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Expression and prognostic value of FOXP1 in esophageal squamous cell carcinoma

Qiao Wei[a], Xiyi Li[b,c], Zhengfei Zhu[c,d], Weiwei Yu[d,e], Guangqi Qin[b,c], Huan Chen[b,c], Yanzi Gu[b,c], Kuaile Zhao[c,d], Xiaolong Fu[d,f], Menghong Sun[b,c,*]

[a] Department of Radiation Oncology, The Second Hospital of Tianjin Medical University, Tianjin, China
[b] Department of Pathology Oncology, Fudan University Shanghai Cancer Center, Shanghai, China
[c] Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, 200032, China
[d] Department of Radiation Oncology, Fudan University Shanghai Cancer Center, Shanghai, China
[e] Department of Radiation Oncology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China
[f] Department of Radiation Oncology, Shanghai Chest Hospital, Shanghai Jiao Tong University, Shanghai, China

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ABSTRACT

Background: Forkhead box protein P1 (FOXP1) has been suggested as a prognostic marker in several malignant tumors. However, the significance of FOXP1 in esophageal squamous cell carcinoma (ESCC) is still unclear. The purpose of this study was to investigate the expression pattern of FOXP1 in normal esophageal tissue and ESCC and to analyze the clinicopathological significance and prognostic value of FOXP1 in ESCC.

Methods: FOXP1 was detected by immunohistochemistry using tissue microarrays containing tumor tissues and adjacent normal tissues from 270 ESCC patients with oncological follow-up data.

Results: Normal esophageal tissues predominantly showed an exclusive nuclear FOXP1 (n-FOXP1) expression pattern, and no exclusive cytoplasmic FOXP1 (c-FOXP1) staining was found. In ESCC, the expression rates of exclusive n-FOXP1-positive, exclusive c-FOXP1-positive, both nuclear and cytoplasmic positive and complete negative were 14.4%, 28.9%, 10.4% and 46.3%, respectively. High n-FOXP1 expression was significantly correlated with decreased postoperative recurrence and distant metastasis ($P < 0.05$). Furthermore, elevated c-FOXP1 expression was significantly associated with regional lymph node metastasis and distant metastasis ($P < 0.05$). High c-FOXP1 expression had an effect on shorter overall survival (OS) time, but the difference was not statistically significant ($P > 0.05$). Kaplan–Meier analysis showed that ESCC patients with high n-FOXP1 expression survived significantly longer than patients with low n-FOXP1 expression. Multivariate analysis confirmed that patients with high n-FOXP1 staining exhibit good prognosis and n-FOXP1 was an independent factor for ESCC prognosis.

Conclusions: Our results suggest that FOXP1 plays an essential role in ESCC progression and prognosis and may be a useful biomarker for predicting survival.

1. Introduction

Esophageal cancer (EC) is the sixth leading cause of cancer death worldwide and is usually with aggressive progression and poor prognosis [1]. In China, EC is one of the most common malignant tumors, with a unique regional distribution, and esophageal squamous cell carcinoma (ESCC) is its primary histological subtype [2]. The latest statistics show that the incidence and mortality of EC in China are both on the decline, but due to population growth and aging, the number of newly diagnosed EC patients is still significant and on the rise [3]. Although the treatment methods have been improving over the past few decades, the prognosis of EC patients is still poor, especially those with advanced disease. The 5-year overall survival (OS) rates of EC patients after surgeries range from 10% to 30%, and the survival rate in ESCC patients was significantly lower than that in esophageal adenocarcinoma (EAC) patients [4]. At present, prognosis prediction relies on the TNM classification system to stratify patients, but it has limited utility and does not consider the biological factors of this fatal disease [5]. Therefore, it is necessary to identify reliable prognostic biomarkers for the effective postoperative monitoring and treatment of ESCC.

The forkhead box protein P1 (FOXP1) gene is located on chromosome 3p14.1 and is a member of the forkhead/winged-helix

* Corresponding author at: Department of Pathology Oncology, Fudan University Shanghai Cancer Center, 270 Dong An Road, Shanghai, 200032, China.
E-mail address: menghongs@hotmail.com (M. Sun).

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transcription factor family [6]. The full-length human FOXP1 gene was cloned for the first time by using a mouse monoclonal antibody (JC12), which recognizes proteins with inconsistent expression in normal and malignant B cells [6]. As one of the widely expressed transcription factors, FOXP1 has a great diversity of functions ranging from the regulation of cardiac development, jaw, lung and esophagus development to monocyte, macrophage and B-cell differentiation [7–10]. In addition, FOXP1 is also a crucial regulator in the development of the learning ability, speech and language [11].

FOXP1 not only plays an vital role in regulating the development of normal human tissues, but also participates in tumorigenesis. Previous studies have shown that FOXP1 behave as both a potential oncogene and a tumor suppressor candidate gene [12]. For example, in diffuse large B-cell lymphoma (DLBCL), FOXP1 acts as an oncogene that suppresses immune response and promotes cancer cell survival [13]. In hepatocellular carcinoma, silencing FOXP1 inhibits cell proliferation and migratory by inducing G1/S phase cell arrest [14]. By contrast, FOXP1 shows tumor suppressive function by inhibiting cell growth and attenuating tumorigenicity in neuroblastoma, prostate cancer and lung adenocarcinoma [15–17]. Therefore, the functional role of FOXP1 in tumorigenesis and development is inconsistent, and its molecular mechanisms need to be further elucidated.

Recent studies have also confirmed the tremendous prognostic value of FOXP1 protein expression in tumor patients, but there is still controversy in different tumors. High expression of FOXP1 was related to short survival in patients with DLBCL, Mucosa-Associated Lymphoid Tissue Lymphomas (MALT), hepatocellular carcinoma and cutaneous melanoma [18–21]. Conversely, high levels of FOXP1 expression were linked to improved survival in non–small cell lung cancer patients [22]. Loss or low FOXP1 protein expression was correlated with worse prognosis in colorectal cancer, pancreatic ductal adenocarcinoma and epithelial ovarian cancer [23–25]. In breast cancer, positive nuclear expression of FOXP1 is associated with favorable prognosis, but positive cytoplasmic FOXP1 expression is correlated with a short survival [26,27]. However, the expression and clinical significance of FOXP1 in ESCC have not been reported.

This study intended to investigate the expression of FOXP1 in a large cohort of ESCC patients who underwent radical surgery without preoperative treatment by immunohistochemistry. We analyzed the expression of FOXP1 in matched ESCC tumor tissues and normal tissues and its correlation with detailed clinicopathologic characteristics. Moreover, we evaluated the correlation between FOXP1 expression levels and the OS of ESCC. As far as we know, no previous studies have reported that FOXP1 expression is associated with the prognosis of ESCC.

2. Materials and methods

2.1. Patients and tumor tissue microarray (TMA)

A total of 270 ESCC patients analyzed in this study underwent radical surgery at Fudan University Shanghai Cancer Center from 2002 to 2009. No patient was treated preoperatively. Two pathologists confirmed the diagnosis of ESCC. Tumors were staged following the standard criteria of the 7th AJCC cancer staging manual. Data were collected on all subjects, including age, gender, overall survival and ESCC features, such as tumor location, size, depth of invasion, histologic stage, nervous invasion, venous invasion, the status of lymph node metastasis and distant metastasis. Patients were followed up every three months in the first year after the operation, and then six months until 30 December 2015. The average follow-up time was 40 months (median, 44 months; range, 2–127 months), with 124 recurrences, 52 metastases, and 143 deaths at the end of follow-up. Detail clinical pathological data are shown in Table 2. This study was approved by the Clinical Research Ethics Committee of Fudan University, and written informed consent was obtained from each participant.

Formalin-fixed, paraffin-embedded (FFPE) tissue blocks and the corresponding original hematoxylin and eosin staining (HE) slides of 270 patients with ESCC were retrieved from Tissue Bank of Fudan University Shanghai Cancer Center. HE slides were reviewed, tumor tissues and corresponding adjacent normal tissues were selected to construct TMA. For each patient, two 1 mm tumor cores and two 1 mm adjacent normal epithelial cores were inserted into the 10 × 12 receptor blocks (Unitma, Seoul, Korea). The receptor blocks were smoothed, dewaxed, precooled, sliced, and then stained with HE. Pathologists confirmed that the histological and cellular structure of each site was identifiable.

2.2. Immunohistochemistry (IHC) and scoring

IHC staining was performed according to the standard Envision (two-step method). Briefly, the tissue section was dewaxed and rehydrated in an alcohol gradient. Antigen retrieval was performed by heat induction in citrate buffer (PH 6), and blocking was in 10% serum for 30 min at room temperature. Then, the polyclonal rabbit anti-FOXP1 antibody (ab16645, Abcam, Cambridge, USA) was incubated at a dilution of 1/200 overnight at 4 degrees Celsius. The antibody reactions were visualized using the Envision kit (Dako, Carpentaria, CA, USA) according to the manufacture's protocols. Finally, each TMA slide was colored with DAB, dehydrated in alcohol and xylene, and mounted with neutral gel. We used two sections form breast cancer with known high FOXP1 expression as the positive control. For negative control, FOXP1 primary antibody was replaced with phosphate-buffered saline (PBS).

The staining results were evaluated by two independent pathologists who were blind to the clinical and pathological features associated with the specimens. The FOXP1 expression in nuclei was defined as negative (score 0, < 10% of tumor cell with nuclear staining), weakly positive (score 1, 10%–30% staining of ESCC cells), moderately positive (score 2, 31%–50% nuclear staining of ESCC cells), or strongly positive (score 3, > 50% nuclear staining of ESCC cells). In the statistical analysis, low n-FOXP1 expression was referred to as the IHC score 0 and 1, and high n-FOXP1 expression was referred to as the IHC score 2 and 3. The FOXP1 expression in cytoplasm was represented by the semi-quantitative immunoreactive score (IRS), which was calculated by multiplying the staining intensity by the percentage of positive cells, and the final IRS score was obtained to define expression level as either low (-) expression ≤ 4 or high (+) expression > 4, as previously described [28]. The staining intensity was classified as negative (0) for no stain, weak (1) for faint yellow, intermediate (2) for yellowish-brown, and strong (3) for brown. The percentage of positive cells was scored as follows: (0) 0%, (1) 1%–24%, (2) 25%–50%, (3) 50%–75%, and (4) > 75%.

2.3. Statistical analysis

All statistical analyses were performed using SPSS 21.0 (IBM, SPSS, Chicago, USA). The Chi-square (χ^2) test was used to determine the relationships between clinical parameters and expressions of FOXP1 of immunochemistry. OS time was defined as time from date of surgery to date of death from any cause or the last follow-up. Univariate survival analysis was performed using the Kaplan-Meier method with Log-rank test performed for comparisons. To examine the independent effect of potential prognostic factors on OS, variables with a $P < 0.05$ in univariate analysis were performed using the multivariate Cox regression. $P < 0.05$ in a two-tailed test was considered statistically significant.

3. Results

3.1. FOXP1 expression in normal esophageal tissues and ESCC tissues

Most normal esophageal squamous epithelium tissues (169/270, 62.6%) showed uniform strong n-FOXP1 staining and solely c-FOXP1

Table 1
FOXP1 expression in ESCC tissues and normal esophageal squamous epithelium tissues.

	Exclusive nuclear expression	Exclusive cytoplasmic expression	Both nuclear and cytoplasmic expression	Complete loss of expression	χ^2	P
Tumor tissue	39(14.4%)	78(28.9%)	28(10.4%)	125(46.3%)	189.799	< 0.001*
Normal tissue	169(62.6%)	0(0.0%)	0(0.0%)	101(37.4%)		

Abbreviations: ESCC, Esophageal squamous cell carcinoma.
* P < 0.05was considered statistically significant.

expression was not found in normal tissues (Table 1). However, in ESCC tissues, exclusive c-FOXP1 expression (78/270, 28.9%) was more frequently observed than solely nuclear expression (39/270, 14.4%), both nuclear and cytoplasmic expression accounted for 10.4% (28/270) of cases and complete loss of expression was observed in 125 cases (46.3%) (Table 1). Representative images of FOXP1 expression were presented in Fig. 1. The FOXP1 expression patterns were significantly different in normal esophageal tissues and ESCC tissues ($\chi^2 = 189.799$, $P < 0.001$; Table 1).

3.2. Correlation between FOXP1 expression and clinicopathological characteristics in ESCC

The associations between FOXP1 expression and basic clinical variables and histopathological characteristics in ESCC were shown in Table 2. High n-FOXP1 expression was significantly correlated with decreased postoperative recurrence and distant metastases ($P = 0.010$ and $P = 0.049$). Nevertheless, there were no statistically significant differences between n-FOXP1 expression and patient age, sex, tumor length, tumor differentiation, vascular invasion, nervous invasion and tumor stage (all $P > 0.05$).

C-FOXP1 expression in male patients was higher than that in female patients, and the difference was statistically significant ($P = 0.043$). High c-FOXP1 staining was observed in 69.6% (71/102) of patients with regional lymph node metastasis, which was higher than that in patients without regional lymph node metastasis (31/102, 30.4%) ($P = 0.020$). Furthermore, there was a significant association between elevated c-FOXP1 expression and distant metastasis ($P = 0.026$). Among 52 subjects with distant metastasis, the high c-FOXP1 expression rate was 57.7% (30/52). No significant relevance was found between c-FOXP1 expression and other clinicopathologic factors (i.e., age, location, length, pT, differentiation, recurrence, vascular invasion and nervous invasion; all $P > 0.05$) in ESCC.

3.3. Correlation between FOXP1 expression and survival prognosis in patients with ESCC

This study included 270 ESCC patients, 127 patients survived, the median survival time was 44 months, and the OS rate was 47.0%. Univariate survival analysis revealed that sex, vascular invasion, pathologic T stage, lymph node metastasis, TNM stage, adjuvant therapy and n-FOXP1 were significantly associated with the overall survival time of patients with ESCC (all $P < 0.05$; Table 3). The average and median survival time in patients with high n-FOXP1 expression were significantly longer than patients with low n-FOXP1 expression ($\chi^2 = 5.012$, $P = 0.025$; Fig. 2A). Patients with low n-FOXP1 had median overall survival time of only 61.3 months, compared to 82.2 months for patients with high n-FOXP1. Interestingly, high c-FOXP1 expression had an effect on shorter overall survival time, but the difference was not statistically significant ($\chi^2 = 3.166$, $P = 0.075$; Fig.2B). Multivariate Cox regression analysis showed that high n-FOXP1 expression was positively correlated with overall survival and negatively correlated with mortality, and high TNM stage (III) was negatively linked with overall survival time (Table 3).

4. Discussion

Forkhead box protein P1 (FOXP1) is a protein encoded by the FOXP1 gene that belongs to the P subfamily of forkhead box transcription factors. FOXP1 protein is broadly expressed in normal tissues and involved in the regulation of cell growth, differentiation, metabolism and survival [6]. The intracellular localization of FOXP1 protein varies between different tissues. The predominant nuclear FOXP1 distribution was observed in most normal human tissues such as blood, kidney, thyroid and tonsil, whereas the cytoplasmic FOXP1 expression was found in other epithelial tissues such as the stomach and colon [6]. In addition, exclusively nuclear, exclusively cytoplasmic, both nuclear and cytoplasmic, and complete loss of expression were observed in a range of tumor types. However, the expression of FOXP1 in normal esophageal epithelial tissue and ESCC tissue has not been documented. In the present study, we investigated the expression pattern of FOXP1 in ESCC tissue and its adjacent normal tissue. In normal tissues, only the strong nuclear staining of FOXP1 was observed, while FOXP1 protein expression was detected in different locations among the 270 ESCC tissue samples. 39 cases were positive in the nucleus; 78 cases were positive in the cytoplasm; 28 cases were positive in both the nucleus and cytoplasm; 125 cases were negative. Taken together, FOXP1 protein has decreased nuclear expression and increased cytoplasmic expression in ESCC tissues compared with matched normal tissues. Therefore, we speculate that the cytoplasmic localization and nuclear localization of FOXP1 may play different roles in the development and progression of ESCC, as its nuclear expression is characteristic of normal esophageal tissue.

Previous studies also indicated that the subcellular localization of FOXP1 played a unique role in the pathogenesis of malignant tumors, and had important clinical significance. In endometrial cancer, overexpression of FOXP1 protein in cytoplasm resulted in deeper myometrial invasion and increased HIF1 expression, whereas nuclear immunoactivity of FOXP1 was associated with the expression of estrogen receptor [29]. In hepatocellular carcinoma, FOXP1 was predominantly expressed in the cytoplasm, and its high expression was associated with high serum α-fetoprotein, large tumor diameter, later TNM stage and poor clinical outcome [20]. Donizy et al. observed that the exclusive cytoplasmic expression of FOXP1 protein in tumor cells and stromal cells of cutaneous melanoma [21]. Elevated expression of FOXP1 protein in tumor cells was strongly associated with more advanced tumor stage and poorer prognosis, while FOXP1 overexpression in stromal cells was related to lower tumor stage, so FOXP1 was considered as a new independent adverse prognostic factor and a specific predictor of lymphatic dissemination of this neoplasm [21]. In epithelial ovarian cancer, decreased nuclear and increased cytoplasmic of FOXP1 protein expression were related to the increase of tumor grade, while decreased nuclear FOXP1 expression was an independent risk factor correlated with chemotherapy resistance and unfavorable prognosis of patients with EOC, but cytoplasmic FOXP1 expression had no correlation with chemosensitivity and prognosis [25]. In breast cancer, FOXP1 nuclear expression was considered to be a favorable prognosis factor and a potential Estrogen receptor (ER) coregulator that regulate important pathways [26]. Moreover, FOXP1 immunoreactivity may predict a good prognosis for breast cancer patients treated with tamoxifen [30]. However, Yang et al. found that FOXP1 expression shifted from the

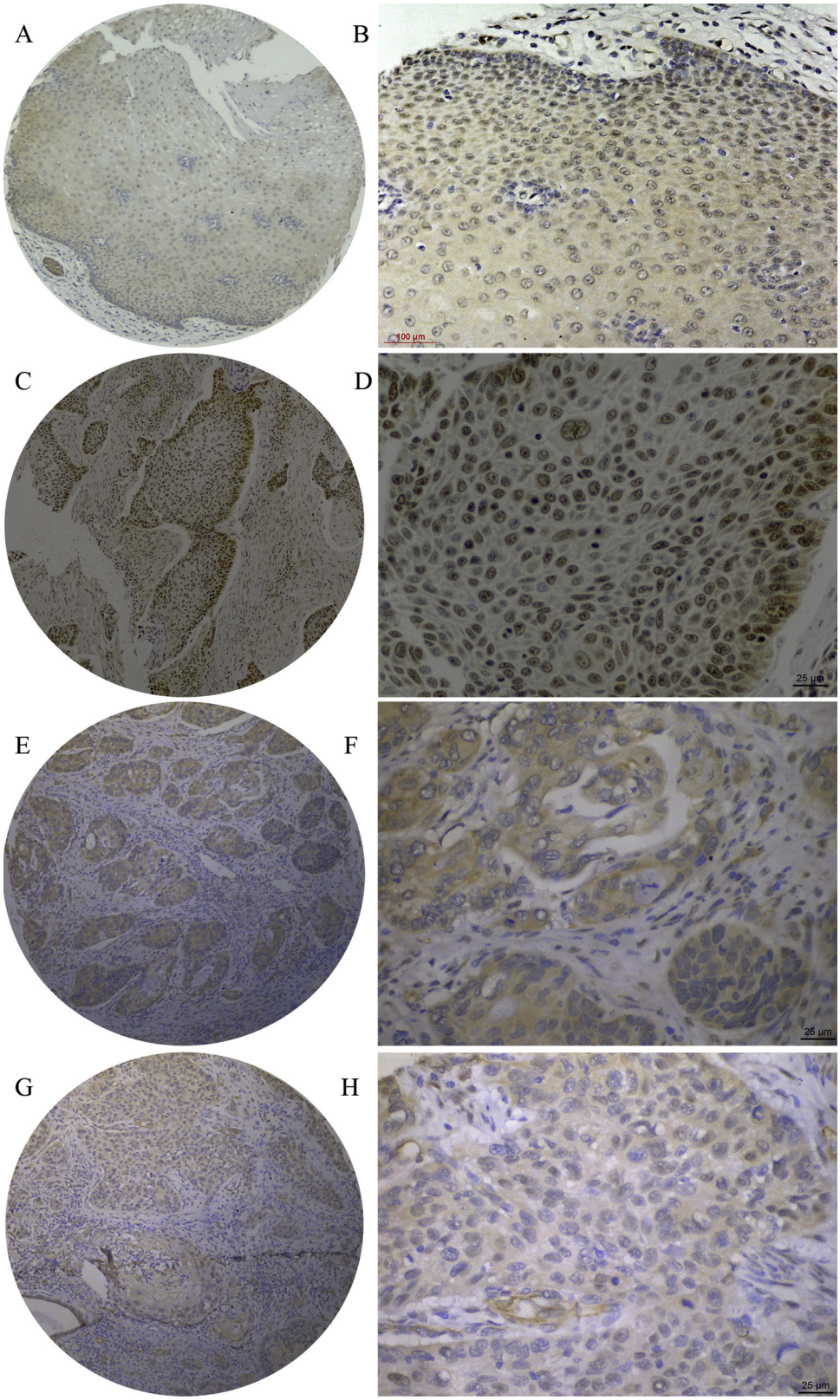

Fig. 1. Representative IHC staining of FOXP1 in esophageal squamous epithelium tissues and ESCC tissues. (A, B) Expression of FOXP1 in healthy esophageal tissue (A,TMA; B × 200). (C, D) Exclusive nuclear expression of FOXP1 protein in ESCC tissue (C, TMA; D × 400). (E, F) Tumor with exclusive cytoplasmic FOXP1 expression (E, TMA; F × 400). (G, H) Tumor specimen with mixed nuclear /cytoplasmic FOXP1 expression (G,TMA; H × 400).

nucleus to the cytoplasm during breast tumorigenesis, and the exclusive cytoplasmic FOXP1 expression was strongly linked with ER and Calpain II expression and predicted a poor outcome in breast cancer [27]. Similarly, in this study, we found that the expression level of FOXP1 protein decreased in the nucleus and increased in the cytoplasm, both of which were related to the increased distant metastases of ESCC. We

Table 2
Associations of n-FOXP1 and c-FOXP1 expression with clinicopathologic features in ESCC.

Parameters	n-FOXP1		χ^2	*P*	c-FOXP1		χ^2	*P*
	High	Low			High	Low		
All cases	67(24.8%)	203(75.2%)			106(39.3%)	164(60.7%)		
Age(year)			0.592	0.442			0.207	0.649
≤60	47(26.3%)	132(73.3%)			72(40.2%)	107(59.8%)		
>60	20(22.0%)	71(78.0%)			34(37.4%)	57(62.6%)		
Gender			0.153	0.695			4.110	0.043*
Male	53(24.3%)	165(75.7%)			92(42.2%)	126(57.8%)		
Female	14(26.9%)	38(73.1%)			14(26.9%)	38(73.1%)		
Tumor Location			1.031	0.597			0.066	0.968
Upper	9(29.0%)	22(71.0%)			12(38.7%)	19(61.3%)		
Middle	40(22.9%)	135(77.1%)			68(38.9%)	107(61.1%)		
Lower	18(28.1%)	46(71.9%)			26(40.6%)	38(59.4%)		
Tumor length (cm)			1.836	0.175			2.655	0.103
≤4 cm	36(22.0%)	128(78.0%)			58(35.4%)	106(64.6%)		
>4 cm	31(29.2%)	75(70.8%)			48(45.3%)	58(54.7%)		
Tumor differentiation			0.075	0.963			0.913	0.634
Well	9(25.7%)	26(74.3%)			13(37.1%)	22(62.9%)		
Moderate	43(24.3%)	134(75.7%)			73(41.2%)	104(58.8%)		
Poor	15(25.9%)	43(74.1%)			20(34.5%)	38(65.5%)		
Vascular invasion			0.016	0.900			0.359	0.549
No	24(25.3%)	71(74.7%)			35(36.8%)	60(63.2%)		
Yes	43(24.6%)	132(75.4%)			71(40.6%)	104(59.4%)		
Nerverous invision			2.208	0.137				
No	58(23.8%)	186(76.2%)			93(38.1%)	151(61.9%)	1.392	0.238
Yes	9(34.6%)	17(65.4%)			13(50.0%)	13(50.0%)		
Lymph node metastasis			0.008	0.928			5.405	0.020*
No	25(24.5%)	77(75.5%)			31(30.4%)	71(69.6%)		
Yes	42(25.0%)	126(75.0%)			75(44.6%)	93(55.4%)		
Tumor relapse (240 cases)			6.579	0.010*			2.799	0.094
No	36(31.0%)	80(69.0%)			41(35.3%)	75(64.7%)		
Yes	21(16.9%)	103(83.1%			57(46.0%)	67(54.0%)		
Pathologic TNM stage			0.634	0.728			0.451	0.798
I	3(25.0%)	9(75.0%)			4(33.3%)	8(66.7%)		
II	28(22.6%)	96(77.4%)			47(37.9%)	77(62.1%)		
III	36(26.9%)	98(73.1%)			55(41.0%)	79(59.0%)		
Distant metastasis(240 cases)			3.880	0.049*			4.925	0.026*
No	50(26.6%)	138(73.4%)			76(40.4%)	112(59.6%)		
Yes	7(13.5%)	45(86.5%)			30(57.7%)	22(42.3%)		

Abbreviations: n-FOXP1, nuclear FOXP1; c-FOXP1, cytoplasmic FOXP1.
* $P < 0.05$ was considered statistically significant.

also identified that the high expression of nuclear FOXP1 resulted in the decreased postoperative recurrence, and also was correlated with the longer average and median survival time in ESCC patients. Moreover, the exclusive cytoplasmic FOXP1 positive expression was related to positive regional lymph node metastasis and conferred a slightly worse prognosis, despite an insignificant difference. Further large-scale studies are needed to validate the prognostic value of cytoplasmic FOXP1 expression in ESCC. Taken together, the abovementioned findings combined with our results suggest that the function of FOXP1 in solid tumors may rely on its subcellular distribution, that is, the nuclear localization may inhibit oncogenesis, whereas the cytoplasmic one may lead to tumorigenesis.

Table 3
Univariate and multivariate Cox regression analyses of overall survival (OS).

Variables	Univariate analysis	Multivariate analysis	
	P Value	*P* Value	HR(95% CI)
Age(≤60y vs >60y)	0.219		
Sex(Male vs Female)	0.014*		
Tumor length(≤4 cm vs >4 cm)	0.530		
Tumor location(Upper vs Middle vs Lower)	0.119		
Tumor differentiation(Well vs Moderate vs Poor)	0.736		
Vascular invasion(No vs Yes)	< 0.001*		
Nerverous invision(No vs Yes)	0.543		
Pathologic T stage(T1 vs T2 vs T3 vs T4)	0.006*		
Lymph node metastasis(No vs Yes)	< 0.001*		
Adjuvant therapy(No vs Yes vs Unknown)	0.042*		
Pathologic TNM stage(I/II vs III)	< 0.001*	0.008*	0.489(0.288-0.830)
n-FOXP1(Low vs High)	0.025*	0.020*	1.672(1.086-2.574)
c-FOXP1(Low vs High)	0.075		

Abbreviations: CI, confidence interval; HR, hazard ratio.
* $P < 0.05$ was considered statistically significant.

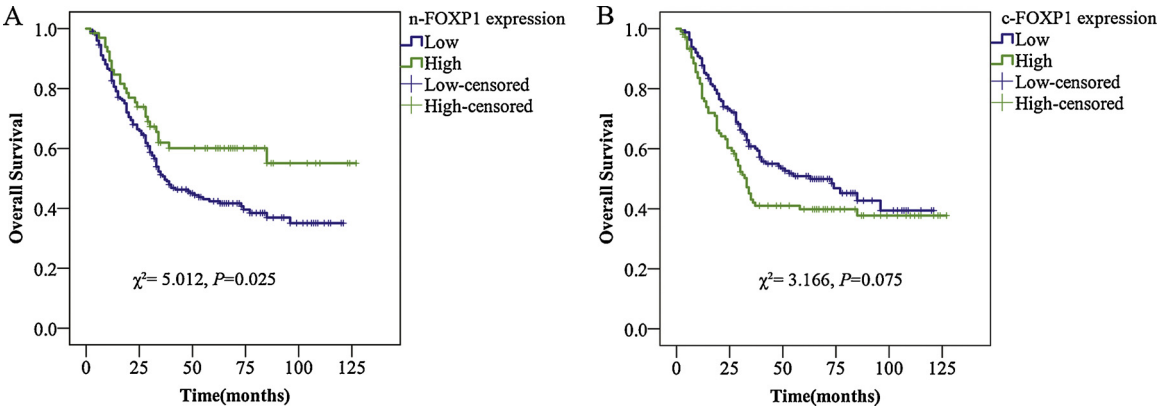

Fig. 2. Kaplan-Meier curves for overall survival of ESCC patients according to nuclear FOXP1 expression level (A) and cytoplasmic FOXP1 expression level (B).The *P*-values were calculated by Log-Rank test. $P < 0.05$ was considered statistically significant.

The underlying mechanisms of the different prognostic significance of nuclear and cytoplasmic FOXP1 expression in tumors remain mostly unknown. One possible explanation for the above-mentioned contradictory role of FOXP1 as an oncogene or a tumor suppressor gene was presented with the recognition of shorter FOXP1 isoforms. Smaller FOXP1 isoforms are N-terminally truncated proteins encoded by spliced FOXP1 mRNA subtypes, which are preferentially expressed in the activated B-cell like subtype of DLBCL (ABC-DLBCL) and primary DLBCL [31]. It was proposed that these smaller FOXP1 isoforms function as oncogenes in DLBCL and MALT, while the full-length FOXP1 acts as a tumor suppressor gene in many tissues or organs [31,32]. Numerous studies have reported that proteins with different bio-activities are generated from a single gene by alternative splicing, and extensive selective splicing of the FOX family members, such as FOXP2 and FOXA1, has also been reported [33,34]. Therefore, the expression of alternatively spliced FOXP1 proteins may explain the presence of cytoplasmic staining that was observed in some tumors but not in normal tissues. In addition to shorter isoforms, cell lineage-or tumor type-specific transcriptional program also affects the behavior of FOXP1 during carcinogenesis. Yang at al. observed that FOXP1 expression shift from the nucleus to the cytoplasm during breast tumorigenesis, and they also found that cytoplasmic FOXP1 expression was remarkably correlated with calpain II in breast cancer [27]. Calpain II can promote breast cancer cell proliferation through the PI3K/AKT signaling pathway [35]. The authors hypothesized that calpain II and the AKT pathway might be involved in the subcellular regulation of FOXP1 [27]. In another breast cancer study, cytoplasmic staining of FOXP1 was found in some tumor tissues, but not in normal breast tissues [26]. Besides, FOXP1 and ER were commonly co-expressed in cell lines, so the investigators concluded that FOXP1 might be a co-regulator of ER receptor that controls the progression of breast cancer [26]. Similarly, subcellular localization of FOXP1 has been reported to play an important role in the pathogenesis of endometrial cancer through KRAS pathway [29]. Moreover, FOXP1 expression is also regulated by translocation, gene amplification and some miRNAs [36–38]. Taken together, FOXP1 are bi-functional cancer-associated genes, which are oncogenic or tumor suppressive in a context-dependent manner as mentioned above. Further studies are required to determine the underlying mechanisms.

In summary, we believe that this is the first report on the expression pattern of FOXP1 protein in ESCC, and FOXP1 may be used as a prognostic marker for ESCC. Our study indicates that high nuclear FOXP1 expression is associated with better survival in patients with ESCC, while high cytoplasmic FOXP1 expression is correlated with aggressive tumor features in ESCC but not significantly linked with patient outcome. Taken together, these results suggest that the nature of FOXP1 is far more complicated than previously thought, and its role in tumorigenesis deserves further investigation, especially in the development of better prognosis markers and therapeutic intervention strategies.

Declaration of Competing Interest

The authors declare that they have no conflict of interests.

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References

[1] L.A. Torre, F. Bray, R.L. Siegel, J. Ferlay, J. Lortet-Tieulent, A. Jemal, Global cancer statistics, 2012, CA Cancer J. Clin. 65 (2015) 87–108 PubMed: 25651787.
[2] Y. Lin, Y. Totsuka, Y. He, S. Kikuchi, Y. Qiao, J. Ueda, W. Wei, M. Inoue, H. Tanaka, Epidemiology of esophageal cancer in Japan and China, J. Epidemiol. 23 (2013) 233–242 PubMed: 23629646.
[3] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, CA Cancer J. Clin. 69 (2019) (2019) 7–34 PubMed: 30620402.
[4] S. Paul, N. Altorki, Outcomes in the management of esophageal cancer, J. Surg. Oncol. 110 (2014) 599–610 PubMed: 25146593.
[5] T.W. Rice, V.W. Rusch, H. Ishwaran, E.H. Blackstone, C. Worldwide esophageal Cancer, Cancer of the esophagus and esophagogastric junction: data-driven staging for the seventh edition of the american joint committee on Cancer/International union against Cancer Cancer Staging manuals, Cancer 116 (2010) 3763–3773 PubMed: 20564099.
[6] A.H. Banham, N. Beasley, E. Campo, P.L. Fernandez, C. Fidler, K. Gatter, M. Jones, D.Y. Mason, J.E. Prime, P. Trougouboff, K. Wood, J.L. Cordell, The FOXP1 winged helix transcription factor is a novel candidate tumor suppressor gene on chromosome 3p, Cancer Res. 61 (2001) 8820–8829 PubMed: 11751404.
[7] Y. Zhang, S. Li, L. Yuan, Y. Tian, J. Weidenfeld, J. Yang, F. Liu, A.L. Chokas, E.E. Morrisey, Foxp1 coordinates cardiomyocyte proliferation through both cell-autonomous and nonautonomous mechanisms, Genes Dev. 24 (2010) 1746–1757 PubMed: 20713518.
[8] W. Shu, M.M. Lu, Y. Zhang, P.W. Tucker, D. Zhou, E.E. Morrisey, Foxp2 and Foxp1 cooperatively regulate lung and esophagus development, Development 134 (2007) 1991–2000 PubMed: 17428829.
[9] J.M. Cesario, A.A. Almaidhan, J. Jeong, Expression of forkhead box transcription factor genes Foxp1 and Foxp2 during jaw development, Gene Expr. Patterns 20 (2016) 111–119 PubMed: 26969076.
[10] T. Patzelt, S.J. Keppler, O. Gorka, S. Thoene, T. Wartewig, M. Reth, I. Forster, R. Lang, M. Buchner, J. Ruland, Foxp1 controls mature B cell survival and the development of follicular and B-1 B cells, Proc. Natl. Acad. Sci. U. S. A. 115 (2018) 3120–3125 PubMed: 29507226.
[11] B.A. Benayoun, S. Caburet, R.A. Veitia, Forkhead transcription factors: key players in health and disease, Trends Genet. 27 (2011) 224–232 PubMed: 21507500.
[12] H.B. Koon, G.C. Ippolito, A.H. Banham, P.W. Tucker, FOXP1: a potential therapeutic target in cancer, Expert Opin. Ther. Targets 11 (2007) 955–965 PubMed: 17614763.
[13] P.J. Brown, K.K. Wong, S.L. Felce, L. Lyne, H. Spearman, E.J. Soilleux, L.M. Pedersen, M.B. Moller, T.M. Green, D.M. Gascoyne, A.H. Banham, FOXP1 suppresses immune response signatures and MHC class II expression in activated B-cell-like diffuse large B-cell lymphomas, Leukemia 30 (2016) 605–616 PubMed: 26500140.

[14] X. Wang, J. Sun, M. Cui, F. Zhao, C. Ge, T. Chen, M. Yao, J. Li, Downregulation of FOXP1 inhibits cell proliferation in hepatocellular carcinoma by inducing G1/S phase cell cycle arrest, Int. J. Mol. Sci. 17 (2016) 1501 PubMed: 27618020.
[15] S. Ackermann, H. Kocak, B. Hero, V. Ehemann, Y. Kahlert, A. Oberthuer, F. Roels, J. Theissen, M. Odenthal, F. Berthold, M. Fischer, FOXP1 inhibits cell growth and attenuates tumorigenicity of neuroblastoma, BMC Cancer 14 (2014) 840 PubMed: 25406647.
[16] K. Takayama, T. Suzuki, S. Tsutsumi, T. Fujimura, S. Takahashi, Y. Homma, T. Urano, H. Aburatani, S. Inoue, Integrative analysis of FOXP1 function reveals a tumor-suppressive effect in prostate cancer, Mol. Endocrinol. 28 (2014) 2012–2024 PubMed: 25329375.
[17] H. Sheng, X. Li, Y. Xu, Knockdown of FOXP1 promotes the development of lung adenocarcinoma, Cancer Biol. Ther. 20 (2019) 537–545 PubMed: 30409062.
[18] A.H. Banham, J.M. Connors, P.J. Brown, J.L. Cordell, G. Ott, G. Sreenivasan, P. Farinha, D.E. Horsman, R.D. Gascoyne, Expression of the FOXP1 transcription factor is strongly associated with inferior survival in patients with diffuse large B-cell lymphoma, Clin. Cancer Res. 11 (2005) 1065–1072 PubMed: 15709173.
[19] X. Sagaert, P. de Paepe, L. Libbrecht, V. Vanhentenrijk, G. Verhoef, J. Thomas, I. Wlodarska, C. De Wolf-Peeters, Forkhead box protein P1 expression in mucosa-associated lymphoid tissue lymphomas predicts poor prognosis and transformation to diffuse large B-cell lymphoma, J. Clin. Oncol. 24 (2006) 2490–2497 PubMed: 16636337.
[20] Y. Zhang, S. Zhang, X. Wang, J. Liu, L. Yang, S. He, L. Chen, J. Huang, Prognostic significance of FOXP1 as an oncogene in hepatocellular carcinoma, J. Clin. Pathol. 65 (2012) 528–533 PubMed: 22422806.
[21] P. Donizy, K. Pagacz, J. Marczuk, W. Fendler, A. Maciejczyk, A. Halon, R. Matkowski, Upregulation of FOXP1 is a new independent unfavorable prognosticator and a specific predictor of lymphatic dissemination in cutaneous melanoma patients, Onco. Ther. 11 (2018) 1413–1422 PubMed: 29559799.
[22] J. Feng, X. Zhang, H. Zhu, X. Wang, S. Ni, J. Huang, High expression of FoxP1 is associated with improved survival in patients with non-small cell lung cancer, Am. J. Clin. Pathol. 138 (2012) 230–235 PubMed: 22904134.
[23] L. De Smedt, S. Palmans, O. Govaere, M. Moisse, B. Boeckx, G. De Hertogh, H. Prenen, E. Van Cutsem, S. Tejpar, T. Tousseyn, X. Sagaert, Expression of FOXP1 and colorectal Cancer prognosis, Lab. Med. 46 (2015) 299–311 PubMed: 26489674.
[24] X. Luo, Z. Yang, X. Liu, Z. Liu, X. Miao, D. Li, Q. Zou, Y. Yuan, The clinicopathological significance of forkhead box P1 and forkhead box O3a in pancreatic ductal adenocarcinomas, Tumour Biol. 39 (2017) 1010428317699129 PubMed: 28466777.
[25] Z. Hu, L. Zhu, J. Gao, M. Cai, M. Tan, J. Liu, B. Lin, Expression of FOXP1 in epithelial ovarian cancer (EOC) and its correlation with chemotherapy resistance and prognosis, Tumour Biol. 36 (2015) 7269–7275 PubMed: 25895457.
[26] S.B. Fox, P. Brown, C. Han, S. Ashe, R.D. Leek, A.L. Harris, A.H. Banham, Expression of the forkhead transcription factor FOXP1 is associated with estrogen receptor alpha and improved survival in primary human breast carcinomas, Clin. Cancer Res. 10 (2004) 3521–3527 PubMed: 15161711.
[27] B.H. Yu, B.Z. Li, X.Y. Zhou, D.R. Shi, W.T. Yang, Cytoplasmic FOXP1 expression is correlated with ER and calpain II expression and predicts a poor outcome in breast cancer, Diagn. Pathol. 13 (2018) PubMed: WOS:000433972500001.
[28] W. Remmele, H.E. Stegner, [Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue], Pathologe 8 (1987) 138–140 PubMed: 3303008.
[29] M. Mizunuma, Y. Yokoyama, M. Futagami, K. Horie, J. Watanabe, H. Mizunuma, FOXP1 forkhead transcription factor is associated with the pathogenesis of endometrial cancer, Heliyon 2 (2016) e00116 PubMed: 27441287.
[30] N. Ijichi, T. Shigekawa, K. Ikeda, K. Horie-Inoue, C. Shimizu, S. Saji, K. Aogi, H. Tsuda, A. Osaki, T. Saeki, S. Inoue, Association of double-positive FOXA1 and FOXP1 immunoreactivities with favorable prognosis of tamoxifen-treated breast cancer patients, Horm. Cancer 3 (2012) 147–159 PubMed: 22476979.
[31] P.J. Brown, S.L. Ashe, E. Leich, C. Burek, S. Barrans, J.A. Fenton, A.S. Jack, K. Pulford, A. Rosenwald, A.H. Banham, Potentially oncogenic B-cell activation-induced smaller isoforms of FOXP1 are highly expressed in the activated B cell-like subtype of DLBCL, Blood 111 (2008) 2816–2824 PubMed: 18077790.
[32] B. Wang, D. Lin, C. Li, P. Tucker, Multiple domains define the expression and regulatory properties of Foxp1 forkhead transcriptional repressors, J. Biol. Chem. 278 (2003) 24259–24268 PubMed: 12692134.
[33] H.A. Bruce, R.L. Margolis, FOXP2: novel exons, splice variants, and CAG repeat length stability, Hum. Genet. 111 (2002) 136–144 PubMed: 12189486.
[34] J.R. Friedman, K.H. Kaestner, The Foxa family of transcription factors in development and metabolism, Cell. Mol. Life Sci. 63 (2006) 2317–2328 PubMed: 16909212.
[35] S.O. Halacli, A.L. Dogan, FOXP1 regulation via the PI3K/Akt/p70S6K signaling pathway in breast cancer cells, Oncol. Lett. 9 (2015) 1482–1488 PubMed: 25663935.
[36] B. Streubel, U. Vinatzer, A. Lamprecht, M. Raderer, A. Chott, T(3;14)(p14.1;q32) involving IGH and FOXP1 is a novel recurrent chromosomal aberration in MALT lymphoma, Leukemia 19 (2005) 652–658 PubMed: 15703784.
[37] A. Goatly, C.M. Bacon, S. Nakamura, H. Ye, I. Kim, P.J. Brown, A. Ruskone-Fourmestraux, P. Cervera, B. Streubel, A.H. Banham, M.Q. Du, FOXP1 abnormalities in lymphoma: translocation breakpoint mapping reveals insights into deregulated transcriptional control, Mod. Pathol. 21 (2008) 902–911 PubMed: 18487996.
[38] V.J. Craig, S.B. Cogliatti, J. Imig, C. Renner, S. Neuenschwander, H. Rehrauer, R. Schlapbach, S. Dirnhofer, A. Tzankov, A. Muller, Myc-mediated repression of microRNA-34a promotes high-grade transformation of B-cell lymphoma by dysregulation of FoxP1, Blood 117 (2011) 6227–6236 PubMed: 21460242.