



BRF2 as a promising indicator for radical lymph-node dissection surgery in patients with cN0 squamous cell carcinoma of the middle thoracic esophagus

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Abstract

Purpose Radical lymph-node dissection surgery in patients with cN0 middle thoracic esophageal squamous cell carcinoma (ESCC) remains controversial. We sought a novel biomarker that could be used for decision-making in relation to radical lymph-node dissection.

Methods One hundred and nineteen patients with cN0 middle thoracic ESCC undergoing three-field lymph-node dissection (3FLND) or two-field lymph-node dissection (Ivor Lewis) esophagectomy were reviewed. A survival analysis, and Chi-square and parametric tests were performed.

Results A Cox regression analysis revealed that the expression of BRF2 was an independent prognostic factor for overall survival ($P=0.014$) and progression-free survival ($P=0.014$). The survival of patients who underwent 3FLND was better than that of patients who underwent Ivor Lewis esophagectomy in the BRF2 overexpression group ($P=0.002$), but not in the BRF2 nonoverexpression group ($P=0.386$). The risk of lymph-node recurrence and the number of recurrent lymph nodes in patients with the overexpression of BRF2 were increased in the Ivor Lewis group in comparison to the 3FLND group ($P=0.01$ and $P<0.001$). The risk of cervical and superior mediastinal lymph-node recurrence was positively correlated with the overexpression of BRF2 ($P=0.027$). Furthermore, in the Ivor Lewis group, a significant correlation was found between the risk of lymph-node recurrence or the number of recurrent lymph nodes and the expression of BRF2 ($P=0.002$ and $P=0.004$), but not in the 3FLND group ($P=0.193$ and $P=0.694$).

Conclusions 3FLND generated better survival outcomes and reduced the rate of lymph-node recurrence in comparison to Ivor Lewis in patients with the overexpression of BRF2. BRF2 can be used as an indicator for radical lymph-node dissection surgery in cN0 ESCC patients.

Keywords BRF2 · Esophageal squamous cell cancer · 3FLND · Ivor Lewis

Abbreviations

BRF2 TFIIB-related factor 2
ESCC Esophageal squamous cell cancer
3FLND Three-field lymph-node dissection esophagectomy

Ivor Lewis Two-field lymph-node dissection esophagectomy
OS Overall survival
PFS Progression-free survival

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Introduction

Esophageal cancer is one of the most malignant tumors throughout the world. It is the sixth most common cause of death, with occurrence rates varying greatly according to geographic location [1]. Squamous cell carcinoma and adenocarcinoma are the main histologic types of esophageal cancer. Esophageal squamous cell carcinoma (ESCC) accounts for the majority of esophageal cancer in China [2]. Although treatment strategies for ESCC have recently

made great progress, most patients die of advanced disease, local relapse, distant metastasis, or resistance to adjuvant therapy [3, 4]. Among the various treatment modalities, surgical resection remains the primary treatment for achieving a better survival.

The structure of the esophagus is different from other parts of the digestive tract due to the presence of lymph vessels in the mucoderm and the muscularis mucosa; consequently, the early recurrence can be observed in patients with positive nodes [5]. To prevent postoperative recurrence and lymph-node metastasis and improve long-term survival, extensive radical lymphadenectomy, such as superior mediastinal lymph-node dissection or three-field lymph-node dissection (3FLND), was introduced for patients with thoracic esophageal carcinoma. However, radical surgery is also associated with a higher mortality rate and a higher incidence of postoperative complications. There is an urgent need to identify novel biomarkers that can help to select patients with a high risk of esophageal carcinoma recurrence. Such biomarkers could provide clinical clues that can be used for the individualization of treatment.

In recent years, many studies have revealed molecular markers associated with lymph-node metastasis and the recurrence of esophageal cancer, including CC chemokine receptor 7, caveolin-1, Sphingosine-1-phosphate, and sphingosine kinase 1 [6–8]. However, they did not report their application in clinical treatment strategies. The TFIIB-related factor 2 (BRF2) gene is located on chromosome 8p12. BRF2 protein is a subunit located on transcription factor IIIIB and plays a role in the production of small RNA, which is catalyzed by RNA pol III [9]. It was recently demonstrated that the dysregulation of TFIIB-mediated transcription may be an important step in tumor development [10]. The relationship between the BRF2 gene and TFIIB determines its important role in tumorigenesis and the development process. BRF2 protein has been shown to be overexpressed in various cancers including gastric, kidney, and melanoma cancers [11, 12]. Lockwood et al. reported that the overexpression of BRF2 could drive the expression of RNA pol III transcripts, contributing to squamous cell carcinoma tumorigenesis [12]. Our previous studies revealed that it is associated with a poor prognosis in non-small-cell lung cancer patients due to its promotion of tumor epithelial–mesenchymal transition [13, 14]. We also found that the overexpression of BRF2 protein is common in the early stage ESCC, and is significantly correlated with the tumor prognosis and relapse [15]. The objective of this study was to investigate the expression of BRF2 in ESCC by immunohistochemistry, and to further explore its correlation with individualized esophagectomy treatment strategies for cN0 ESCC.

Materials and methods

Patients

The study included 119 patients with squamous cell carcinoma of the middle thoracic esophagus. All patients underwent R0 esophagectomy in the Department of Thoracic Surgery, Qilu Hospital of Shandong University between Jan 2008 and Aug 2009. Among these patients (without a residual tumor according to the AJCC classification), 52 patients underwent extended 3FLND esophagectomy and the remaining patients underwent Ivor Lewis esophagectomy. After surgery, all patients underwent clinical and imaging examinations regularly until the end of the 5-year follow-up period or death. No patient received chemotherapy or radiotherapy before or after surgery. The clinical TNM stage was determined based on CT. The pathological diagnosis of ESCC was confirmed via a pathological examination after surgery; no residual cancer cells were detected under the upper and lower cutting edge or the lateral margin. The clinical and postoperative pathological stages were determined according to the UICC TNM staging system. The study population included patients without lymph-node metastasis and distant metastasis (cT_{1–4}N0M0 as well as pT_{1–4}N0M0). Patients with diabetes mellitus, hypertension, or psychiatric disorders were excluded. None of the selected patients suffered serious postoperative complications.

Surgical procedure

The standard procedures for 3FLND and Ivor Lewis esophagectomy have been described previously [16, 17]. For 3FLND, the arch of the azygous vein was resected, and the right bronchial artery was preserved. The superior vena cava, innominate artery, right subclavian artery, and pulmonary branch of the right vagal nerve and the bilateral recurrent laryngeal nerves were carefully exposed and preserved. Full 3FLND involves the removal of five anatomical groups of lymph nodes, including the cervical, upper mediastinal, middle mediastinal, lower mediastinal, and abdominal lymph nodes. Right thoracotomy followed by a laparotomy incision, and thoracic and abdominal lymph-node dissection were undertaken for Ivor Lewis esophagectomy according to the American Joint Committee on Cancer lymph-node mapping system for esophageal cancer [18]. The mean number of dissected lymph nodes in patients who underwent two-field and three-field lymph-node dissection is 20 ± 5 and 23 ± 3 , respectively. This study was approved by the Ethics Committee of Qilu Hospital of Shandong University and each patient gave their informed consent for the publication of this study.

Immunohistochemistry and the evaluation of the BRF2 protein expression

All specimens were collected during surgery, fixed in 10% formalin, and embedded in paraffin. The tissue specimens were cut into 4- μ m serial sections, deparaffinized in xylene, and rehydrated through an ethanol series to water. High-temperature antigen retrieval was carried out in citrate buffer for 25 min in a microwave oven. Then, the endogenous peroxidase enzyme activity was blocked using 3% H₂O₂ in methanol for 20 min at room temperature. The slides were then incubated with primary rabbit anti-BRF2 polyclonal antibody (1:1000, Abcam, Cambridge, MA, USA) overnight at 4 °C in a high humidity chamber, followed by incubation for 30 min at 37 °C with biotinylated secondary antibodies and streptavidin–peroxidase complex. Finally, a 3,30-diaminobenzidine solution was added, and the slides were counterstained with hematoxylin and mounted with neutral balsam. For negative controls, sections were incubated with PBS instead of the primary antibodies.

For BRF2 staining, the area within the diagnostic area was scored by three independent observers and a reproducible semi-quantitative method that considered both the staining intensity (0, negative; 1, weak; 2, moderate; 3, strong) and the percentage of positively stained cells (0, 0–10%; 1, 11–25%; 2, 26–50%; 3, 51–75%; 4, > 76%). For the evaluation of the positive BRF2 staining, at least three sections or areas from each sample should be scored. Conflicting scores were resolved by agreement between two observers or by averaging the scores. The final staining score was determined by multiplying the staining intensity by the percentage of positive cells, and was further graded as follows: 0–1, (–); 2–3, (+); 4–5, (++) ; 6–7, (+++). The expression of BRF2 was divided into a nonoverexpression group (– or +) and an overexpression group (++ or +++) [19, 20].

Postoperative follow-up and the diagnosis of lymph-node recurrence

All patients underwent follow-up examinations every 3–6 months after surgery. These examinations included Doppler ultrasound, chest enhanced CT, and abdominal CT examination, and, if necessary, PET-CT and endoscopy. Recurrence was defined as apparent recurrence on imaging studies during follow-up. The diagnosis of cervical lymph-node recurrence was mainly made based on the results of a physical examination, Doppler ultrasound, and fine-needle aspiration cytology. The diagnosis of mediastinal lymph-node recurrence was mainly based on CT findings. The diagnosis of abdominal lymph-node recurrence was mainly based on Doppler ultrasound and CT findings [21].

Statistical analysis

The correlation between lymph-node recurrence and a patient's clinicopathological factors or BRF2 protein immunoreactivity was analyzed by Chi-squared test or nonparametric test (Mann–Whitney *U* test). The Kaplan–Meier method was used to calculate the survival curves, and a log-rank test was used to compare the survival of subgroups. A multivariate Cox regression analysis was used to identify significant independent prognostic factors. *P* values of < 0.05 were considered to indicate statistical significance. All statistical analyses were performed using the SPSS software program (version 19.0, SPSS Inc., Chicago, IL, USA).

Results

The BRF2 expression in ESCC

The clinicopathological characteristics of the patients are listed in Table 1. The study population included 77 male patients and 42 female patients. The median age of the patients was 63 years (range 35–79 years). The stages of the patients were classified as T1–2 (*n* = 36), T3 (*n* = 69) and T4a (*n* = 14). The tumors were classified as well differentiated (*n* = 35), moderately differentiated (*n* = 45), and poorly differentiated (*n* = 39). The BRF2 protein expression in the tumor tissues was detected by immunohistochemistry. As shown in Fig. 1, various intensities of diffuse nuclear staining of BRF2 protein were observed in cancer cells. In addition, some staining was observed in the cytoplasm of cancer cells. No significant associations were observed between the BRF2 protein expression and any of the clinicopathological features of the ESCC patients (all *P* > 0.05, Table 1). Furthermore, the surgical procedures were not significantly associated with any of the patients' clinicopathological variables (all *P* > 0.05, Table 1).

The association between lymph-node recurrence and the clinicopathological factors of ESCC

Table 2 summarizes the association between lymph-node recurrence and various clinicopathological features of ESCC patients. The numbers of patients and recurrent lymph nodes were both analyzed. As shown in Table 2, deeper tumor invasion (*P* = 0.005 and *P* = 0.003), longer tumor length (both *P* < 0.001), conservative lymph-node dissection surgery (*P* = 0.027 and *P* = 0.001), and BRF2 overexpression (*P* = 0.047 and *P* = 0.038) were associated with a higher risk of lymph-node recurrence and a greater number of recurrent lymph nodes, respectively. Lymph-node recurrence after surgery was not associated with age, gender, or tumor differentiation (all *P* > 0.05, Table 2).

Table 1 Association of the clinicopathological variables of ESCC patients with BRF2 expression level and with the surgical procedure

Variable	No. of patients	BRF2 (overexpression)			Surgical procedure		
		No	Yes	<i>P</i> ^a	3FLND	Ivor Lewis	<i>P</i> ^a
Age				0.580			0.165
≤65 years	52	22	30		19	33	
>65 years	67	25	42		33	34	
Gender				0.344			0.524
Male	77	28	49		32	45	
Female	42	19	23		20	22	
T status				0.940			0.712
T1–2	36	14	22		17	19	
T3	69	28	41		28	41	
T4a	14	5	9		7	7	
Tumor diameter (cm)				0.662			0.774
<3	40	17	23		18	22	
3–5	36	12	24		17	19	
>5	43	18	25		17	26	
Differentiation				0.180			0.073
Well	35	18	17		15	20	
Moderate	45	14	31		25	20	
Poor	39	15	24		12	27	
Surgical procedure				0.337			–
3FLND	52	18	34		–	–	
Ivor Lewis	67	29	38		–	–	
BRF2 overexpression				–			0.337
No	47	–	–		18	29	
Yes	72	–	–		34	38	

P^a Chi-squared test

The univariate and multivariate survival analyses

Of the 119 patients, 65 (54.6%) patients died within 5 years after surgery, and recurrent disease developed in 70 (58.8%) patients during the follow-up period. The Kaplan–Meier analysis and log-rank test were used to calculate the effects of clinicopathological factors on overall survival (OS) and progression-free survival (PFS). A univariate analysis demonstrated that the overexpression of BRF2 protein ($P=0.032$ for OS and $P=0.027$ for PFS), deeper tumor invasion ($P=0.017$ for OS and $P=0.029$ for PFS), and longer tumor diameter ($P=0.012$ for OS and PFS) were significantly associated with decreased overall 5-year survival and a higher risk of recurrence. Furthermore, a multivariate analysis revealed that the expression of BRF2 ($P=0.014$) and T status ($P=0.032$) were independent prognostic factors for overall survival. Furthermore, the overexpression of BRF2 remained a significant independent prognostic factor for PFS ($P=0.014$, Table 3; Fig. 2).

The univariate analyses of the surgical procedures in selected patient subgroups

To evaluate the impact of two different surgeries on 5-year overall survival, we further examined the difference in survival of patients who underwent 3FLND and Ivor Lewis esophagectomy according to the T status, differentiation, diameter of the tumor, and the BRF2 protein expression status. The OS of patients with the overexpression of BRF2 who underwent 3FLND was significantly better than that of patients who underwent Ivor Lewis esophagectomy ($P=0.002$, Table 4; Fig. 3). No significant differences between 3FLND and Ivor Lewis esophagectomy groups were found in the other subgroups (all $P>0.05$, Table 4).

The association between the surgical procedure and lymph-node recurrence

We investigated the difference in the incidence of lymph-node recurrence between the 3FLND and Ivor Lewis

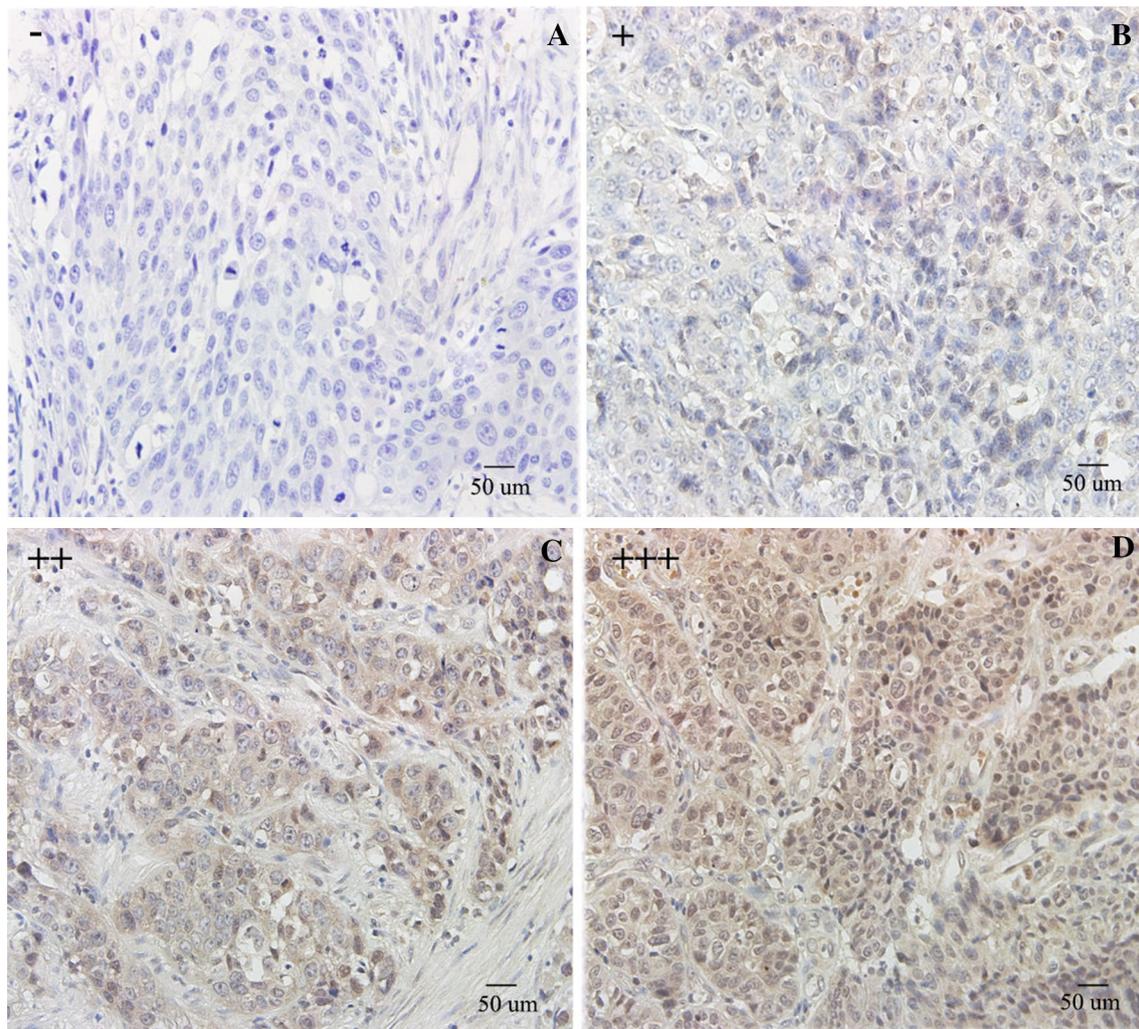


Fig. 1 Immunohistochemical staining of BRF2 protein and microvessels, which were graded as (-), (+), (++) or (+++) ($\times 400$)

esophagectomy subgroups in the BRF2 overexpression group. As shown in Table 5, 13 of 34 (38.2%) patients in the 3FLND group relapsed, while 31 of the 38 (81.6%) patients in the Ivor Lewis group relapsed ($P=0.010$). In the 3FLND group, 2 (5.9%) patients had cervical and superior mediastinal lymph-node recurrence and 1 (2.9%) patient had transregional recurrence including this area. In the Ivor Lewis group, the risk of lymph-node recurrence increased to 18.4% (7/38) in the cervical and superior mediastinal area alone and 10.5% (4/38) in the transregional case. Moreover, we calculated the amount of recurrent lymph nodes. The number in the Ivor Lewis group was higher than that in the 3FLND group (60 vs. 18, $P<0.001$), especially in the cervical and superior mediastinal region. 3FLND was associated with a reduced number of recurrent cervical and superior mediastinal lymph nodes (recurrence in this area alone and transregional cases including this area) from 22 to 4 in the Ivor Lewis group. However, in the BRF2 nonoverexpression

group, the difference in the risk of lymph-node relapse and the number of recurrent lymph nodes in the 3FLND and Ivor Lewis groups did not differ to a statistically significant extent ($P=0.702$ and 0.197, respectively) (Table 6).

Association between the BRF2 expression level and lymph-node recurrence

We performed a Chi-squared test to analyze the association between cervical and superior mediastinum lymph-node recurrence and the BRF2 expression level. We found that the overexpression of BRF2 was associated with a higher risk of cervical and superior mediastinal lymph-node recurrence ($P=0.027$, Table 7). The association remained significant in the Ivor Lewis group ($P=0.012$, Table 8) but not in the 3FLND group ($P=0.674$, Table 9). Furthermore, we analyzed the association between the BRF2 expression level and the site of lymph-node relapse in the Ivor Lewis group

Table 2 Association between the clinicopathological variables and lymph node recurrence

Variable	Number of patients	Number of patients with lymph-node recurrence (%)	P^a	Number of recurrent lymph nodes	P^b
Age			0.702		0.544
< 65 years	52	29 (55.8%)		52	
≥ 65 years	67	35 (52.2%)		59	
Gender			0.541		0.443
Male	77	43 (55.8%)		76	
Female	42	21 (50.0%)		35	
T status			0.005		0.003
T1–2	36	11 (30.5%)		17	
T3	69	44 (63.8%)		80	
T4a	14	9 (64.3%)		14	
Tumor diameter			<0.001		P < 0.001
< 3 cm	40	11 (27.5%)		17	
3–5 cm	36	23 (63.9%)		39	
> 5 cm	43	30 (69.8%)		55	
Surgical procedure			0.027		0.001
3FLND	52	22 (42.3%)		30	
Ivor Lewis	67	42 (62.7%)		81	
Differentiation			0.262		0.112
Well	35	20 (57.1%)		35	
Moderate	45	20 (44.4%)		31	
Poor	39	24 (61.5%)		45	
BRF2 overexpression			0.047		0.038
No	47	20 (42.6%)		33	
Yes	72	44 (61.1%)		78	

Significant values are in bold ($P < 0.05$)

P^a Chi-squared test

P^b Nonparametric test

(Table 10). Among 38 patients with the overexpression of BRF2, 31 patients (81.6%) had lymph-node recurrence. Six patients (15.7%) had cervical and superior mediastinal recurrence and four (10.5%) had transregional recurrence that included this area. Fifteen (39.5%) patients had recurrence in the middle and inferior mediastinum, 1 (2.6%) had abdominal recurrence, and 5 (13.2%) had transregional recurrence excluding the cervical and superior mediastinal areas. Meanwhile, the risk of recurrence in patients with the nonoverexpression of BRF2 in the above-mentioned areas was 3.4% (1/29), 0.0% (0/29), 20.7% (6/29), 10.3% (3/29), and 3.4% (1/29), respectively. The difference between these two groups was statistically significant ($P=0.002$). Furthermore, the number of recurrent lymph nodes in the BRF2 overexpression group was significantly greater than that in the BRF2 nonoverexpression group (60 vs. 21, $P=0.004$), especially in cervical and superior mediastinal and transregional recurrence that included this area (20 vs. 3). However, in the 3FLND group, no significant association was observed between the risk of lymph-node recurrence or the number

of recurrent lymph nodes and the BRF2 expression level ($P=0.193$ and 0.694 , respectively, Table 11).

Discussion

Esophageal cancer frequently shows a high degree of invasiveness and is associated with a high rate of lymph-node metastasis, especially in the cervical and abdominal lymph nodes. In addition, the incidence of metastasis to the extra-mediastinal nodes in the absence of mediastinal metastasis (jumping metastasis) is high. Moreover, this high rate of jumping metastasis is considered to be a characteristic of lymph-node metastasis in thoracic esophageal cancer [22]. Thus, the enlargement of the area of lymph-node resection seems to be important for the long-term survival of ESCC patients. However, controversy still exists regarding the resection strategy and the extent of lymphadenectomy. In general, the removal of metastatic regional nodes may be effective for improving long-term survival after radical esophagectomy. However, the cervical nodes are not

Table 3 Univariate and multivariate analyses of the prognostic factors

Variable	OS univariate analysis	OS multivariate analysis		
	<i>P</i> value (log-rank test)	HR	95% confidence interval	<i>P</i> value
Gender: male vs. female	0.509	0.953	0.556–1.633	0.860
Age: < 65 vs. ≥ 65 years	0.882	1.093	0.660–1.810	0.730
T status: T1–2 vs. T3–4	0.017	1.635	1.042–2.564	0.032
Tumor diameter: < 3 cm vs. ≥ 3 cm	0.012	1.282	0.897–1.833	0.173
Surgical procedure: 3FLND vs. Ivor Lewis	0.112	1.667	0.978–2.842	0.061
Differentiation: well vs. moderate vs. poor	0.114	0.761	0.542–1.069	0.115
BRF2 overexpression: no vs. yes	0.032	0.501	0.289–0.868	0.014

Variable	PFS univariate analysis	PFS multivariate analysis		
	<i>P</i> value (log-rank test)	HR	95% confidence interval	<i>P</i> value
Gender: male vs. female	0.548	0.991	0.590–1.663	0.972
Age: < 65 vs. ≥ 65 years	0.907	0.973	0.599–1.582	0.912
T status: T1–2 vs. T3–4	0.029	1.472	0.961–2.254	0.075
Tumor diameter < 3 cm vs. ≥ 3 cm	0.012	1.292	0.913–1.828	0.148
Surgical procedure: 3FLND vs. Ivor Lewis	0.302	1.402	0.842–2.336	0.194
Differentiation: well vs. moderate vs. poor	0.193	0.827	0.597–1.146	0.254
BRF2 overexpression: no vs. yes	0.027	0.518	0.306–0.878	0.014

Significant values are in bold (*P* < 0.05)

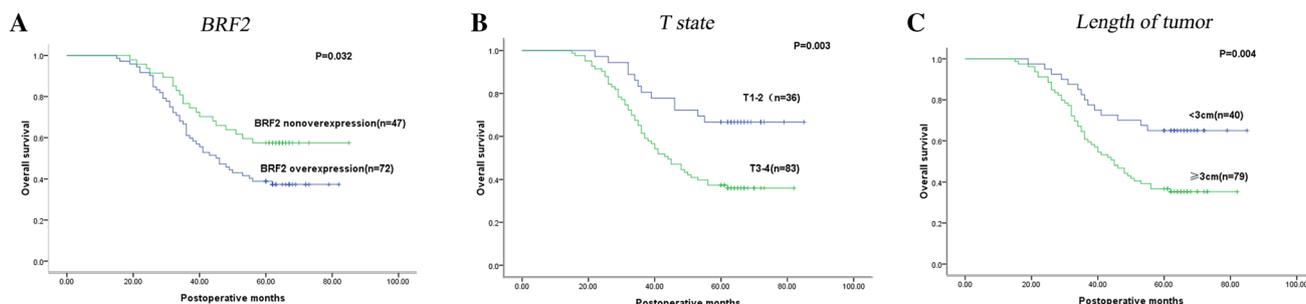


Fig. 2 Kaplan–Meier curves for overall survival stratified according to the expression of BRF2 protein (a), invasion depth (b), and the diameter of the tumor (c)

considered to be regional nodes for the thoracic esophagus. Thus, positive cervical nodes are classified as distant metastases, suggesting that the disease is systemic or that

it is not curable with surgical resection [23]. At the time of writing, this remains a topic of debate. One reason for this is that there are no reasonable criteria for selecting patients

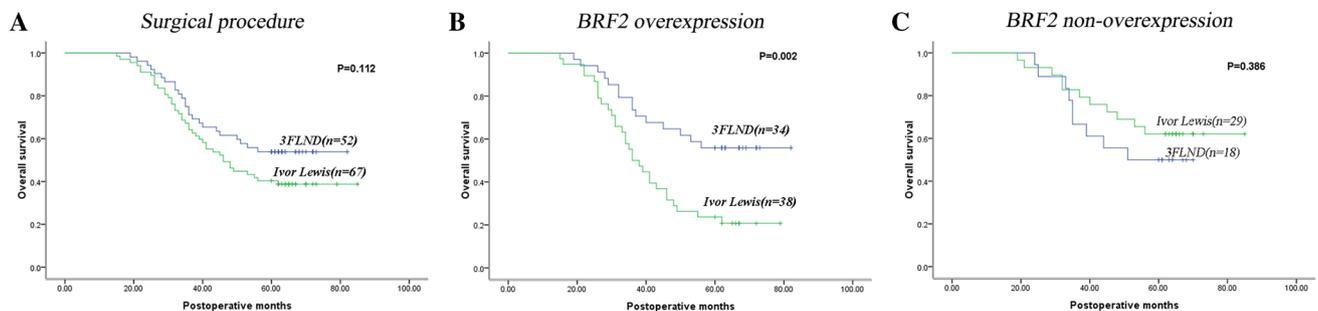
Table 4 Univariate analysis of the association between the surgical procedure and OS in selected patient subgroups

Variable	3FLND (no. of patients)	Ivor Lewis (no. of patients)	OS univariate analysis <i>P</i> value (log-rank test)
T status			
T1–2	17	19	0.615
T3–4	35	48	0.154
Tumor diameter			
< 3 cm	18	22	0.164
≥ 3 cm	34	45	0.318
Differentiation			
Well	15	20	0.062
Moderate	25	20	0.307
Poor	12	27	0.303
BRF2 overexpression			
No	18	29	0.386
Yes	34	38	0.002

Significant values are in bold ($P < 0.05$)

who will benefit from surgery. Some studies have suggested that the cervical node groups should be considered part of the regional nodes of the thoracic esophagus, because they are frequently involved by metastasis from primary tumors, occasionally as jumping metastasis in the early stage of the lymphatic spread because of the location of the cervical node groups. Patients who undergo radical lymph-node dissection were reported to have a low risk of loco-regional recurrence and a better survival [24]. In contrast, other studies suggested that radical lymph-node dissection may increase postoperative complication rates and the recovery time without improving the prognosis [25].

It has been reported that the rate of lymph-node metastasis in patients with esophageal carcinoma invading the mucosa muscularis is approximately 9.3%. With the increase in the invasion depth, the rate of lymph-node metastasis will also increase. For submucosal lesions, the lymph-node metastasis rate can be as high as 50%. In general, lymph-node dissection should only be performed if lymph-node metastasis is suspected, even in patients with superficial cancer such as T2. The characteristics of jumping metastasis in esophageal cancer reduce the effectiveness of sentinel lymph-node

**Fig. 3** Kaplan–Meier survival curves stratified according to surgical procedure in all patients (a), patients in the BRF2 overexpression group (b), and patients in the BRF2 nonoverexpression group (c)**Table 5** Association between the site of recurrent lymph nodes and surgery in the BRF2 overexpression group

Site of lymph-node recurrence	Number of relapsed patients			Number of recurrent lymph nodes		
	3FLND	Ivor Lewis	<i>P</i> value	3FLND	Ivor Lewis	<i>P</i> value
Cervical and superior mediastinum	2 (5.9%)	7 (18.4%)	0.010	2 (11.1%)	12 (20.0%)	<0.001
Middle and inferior mediastinum	7 (20.6%)	14 (36.8%)		8 (44.4%)	24 (40.0%)	
Abdomen	0 (0.0%)	1 (2.6%)		0 (0%)	1 (1.7%)	
Transregional recurrence including cervical and superior mediastinal lymph nodes	1 (2.9%)	4 (10.5%)		2 (11.1%)	10 (16.7%)	
Transregional recurrence excluding cervical and superior mediastinal lymph nodes	3 (8.8%)	5 (13.2%)		6 (33.3%)	13 (21.6%)	
None	21 (61.8%)	7 (18.4%)				
Total	34	38		18	60	

Table 6 Association between site of recurrent lymph nodes and surgery in the BRF2 nonoverexpression group

Site of lymph-node recurrence	Number of relapsed patients			Number of recurrent lymph nodes		
	3FLND	Ivor Lewis	<i>P</i> value	3FLND	Ivor Lewis	<i>P</i> value
Cervical and superior mediastinum	0 (0.0%)	1 (3.4%)	0.702	0 (0.0%)	3 (14.2%)	0.197
Middle and inferior mediastinum	4 (22.2%)	6 (20.7%)		4 (33.3%)	12 (57.1%)	
Abdomen	3 (16.7%)	3 (10.3%)		5 (41.7%)	4 (19.0%)	
Transregional recurrence including cervical and superior mediastinal lymph nodes	1 (5.6%)	0 (0.0%)		1 (8.3%)	0 (0.0%)	
Transregional recurrence excluding cervical and superior mediastinal lymph nodes	1 (5.6%)	1 (3.4%)		2 (16.7%)	2 (9.5%)	
None	9 (50.0%)	18 (62.1%)				
Total	18	29		12	21	

Table 7 Association between cervical and superior mediastinal lymph-node recurrence and the BRF2 expression level

Site of lymph-node recurrence	BRF2 overexpression		<i>P</i>
	Yes	No	
Cervical and superior mediastinal	13	2	0.027
Others	59	45	

Table 8 Association between cervical and superior mediastinal lymph-node recurrence and the BRF2 expression level in the Ivor Lewis group

Site of lymph nodes recurrence	BRF2 overexpression		<i>P</i>
	Yes	No	
Cervical and superior mediastinal	10	1	0.012
Others	28	28	

Table 9 Association between cervical and superior mediastinal lymph-node recurrence and the BRF2 expression level in the 3FLND group

Site of lymph node recurrence	BRF2 overexpression		<i>P</i>
	Yes	No	
Cervical and superior mediastinal	3	1	0.674
Others	31	17	

biopsy in predicting overall lymph-node metastasis. In middle thoracic esophageal carcinoma, lymph-node metastasis is more likely to involve the three regions of the neck, chest and abdomen. Because most sites of cervical lymph-node metastasis are located next to the cervical esophagus, it is recommended that the neck lymph nodes should be cleared through the thoracic approach. Although it is worthwhile to determine dissection of the lymph nodes in the neck which is necessary via the neck approach, there is no consensus as to whether sentinel lymph-node biopsy of the esophagus can serve as an effective criterion.

In China, the consensus approach for esophageal cancer surgery is esophagectomy and esophagogastrotomy via a left thoracic lateral incision pathway, right thoracotomy anterolateral and abdominal pathways, or right thoracotomy anterolateral, abdominal, and cervical pathways. Lymphadenectomy includes traditional two-field, improved two-field, and three-field lymph-node dissection. Chinese surgeons perform esophagectomy via left thoracic lateral incision for lower and middle thoracic ESCC, esophagectomy and esophagogastrotomy via right thoracotomy anterolateral and abdominal pathways or right thoracotomy anterolateral, abdominal, and cervical pathways for upper and middle thoracic ESCC. 3FLND results in a larger surgery field and a greater risk of postoperative complications. However, 3FLND can reduce the incidence of lymph-node metastasis and recurrence as it allows for the dissection of a greater number of lymph nodes. The tumor location is one of the most important parameters to be considered for lymphadenectomy. For cN0 ESCC patients, 3FLND is used for cervical and upper thoracic ESCC and 2FLND is performed for lower thoracic ESCC. However, there is no consensus on the best approach for middle thoracic ESCC in our country. We aimed to evaluate the significance of BRF2 for predicting the prognosis of patients undergoing 3FLND or 2FLND. We excluded the effect of the tumor location, and only patients with stage cN0 middle thoracic ESCC were included in this

Table 10 Association between the expression of BRF2 and lymph-node recurrence in the Ivor Lewis group

Site of lymph-node recurrence	Number of relapsed patients		<i>P</i> value	Number of recurrent lymph nodes		<i>P</i> value
	BRF2 overexpression			BRF2 overexpression		
	Yes	No		Yes	No	
Cervical and superior mediastinal	6 (15.7%)	1 (3.4%)	0.002	10 (16.7%)	3 (15.3%)	0.004
Middle and inferior mediastinal	15 (39.5%)	6 (20.7%)		26 (43.3%)	12 (57.1%)	
Abdomen	1 (2.6%)	3 (10.3%)		1 (1.67%)	4 (19.0%)	
Transregional recurrence including cervical and superior mediastinal	4 (10.5%)	0 (0.0%)		10 (16.71%)	0 (0.0%)	
Transregional recurrence excluding cervical and superior mediastinal	5 (13.2%)	1 (3.4%)		13 (21.7%)	2 (9.5%)	
None	7 (18.4%)	18 (62.1%)				
Total	38 (100%)	29 (100%)		60 (100%)	21 (100%)	

Table 11 Association between the BRF2 expression level and lymph-node recurrence in the 3FLND group

Site of lymph-node recurrence	Number of relapsed patients		<i>P</i> value	Number of recurrent lymph nodes		<i>P</i> value
	BRF2 overexpression			BRF2 overexpression		
	Yes	No		Yes	No	
Cervical and superior mediastinal	2 (5.9%)	0 (0.0%)	0.193	2 (11.1%)	0 (0.0%)	0.694
Middle and inferior mediastinal	7 (20.6%)	4 (22.2%)		8 (44.4%)	4 (33.3%)	
Abdomen	0 (0.0%)	3 (16.7%)		0 (0%)	5 (41.7%)	
Transregional recurrence including cervical and superior mediastinal	1 (2.9%)	1 (5.6%)		2 (11.1%)	1 (8.3%)	
Transregional recurrence excluding cervical and superior mediastinal	3 (8.8%)	1 (5.6%)		6 (33.3%)	2 (16.7%)	
None	21 (61.8%)	9 (50.0%)				
Total	34 (100%)	18 (100%)		18 (100%)	12 (100%)	

study. For these patients, the choice of 3FLND and 2FLND depends on the skill of the surgeon, the physical condition of patient and the willingness of the patient.

For individualized strategies, biomarkers that predict the severity of ESCC and the prognosis of ESCC patients are very important. The relationship between the BRF2 gene and TFIIB is responsible for its important role in tumorigenesis and the development process. We have reported the roles in predicting the prognosis and the mechanisms of lung cancer. In this study, our results showed that the overexpression of BRF2 protein is significantly associated with a higher rate of lymph-node recurrence. Furthermore, deeper tumor invasion, a longer tumor diameter, and conservative lymph-node dissection were associated with a higher risk of lymph-node recurrence. A further analysis using a Cox regression model confirmed that the expression of BRF2 was independently associated with OS and PFS in ESCC patients, suggesting that the BRF2 protein may be a prognostic factor that can be used to predict relapse in ESCC patients. The findings suggest that BRF2 protein may potentially be used as biomarker

for selecting surgical strategies. Furthermore, the T status and diameter of the tumor were found to be associated with OS. Stratified analysis methods were used to detect the significance of these parameters in decision-making in relation to surgical strategies. The survival of the patients in the 3FLND and Ivor Lewis groups according to their BRF2 expression levels. As expected, a statistically significant difference was found in the BRF2 overexpression group. No differences were found in the other stratified groups. We also found that the risk of lymph-node recurrence in the Ivor Lewis dissection group was higher than that in the 3FLND group, especially in the cervical and superior mediastinal region, mainly due to the fact that the lymph nodes in this area were not dissected in the Ivor Lewis group. This is a possible reason for why the survival of the patients with the overexpression of BRF2 in the 3FLND group was superior to that of the patients in the Ivor Lewis dissection group. We also found that patients with the overexpression of BRF2 showed a higher rate of lymph-node recurrence in comparison to the BRF2 nonoverexpression subgroup in the Ivor

Lewis dissection group but not in the 3FLND group. This reflected that the overexpression of BRF2 is associated with a higher risk of lymph-node recurrence and that this could be mitigated by radical lymph-node dissection surgery.

As a central redox-sensing transcription factor involved in the oxidative stress pathway, increased levels of BRF2 allow cancer cells to tolerate high ROS levels, allowing for the accumulation of DNA damage and, as a consequence, enhancing their mutagenic rate [26, 27]. Our results also revealed that the high expression of BRF2 protein was associated with increased angiogenic activity, suggesting that BRF2 plays a crucial role in ESCC tumorigenesis through the induction and/or promotion of tumor angiogenesis [15]. Besides, it was reported that the overexpression of miR-373 can suppress cell EMT, and inhibit the proliferation, migration, and invasion of NSCLC cells by targeting BRF2 [28], which provides new clues as to the roles of BRF2 in cancer. BRF2 has been reported to be involved in tumorigenesis, angiogenesis, proliferation, and metastasis as an oncogene. The overexpression of BRF2 indicates a higher risk of metastasis and cancer recurrence. From this study, cN0 patients with the overexpression of BRF2, who may be at high risk for lymph-node recurrence, should undergo 3FLND esophagectomy. This approach may lead to a better survival among such patients.

The present study was associated with some limitations. First, it was a retrospective investigation with a relatively small population. Randomized controlled trials with a large cohort of patients will be needed to validate the conclusion. Second, only cN0 ESCC patients were included. It is also important to investigate the association between lymph-node metastasis and the BRF2 expression level.

In summary, our data support the assumption that the overexpression of BRF2 protein is associated with a high risk of lymph-node recurrence and that it has potential application as an indicator for radical lymph-node dissection surgery in patients with cN0 middle thoracic ESCC.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest in association with the present study.

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