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Tumor Forkhead Box J2 as a Biomarker Reflecting Risks of Recurrence and Death in Non-Small Cell Lung Cancer Receiving Surgical Resection

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Forkhead box J2 (FOXJ2) induces cell apoptosis and restrains epithelial-mesenchymal transition in lung cancer, but its capability to serve as a prognostic biomarker in non-small cell lung cancer (NSCLC) remains unclear. Hence, this study intended to investigate the association of FOXJ2 with clinical characteristics, disease-free survival (DFS), and overall survival (OS) in NSCLC patients who received surgical resection. Totally, 182 NSCLC patients who received surgical resection were retrospectively enrolled. Their tumor FOXJ2 expression was quantified by immunohistochemistry (IHC). FOXJ2 IHC score = s taining intensity × density, with a total score of 12. FOXJ2 IHC score was 0 in 128 (70.3%) patients and > 0 in the remaining 54 (29.7%) patients; meanwhile, it was ≤ 3 in 157 (86.3%) patients and > 3 in 25 (13.7%) patients. FOXJ2 was negatively related to node (N) stage (P = 0.013) and tumor-nodes-metastasis (TNM) stage (P = 0.034). Intriguingly, FOXJ2 IHC score was reduced in patients with adjuvant chemotherapy than in patients without adjuvant chemotherapy (P = 0.036). The median DFS and OS (95% confidence interval) were 35.0 (31.3-38.7) months and 48.8 (43.7-53.9) months, respectively. Notably, FOXJ2 IHC score > 0 (P = 0.006) and > 3 (P = 0.002) was correlated with prolonged DFS. Also, FOXJ2 IHC score > 0 (P = 0.027) and > 3 (P = 0.028) was associated with longer OS. After adjustment by backward stepwise multivariate model, FOXJ2 IHC score > 3 was independently associated with prolonged DFS (hazard ratio = 0.367, P = 0.009). In conclusion, tumor FOXJ2 negatively links with N stage and TNM stage; moreover, FOXJ2 IHC score > 3 estimates prolonged DFS and OS in NSCLC patients who received surgical resection.

Keywords: clinical characteristics; disease-free survival; Forkhead box J2; non-small cell lung cancer; overall survival

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Introduction

Non-small cell lung cancer (NSCLC) is the main subtype of lung cancer accounting for 80%-85% of cases, with approximately 820,000 new diagnoses and 715,000 cancer-related deaths in China in 2020 (Chen et al. 2022). The major risk factors of NSCLC include tobacco smoking, environmental pollution, family history (heredity), etc. (Alduais et al. 2023). At present, curative surgical excision is recommended for NSCLC patients assessed as tumor nodes metastasis (TNM) stage I, II, and a part of patients at stage III (Alexander et al. 2020; Ettinger et al. 2022). However, it remains a great concern that many NSCLC patients who receive surgery are likely to develop local recurrence or distant metastases and subsequently suffer from unfavorable survival (Uramoto and Tanaka 2014; Cruz et al. 2017; Torrente et al. 2022). Therefore, it would be helpful to explore some markers that assist in monitoring the risk of recurrence and death in NSCLC patients who receive surgical resection. This can further conduce to the improvement of individualized post-operative management and long-term survival (Tang et al. 2017).

Forkhead box J2 (FOXJ2), also named as Forkhead homologous X (FHX), is constitutively localized at the cell nucleus and widely present in different tissues (Martin-de-Lara et al. 2008). As a Forkhead transcriptional activator

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with dual DNA-binding specificity, FOXJ2 participates in multiple biological processes, such as signal transduction, metabolism, cell cycle, etc. (Martin-de-Lara et al. 2008). Inspiringly, it is reported that FOXJ2 is a regulator of the progression and metastasis of several cancers (Qiang et al. 2015; Shan et al. 2017; Guo and Zhu 2022). For example, one study elucidates that FOXJ2 suppresses the proliferation, migration, and epithelial-mesenchymal transition (EMT) of prostate carcinoma cells by restraining the Jagged-1/Notch-1/Hes-1 pathway (Guo and Zhu 2022). Notably, one study identifies the lower FOXJ2 expression in NSCLC samples compared to matched peritumoral lung tissues; also, FOXJ2 knockdown promotes the EMT process in NSCLC by regulating Notch 1 and Notch intracellular domain (NICD) (Yang et al. 2017). From the clinical aspect, FOXJ2 is negatively linked with the pathological stage and positively related to overall survival (OS) in patients with epithelial ovarian cancer (Li et al. 2021). Another study notices that hepatocellular carcinoma patients with low FOXJ2 expression have worse survival compared to those with high FOXJ2 expression (Zhang et al. 2016). Subsequently, it is speculated that FOXJ2 may also serve as a useful biomarker that estimates clinical outcomes of NSCLC patients, while this assumption has not been verified yet.

Hence, this study intended to investigate the association of FOXJ2 with clinical characteristics, disease-free survival (DFS), and OS in NSCLC patients who received surgical resection.

Methods

Patients

One hundred eighty-two NSCLC patients who received surgical resection in the last 5 years (July 2018 to June 2023) were retrospectively screened for this study analysis. Patients who met the following criteria were eligible for screening: 1) pathologically diagnosed as NSCLC; 2) age ≥ 18 years; 3) received surgical resection; 4) had tumor tissue samples that could be used for immunohistochemistry (IHC) detection; 5) had complete clinical data that were retrievable and analyzable. Patients who had other primary malignant diseases were excluded. Besides, this was a retrospective study that did not intervene the treatment. The application of neoadjuvant chemotherapy was based on the Chinese Society of Clinical Oncology guideline combined with the patient's condition and willingness. The Ethics Committee of Inner Mongolia Medicine University Affiliated Hospital permitted the study. Patients or guardians signed the informed consents.

Sample detection

Tumor tissue samples (biopsy specimens of patients who scheduled for neoadjuvant chemotherapy were obtained before neoadjuvant treatment, and the samples of the other patients were collected by surgical resection) fixed by formalin and embedded by paraffin were collected to

Table 1. Clinical characteristics.

Items	NSCLC patients (N = 182)
Age (years)	59.2 ± 11.1
Age > 60 years	95 (52.2)
Male	129 (70.9)
Former or current smoker	74 (40.7)
Hypertension	53 (29.1)
Hyperlipidemia	28 (15.4)
Diabetes	21 (11.5)
Subtype	
AC	82 (45.1)
SCC	88 (48.4)
ASC	12 (6.6)
ECOG PS score	
0	122 (67.0)
1	60 (33.0)
Pathological grade	
Grade I	47 (25.8)
Grade II	79 (43.4)
Grade III	56 (30.8)
T stage	
T1	40 (22.0)
T2	54 (29.7)
T3	60 (33.0)
T4	28 (15.4)
N stage	
N0	98 (53.8)
N1	64 (35.2)
N2	20 (11.0)
M stage	
M0	182 (100.0)
TNM stage	
Stage I	34 (18.7)
Stage II	82 (45.1)
Stage III	66 (36.3)
EGFR mutation	45 (24.7)
Lymph node metastasis	84 (46.2)
Vascular invasion	66 (36.3)
Pleural invasion	87 (47.8)
CEA (ng/mL)	16.2 (3.2-47.2)
Abnormal CEA (> 5 ng/mL)	119 (65.4)
CA125 (U/mL)	31.7 (15.1-94.5)
Abnormal CA125 (> 35 U/mL)	87 (47.8)
CA19-9 (U/mL)	54.2 (20.9-222.7)
Abnormal CA19-9 (> 37 U/mL)	99 (54.4)

Age was shown using mean ± standard deviation; CEA, CA125, and CA19-9 were shown using median (interquartile range); other counting variables were shown using count (percentage). Grade I of pathological grade means well differentiated; Grade II means moderately differentiated; Grade III means poorly differentiated. NSCLC, non-small cell lung cancer; AC, adenocarcinoma; SCC, squamous cell carcinoma; ASC, adenosquamous carcinoma; ECOG PS, Eastern Cooperative Oncology Group Performance Status; TNM, tumor nodes metastasis; EGFR, epidermal growth factor receptor; CEA, carcinoembryonic antigen; CA125, cancer antigen 125; CA19-9, cancer antigen 19-9.

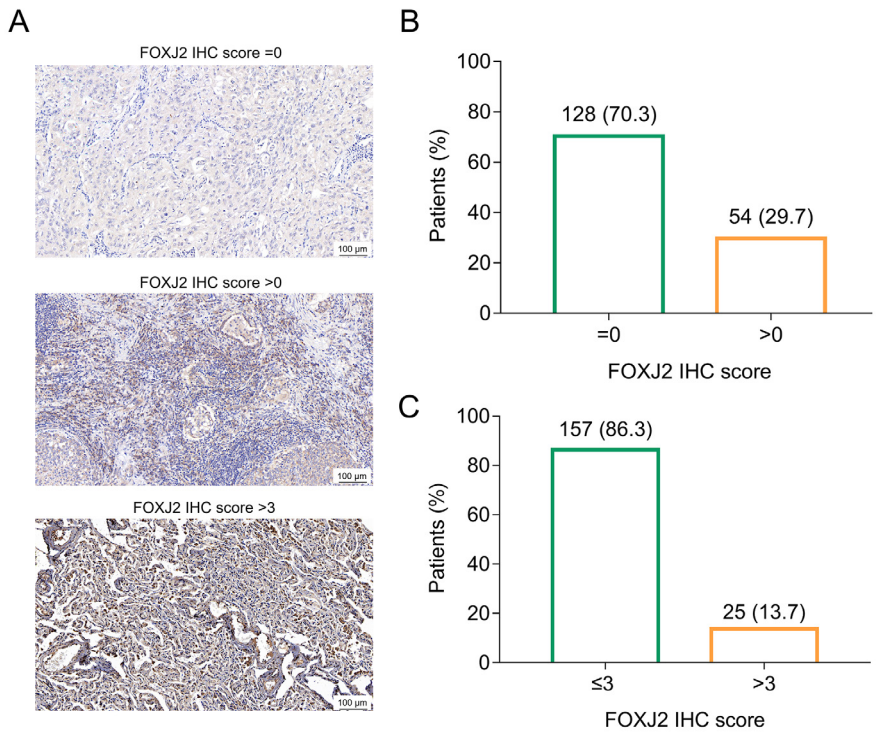

Fig. 1. FOXJ2 was insufficient in tumor tissues of NSCLC patients who received surgical resection. IHC images of FOXJ2 IHC score = 0, > 0, and > 3 (A). The proportion of patients with FOXJ2 IHC score = 0 and > 0 (B). The proportion of patients with FOXJ2 IHC score ≤ 3 and > 3 (C).

detect FOXJ2 level by IHC assay as previously reported (Shan et al. 2017). The samples were treated with the rabbit anti-FOXJ2 polyclonal primary antibody (1:50; Santa Cruz, Dallas, Texas, America) and then were incubated overnight at 4°C. Following that, the samples were treated with and peroxidase-conjugated goat anti-rabbit antibody (Dako, Carpinteria, California, America) for 15 minutes. The general process of IHC was showed in Supplementary Fig. S1. For evaluation, the FOXJ2 IHC score was evaluated from the product of the staining intensity and density. In detail, the intensity score was assessed as follows: negative staining = 0, weak staining = 1, moderate staining = 2, and strong staining = 3. Meanwhile, the density score was evaluated as follows: 0% of positive cells = 0, 1%-10% of positive cells = 1, 11%-50% of positive cells = 2, 51%-75% of positive cells = 3, 76%-100% of positive cells = 4. The total score was 12. Then, FOXJ2 IHC score was classified as = 0 vs. > 0 and ≤ 3 vs. > 3 for further exploration.

Documentations

Clinical characteristics, treatment information, and follow-up data were retrieved. The median and range of follow-up were 30.2 and 3.1-57.5 months, respectively. During the period, 100 (54.9%) patients had disease progression, had disease recurrence, or died, and 53 (29.1%) patients died. Based on patient status, DFS, and OS were imputed.

Statistics

SPSS 26.0 (IBM Corp., Armonk, New York, America) was used to complete data analyses. Comparisons were analyzed using Wilcoxon rank sum and Kruskal-Wallis H rank sum tests. Correlations were determined using Spearman's rank test. Associations of FOXJ2 with DFS and OS were analyzed using Kaplan-Meier curve with log-rank test. Factors associated with DFS and OS were identified using univariate and backward-stepwise multivariate Cox proportional hazard regression models. $P < 0.05$ was significant.

Results

Clinical features

The mean age of 182 NSCLC patients who received surgical resection was 59.2 ± 11.1 years, and 95 (52.2%) patients were > 60 years. One hundred twenty-nine (70.9%) patients were male. Besides, 47 (25.8%), 79 (43.4%), and 56 (30.8%) patients were assessed as pathological grade I, II, and III, correspondingly. Concerning the TNM stage, a respective of 34 (18.7%), 82 (45.1%), and 66 (36.3%) patients were evaluated as stage I, II, and III. Besides, 45 (24.7%) patients had epidermal growth factor receptor (EGFR) mutation; a total of 84 (46.2%), 66 (36.3%), and 87 (47.8%) patients suffered lymph node metastasis, vascular invasion, and pleural invasion, respectively. More detailed clinical features were shown in Table 1.

Table 2. Correlation of FOXJ2 IHC score with clinical features.

Items	FOXJ2 IHC score		*Z/H/ρ* value	*P* value
	Median (IQR)	Range		
Age			−1.410	0.159
≤ 60 years	0.0 (0.0-3.0)	0.0-12.0		
> 60 years	0.0 (0.0-1.0)	0.0-8.0		
Sex			−0.439	0.660
Female	0.0 (0.0-1.0)	0.0-9.0		
Male	0.0 (0.0-1.5)	0.0-12.0		
Former or current smoker			−0.857	0.391
No	0.0 (0.0-1.0)	0.0-9.0		
Yes	0.0 (0.0-2.0)	0.0-12.0		
Hypertension			−0.319	0.750
No	0.0 (0.0-1.0)	0.0-9.0		
Yes	0.0 (0.0-2.0)	0.0-12.0		
Hyperlipidemia			−0.519	0.603
No	0.0 (0.0-1.3)	0.0-12.0		
Yes	0.0 (0.0-1.0)	0.0-8.0		
Diabetes			−1.566	0.117
No	0.0 (0.0-2.0)	0.0-12.0		
Yes	0.0 (0.0-0.0)	0.0-8.0		
Subtype			2.864	0.239
AC	0.0 (0.0-0.0)	0.0-12.0		
SCC	0.0 (0.0-2.0)	0.0-9.0		
ASC	0.0 (0.0-2.8)	0.0-6.0		
ECOG PS score			−1.443	0.149
0	0.0 (0.0-2.0)	0.0-12.0		
1	0.0 (0.0-0.0)	0.0-8.0		
Pathological grade			−0.090	0.225
Grade I	0.0 (0.0-4.0)	0.0-12.0		
Grade II	0.0 (0.0-1.0)	0.0-9.0		
Grade III	0.0 (0.0-1.8)	0.0-6.0		
T stage			−0.057	0.443
T1	0.0 (0.0-2.0)	0.0-8.0		
T2	0.0 (0.0-1.3)	0.0-12.0		
T3	0.0 (0.0-2.0)	0.0-9.0		
T4	0.0 (0.0-0.0)	0.0-6.0		
N stage			−0.183	0.013
N0	0.0 (0.0-3.0)	0.0-12.0		
N1	0.0 (0.0-0.0)	0.0-4.0		
N2	0.0 (0.0-1.0)	0.0-8.0		
TNM stage			−0.157	0.034
Stage I	0.0 (0.0-3.0)	0.0-12.0		
Stage II	0.0 (0.0-2.0)	0.0-9.0		
Stage III	0.0 (0.0-0.0)	0.0-8.0		
CEA (ng/mL)			−0.718	0.473
Normal	0.0 (0.0-3.0)	0.0-12.0		
Abnormal	0.0 (0.0-1.0)	0.0-9.0		
CA125 (U/mL)			−0.407	0.684
Normal	0.0 (0.0-2.0)	0.0-9.0		
Abnormal	0.0 (0.0-1.0)	0.0-12.0		
CA19-9 (U/mL)			−0.627	0.531

Normal	0.0 (0.0-2.0)	0.0-12.0
Abnormal	0.0 (0.0-1.0)	0.0-8.0

FOXJ2, fork head box J2; IQR, interquartile range; AC, adenocarcinoma; SCC, squamous cell carcinoma; ASC, adenosquamous carcinoma; ECOG PS, Eastern Cooperative Oncology Group Performance Status; TNM, tumor nodes metastasis; CEA, carcinoembryonic antigen; CA125, cancer antigen 125; CA19-9; cancer antigen 19-9.

Table 3. Neoadjuvant and adjuvant chemotherapy information.

Items	NSCLC patients (N = 182)
Neoadjuvant chemotherapy	64 (35.2)
Neoadjuvant chemotherapy regimen	
NP	42 (23.1)
DP	12 (6.6)
GP	6 (3.3)
TP	4 (2.2)
Adjuvant chemotherapy	137 (75.3)
Adjuvant chemotherapy regimen	
NP	86 (47.3)
DP	25 (13.7)
GP	13 (7.1)
TP	13 (7.1)

The variables were shown using count (percentage). NSCLC, non-small cell lung cancer; NP, navelbine plus cisplatin; DP, docetaxel plus cisplatin; GP, gemcitabine plus cisplatin; TP, paclitaxel plus cisplatin.

FOXJ2 IHC score

The imaging examples of FOXJ2 IHC score = 0, > 0, and > 3 were displayed in Fig. 1A. FOXJ2 IHC score was 0 in 128 (70.3%) patients and it was > 0 in the remaining 54 (29.7%) patients (Fig. 1B). Additionally, FOXJ2 IHC score was ≤3 in 157 (86.3%) patients and > 3 in the other 25 (13.7%) patients (Fig. 1C).

Linkage of FOXJ2 IHC score with clinical features

FOXJ2 IHC score was not associated with age (P = 0.159), sex (P = 0.660), smoke (P = 0.391), hypertension (P = 0.750), hyperlipidemia (P = 0.603), diabetes (P = 0.117), disease subtype (P = 0.239), Eastern Cooperative Oncology Group Performance Status (ECOG PS) score (P = 0.149), pathological grade (P = 0.225), or tumor (T) stage (P = 0.443). While FOXJ2 was negatively related to the node (N) stage (P = 0.013) and TNM stage (P = 0.034). Besides, FOXJ2 was not linked with carcinoembryonic antigen (CEA) (P = 0.473), cancer antigen 125 (CA125) (P = 0.684), or cancer antigen 199 (CA19-9) (P = 0.531) (Table 2, Supplementary Fig. S2A-O).

Comparison of FOXJ2 IHC score between patients with and without adjuvant/neoadjuvant therapy as well as among patients with different regimens

A total of 64 (35.2%) patients received neoadjuvant chemotherapy. In detail, 42 (23.1%), 12 (6.6%), 6 (3.3%), and 4 (2.2%) patients were treated with navelbine plus cisplatin (NP), docetaxel plus cisplatin (DP), gemcitabine plus cisplatin (GP), and paclitaxel plus cisplatin (TP), accordingly. Moreover, 137 (75.3%) patients received adjuvant chemotherapy. Among them, 86 (47.3%), 25 (13.7%), 13 (7.1%), and 13 (7.1%) patients received NP, DP, GP, and TP, respectively (Table 3). Among patients who receive neoadjuvant (n = 64), 8 (12.5%) patients achieved pathological complete response; additionally, 0 (0.0%), 29 (45.3%), 35 (54.7%), 0 (0.0%) patients reached clinical complete response, partial response (PR), stable disease (SD), and progressive disease, respectively. FOXJ2 IHC score was not different between PR and SD patients (P = 0.905) (Supplementary Table S1).

FOXJ2 IHC score was not different between patients with and without neoadjuvant chemotherapy (P = 0.731, Fig. 2A). No difference was seen in FOXJ2 IHC score among patients with different neoadjuvant chemotherapy regimens, including NP, DP, GP, and TP (P = 0.635, Fig. 2B). Intriguingly, FOXJ2 IHC score was reduced in patients with adjuvant chemotherapy than in patients without adjuvant chemotherapy (P = 0.036, Fig. 2C). However, FOXJ2 IHC score did not vary among patients with different adjuvant chemotherapy regimens, including NP, DP, GP, and TP (P = 0.294, Fig. 2D).

Linkage of FOXJ2 IHC score with DFS and OS

The median DFS (mDFS) (95% confidence interval (CI)) of NSCLC patients who received surgical resection was 35.0 (31.3-38.7) months (Fig. 3A). FOXJ2 IHC score > 0 was correlated with prolonged DFS (P = 0.006). Specifically, the mDFS (95%CI) was 37.3 (28.1-46.5) months and 30.7 (29.0-32.4) months in patients with FOXJ2 IHC score > 0 and = 0, correspondingly (Fig. 3B). Furthermore, FOXJ2 IHC score > 3 was related to prolonged DFS (P = 0.002). In detail, the mDFS (95%CI) was not reached in patients with FOXJ2 IHC score > 3 and 31.3 (27.3-35.3) months in patients with FOXJ2 IHC score ≤ 3 (Fig. 3C).

The median OS (mOS) (95%CI) was 48.8 (43.7-53.9) months (Fig. 4A). FOXJ2 IHC score > 0 was linked with longer OS (P = 0.027). Detailly, the mOS (95%CI) was not reached and 44.6 (38.7-50.5) months in patients with FOXJ2 IHC score > 0 and = 0, correspondingly (Fig. 4B). Meanwhile, FOXJ2 IHC score > 3 was related to prolonged OS (P = 0.028). In detail, the mOS (95%CI) was not reached in patients with FOXJ2 IHC score > 3 and 47.9

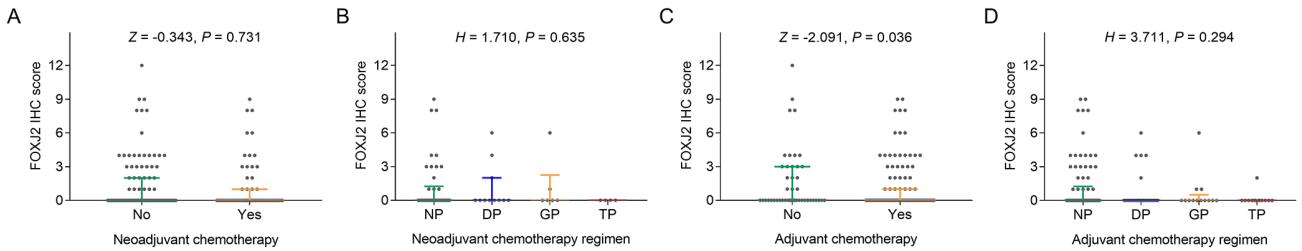

Fig. 2. FOXJ2 IHC score was declined in NSCLC patients who received surgical resection with adjuvant chemotherapy. Comparison of FOXJ2 IHC score between patients with and without neoadjuvant chemotherapy (A). Comparison of FOXJ2 IHC score among patients with different neoadjuvant chemotherapy regimens (B). Comparison of FOXJ2 IHC score between patients with and without adjuvant chemotherapy (C). Comparison of FOXJ2 IHC score among patients with different adjuvant chemotherapy regimens (D).

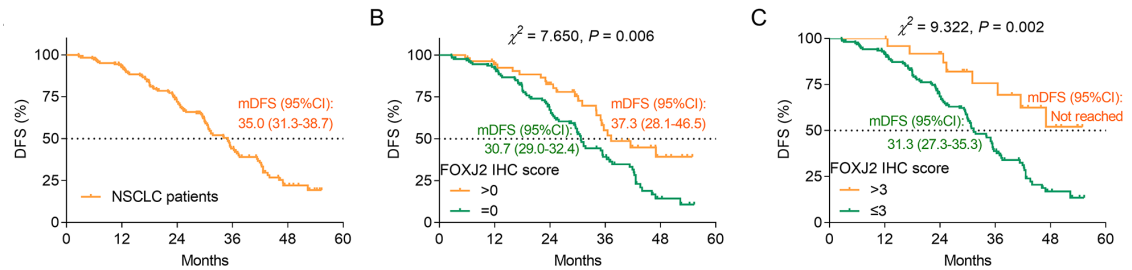

Fig. 3. FOXJ2 IHC score > 0 and > 3 was linked with prolonged DFS in NSCLC patients who received surgical resection. The mDFS (95%CI) in NSCLC patients who received surgical resection (A). Linkage of FOXJ2 IHC score > 0 (B) and > 3 (C) with DFS.

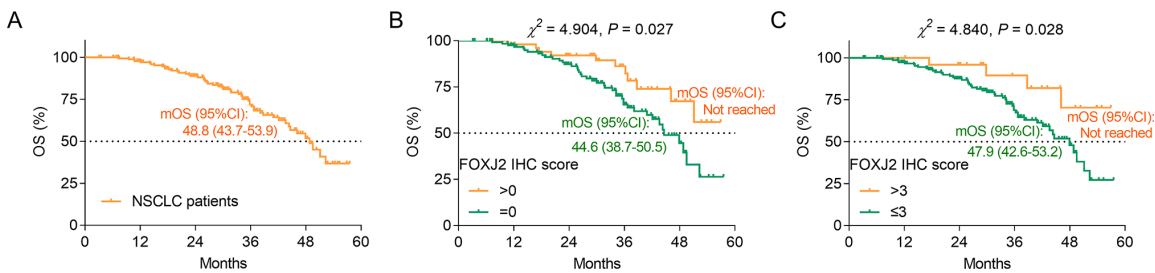

Fig. 4. FOXJ2 IHC score > 0 and > 3 was linked with prolonged OS in NSCLC patients who received surgical resection. The mOS (95%CI) in NSCLC patients who received surgical resection (A). Linkage of FOXJ2 IHC score > 0 (B) and > 3 (C) with OS.

(42.6-53.2) months in patients with FOXJ2 IHC score ≤ 3 (Fig. 4C).

Subgroup analyses

In patients with subtype of adenocarcinoma (AC), FOXJ2 IHC score > 0 ($P = 0.182$) and > 3 ($P = 0.205$) was not associated with DFS, while FOXJ2 IHC score > 0 was related to prolonged OS ($P = 0.017$), and its score > 3 exhibited a correlation trend with longer OS (but lacked statistical significance) ($P = 0.052$) (Supplementary Fig. S3A-D). In patients with subtype of squamous cell carcinoma (SCC), both FOXJ2 IHC score > 0 ($P = 0.011$) and > 3 ($P = 0.003$) was related to longer DFS, whereas they were not linked with OS (both $P > 0.050$) (Supplementary Fig. S3E-H). In patients with subtype of adenosquamous carcinoma (ASC), FOXJ2 IHC score > 0 and > 3 was not associated with DFS or OS (all $P > 0.050$) (Supplementary Fig. S3I-L).

In NSCLC patients with neoadjuvant chemotherapy, FOXJ2 IHC score > 0 and > 3 was not linked with DFS or OS (all $P > 0.050$) (Supplementary Fig. S4A-D); in NSCLC patients without neoadjuvant chemotherapy, FOXJ2 IHC score > 0 ($P = 0.021$) (Supplementary Fig. S4E) and > 3 ($P = 0.009$) (Supplementary Fig. S4F) was correlated with prolonged DFS, but not OS (both $P > 0.050$) (Supplementary Fig. S4G-H).

Table 4. Cox proportional hazard regression models for DFS.

Items	*P* value	HR	95% CI
Univariate models			
FOXJ2 IHC score > 0	0.007	0.523	0.328-0.836
FOXJ2 IHC score > 3	0.004	0.341	0.165-0.704
Age > 60 years	0.290	1.239	0.833-1.845
Male	0.514	1.159	0.744-1.805
Former or current smoker	0.422	1.178	0.790-1.757
Hypertension	0.495	1.164	0.753-1.800
Hyperlipidemia	0.136	1.455	0.889-2.382
Diabetes	0.679	0.865	0.434-1.722
Subtype			
AC	Reference		
SCC	0.921	0.979	0.646-1.484
ASC	0.709	1.148	0.556-2.372
ECOG PS score	0.025	1.587	1.058-2.379
Pathological grade	0.022	1.356	1.044-1.760
T stage	0.015	1.290	1.050-1.584
N stage	0.110	1.256	0.950-1.660
TNM stage	0.005	1.538	1.139-2.076
EGFR mutation	0.214	0.746	0.470-1.184
Lymph node metastasis	0.312	1.225	0.826-1.816
Vascular invasion	0.013	1.644	1.109-2.436
Pleural invasion	0.107	1.382	0.932-2.049
Abnormal CEA	0.142	1.378	0.898-2.115
Abnormal CA125	0.074	1.434	0.965-2.131
Abnormal CA19-9	0.411	1.180	0.795-1.753
Neoadjuvant chemotherapy	0.535	1.136	0.760-1.698
Adjuvant chemotherapy	0.188	1.409	0.845-2.348
Backward stepwise multivariate model			
FOXJ2 IHC score > 3	0.009	0.367	0.173-0.780
ECOG PS score	0.014	1.761	1.119-2.773
Pathological grade	0.101	1.257	0.957-1.651
N stage	0.070	0.636	0.390-1.038
TNM stage	0.019	1.750	1.097-2.793
Vascular invasion	0.068	1.652	0.964-2.832
Neoadjuvant chemotherapy	0.079	0.622	0.367-1.056

DFS, disease-free survival; HR, hazard ratio; CI, confidence interval; FOXJ2, fork head box J2; AC, adenocarcinoma; SCC, squamous cell carcinoma; ASC, adenosquamous carcinoma; ECOG PS, Eastern Cooperative Oncology Group Performance Status; TNM, tumor nodes metastasis; EGFR, epidermal growth factor receptor; CEA, carcinoembryonic antigen; CA125, cancer antigen 125; CA19-9, cancer antigen 19-9.

Table 5. Cox proportional hazard regression models for OS.

Items	*P* value	HR	95% CI
Univariate models			
FOXJ2 IHC score > 0	0.030	0.479	0.246-0.933
FOXJ2 IHC score > 3	0.036	0.334	0.120-0.931
Age > 60 years	0.010	2.148	1.197-3.856
Male	0.987	0.995	0.553-1.790
Former or current smoker	0.776	0.921	0.524-1.619
Hypertension	0.958	0.983	0.522-1.853
Hyperlipidemia	0.958	0.983	0.522-1.853
Diabetes	0.993	1.004	0.395-2.551
Subtype			
AC	Reference		
SCC	0.956	0.983	0.544-1.778
ASC	0.298	1.588	0.664-3.799
ECOG PS score	< 0.001	3.501	2.029-6.041
Pathological grade	0.012	1.610	1.112-2.333
T stage	0.002	1.598	1.186-2.152
N stage	< 0.001	2.018	1.400-2.909
TNM stage	< 0.001	2.747	1.715-4.399
EGFR mutation	0.171	0.626	0.320-1.225
Lymph node metastasis	0.006	2.209	1.254-3.891
Vascular invasion	0.002	2.365	1.372-4.079
Pleural invasion	0.027	1.868	1.074-3.248
Abnormal CEA	0.003	2.830	1.413-5.669
Abnormal CA125	0.006	2.203	1.257-3.860
Abnormal CA19-9	0.180	1.458	0.840-2.530
Neoadjuvant chemotherapy	0.001	2.468	1.428-4.265
Adjuvant chemotherapy	0.026	2.852	1.134-7.169
Backward stepwise multivariate model			
Age > 60 years	0.028	2.020	1.080-3.781
ECOG PS score	< 0.001	2.958	1.677-5.217
Pathological grade	0.004	1.810	1.202-2.723
EGFR mutation	0.063	0.498	0.239-1.039
Vascular invasion	0.053	1.747	0.992-3.075
Pleural invasion	0.054	1.767	0.991-3.150
Abnormal CEA	0.029	2.256	1.088-4.678
Abnormal CA125	0.101	1.639	0.909-2.957

OS, overall survival; HR, hazard ratio; CI, confidence interval; FOXJ2, fork head box J2; AC, adenocarcinoma; SCC, squamous cell carcinoma; ASC, adenosquamous carcinoma; ECOG PS, Eastern Cooperative Oncology Group Performance Status; TNM, tumor nodes metastasis; EGFR, epidermal growth factor receptor; CEA, carcinoembryonic antigen; CA125, cancer antigen 125; CA19-9; cancer antigen 19-9.

Associating factors of DFS

Univariate Cox proportional hazard regression model exhibited that FOXJ2 IHC score > 0 (hazard ratio (HR) = 0.523, $P = 0.007$) and > 3 (HR = 0.341, $P = 0.004$) were linked with longer DFS. Moreover, ECOG PS score (HR = 1.587, $P = 0.025$), pathological grade (HR = 1.356, $P = 0.022$), T stage (HR = 1.290, $P = 0.015$), TNM stage (HR = 1.538, $P = 0.005$), and vascular invasion (HR = 1.644, $P = 0.013$) were related to shorter DFS.

After adjustment by backward stepwise multivariate model, FOXJ2 IHC score > 3 was independently associated with prolonged DFS (HR = 0.367, $P = 0.009$), while ECOG PS score (HR = 1.761, $P = 0.014$) and TNM stage (HR =

1.750, $P = 0.019$) were independently correlated with shortened DFS (Table 4).

Associating factors of OS

FOXJ2 IHC score > 0 (HR = 0.479, $P = 0.030$) and > 3 (HR = 0.334, $P = 0.036$) were correlated with longer OS. Reversely, age > 60 years (HR = 2.148, $P = 0.010$), ECOG PS score (HR = 3.501, $P < 0.001$), pathological grade (HR = 1.610, $P = 0.012$), T stage (HR = 1.598, $P = 0.002$), N stage (HR = 2.018, $P < 0.001$), TNM stage (HR = 2.747, $P < 0.001$), lymph node metastasis (HR = 2.209, $P = 0.006$), vascular invasion (HR = 2.365, $P = 0.002$), pleural invasion (HR = 1.868, $P = 0.027$), abnormal CEA (HR = 2.830, $P = 0.003$), abnormal CA125 (HR = 2.203, $P = 0.006$), neoadjuvant chemotherapy (HR = 2.468, $P = 0.001$), and adjuvant chemotherapy (HR = 2.852, $P = 0.026$) were related to shorter OS.

After adjustment, age > 60 years (HR = 2.020, $P = 0.028$), ECOG PS score (HR = 2.958, $P < 0.001$), pathological grade (HR = 1.810, $P = 0.004$), and abnormal CEA (HR = 2.256, $P = 0.029$) were independently correlated with shortened OS (Table 5).

Discussion

FOX family, containing over 80 members of transcription factors, shares a similar DNA-binding domain termed the Forkhead box or winged helix domain; among them, FOXJ2 is a representative FOX subfamily member with an essential role in regulating tumor growth and metastasis (Jin et al. 2020; Qiang et al. 2020; Guo and Zhu 2022). Clinically, several studies have quantified the expression of FOXJ2 in cancer patients (Qiang et al. 2015; Zhang et al. 2016). For instance, one study observes that the level of FOXJ2 mRNA and protein are both decreased in extrahepatic cholangiocarcinoma tissues compared to adjacent normal bile duct tissues (Qiang et al. 2015). Another study exhibits reduced FOXJ2 expression in hepatocellular carcinoma tissues compared with adjacent normal liver tissues (Zhang et al. 2016). In the present study, FOXJ2 IHC score was 0 in 128 (70.3%) patients and it was > 0 in the remaining 54 (29.7%) patients; meanwhile, it was ≤ 3 and > 3 in 157 (86.3%) and 25 (13.7%) patients, respectively. The findings of this study, combing the abnormally decreased FOXJ2 expression in some cancer tissues as previously reported (Qiang et al. 2015; Zhang et al. 2016), reflected the tumor suppressor role of FOXJ2.

Regarding the association of FOXJ2 with tumor features, one previous study observes the negative association of FOXJ2 with International Federation of Gynecology and Obstetrics (FIGO) stage in patients with epithelial ovarian cancer (Li et al. 2021). Another study shows that high FOXJ2 is related to tumor size ≤ 5 and well histological differentiation in hepatocellular carcinoma patients (Zhang et al. 2016). The current study identified that tumor FOXJ2 was negatively linked with N stage and TNM stage in NSCLC patients who received surgical resection. The possible explanations were as follows: (i) FOXJ2 retarded cancer migration and invasion via inactivating Jagged-1/Notch-1/Hes-1 pathway as well as upregulating phosphoglucomutase 1 (PGM1) (Jin et al. 2018; Guo and Zhu 2022; Zheng et al. 2022). Consequently, FOXJ2 was negatively related to the N stage in NSCLC patients who received surgical resection; and (ii) it was assumed that the negative association of FOXJ2 with TNM stage was mainly due to its negative linkage with N stage. Also, it was noticed that declined tumor FOXJ2 was related to adjuvant chemotherapy in NSCLC patients who received surgical resection. A likely explanation was that: adjuvant chemotherapy was frequently offered to patients with elevated TNM stage (Chaft et al. 2021); in detail, it was recommended for stage I and stage II patients with incomplete excision, and all stage III patients who received tumor resection. Meanwhile, elevated TNM stage was correlated with reduced FOXJ2 as aforementioned. Consequently, FOXJ2 was reduced in patients with adjuvant chemotherapy than in those without adjuvant chemotherapy.

The prognostic value of FOXJ2 remains debated depending on different cancer types (Zhang et al. 2016; Shan et al. 2017). It is previously reported that the high FOXJ2 expression is correlated with reduced OS rate in patients with nasopharyngeal carcinoma (Shan et al. 2017). Nonetheless, in another study, hepatocellular carcinoma patients with high FOXJ2 expression achieve better survival than those with low FOXJ2 expression (Zhang et al. 2016). In the present study, FOXJ2 IHC score > 0 and > 3 were both correlated with longer DFS and OS in NSCLC patients who received surgical resection. More importantly, FOXJ2 IHC score > 3 was independently associated with longer DFS in these patients. The probable reason was that: FOXJ2 was recognized as a tumor suppressor (Yang et al. 2017), whose increased expression led to relieved tumor burden, whereafter, the risk of disease recurrence and dismal survival was reduced. As a result, NSCLC patients with FOXJ2 IHC score > 0 (versus = 0) and > 3 (versus ≤ 3) had prolonged DFS and OS. Moreover, this study intended to evaluate the prognostic value of FOXJ2 in the entire surgical NSCLC patients, and therefore, patients who received neoadjuvant chemotherapy were not excluded. While the inclusion of patients who underwent neoadjuvant chemotherapy would introduce a potential bias, thus we further performed subgroup analyses in patients with and without neoadjuvant chemotherapy, which show that in NSCLC patients with neoadjuvant chemotherapy, FOXJ2 IHC score > 0 and > 3 was not linked with DFS or OS; in NSCLC patients without neoadjuvant chemotherapy, FOXJ2 IHC score > 0 and > 3 was correlated with prolonged DFS, but not OS. The findings indicated that the prognostic value was relatively weak in NSCLC patients with neoadjuvant chemotherapy.

Some inevitable limitations should be mentioned here: (i) the sample size (N = 182) partially reduced the statistical power. Thus, the findings of this study needed more valida-

tions in a study with a larger sample size; (ii) this was a single-center study, leading to unavoidable selective bias; (iii) this study failed to match paired tissue samples, and the tumor specificity of FOXJ2 in NSCLC patients should be further analyzed; (iv) this was a retrospective study that was finished, and the data of spread through air spaces (STAS) were unavailable in partial patients. Therefore, STAS could not be included in the Cox proportional hazard regression model, which required investigation in the future; and (v) the pathological grade of patients who visited the hospital before December 2021 was determined following the 2015 World Health Organization Classification of Lung Tumors (Travis et al. 2015) and those who visited the hospital after December 2021 were graded following the 2021 updated version (Nicholson et al. 2022), which influenced the consistency to some extent.

In summary, tumor FOXJ2 links with reduced N stage and TNM stage, whose IHC score > 3 estimates prolonged DFS and OS in NSCLC patients who received surgical resection. The findings suggest that it may be a candidate prognostic marker in these patients to help the treatment decision-making process, while more validations are necessary.

Conflict of Interest

The authors declare no conflict of interest.

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Supplementary Files

Please find supplementary file(s);
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