High expression of SRY-box transcription factor 30 associates with well differentiation, absent lymph node metastasis and predicts longer survival in nonsmall-cell lung cancer patients

Cui Liu, MM, Yameng Liu, MM, Jun Tian, MB, Shengli Zhang, MB, Xinmiao Li, MM, Xiemin Zhai, MB, Qiang Feng, MM

Abstract
The present study aimed to investigate SRY-box transcription factor 30 (SOX30) expression in nonsmall-cell lung cancer (NSCLC) tumor tissues and adjacent noncancerous tissues, and further explore the correlation of tumor SOX30 expression with clinical characteristics and survival profiles in patients with NSCLC.

Totally, 365 patients with NSCLC who underwent resection were screened, and SOX30 expression was detected in their tumor tissues and adjacent noncancerous tissues via immunohistochemistry (IHC) assay, which was assessed by a semiquantitative method considering the multiplying staining intensity score and staining density score. According to the tumor SOX30 expression, patients were categorized as tumor SOX30 low (IHC score ≤ 3) and high (IHC score 4–12) patients, the latter were further divided into tumor SOX30 high+ (IHC score 4–6), high++ (IHC score 7–9), and high+++ (IHC score 10–12) patients.

SOX30 was downregulated in NSCLC tumor tissues compared with adjacent noncancerous tissues. Meanwhile, tumor SOX30 high expression associated with well differentiation, absent lymph node metastasis, decreased TNM stage, but did not associated with age, gender, history of smoke and drink, hypertension, hyperlipidemia, diabetes, tumor size, or carcinoembryonic antigen level. Both accumulating disease-free survival and overall survival were the longest in tumor SOX30 high+++ patients, followed by tumor SOX30 high++ patients, and tumor SOX30 high+ patients, and the shortest in tumor SOX30 low patients. Besides, tumor SOX30 high expression was an independent predictor for longer disease-free survival and overall survival.

Tumor SOX30 exhibits the potential to be a novel biomarker for survival prediction of patients with NSCLC.

Abbreviations: DFS = disease-free survival, IHC = immunohistochemistry, NSCLC = nonsmall-cell lung cancer, OS = overall survival, SOX30 = SRY-box transcription factor 30, TNM = Tumor-Node-Metastasis.

Keywords: clinical characteristics, immunohistochemistry, nonsmall-cell lung cancer, SRY-box transcription factor 30, survival

1. Introduction
Nonsmall-cell lung cancer (NSCLC) accounts for approximately 83% of total lung cancer cases, and one recent global cancer statistics report indicates that NSCLC is one of the main causes of cancer-related mortality with only a 5-year survival rate of 23%.1,2 The treatment for NSCLC, including surgery, chemotherapy, radiotherapy, and target treatment, has undergone a great improvement and achieved prolonged survival in a proportion of patients with NSCLC; however, the treatment efficacy is still impaired by drug resistance and the survival data remains unsatisfied owing to the tumor locoregional recurrence and distant metastasis.1,3,4 Hence, it is important to pay attention to the cancer surveillance in the long-term follow-up care, and meanwhile, to further investigate into the underlying mechanisms of NSCLC as well as novel biomarkers, which helps to improve the prognosis in NSCLC management.

SRY-box transcription factor 30 (SOX30), as a member of SOX family, is characterized as a sex-determining factor existing throughout the mammals, and it is involved in the spermatogenesis differentiation and has regulatory effects on spermatogenesis function.5,6 Recently, SOX30 is shown to function as a tumor
suppressor through attenuating oncogenic pathway in various cancers, such as lung cancer, hepatocellular carcinoma, and ovarian cancer.\textsuperscript{[7–10]} For example, in vitro experiments of lung cancer show that SOX30 promotes cancer cell apoptosis but inhibits cancer cell metastasis via diminishing Wnt-signaling and attenuating β-catenin transcriptional activity.\textsuperscript{[11,12]} In another study of NSCLC, SOX30 induces expression of desmosomal genes via directly binding to the desmosomal genes promoter region, which inactivates Wnt signaling and ERK signaling and further inhibits cell proliferation, migration, and invasion.\textsuperscript{[13]} According to the aforementioned studies, we hypothesized that SOX30 might be of clinical significance in NSCLC management. However, the related research is still limited. Therefore, we conducted this study to detect the SOX30 expression in the tumor tissues as well as the noncancerous tissues of patients with NSCLC, and further investigate the correlation of tumor SOX30 expression with clinical characteristics and survival profiles in patients with NSCLC.

2. Methods

2.1. Patients

In this retrospective study, a total of 365 eligible patients with NSCLC who underwent resection were screened from our hospital between January 2015 and December 2019. The main inclusion criteria were: histologically confirmed as primary NSCLC; age ranging from 18 to 80 years old; TNM stage I–IIIa; tumor tissues and adjacent noncancerous tissues excised from surgery were well preserved; the records of tumor features before resection were complete; and follow-up data were eligible for calculating disease-free survival (DFS) and overall survival (OS). The main exclusion criteria were: relapse or metastasis; death before surgery were well preserved; the records of tumor features before resection were complete; and follow-up data were eligible for calculating disease-free survival (DFS) and overall survival (OS). The main exclusion criteria were: relapse or metastasis; death during or after surgery; poorly controlled hepatorenal disorders; and severe abnormalities in hematologic indexes. This study was approved by the Ethics Committee of our hospital. All patients or their family members proved the written informed consents.

2.2. Immunohistochemistry assay

After acquiring agreement from the Pathology Department of our hospital, the expressions of SOX30 in the formalin-fixed and paraffin-embedded tumor tissues and formalin-fixed and paraffin-embedded adjacent noncancerous tissues were detected by immunohistochemistry (IHC) assay, anti-SOX30 antibody (1:100; Abcam, Cambridge, MA) and goat anti-rabbit IgG H&L (HRP) (1:5000; Abcam) were used in IHC assay. All procedures were carried out according to application manuals of the antibodies. The total IHC score was assessed by multiplying staining intensity score and staining density score as previously described.\textsuperscript{[14]} Briefly, the staining intensity was scored as: 0 (no staining), 1 (weak staining), 2 (moderate staining), and 3 (strong staining). The staining density was scored as: 0 (no positively stained cells), 1 (1–25% of positively stained cells), 2 (26–50% of positively stained cells), 3 (51–75% of positively stained cells), and 4 (76–100% of positively stained cells). The total IHC score was ranging from 0 to 12. SOX30 high expression was defined as total IHC score ≥3, and SOX30 low expression was defined as the total IHC score <3. The SOX30 high expression were further divided into SOX30 high+ (IHC score 4–6), SOX30 high++ (total IHC score 7–9), and SOX30 high+++ (total IHC score 10–12).\textsuperscript{[14]}

2.3. Data collection and follow-up

The demographics and major preoperative tumor features were extracted from database of our hospital, and the survival data were obtained from survival data of our hospital. The last follow-up date was December 31, 2019, and the median follow-up duration was 30.0 months. DFS was defined as the duration from surgery to disease relapse, disease progression, or death, and OS was defined as the duration from surgery to death.

2.4. Statistical analysis

All statistical analyses were performed using SPSS version 22.0 (IBM, Chicago, IL), and all figures were plotted using GraphPad Prism version 7.00 (GraphPad Software, La Jolla, CA). Comparison of SOX30 expression between tumor tissues and noncancerous tissues was determined by paired-samples t test, McNemar test, or McNemar–Bowker test. Comparison of clinical characteristics between SOX30 high expression group and SOX30 low expression group was determined by Chi-squared test, Fisher exact test, or Wilcoxon rank sum test. DFS and OS were displayed using Kaplan–Meier curves. Comparison of DFS and OS between 2 groups or among 4 groups was determined by Log-rank test. Factors predicting DFS and OS were analyzed by univariate Cox proportional hazard regression model, and factors with P < .05 in the univariate Cox regression were included in the forward stepwise multivariate Cox regression analysis. P value < .05 was considered as significant.

3. Results

3.1. Clinical characteristics of patients with NSCLC

The average age of patients with NSCLC (N=365) was 61.6 ± 10.0 years (Table 1). The number of males and females were 298 (81.6%) and 67 (18.4%), respectively. Regarding tumor features, the number of patients with well, moderate, and poor differentiations were 67 (18.3%), 213 (58.4%), and 85 (23.3%), respectively. The mean tumor size was 5.3 ± 2.1 cm, and there were 213 (58.4%) patients with ≤5 cm tumor and 152 (41.6%) patients with >5 cm tumor. Besides, there were 121 (33.2%) patients with lymph node (LYN) metastasis. The number of patients with TNM stage I, II, III were 115 (31.5%), 133 (36.4%), and 117 (32.1%), respectively. More detailed information of clinical characteristics of patients with NSCLC is exhibited in Table 1.

3.2. SOX30 expression in NSCLC tumor tissues and noncancerous tissues

The expression of SOX30 in the tumor tissues and adjacent noncancerous tissues was detected by IHC assay, and all tissues were classified as SOX30 high expression (total IHC score >3) and SOX30 low expression (total IHC score ≤3). The SOX30 high expression were further divided into SOX30 high+ (total IHC score 4–6), SOX30 high++ (total IHC score 7–9), and SOX30 high+++ (total IHC score 10–12). Representative IHC images of SOX30 low expression in tumor tissue and SOX30 high expression in noncancerous tissue are shown in Figure 1A. The average SOX30 IHC score was decreased in NSCLC tumor tissue (2.9 ± 2.5) compared with noncancerous tissue (4.7 ± 3.1) (P < .001), suggesting that SOX30 was downregulated in NSCLC tumor tissues compared with noncancerous tissues (Fig. 1B).
There were 278 (76.2%) patients with tumor SOX30 low expression and 87 (23.8%) patients with tumor SOX30 high expression; meanwhile, there were 205 (56.2%) patients with noncancerous SOX30 low expression and 160 (43.8%) patients with noncancerous SOX30 high expression; further comparison analysis indicated that SOX30 was downregulated in NSCLC tumor tissues compared with noncancerous tissues \( (P < 0.001) \) (Fig. 1C). In addition, there were 278 (76.2%), 54 (14.8%), 22 (6.0%), and 11 (3.0%) patients with tumor SOX30 low, high+, high++, and high+++ expression, respectively; while there were 205 (56.2%), 77 (21.1%), 56 (15.3%), and 27 (7.4%) patients with noncancerous SOX30 low, high+, high++, and high+++ expression, respectively; further comparison analysis also indicated that SOX30 was downregulated in NSCLC tumor tissues compared with noncancerous tissues \( (P < 0.001) \) (Fig. 1D).

3.3. Correlation of tumor SOX30 with clinical characteristics in patients with NSCLC

There was no correlation of tumor SOX30 with age \( (P = 0.529) \), gender \( (P = 0.336) \), history of smoke \( (P = 0.596) \), history of drink \( (P = 0.888) \), hypertension \( (P = 0.529) \), hyperlipidemia \( (P = 0.535) \), or diabetes \( (P = 0.465) \) in patients with NSCLC (Table 2). As for the correlation of tumor SOX30 with tumor features, it was associated with well differentiation \( (P = 0.020) \) (Fig. 2A), absent LYN metastasis \( (P = 0.047) \) (Fig. 2C) and decreased TNM stage \( (P = 0.003) \) (Fig. 2D); however, there was no correlation of tumor SOX30 with tumor size \( (P = 0.192) \) (Fig. 2B) or carcinoembryonic antigen (CEA) level \( (P = 0.270) \) (Fig. 2E).

3.4. Correlation of tumor SOX30 with DFS in patients with NSCLC

Accumulating DFS was increased in tumor SOX30 high expression patients compared with tumor SOX30 low expression patients \( (P = 0.002) \) (Fig. 3A). Furthermore, accumulating DFS was the longest in tumor SOX30 high+++ expression patients, followed by tumor SOX30 high++ patients and tumor SOX30 high+ expression patients, and the shortest in tumor SOX30 low expression patients \( (P = 0.014) \) (Fig. 3B).

3.5. Factors affecting DFS in patients with NSCLC

Univariate Cox regression revealed that tumor SOX30 high expression (hazard ratio [HR] = 0.612, \( P = 0.003 \)) was positive-

---

**Table 1**
Features of patients with NSCL.

| Items                      | NSCLC patients \( (N = 365) \) |
|----------------------------|---------------------------------|
| Demographic features       |                                 |
| Age, yrs, mean ± SD        | 61.6 ± 10.0                     |
| Gender (male/female), n    | 298/67                          |
| History of smoke, n (%)    | 202 (55.3)                      |
| History of drink, n (%)    | 145 (39.7)                      |
| Commonly chronic complications |                                |
| Hypertension, n (%)        | 132 (36.2)                      |
| Hyperlipidemia, n (%)      | 116 (31.8)                      |
| Diabetes, n (%)            | 59 (15.9)                       |
| Tumor features             |                                 |
| Differentiation            |                                 |
| Well                       | 67 (18.4)                       |
| Moderate                   | 213 (58.4)                      |
| Poor                       | 85 (23.3)                       |
| Tumor size, cm, mean ± SD | 5.3 ± 2.1                       |
| ≤5                        | 213 (58.4)                      |
| >5                        | 152 (41.6)                      |
| LYN metastasis, n (%)      |                                 |
| Absent                     | 244 (66.8)                      |
| Present                    | 121 (33.2)                      |
| TNM stage, n (%)           |                                 |
| I                          | 115 (31.5)                      |
| II                         | 133 (36.4)                      |
| III                        | 117 (32.1)                      |
| CEA, ng/mL, median (IQR)   | 5.9 (2.7–20.9)                  |
| Normal*                   | 170 (46.6)                      |
| Abnormal*                  | 195 (53.4)                      |

CEA = carcinoembryonic antigen, IQR = interquartile range, LYN = lymph node, NSCLC = nonsmall-cell lung cancer, SD = standard deviation.

* CEA abnormal level > 5.0 ng/mL, CEA normal level ≤ 5.0 ng/mL.

There were 278 (76.2%) patients with tumor SOX30 low expression and 87 (23.8%) patients with tumor SOX30 high expression; meanwhile, there were 205 (56.2%) patients with noncancerous SOX30 low expression and 160 (43.8%) patients with noncancerous SOX30 high expression; further comparison analysis indicated that SOX30 was downregulated in NSCLC tumor tissues compared with noncancerous tissues \( (P < .001) \) (Fig. 1C). In addition, there were 278 (76.2%), 54 (14.8%), 22 (6.0%), and 11 (3.0%) patients with tumor SOX30 low, high+, high++, and high+++ expression, respectively; while there were 205 (56.2%), 77 (21.1%), 56 (15.3%), and 27 (7.4%) patients with noncancerous SOX30 low, high+, high++, and high+++ expression, respectively; further comparison analysis also indicated that SOX30 was downregulated in NSCLC tumor tissues compared with noncancerous tissues \( (P < 0.001) \) (Fig. 1D).

---

Figure 1. SRY-box transcription factor 30 (SOX30) expression in nonsmall-cell lung cancer (NSCLC). Representative immunohistochemistry (IHC) images of SOX30 low expression in NSCLC tumor tissue and SOX30 high expression in noncancerous tissue (A). Comparison of SOX30 IHC score between tumor tissues and noncancerous tissues (B). Comparison of the percentage of SOX30 high/low expressions between tumor tissues and noncancerous tissues (C). Comparison of the percentage of SOX30 low/SOX30 high+/SOX30 high++/SOX30 high+++ expressions between tumor tissues and noncancerous tissues (D).
ly associated with DFS, while poor differentiation (HR = 1.461, \( P = .012 \)), larger tumor size (>5 cm) (HR = 1.371, \( P = .016 \)), LYN metastasis (HR = 2.257, \( P < .001 \)), advanced TNM stage (HR = 2.105, \( P < .001 \)), and abnormal CEA (HR = 1.509, \( P = .002 \)) were negatively associated with DFS (Table 3). Forward stepwise multivariate Cox regression further indicated that tumor SOX30 high expression (HR = 0.702, \( P = .035 \)) was an independent predictive factor for increased DFS, while LYN metastasis (HR = 1.876, \( P < .001 \)), advanced TNM stage (HR = 1.471, \( P = .014 \)), and abnormal

### 3.6. Correlation of tumor SOX30 with OS in patients with NSCLC

Accumulating OS was increased in tumor SOX30 high expression patients compared with tumor SOX30 low expression patients (\( P = .002 \)) (Fig. 4A). Furthermore, accumulating OS was longest in tumor SOX30 high+++ expression patients, followed by tumor SOX30 high++ expression patients and tumor SOX30 high+ expression patients, and the shortest in tumor SOX30 low expression patients (\( P = .007 \)) (Fig. 4B).

### 3.7. Factor affecting OS in patients with NSCLC

Univariate Cox regression revealed that tumor SOX30 (HR = 0.531, \( P = .002 \)), poor differentiation (HR = 1.586, \( P = .005 \)), larger tumor size (>5 cm) (HR = 1.457, \( P = .013 \)), LYN metastasis (HR = 2.871, \( P < .001 \)), advanced TNM stage (HR = 1.883, \( P < .001 \)), and abnormal CEA (HR = 1.647, \( P = .001 \)) were negatively associated with OS (Table 4). Forward stepwise multivariate Cox regression revealed that tumor SOX30 high expression (HR = 0.625, \( P = .022 \)) was an independent predictive factor for increased OS, but poor differentiation (HR = 1.446, \( P = .026 \)), LYN metastasis (HR = 2.859, \( P < .001 \)), and abnormal CEA (HR = 1.599, \( P = .003 \)) were independent predictive factors for decreased OS.

## 4. Discussion

In the present study, we found that SOX30 was downregulated in NSCLC tumor tissues compared with noncancerous tissues. SOX30 was associated with well differentiation, absent LYN metastasis and decreased TNM stage in patients with NSCLC. SOX30 high expression was an independent predictive factor for longer DFS and OS in patients with NSCLC.

Emerging evidence has been published that SOX protein family mediates DNA binding via high-mobility group domain and

### Table 2

| Items                        | SOX30 low (n = 278) | SOX30 high (n = 87) | \( P \) value |
|------------------------------|---------------------|---------------------|--------------|
| Age, yr, n (%)               |                     |                     | .520         |
| <65                         | 164 (59.0)          | 48 (55.2)           |              |
| >65                         | 114 (41.0)          | 39 (44.8)           |              |
| Gender, n (%)                |                     |                     | .336         |
| Female                      | 48 (17.3)           | 19 (21.8)           |              |
| Male                        | 230 (82.7)          | 68 (78.2)           |              |
| History of smoke, n (%)     |                     |                     | .596         |
| No                          | 122 (43.9)          | 41 (47.1)           |              |
| Yes                         | 156 (56.1)          | 46 (52.9)           |              |
| History of drink, n (%)     |                     |                     | .888         |
| No                          | 167 (60.1)          | 53 (60.9)           |              |
| Yes                         | 111 (39.9)          | 34 (39.1)           |              |
| Hypertension, n (%)          |                     |                     | .529         |
| No                          | 175 (62.9)          | 58 (66.7)           |              |
| Yes                         | 103 (37.1)          | 29 (33.3)           |              |
| Hyperlipidemia, n (%)        |                     |                     | .535         |
| No                          | 192 (69.1)          | 57 (65.5)           |              |
| Yes                         | 86 (30.9)           | 30 (34.5)           |              |
| Diabetes, n (%)              |                     |                     | .465         |
| No                          | 236 (84.9)          | 71 (81.6)           |              |
| Yes                         | 42 (15.1)           | 16 (18.4)           |              |

Comparison was determined by Chi-squared test.
SOX30 = sex-determining region Y-box 30.
exerts various functions in cell development and differentiation. SOX30 is located on the chromosome 5, and is of physiologic and biologic function during the tumorigenesis, tumor metastasis has also been disclosed in several cancers. For example, one study exhibits that, in bladder cancer, SOX30 expression is markedly decreased in tumor tissues compared to the adjacent noncancerous tissues, and in vitro experiments disclose that SOX30 overexpression attenuates cell proliferation, invasion, and migration, but promotes apoptosis. As for the implication of SOX30 in lung cancer, one study indicates that SOX30 suppresses cancer cell migration and invasion, and at transcriptional level, SOX30 represses the activity of β-catenin promoter via C-terminus, which inactivates the Wnt-signaling pathway. Another study reveals that SOX30 upregulates the expression of 3 known tumor suppressor, including desmoplakin, junction plakoglobin and desmocollin 3, via directly binding to their promotor region, which inactivates Wnt/β-catenin signaling pathway, and further inhibits cell proliferation, migration, and invasion in lung adenocarcinoma. According to these previous studies, we hypothesized that SOX30 might have clinical significance in NSCLC. To test this hypothesis, we performed the present study and found that SOX30 was downregulated in various cancer tissues (bladder cancer, lung cancer, and NSCLC) compared with adjacent noncancerous tissues. We further explored the association of tumor SOX30 with clinical characteristics in patients with NSCLC, and observed that high expression of tumor SOX30 was associated with well differentiation, absent LYN metastasis, and decreased TNM stage in patient with NSCLC. The possible reasons might include that based on the prior study, the anti-tumor effect of SOX30 is exerted via directly activating p53.

| Items                              | Cox proportional hazard regression model analyses of factors affecting DFS. |
|------------------------------------|--------------------------------------------------------------------------|
|                                    | Cox proportional hazard regression model                                   |
|                                    | P value | HR | Lower | Higher |
|------------------------------------|---------|----|-------|--------|
| SOX30 high                         | .003    | 0.612 | 0.442 | 0.847  |
| Age (>65 yr)                       | .081    | 0.792 | 0.609 | 1.029  |
| Male                               | .232    | 0.820 | 0.592 | 1.135  |
| History of smoke                   | .918    | 1.014 | 0.783 | 1.312  |
| History of drink                   | .517    | 1.090 | 0.840 | 1.416  |
| Hypertension                       | .382    | 0.886 | 0.677 | 1.161  |
| Hyperlipidemia                     | .339    | 1.150 | 0.873 | 1.514  |
| Diabetes                           | .233    | 0.801 | 0.557 | 1.153  |
| Differentiation (poor vs moderate/well) | .012    | 1.461 | 1.089 | 1.961  |
| Tumor size (>5 cm)                 | .016    | 1.371 | 1.060 | 1.737  |
| LYN metastasis                     | <.001   | 2.257 | 1.738 | 2.932  |
| TNM stage (III vs II/I)            | <.001   | 2.105 | 1.619 | 2.736  |
| CEA abnormal*                      | .002    | 1.509 | 1.159 | 1.963  |
| Forward stepwise multivariate Cox regression | .035    | 0.702 | 0.504 | 0.976  |
| CEA abnormal*                      | .002    | 1.523 | 1.168 | 1.984  |

Only factors with P < .05 in the univariate Cox regression were included in the forward stepwise multivariate Cox regression analysis.

CEA = carcinoembryonic antigen, CI = confidence interval, DFS = disease-free survival, HR = hazard ratio, LYN = lymph node, SOX30 = sex-determining region Y-box 30.

*pCEA abnormal level > 5.0 ng/mL; CEA normal level ≤ 5.0 ng/mL.
transcription, and the restoration of p53 is suggested to restrain tumor progression against the Ras oncogenic signaling path-
way.\cite{10,17} Therefore, tumor SOX30 is associated with favorable tumor features in patients with NSCLC. In addition, as previously stated, SOX30 prevents the characteristics of epithelial-mesenchymal transition (EMT) via regulating the markers of EMT, such as E-cadherin and fibronectin, and EMT is reported to cause organ fibrosis and lead to initiation and progression of cancers via through diverse mechanisms.\cite{10,18,19} The activation of EMT permits NSCLC cells to be of migratory, invasive and stem-like properties; therefore, patients with tumor SOX30 low expression has decreased rate of LYN metastasis and advanced TNM stage. However, the detailed underlying molecular mechanism of the regulatory role of SOX30 in NSCLC needed further exploration.

Existing evidence demonstrate that SOX30 is positively correlated with favorable prognosis in patients with various cancers.\cite{10,13,16} For example, in bladder cancer, low SOX30 expression is correlated with decreased survival rate.\cite{16} In another study, high SOX30 expression exhibits value in predicting desirable OS and serves as an independent prognostic factor in advanced-stage patients with ovarian cancer.\cite{10} Consistently, in the present study, we also found that accumulating DFS was the longest in tumor SOX30 high+++ expression patients, followed by tumor SOX30 high++ expression patients and tumor SOX30 high+ expression patients, and the shortest in tumor SOX30 low expression patients. More notably, tumor SOX30 high expression was an independent predictive factor for increased DFS and OS in patients with NSCLC. The possible reasons might include that according to

Table 4
Cox proportional hazard regression model analyses of factors affecting OS.

| Items                                      | Cox proportional hazard regression model | 95% CI       |
|--------------------------------------------|-----------------------------------------|--------------|
|                                            | P value | HR | Lower | Higher |
| Univariate Cox regression                  |         |    |       |        |
| SOX30 high                                 | .002    | 0.531 | 0.356 | 0.791  |
| Age (>65 yrs)                              | .243    | 0.836 | 0.618 | 1.130  |
| Male                                       | .683    | 0.922 | 0.626 | 1.360  |
| History of smoke                           | .703    | 0.944 | 0.702 | 1.270  |
| History of drink                           | .278    | 1.179 | 0.875 | 1.589  |
| Hypertension                               | .358    | 0.864 | 0.632 | 1.180  |
| Hyperlipidemia                             | .207    | 1.224 | 0.894 | 1.674  |
| Diabetes                                   | .192    | 0.757 | 0.499 | 1.150  |
| Differentiation (poor vs moderate/well)    | .005    | 1.586 | 1.149 | 2.190  |
| Tumor size (>5 cm)                         | .013    | 1.457 | 1.083 | 1.960  |
| LYN metastasis                             | <.001   | 2.871 | 2.129 | 3.872  |
| TNM stage (III vs II/I)                    | <.001   | 1.883 | 1.390 | 2.551  |
| CEA abnormal\*                             | .001    | 1.647 | 1.212 | 2.239  |
| Forward stepwise multivariate Cox regression|         |    |       |        |
| SOX30 high                                 | .022    | 0.625 | 0.418 | 0.934  |
| Differentiation (poor vs moderate/well)    | .026    | 1.446 | 1.044 | 2.002  |
| LYN metastasis                             | <.001   | 2.859 | 2.117 | 3.860  |
| CEA abnormal\*                             | .003    | 1.599 | 1.172 | 2.182  |

Only factors with P < .05 in the univariate Cox regression were included in the forward stepwise multivariate Cox regression analysis.

CEA = carcinoembryonic antigen, CI = confidence interval, HR = hazard ratio, LYN = lymph node, OS = overall survival, SOX30 = sex-determining region Y-box 30.

\*CEA abnormal level > 5.0 ng/mL, CEA normal level ≤ 5.0 ng/mL.
aforementioned results, tumor SOX30 was associated with well differentiation, absent LYN metastasis and decreased TNM stage in patients with NSCLC, and well differentiation, absent LYN metastasis were independent predictive factors for increased survival; therefore, SOX30 could predict favorable prognosis in patients with NSCLC. Based on the previous studies, SOX30 could suppress the EMT progression, and EMT was reported to possess the NSCLC cell with various cellular characteristics, including invasiveness, drug resistance, stemness, and the ability to form metastases, which led to increased chemoresistance during the cancer treatment as well as cancer recurrence, further resulting to unfavorable prognosis.[20,21] Hence, SOX30 conferred favorable prognosis in patients with NSCLC.

However, our study still existed some limitations including: as our study was a retrospective study, there might be some selective bias and substantial compounding factors. (2) Since the underlying molecular mechanism of SOX30 at transcriptional level of NSCLC cell was not included in our study, further cellular experiments were needed. The follow-up period was relative short; therefore, the long-term prognostic significance of SOX30 needed further investigation.

In conclusion, tumor SOX30 is associated with well differentiation, absent LYN metastasis, decreased TNM stage, and independently predicts longer DFS and OS in patients with NSCLC, which suggests its potential role as a prognostic biomarker in NSCLC management.

Author contributions
Conception and design: C Liu and X Li; Administrative support: Q Feng; Provision of study materials or patients: Y Liu, J Tian and S Zhang; Collection and assembly of data: X Zhai and Q Feng; Data analysis and interpretation: C Liu, Y Liu, J Tian, S Zhang and X Li; Manuscript writing: All authors. Final approval of manuscript: All authors.

References
[1] Miller KD, Nogueira L, Mariotto AB, et al. Cancer treatment and survivorship statistics, 2019. CA Cancer J Clin 2019;69:363–85.
[2] Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394–424.
[3] La-Beck NM, Nguyen DT, Le AE, et al. Optimizing patient outcomes with PD-1/PD-L1 immune checkpoint inhibitors for the first-line treatment of advanced non-small cell lung cancer. Pharmacotherapy 2020;40:239–55.
[4] Passiglia F, Pioletto S, Facchinetti F, et al. Treatment of advanced non-small-cell lung cancer: The 2019 AIOM (Italian Association of Medical Oncology) clinical practice guidelines. Crit Rev Oncol Hematol 2019;146:10288.
[5] Han F, Wang Z, Wu F, et al. Characterization, phylogeny, alternative splicing and expression of Sox30 gene. BMC Mol Biol 2010;11:98.
[6] Osaki E, Nishina Y, Inazawa J, et al. Identification of a novel Sry-related gene and its germ cell-specific expression. Nucleic Acids Res 1999;27:2503–10.
[7] Tao J, Liu Z, Wang Y, et al. MicroRNA-645 represses hepatocellular carcinoma progression by inhibiting SOX30-mediated p53 transcriptional activation. Int J Biol Macromol 2019;121:214–22.
[8] Han F, Liu W, Jiang X, et al. SOX30, a novel epigenetic silenced tumor suppressor, promotes tumor cell apoptosis by transcriptional activating p53 in lung cancer. Oncogene 2015;34:4391–402.
[9] Fu Q, Sun Z, Yang F, et al. SOX30, a target gene of miR-653-5p, represses the proliferation and invasion of prostate cancer cells through inhibition of Wnt/beta-catenin signaling. Cell Mol Biol Lett 2019;24:71.
[10] Han F, Liu WB, Li JJ, et al. SOX30 is a prognostic biomarker and chemotherapeutic indicator for advanced-stage ovarian cancer. Endocr Relat Cancer 2019;26:303–19.
[11] Zhan C, Wang T, You H, et al. Different expressions of miR-125b and SOX30 in malignant lymphomas and their significance. J BUON 2018;23:1179–84.
[12] Han F, Liu WB, Shi XY, et al. SOX30 inhibits tumor metastasis through attenuating Wnt-signaling via transcriptional and posttranslational regulation of beta-catenin in lung cancer. EBioMedicine 2018;31:253–66.
[13] Han X, Han F, Ma B, et al. SOX30 is a key regulator of desmosomal gene suppressing tumor growth and metastasis in lung adenocarcinoma. J Exp Clin Cancer Res 2018;37:111.
[14] Fu H, Jin C, Zhu Q, et al. Dysregulated expressions of PTEN, NF-kappaB, WWP2, p53 and e-Myc in different subtypes of B cell lymphomas and reactive follicular hyperplasia. Am J Transl Res 2019;11:1092–101.
[15] Grimm D, Bauer J, Wise P, et al. The role of SOX family members in solid tumours and metastasis. Semin Cancer Biol 2019;https://doi.org/10.1016/j.semcancer.2019.03.004.
[16] Liu Y, Wang H, Zhong J, et al. Decreased expression of SRY-box containing gene 30 is related to malignant phenotypes of human bladder cancer and correlates with poor prognosis. BMC Cancer 2018;18:642.
[17] Junttila MR, Karmezis AN, Garcia D, et al. Selective activation of p33-mediated tumour suppression in high-grade tumours. Nature 2010;468:567–71.
[18] Thierry JP, Acloque H, Huang RY, et al. Epithelial-mesenchymal transitions in development and disease. Cell 2009;139:871–90.
[19] De Craene B, Berx G. Regulatory networks de

7