs on prognosis, which will be addressed in future studies.

In conclusion, TB and TILs are closely associated with lymph node metastasis in esophageal squamous cell carcinoma and demonstrate good predictive value. These findings provide valuable guidance for treatment decisions in early ESCC patients undergoing ESD and in patients who have undergone radical esophagectomy but have insufficient lymph node dissection.

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Author Contributions: ZHANG Jifang conceptualized the study, designed the research, performed statistical analysis, prepared figures and tables, and drafted the manuscript. TANG Jiawen collected and organized data. ZHANG Jifang and CHEN Fang evaluated pathological morphology and revised the manuscript. LI Hongliang supervised quality control and review, and assumed overall responsibility for the manuscript.

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