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Original Article

Elevated expression of SOX2 and FGFR1 in correlation with poor prognosis in patients with small cell lung cancer

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Abstract: *Objectives*: The central issue in this study is to investigate the expression of Sex determining region Y-BOX2 (SOX2) and fibroblast growth factor receptor 1 (FGFR1), evaluate their clinicopathological variables and prognostic significance in small cell lung cancer (SCLC). *Methods*: Specimens from 222 SCLC patients and 53 adjacent normal lung tissues were detected by the immunohistochemistry for SOX2 and FGFR1 expression. The relationship between the expression of both markers and survival status was determined. *Results:* Overexpression of SOX2 and FGFR1 were revealed in SCLC tumors than in normal tissues (P<0.05). SOX2 expression was associated with clinical stage (P=0.014) and lymph node status (P=0.041). Besides, FGFR1 expression was significantly higher in ever smokers (P=0.030) and late stage SCLC (P=0.005). SOX2, FGFR1 and TNM stage were independent prognostic factors for overall survival (OS) and Recurrence-free survival (RFS) by multivariate analysis. In stage I patients, only overexpression of SOX2, but not of FGFR1, predicted poor OS (0.027) and RFS (P=0.013). According to the expression of SOX2 and FGFR1, patients were categorized into three groups. Patients with elevated expression of both markers belonged to the group with the shortest RFS (P<0.0001) and OS (P<0.0001). *Conclusions:* Increased expression of SOX2 and FGFR1 may be available as poor prognostic indicators in SCLC patients.

Keywords: SOX2, FGFR1, small cell lung cancer, immunohistochemistry, prognosis

Introduction

SCLC is an extremely aggressive malignancy with particular low five-year survival rates and extraordinary preference for early metastasis [1, 2]. Over the last decade, prognosis for patients with SCLC has changed little and there has been an increase in effort to search novel therapeutic targets in SCLC [3-6]. Unfortunately, up to now, no targeted therapeutics is available in clinical application. Nevertheless, with the development of next-generation sequencing technologies, two comprehensive genomic analyses first furnished the landscape of SCLC and emphasized SOX2, FGFR1, CREBBP, EP300 and MYC family members as the potential therapeutic paradigms for this highly malignant cancer [7, 8].

The detection of novel genomic alterations is attractive. SOX2, which is located at 3q 26.33, has been validated to be a transcription factor that is essential to maintain the pluripotency of embryonic stem cells [9]. Comprehensive genomic characterization of SCLC and squamous cell carcinoma (SCC) each becomes aware of amplification of SOX2 [7, 10]. Particularly, down-regulation of SOX2 in SCLC cell lines markedly suppressed amounts of SOX2 protein and reduced cell proliferation [7]. These findings suggest that SOX2 is a critical driver gene in SCLC.

FGFR1 was considered another candidate target gene in both SCLC and SCC [8, 10]. FGFR1, a member of FGFR family, which was located on chromosome 8p12, plays a significant role in

Table 1. Correlation between SOX2 and FGFR1 expression and Clinicopathological Features

		SOX2 expression			FGFR1 expression		
Characteristics	Cases	Low	High	P	Low	High	P
Gender				0.822			0.608
Male	140	61	79		77	63	
Female	82	37	45		48	34	
Age (years)				0.444			0.058
<60	162	69	93		85	77	
≥60	60	29	31		40	20	
Smoking Status				0.678			0.030
Non-smokers	103	47	56		66	37	
Ever-smokers	119	51	68		59	60	
[1]ECOG status				0.883			0.755
0-1 score	194	86	108		110	84	
≥2 scores	28	12	16		15	13	
TNM stage				0.014			0.005
I	102	51	51		64	38	
II	71	32	39		42	29	
III	35	7	28		17	18	
IV	14	8	6		2	12	
Tumor size				0.920			0.723
≥3 cm	139	61	78		77	62	
<3 cm	83	37	46		48	35	
[2]LNM				0.041			0.287
Positive	110	41	69		58	52	
Negative	112	57	55		67	45	

[1]ECOG Eastern Cooperative Oncology Group. [2]LNM Lymph node metastasis.

tumorigenesis [11]. Moreover, in vitro the FGFR inhibitor can prevent SCLC cell lines from proliferation. In vivo full tumor regression and an increase in apoptotic cell death were revealed [12]. Therefore, FGFR1 inhibition could be efficient as a monotherapy for SCLC patients.

Notably, previous study has shown that blocking FGF signaling with FGFR1 inhibitor SU5402 can reduce the level of SOX2 expression [13]. FGF signaling could control osteoblast differentiation through induction of SOX2 and regulation of the Wnt-β-catenin pathway [14].

Published studies have described the serum antibody titers of SOX2 [15, 16] and FGFR1 inhibitor in the xenograft. However, to our knowledge, the prognostic value of SOX2 and FGFR1 in SCLC is still unknown. The identification of new biomarkers, which predict a high risk of recurrence, may allow a more targeted approach to the therapies for SCLC. Therefore, to identify the expression of SOX2 and FGFR1 in SCLC and conduct correlative analyses with clinicopathological variables and prognosis, we carried out the immunohistochemical expression of SOX2 and FGFR1 in archived SCLC tissue samples and normal lung tissues.

Materials and methods

Patients and samples

Two hundred and twenty-two tumor specimens and fifty-three normal tissue samples from small cell lung cancer patients pathologically confirmed at The Tumor Hospital Affiliated Harbin Medical University, The First Affiliated Hospital of Harbin Medical University and The Second Affiliated Hospital of Harbin Medical University were included in this study. All specimens obtained after surgery were collected between January 2000 and December 2011. Archival material included clinical data and follow-up information were available to all patients. Patients range in age from 26 to 75 years old, with a mean of 53 years. None of the patients received adjuvant chemotherapy, radiotherapy or immunotherapy before surgery. The pathological diagnosis was confirmed by two senior pathologists. The study was approved by Ethical Review Committee of Harbin Medical University, Harbin, China. All patients were provided with written informed consent to participate in the study.

Immunohistochemistry

4-micrometer thickness, formalin-fixed, paraffin-embedded, tissue sections were chosen for immunohistochemical staining. Mouse monoclonal anti-SOX2 antibody and Rabbit polyclonal anti-FGFR1 antibody were purchased from Abcam Company. The tissue sections were deparaffinized in xylene and then hydrat-

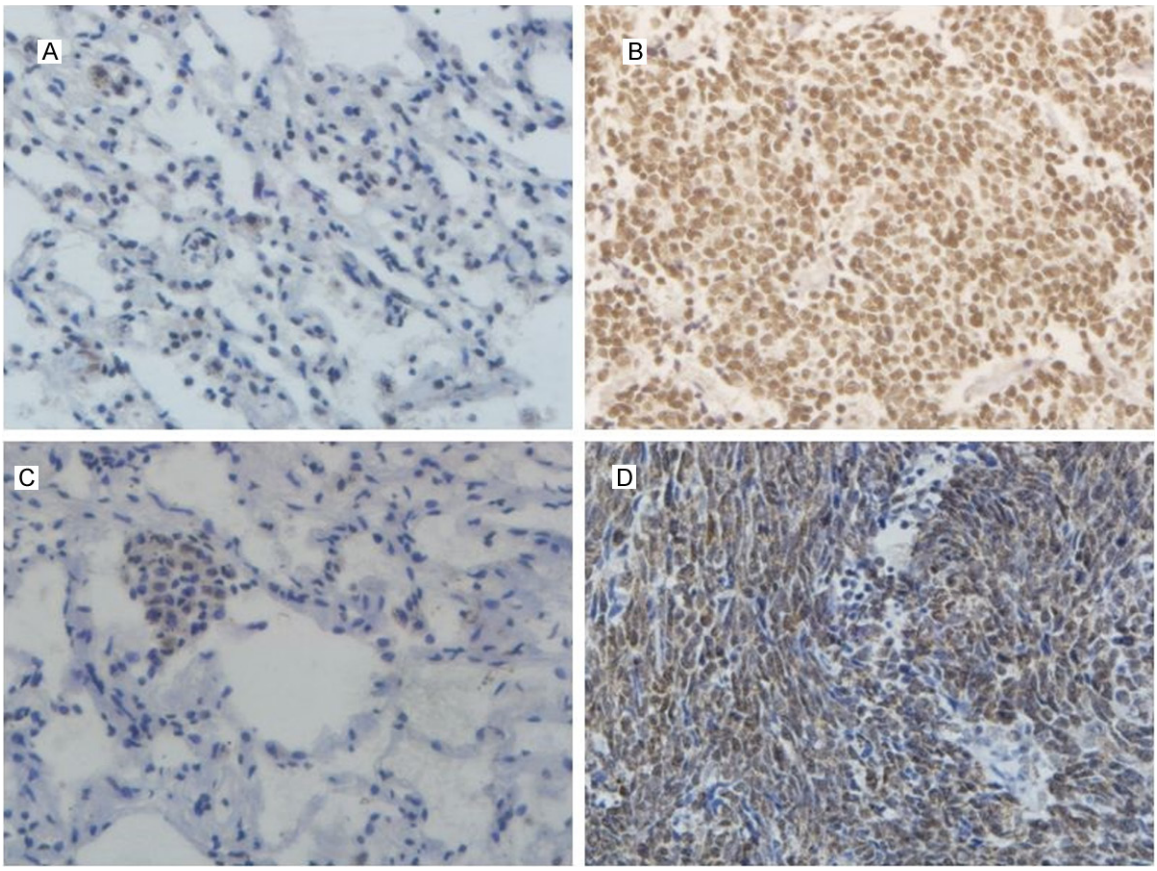

Figure 1. Immunohistochemical staining of SOX2 and FGFR1 in SCLC samples. A: Low expression of SOX2 in normal lung tissue. B: High expression of SOX2 in small cell carcinoma. C: Low expression of FGFR1 in normal lung tissue. D: High expression of FGFR1 in small cell carcinoma.

ed in serially graded alcohols. Specimens were heated in 10 mM sodium citrate buffer (pH 6.0) and EDTA (PH 8.0), respectively prepared for SOX2 and FGFR1, at 120°C for 5 min to expose the antigens. Sections were then washed with phosphate-buffered saline (PBS, PH 7.4), incubated with 3% H_2O_2 for 20 min to eliminate endogenous peroxidase activity and 5% Bovine Serum Albumin (BSA) for 30 min to reduce non-specific binding. The slides were incubated overnight at 4°C with primary antibodies (SOX2 antibody with a dilution of 1:200, FGFR1 antibody with a dilution of 1:250). After washing, the specimens were treated with peroxidase-labelled polymer conjugated to goat anti-mouse (for SOX2), goat anti-rabbit (for FGFR1) immunoglobulins in Tris-HCL buffer at room temperature for 30 min. Signals were visualized with diaminobenzidine (DAB) and the slides were counterstained with hematoxylin. For negative controls, the primary antibody was substituted with PBS.

In cytoplasm, the percentage of the extent of reactivity was scored as follows: 0 (no positive cells), 1 (fewer than 15% positive cells), 2 (15%-50% positive cells) and 3 (more than 50% positive cells). The staining intensity was graded as 0 (no staining), 1 (light yellow = weak staining), 2 (yellow brown = moderate staining), 3 (brown = strong staining). The cytoplasmic expression score was obtained by multiplying the intensity and reactivity extension values. Scores ≥4 were classified as high expression, <4 were classified as low expression. According to the percentage of positive nuclei, the nuclear expression was quantified using a range of 0-100. Scores ≥55 were classified as high expression, the remainders were classified as low expression.

Statistical analysis

Statistical software (SPSS 17.0 for Windows; Inc., Chicago, IL, USA) was used for all analyses. The chi-square was performed to evaluate

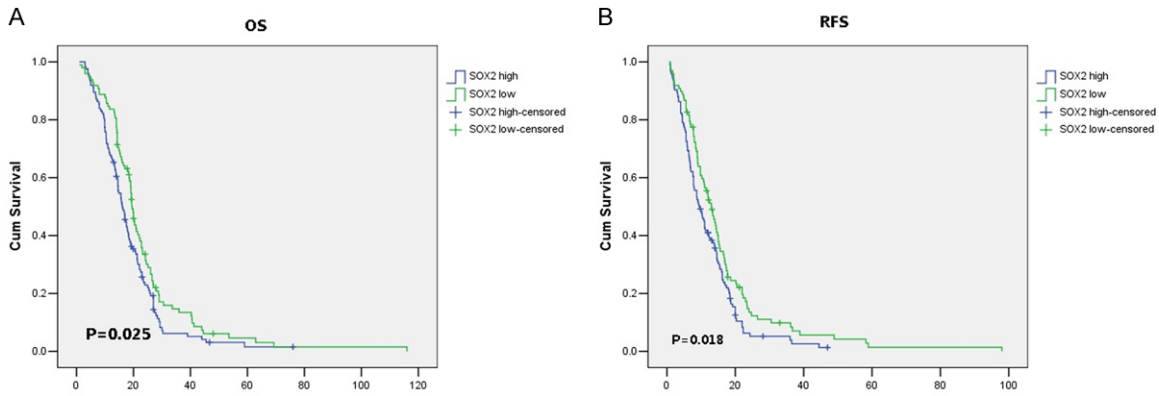

Figure 2. Kaplan-Meier curves of the OS and RFS for SCLC patients with SOX2 expression. *P* values were obtained by log-rank test. A: OS cures of SCLC patients according to the SOX2 expression (P=0.025); B: RFS cures of SCLC patients according to the SOX2 expression (P=0.018).

the correlations between the SOX2 and FGFR1 expression and clinical parameters. The recurrence-free survival (RFS) and overall survival (OS) analysis were evaluated by the Kaplan-Meier method. COX univariate and multivariate regression analyses with 95% confidence interval (CI) were used to asses independent prognostic factors. For all tests, *P*-values<0.05 was considered statistically significant.

Results

Patient characteristics

The main clinical characteristics of the patients are demonstrated in **Table 1**. The median age was 53 years old (range, 26-75). 46.4% patients were never smokers, 53.6% patients were ex-smokers (patients who had smoked at least 100 cigarettes in their lifetime). Of these patients, lymph node metastases were present in 110 patients (49.5%), and absent in 112 patients (50.5%). After staging evaluation (including CT of the chest, brain MRI, upper abdomen ultrasound, bone scan or PET/CT imaging), a total of 102 (45.9%) patients were classified at stage I, 71 (32.0%) were stage II, 35 (15.8%) were stage III, 14 (6.3%) were stage IV.

Expression of SOX2 and FGFR1 in small cell lung cancer compared with that in normal tissues

SOX2 staining in SCLC tissues appeared as brown particles which were located in the nuclei. FGFR1 protein showed cytoplasm and nucleus staining. Of the paraffin-embedded SCLC carcinoma slides examine, we demonstrated the expression rates of SOX2 positive were 124 of 222 (55.9%) and the expression rates of FGFR1 positive was 97 of 222 (43.7%). The paraffin-embedded normal SCLC tissues exhibited weaker expression of SOX2 than carcinoma specimens (P=0.018) while there was no positive staining in PBS control samples. Significant higher FGFR1 expression were observed in tumor than in normal tissues respectively (P=0.005) (**Figure 1**).

Correlation of clinicopathological features and SOX2 and FGFR1 expression

We correlated expression of SOX2 and FGFR1 with clinicopathological characteristics, including gender, age, smoking history, ECOG performance status, tumor size, lymph node metastasis and clinical stage. (**Table 1**) Overexpression of SOX2 was positively related with TNM stage (P=0.014) and lymph node metastasis (P=0.041). FGFR1 expression was significantly lower in never smokers than in ever smokers (P=0.003) and was strongly associated with TNM stage (P=0.005).

Sox2 and FGFR1 expression and clinical outcome of SCLC

We assessed the prognostic value of SOX2 and FGFR1 in SCLC. The Kaplan-Meier survival analysis demonstrated that overexpression of SOX2 were correlated with shorter OS and RFS (P=0.025, P=0.018 respectively) (**Figure 2**). The expression of FGFR1 conferred to patients a worse RFS (P=0.002) and OS (P=0.001) (**Figure 3**). Moreover, Univariate analysis identi-

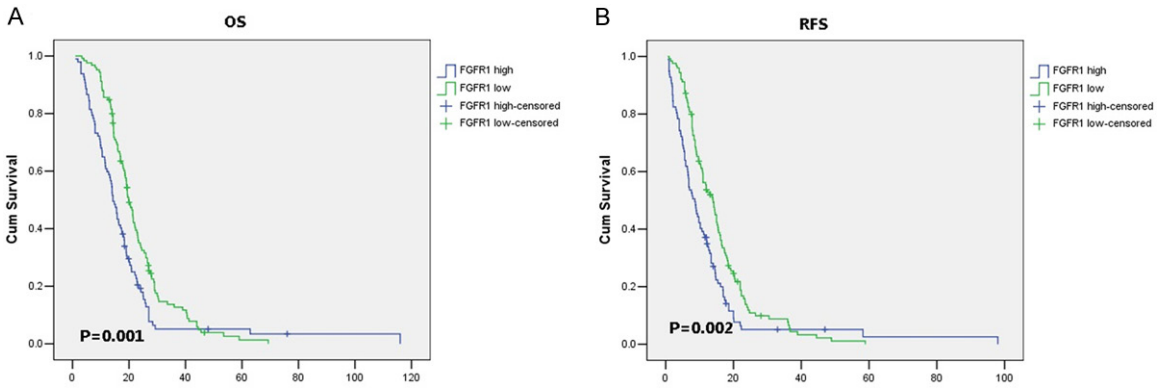

Figure 3. Kaplan-Meier curves of the OS and RFS for SCLC patients with FGFR1 expression. *P* values were obtained by log-rank test. A: OS cures of SCLC patients according to the FGFR1 expression (P=0.001); B: RFS cures of SCLC patients according to the FGFR1 expression (P=0.002).

Table 2. Univariable analysis of RFS and OS of small cell lung cancer patients

Variables	OS			RFS		
	Risk ratio	Univariate 95% CI	P	Risk ratio	Univariate 95% CI	P
Gender						
Male/Female	1.130	0.850-1.502	0.401	0.881	0.662-1.172	0.383
Age (years)						
<60/≥60	1.032	0.757-1.408	0.840	0.939	0.688-1.282	0.693
Smoking Status						
Non-smokers/Ever-smokers	1.326	1.000-1.757	0.050	0.776	0.587-1.026	0.076
[1]ECOG status						
0-1/≥2 scores	0.913	0.594-1.404	0.680	1.008	0.657-1.548	0.970
TNM stage (based on stage IV)						
I	0.176	0.098-0.315	<0.0001	0.174	0.096-0.313	<0.0001
II	0.258	0.143-0.467	<0.0001	0.254	0.140-0.461	<0.0001
III	0.290	0.153-0.547	<0.0001	0.312	0.165-0.589	<0.0001
IV	1.0			1.0		
Tumor size						
≥3 cm/<3 cm	0.830	0.623-1.105	0.201	0.847	0.636-1.126	0.847
[2]LNM						
positive/negative	1.490	1.125-1.973	0.005	1.541	1.161-2.044	0.003
SOX2 expression						
High/Low	0.730	0.552-0.964	0.027	0.714	0.538-0.947	0.019
FGFR1 expression						
High/Low	0.629	0.475-0.833	0.001	0.641	0.485-0.849	0.002

[1]ECOG: Eastern Cooperative Oncology Group. [2]LNM: Lymph node metastasis.

fied SOX2 expression, FGFR1 expression, lymph node metastasis and TNM stage as significant variables affecting RFS and OS (**Table 2**). Variables with *p* value of 0.1 or less were entered in COX regression model for multivariable analysis. TNM stage, SOX2 expression and FGFR1 expression were identified as independent prognostic factors (**Table 3**). In stage I patients, SOX2 high expression was associated with worse RFS (P=0.013) and OS (P=0.027), while FGFR1 has no significant impact on survival (OS: P=0.161, RFS: P=0.185). We performed the analysis between the expression of SOX2 and FGFR1 in tumor samples. No signifi-

Table 3. Multivariable analysis of RFS and OS of small cell lung cancer patients

Variables	OS			RFS		
	Risk ratio	95% CI	P	Risk ratio	95% CI	P
Smoking Status						
Non-smokers/Ever-smokers	1.297	0.968-1.738	0.082	-		
TNM stage (based on stage IV)						
I	0.111	0.048-0.257	<0.0001	0.115	0.049-0.266	<0.0001
II	0.254	0.138-0.468	<0.0001	0.257	0.140-0.471	<0.0001
III	0.283	0.145-0.553	<0.0001	0.302	0.155-0.588	<0.0001
IV	1.0			1.0		
[1]LNM						
positive/negative	1.705	0.863-3.369	0.125	1.660	0.848-3.247	0.139
SOX2 expression						
High/Low	1.359	1.016-1.818	0.039	1.365	1.017-1.831	0.038
FGFR1 expression						
High/Low	1.459	1.096-1.944	0.010	1.457	1.097-1.935	0.009

[1]LNM: Lymph node metastasis.

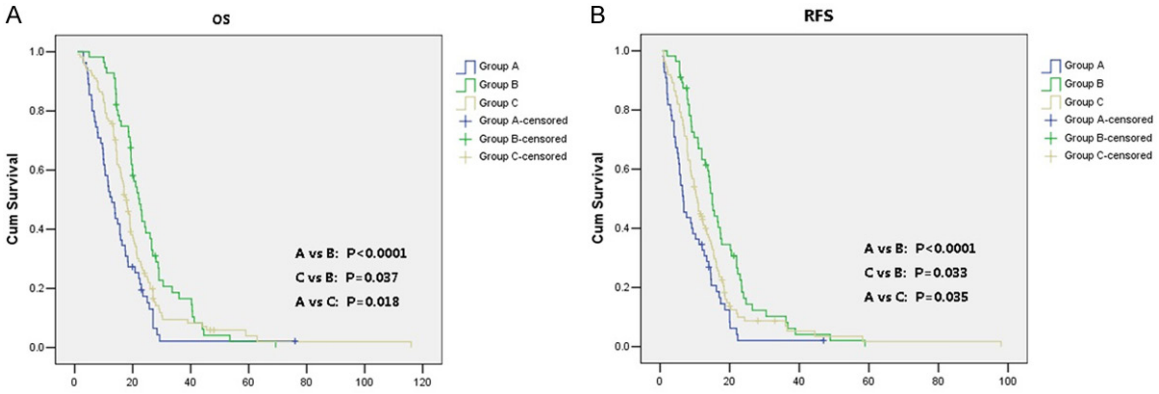

Figure 4. Kaplan-Meier curves of the OS and RFS for SCLC patients based on SOX2 and FGFR1 expression. A: OS cures of SCLC patients based on the expression of SOX2 and FGFR1 (P<0.0001); B: RFS cures of SCLC patients based on the expression of SOX2 and FGFR1 (P<0.0001). Group A = ($SOX2^{high}$/$FGFR1^{high}$) (n=55); Group B = ($SOX2^{low}$/$FGFR1^{low}$) (n=56); Group C = ($SOX2^{high}$/$FGFR1^{low}$ or $SOX2^{low}$/$FGFR1^{high}$) (n=111).

cant correlation between SOX2 expression and FGFR1 expression in SCLC (P=0.823).

Prognostic prediction using combined SOX2 and FGFR1

Meanwhile, we divided the patients into three subgroups according to the expression of SOX2 and FGFR1: Group A = ($SOX2^{high}$/$FGFR1^{high}$) (n=55); Group B = ($SOX2^{low}$/$FGFR1^{low}$) (n=56); Group C = ($SOX2^{high}$/$FGFR1^{low}$ or $SOX2^{low}$/$FGFR1^{high}$) (n=111). Kaplan-Meier survival curves were generated. The results showed that patients with high SOX2 and FGFR1 expression (Group A) had significantly shorter RFS (P<0.0001, P=0.035 respectively) and OS (P<0.0001, P=0.018 respectively) compared with GroupB (double negative) and Group C (any marker positive). Group C displayed shorter RFS (P=0.033) and OS (P=0.037) compared with Group B (**Figure 4**).

Discussion

As is known to all, although SCLC is extremely sensitive to initial chemotherapy and radiotherapy, lots of patients ultimately die from recurrent or progressive disease [17]. Up to the present, no molecular targeted therapy has showed significant activity in SCLC. Nevertheless, it is excited that genomic analyses in SCLC recently have provided several factors worthy to further

study, including SOX2, FGFR1,CREBBP, EP300, MYCN and EPHA7 [7, 8]. SOX2 and FGFR1 have been investigated in many malignancies respectively, for instance breast cancer, non-small cell lung cancer (NSCLC), meningioma, gastric cancer, prostate cancer and colorectal cancer [18-25]. While only a few studies were conducted on the expression of SOX2 in a small samples of SCLC patients by IHC [19, 26], no research was focused on the expression of FGFR1 and prognostic value of the two markers in a large sample of SCLC. We aimed to consummate the data in SCLC.

In the present research, we showed that high expression of SOX2 and FGFR2 by IHC and analyzed the associations between the expression of SOX2 and FGFR1 and clinicopathologic features and prognosis in a large series of SCLC patients. To our knowledge, this is the first comprehensive analysis of SOX2 and FGFR1 in the same set of SCLC tissues. Moreover, we found that high expression of SOX2 and FGFR1 in SCLC tumor cells than in normal tissues, which agreed with the previous studies [19, 23]. Tumors overexpressing both SOX2 and FGFR1 were associated with the worst outcome. In stage I patients, only SOX2 was an independent factor for worse OS and RFS.

SOX2 is a transcriptional factor required for pluripotency during embryogenesis and critical for the maintenance of embryonic stem cell identity [27]. Recently, it has been confirmed to be a genuine SCLC driver gene [7]. Several experiments have demonstrated that SOX2 is highly expressed in NSCLC, especially in SCC [28, 29]. SOX2 is also highly expressed in large cell neuroendocrine carcinomas and carcinoid tumors [26]. In small cell lung cancer, most studied focus on using ELISA methods to examine SOX2 expression [15, 16]. In this study, we describe that higher expression of SOX2 compared to matched normal tissues by IHC (P=0.018). Our findings of a significant increase in the immunohistochemical expression of SOX2 in tumor tissues with late clinical stage and lymph node metastasis, suggest that SOX2 could play a role in the progression and metastasis of SCLC. Down-regulation of SOX2 is associated with reduced cell proliferation [7]. Our finding is consistent with this phenomenon.

FGFR1 belongs to FGFR family, when bFGF bind to the extracellular domain of FGFRs, signal transduction cascade was initiated to promote cell proliferation and angiogenesis [30]. It has been confirmed as a new targetable oncogens in lung squamous carcinoma [10]. There are also amplification of FGFR1 in neuroendocrine tumors consisting of carcinoids of the lung [31]. Moreover, selective FGFR inhibitor PD173074 has demonstrated as a powerful therapeutic strategy in SCLC cell lines and animal model [12]. In current study, we first investigated the expression of FGFR1 in a large series of SCLC by IHC. The fact that high FGFR1 was present in ex-smokers than non-smokers was consistent with the reports of it in NSCLC (P<0.0001) [18]. FGFR1 was strongly associated with TNM stage. Meanwhile, the expression of FGFR1 is elevated in SCLC tumor tissue than normal tissues. Unfortunately, this study did not discover the meaningful relevance of SOX2 and FGFR1 expression.

Furthermore, our results demonstrated that higher SOX2 and FGFR1 expression correlated with poor OS and RFS. This supported the possibility to make them therapeutic targets, which could be a powerful relevance with tumor progression and metastasis. Although two studies have observed the result that SOX2 is not relevant with prognosis by using ELISA [15, 16]. One possible explanation is that serum and tumor tissue have heterogeneity. Combined overexpression of SOX2 and FGFR1 selects the patients from the worst prognostic group. Multivariate analysis for the prognosis of patients with SCLC revealed that clinical stage, SOX2 and FGFR1 were significant independent prognostic predictors. This finding were consistent with the study that patients with advanced stage and metastasis lived a shorter life [32]. However, in stage I patients, only SOX2 expression, rather than FGFR1, was associated with outcome.

In conclusion, the current study revealed for the first time that both SOX2 and FGFR1 were overexpressed in SCLC tissues than in normal tissues by IHC. Based on the relationship between SOX2 and FGFR1 expression and survival, we considered that SOX2 and FGFR1 might be used as prognostic biomarkers and also new targets for SCLC. The expression of markers may well be an efficacious approach that can prospectively confirm patients at high risk of relapse following resection of small cell lung cancer. Indeed, further investigations are

required to illustrate the mechanism of SOX2 and FGFR1.

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Disclosure of conflict of interest

We declare that we have no conflict of interest.

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