Association of SOX2 and Nestin DNA amplification and protein expression with clinical features and overall survival in non-small cell lung cancer: A systematic review and meta-analysis

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ABSTRACT

Up to now, the prognosis of non-small cell lung cancer (NSCLC) is poor. With progress of cancer biology, a number of genes have been investigated for predicting prognosis of NSCLC, such as cancer stem cell markers SRY (sex determining region Y)-box 2 (SOX2) and Nestin. Recently, a series of studies have been performed to examine the associations of SOX2 and Nestin with clinical parameters and prognosis in NSCLC, however, the results were not consistent. In the present study, we conducted a systematic review and meta-analysis to summarize the associations. Four English databases (PubMed, ISI web of science, Embase, and Ovid) were used to search the relevant studies with the last date of November 10, 2015. The pooling analyses were stratified by DNA amplification and protein expression. The pooling ORs or HRs were used to assess the strength of the associations. Finally, we included 19 articles for SOX2 and six articles for Nestin according to the inclusion and exclusion criteria. The pooling analyses revealed that there were significant associations between SOX2 DNA amplification and clinical features of NSCLC, gender, smoking status, squamous cell carcinoma (SCC) histology, and differentiations. And significant associations were also identified between SOX2 protein expression and clinical parameters, smoking status and SCC histology. For Nestin, its protein expression was correlated with lymph node metastasis and stage. Simultaneously, we found that high/positive SOX2 alterations, either DNA amplification or protein expression, were favorable for overall survival (OS) in NSCLC. On the contrary, high/positive Nestin protein expression was poor for OS.

INTRODUCTION

Lung cancer is the leading cause of cancer death worldwide and its 5-year relative survival rate is low [1]. Traditionally, it is classified into two major subtypes, small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). The latter can be subdivided into adenocarcinoma (ADC), squamous cell carcinoma (SCC), and large cell carcinoma (LCC) [2].

In recent years, some activated oncogenes such as Epidermal Growth Factor Receptor (EGFR) mutations and Anaplastic Lymphoma Kinase (ALK) rearrangements have been found and used as novel therapeutic targets [3–5]. All these progresses encourage the researchers to identify new biomarkers or therapeutic targets. Of which, the cancer stem cell markers such as SRY (sex determining region Y)-box 2 (SOX2) and Nestin have gotten researchers interested.
SOX2 locates on chromosome 3q26.33 and encodes a transcription factor of 317 amino acids [6, 7]. It has been reported to be involved in pluripotency regulation in embryonic stem cells and the morphogenesis and homoeostasis of tracheobronchial epithelia [8]. Recently, SOX2 aberrant DNA amplification and protein expression have been found in various types of tumors. Functional experiments suggest that SOX2 is responsible for cellular proliferation, tumor invasion and migration, self-renewal, maintenance in cancer stem cell populations, and lung tumorigenesis [6, 9–12]. It also has been reported that DNA amplification and protein expression of SOX2 are associated with clinicopathological features and prognosis in lung cancers, however, the results are not always consistent [13–16]. Although a meta-analysis in the year of 2013 has been performed to summarize the associations, the included studies were relatively rare and the authors do not distinguish SOX2 DNA amplification, mRNA expression, and protein expression [17].

Nestin is a member of the intermediate filament (IF) family and serves as a potential proliferative and muti-potency marker in progenitor and stem cells [18, 19]. Nestin has been also found to have an anti-apoptotic function through inhibiting caspase activation [20]. Recent observations have revealed a link between Nestin aberrant expression and malignant characteristics and poor prognosis in different cancers [21–25].

In the present study, we performed a systematic review and meta-analysis to investigate the associations of DNA amplification and protein expression of SOX2 and Nestin with clinicopathological features and overall survival in NSCLC.

RESULTS

Study characteristics

The literature selection process was shown in Figure 1. Four English databases were used and a total of 1442 documents were initially identified. After excluding those duplicated records, animal experiments or cellular studies, non-NSCLC related studies, and non-original articles, 36 full texts were left for further evaluation. Subsequently, six articles were excluded due to insufficient data [26–31], and one was excluded because it contained other type of lung cancer besides NSCLC [32]. Here, we only focused on DNA amplification and protein expression. Then another four studies were excluded due to only reporting the SOX2 or Nestin mRNA related data [33–36]. Finally, 25 papers were included in the present study [13–16, 21, 37–56]. Of which, 19 articles reported SOX2 DNA amplification and/or protein expression [13–16, 37–51], six articles reported Nestin protein expression, and none reported Nestin DNA amplification [21, 52–56]. In addition, Velcheti et al. [46] and Iijima et al. [50] reported two independent cohorts, respectively, and each cohort was considered as independent study in the meta-analysis. The included studies were published from 2010 to 2015 and the sample size ranged from 33 to 758. In the original studies, the DNA amplification was determined by PCR or FISH (n = 3 and 6) and the protein expression was determined by IHC or IF (n = 19 and 2). The detailed characteristics of the included studies were shown in Table 1.

Meta-analysis results

SOX2

Significant associations were identified between high/positive SOX2 DNA amplification and clinicopathological features, gender (OR = 1.969, 95% CI = 1.050–3.693, P = 0.035), smoking status (OR = 2.830, 95% CI = 1.269–6.310, P = 0.011), histology (OR = 8.136, 95% CI = 2.136–30.997, P = 0.000), differentiation (OR = 1.644, 95% CI = 1.119–2.415, P = 0.011), and OS (HR = 0.732, 95% CI = 0.593–0.904, P = 0.004) (Figure 2 and Table 2). For SOX2 protein expression, its associations with smoking status (OR = 2.245, 95% CI = 1.008–5.001, P = 0.048), histology (OR = 5.437, 95% CI = 2.344–12.610, P = 0.000), and OS (HR = 0.579, 95% CI = 0.359–0.934, P = 0.025) were found (Figure 2 and Table 2).

Nestin

There was no study reporting Nestin DNA amplification in NSCLC and then only Nestin protein expression was analyzed. The pooling analyses revealed significant associations of Nestin protein expression with lymph node metastasis (OR = 2.732, 95% CI = 1.393–5.376, P = 0.004), stage (OR = 1.996, 95% CI = 1.157–3.445, P = 0.013), and OS (HR = 2.166, 95% CI = 1.437–3.263, P = 0.000) (Figure 3 and Table 2).

Heterogeneity and sensitivity analysis

SOX2

The heterogeneity and sensitivity were analyzed by subgroup analysis according to ethnicity, histology, and sample size or excluding single individual study. The results indicated that the heterogeneity existed in evaluating the associations of SOX2 DNA amplification with gender (F = 55.6%), smoking status (F = 62.2%), histology (F = 89.7%), and differentiation (poor vs. well/moderate, F = 68.2%) (Table 2). For gender, heterogeneity decreased to 31.4% after excluding Wilbertz et al’s study and the pooling OR was not influenced. For smoking status, after grouping by China and non-China studies, heterogeneity of both subgroups decreased to 0% and the associations were still significant. For histology, the heterogeneity could not be removed by subgroup analyses or excluding single individual study. For differentiation, the heterogeneity decreased (F = 19.3%) by excluding the study of Zhang et al. 2015 and the pooling
OR was not influenced. Meanwhile, there were significant heterogeneity in assessment of the associations of SOX2 protein expression with gender ($I^2 = 55.1\%$), age ($I^2 = 90.3\%$), smoking status ($I^2 = 58.9\%$), histology ($I^2 = 68.7\%$), and OS ($I^2 = 84.9\%$). The heterogeneity deceased significantly when deleting single individual studies of Chou et al. 2013 (gender, $I^2 = 38.7\%$), Chen et al. 2012 (age, $I^2 = 15.0\%$), Zheng et al. 2015 (smoking status, $I^2 = 0\%$), and Li et al. 2012 (histology, $I^2 = 34.5\%$), respectively. And the pooled ORs were not influenced, suggesting the results were stable. For OS, the heterogeneity still existed when excluding single individual study one by one. In subgroup analysis stratified by histology (SCC, $n = 4$; ADC, $n = 1$; and SCC/ADC, $n = 4$), heterogeneity was 29.7% in SCC, and 90.3% in SCC/ADC. And the association was significant in SCC but not SCC/ADC.

### Nestin

As shown in Table 2, there was heterogeneity in assessment of the associations of Nestin protein expression with smoking status ($I^2 = 60.7\%$), histology ($I^2 = 92.0\%$), differentiation ($I^2 = 94.6\%$), and OS ($I^2 = 68.4\%$). When the studies of Chen et al. 2010, Ryuge et al. 2011, and Janikova et al. 2012 were excluded, respectively, the heterogeneity significantly decreased (smoking status, $I^2 = 0\%$; differentiation, $I^2 = 0\%$; and OS, $I^2 = 48.2\%$) and the pooling ORs were not influenced except differentiation. As for histology, subgroup analysis suggested that the heterogeneity among the studies performed in China was decreased ($I^2 = 0\%$) and a significant association was presented. But the heterogeneity still existed in Japan group ($I^2 = 95.5\%$).
### Table 1: Characteristics of the included studies

| Reference           | Country          | Patient No. | Age (year) | Method | Cut-off value                                      | Protein/Amplification | Positive/Negative |
|---------------------|------------------|-------------|------------|--------|----------------------------------------------------|-----------------------|-------------------|
| SOX2                |                  |             |            |        |                                                    |                       |                   |
| Yuan et al. 2010    | USA              | 57          | IHC        | SCC (high > 270, low < 140); ADC (high > 193, low < 10) | Protein               | 37/19             |
| Lu et al. 2010      | USA              | 40          | IHC        | 4 copy Amplification | Protein               | 19/21             |
| Sholl et al. 2010   | USA              | 104         | IHC        | 5%     | Protein                                           | 52/52                 |
| Sholl et al. 2010   | USA              | 66          | IHC        | 5%     | Protein                                           | 41/25                 |
| Wilbertz et al. 2011| Switzerland/USA  | 758         | FISH       | 30%    | Amplification                                     | 224/534               |
| Cai et al. 2011     | China            | 115         | PCR        | Ratio > M + 2SD | Amplification | 30/85             |
| Koji et al. 2011    | Japan            | 309         | IHC        | 5%     | Protein                                           | 79/71                 |
| Sasaki et al. 2012  | Japan            | 127         | PCR        | 4 copy Amplification | Protein               | 42/85             |
| Brcic et al. 2012   | American         | 147         | IHC        | 5%     | Protein                                           | 14/52                 |
| Li et al. 2012      | China            | 44          | IHC        | 10%    | Protein                                           | 31/13                 |
| Velcheti et al. 2013| Greek            | 340         | IF         | Score > 193 | Protein               | 418/229              |
| Chou et al. 2013    | China            | 175         | IHC        | No stain in nuclear | Protein               | 51/124             |
| Yusuke et al. 2015  | Japan            | 282         | FISH       | Mean value | Amplification | 34/244               |
| Toschi et al. 2015  | Italy            | 447         | FISH       | 4 copy or presence of gene cluster | Amplification | 105/340             |
| Iijima et al. 2015  | China cohort     | 57          | IHC        | H-score > 0 | Protein               | 40/17                |
| Zheng et al. 2015   | China            | 162         | FISH       | H-score > 0 | Protein               | 45/21                |
| Zheng et al. 2015   | China            | 162         | FISH       | 100 score | Protein               | 85/65                |
| Nestin              |                  |             |            |        |                                                    |                       |                   |
| Chen et al. 2010    | China            | 52          | IHC        | 8.4(median histoscore of Nestin) | Protein               | 25/27               |
| Janikova et al. 2010| Czech            | 121         | IHC        | 10%    | Protein                                           | 74/38                 |
| Ryuge et al. 2011   | Japan            | 173         | IHC        | 5%     | Protein                                           | 27/144                |
| Skarda et al. 2012  | Czech & Israel   | 115         | IHC        | H-score > 0 | Protein               | 40/74                |
| Chen et al. 2014    | China            | 71          | IHC        | 8.4(median histoscore of Nestin) | Protein               | 35/36               |
| Sterlacci et al. 2014| Austria          | 215         | IHC        | Median % positive staining cell | Protein               | 57/269              |
Figure 2: Forest plot for associations of SOX2 with clinicopathological features and overall survival in NSCLC.
Table 2: Meta-analysis results

| Clinical parameters | N   | OR/HR | OR/HR 95% CI | P OR | Model | F   | P hetero | P Egger | P Egger |
|---------------------|-----|-------|--------------|------|-------|-----|----------|---------|---------|
| **SOX2 Amplification** |     |       |              |      |       |     |          |         |         |
| Gender (male vs. female) | 8   | 1.969 | 1.050–3.693 | 0.035 | R     | 55.6 | 0.027   | 0.711   | 0.652   |
| Age (≤ 60 vs. > 60 or ≤ 65 vs. > 65) | 3   | 0.857 | 0.507–1.448 | 0.563 | F     | 0.6  | 0.365   | 1.000   | 0.367   |
| Smoking status (yes vs. no) | 7   | 2.830 | 1.269–6.310 | 0.011 | R     | 62.2 | 0.014   | 0.368   | 0.052   |
| Histology (SCC vs. ADC) | 6   | 16.530 | 5.134–53.221 | 0.000 | R     | 89.7 | 0.000   | 0.707   | 0.885   |
| Differentiation (moderate+poor vs. well) | 5   | 1.644 | 1.119–2.415 | 0.011 | F     | 0.0  | 0.935   | 0.462   | 0.629   |
| Differentiation (poor vs. well+moderate) | 3   | 0.807 | 0.317–2.054 | 0.654 | R     | 68.2 | 0.041   | 1.000   | 0.796   |
| Lymph node metastasis (N0 vs. N1,2) | 5   | 0.943 | 0.678–1.312 | 0.728 | F     | 0.0  | 0.650   | 0.806   | 0.688   |
| Lymph nodemetastasis (N0,1 vs. N2,3) | 3   | 0.903 | 0.468–1.743 | 0.761 | F     | 0.0  | 0.418   | 0.308   | 0.168   |
| Stage (I vs. II–IV) | 5   | 1.222 | 0.860–1.737 | 0.263 | F     | 0.0  | 0.855   | 0.221   | 0.363   |
| Stage (I–II vs. III–IV) | 4   | 1.226 | 0.877–1.714 | 0.232 | F     | 0.0  | 0.849   | 0.734   | 0.690   |
| OS | 6   | 0.732 | 0.593–0.904 | 0.004 | F     | 0.0  | 0.949   | 0.707   | 0.794   |
| **SOX2 Protein expression** |     |       |              |      |       |     |          |         |         |
| Gender (male vs. female) | 9   | 1.345 | 0.726–2.493 | 0.558 | R     | 55.1 | 0.023   | 0.917   | 0.738   |
| Age (≤ 60 vs. > 60 or ≤ 65 vs. > 65) | 6   | 0.439 | 0.104–1.857 | 0.263 | R     | 90.3 | 0.000   | 0.368   | 0.199   |
| Smoking status (yes vs. no) | 5   | 2.245 | 1.008–5.001 | 0.048 | R     | 58.9 | 0.045   | 0.806   | 0.537   |
| Histology (SCC vs. ADC) | 7   | 5.437 | 2.344–12.610 | 0.000 | R     | 68.7 | 0.004   | 1.000   | 0.749   |
| Differentiation (moderate+poor vs. well) | 6   | 1.082 | 0.695–1.685 | 0.726 | F     | 0.0  | 0.694   | 1.000   | 0.471   |
| Differentiation (poor vs. well+moderate) | 9   | 0.723 | 0.517–1.011 | 0.058 | F     | 14.2 | 0.316   | 1.000   | 0.496   |
| Lymph node metastasis (N0 vs. N1,2) | 3   | 1.078 | 0.649–1.789 | 0.772 | F     | 0.0  | 0.693   | 1.000   | 0.952   |
| Lymph nodemetastasis (N0,1 vs. N2,3) | 1   |       |              |      |       |     |          |         |         |
| Stage (I vs. II–IV) | 4   | 1.288 | 0.807–2.057 | 0.289 | F     | 18.4 | 0.298   | 0.734   | 0.959   |
| Stage (I–II vs. III–IV) | 3   | 0.818 | 0.327–2.044 | 0.667 | F     | 5.3  | 0.348   | 1.000   | 0.648   |
| OS | 9   | 0.579 | 0.359–0.934 | 0.025 | R     | 84.9 | 0.000   | 0.466   | 0.109   |
| **Nestin Protein expression** |     |       |              |      |       |     |          |         |         |
| Gender (male vs. female) | 4   | 0.932 | 0.569–1.527 | 0.780 | F     | 11.7 | 0.334   | 0.734   | 0.478   |
| Age (≤ 60 vs. > 60 or ≤ 65 vs. > 65) | 3   | 1.111 | 0.650–1.897 | 0.701 | F     | 5.1  | 0.349   | 0.294   | 0.174   |
| Smoking status (yes vs. no) | 3   | 1.237 | 0.486–3.151 | 0.655 | R     | 60.7 | 0.078   | 1.000   | 0.145   |
| Histology (SCC vs. ADC) | 4   | 2.378 | 0.420–13.462 | 0.327 | R     | 92.0 | 0.000   | 0.734   | 0.542   |
| Differentiation (well+moderate vs. poor) | 3   | 2.671 | 0.170–41.861 | 0.484 | R     | 94.6 | 0.000   | 1.000   | 0.335   |
| Lymph node metastasis (N1,2 vs. N0) | 2   | 2.732 | 1.393–5.376 | 0.004 | F     | 0.0  | 0.694   |         |         |
| Stage (II–IV vs. I) | 3   | 1.966 | 1.157–3.445 | 0.013 | F     | 0.0  | 0.981   | 1.000   | 0.534   |
| OS | 5   | 2.166 | 1.437–3.263 | 0.000 | R     | 68.4 | 0.013   | 0.806   | 0.534   |

**Publication bias**

Furthermore, publication bias was also assessed by Begg’s test and Egger’s test. Symmetrical Begg’s funnel plots and Egger’s test results revealed no publication bias in all comparisons (Figure 4 and Table 2).

**DISCUSSION**

A number of studies have been performed to explore the associations of cancer cell stem cell markers, such as SOX2 and Nestin, with clinical parameters and prognosis in various types of cancers including NSCLC. However, the results in the studies were not consistent.

Up to now, there were two meta-analyses trying to investigate the associations of SOX2 with clinicopathological features and/or overall survival in NSCLC [17, 57]. Chen et al. [17] only searched relevant studies in PubMed, up to May 2013 and included eight studies. Shao et al. [57] pooled seven studies published from 2010 to 2013. Neither of the studies distinguished SOX2 DNA amplification, mRNA expression, and protein expression. In the present study, we analyzed SOX2 DNA amplification and protein expression, respectively,
unlike with the above reports. We searched in more English database and included more articles than the previous meta-analysis (19 vs. 8 and 7) although we did not combined the mRNA related studies. Pooling analyses suggested that both of the DNA amplification and the protein expression of SOX2 were associated with smoking status, histology, and OS. In addition, SOX2 DNA amplification was also associated with gender and differentiation. The discrepancy between DNA amplification and protein expression might be caused by the heterogeneity among studies and the inconsistency between amplification and protein expression. More studies examining amplification and protein expression of SOX2 at the same time should be performed to confirm the conclusions. For Nestin, there was only one meta-analysis examining the associations of Nestin protein expression with TNM in regardless of cancer types. And the authors found that Nestin was positively associated

Figure 3: Forest plot for associations of Nestin with clinicopathological features and overall survival in NSCLC.
with cancer stage and lymph node [58]. In the present meta-analysis, we summarized the associations of Nestin with clinicopathological features and OS in a single type of cancer, NSCLC. The pooling analyses suggested that high/positive Nestin was an indicator of poor prognosis in NSCLC, not as well as SOX2, which was a favorable factor for OS in NSCLC. This might bring us confusion when understanding the role of the two genes in molecular pathogenesis of NSCLC. Because both of them were cancer stem cell markers and mechanisms studies suggested that they all had proliferative and anti-apoptotic effects in vitro and animal model. Combined the results of the previous reports and the present meta-analysis, we proposed a mechanism model that SOX2 was an oncogene and promoted tumorigenesis. Meanwhile, the tumors with SOX2 up-regulation might exhibit a clearer squamous cell differentiation and were associated with better prognosis.

Although we pooled all the potential studies according to the inclusion and exclusion criteria, some limitations existed. Firstly, the number and sample size of Nestin related studies were small. Secondly, the studies of the subgroup of ADC for SOX2 were rare. As the original studies suggested that SOX2 was more frequently upregulated in SCC than ADC, the predictive role of SOX2 in SCC and ADC might be not consistent. Then the impact of SOX2 on prognosis in SCC and ADC should be compared in more studies with larger sample size.

In summary, we got a comprehensive result from the current meta-analysis that SOX2 DNA amplification and protein expression were associated with smoking status and histology, and were favorable for prognosis in NSCLC. And Nestin was associated with cancer stage, lymph node, and poor outcome.

Figure 4: Begg’s funnel plot for publication bias analysis.
MATERIALS AND METHODS

Publication search

A systematic search was performed in four English databases (PubMed, EMBASE, OVID, and Web of science) for published articles on the associations of SOX2 and Nestin with clinical features and/or overall survival (OS) in NSCLC up to November 10, 2015. The following keywords were used: “lung OR pulmonary”, “cancer OR tumor OR carcinoma”, and “Nestin OR Sex determining region Y box-2 OR SRY box-2 OR SOX2”. Two independent investigators screened the retrieved documents by reviewing the article titles, abstracts, or full texts according to the inclusion and exclusion criteria. The review articles and the references of selected articles were also screened to identify additional eligible studies.

Inclusion and exclusion criteria

Inclusion criteria: (1) the histologic type of the tumors was NSCLC and if one study containing multiple types of lung cancer, only the data related to NSCLC was included; (2) evaluating the associations of SOX2 and Nestin DNA amplification and/or protein expression with clinicopathological features and OS; (3) peer reviewed papers that have been published as full texts; (4) the language was limited as English; (5) if multiple studies contained overlap or duplicated data, only the study with larger sample size was included. Exclusion criteria: (1) the frequency of patients with positive/negative/high/low DNA amplification and protein expression was not specific to clinicopathological features; (2) study with insufficient data; (3) abstracts, letters, or review articles.

Data extraction

Two independent investigators collected related data carefully and the following characteristics were extracted from included studies: first author name, year of publication, country, ethnicity, patient number, gender, age, protein expression/amplification, method, cut-off value, smoking status, histologic type, differentiation, lymph node metastasis, stage, and OS.

Statistical analysis

All the statistical analyses were carried out with the software Stata 12.0 (StataCorp, College Station, TX, USA). The crude odds ratios (ORs) with 95% confidence intervals (95% CIs) were calculated to estimate the associations of DNA amplification and protein expression of SOX2 and Nestin with clinicopathological features of NSCLC. The crude hazard ratios (HRs) with 95%CIs were used to assess their clinical significance in predicting prognosis of NSCLC. The statistical significant level was determined by Z-test with P value less than 0.05. If the prognosis was only presented by a Kaplan-Meier plot curve, HR and its 95%CI were calculated according to previous reports [59, 60]. Briefly, the KM plot curves were read by Engauge Digitizer version 2.11 and HR was estimated by the calculation spreadsheet. The spreadsheet could be freely downloaded from http://www.trialsjournal.com/content supplementary/1745-6215-8-16-s1.xls. Inconsistency was solved by discussion. The heterogeneity among studies was explored using the chi-square based on Q statistic test. If P > 0.1 or I² < 50%, fixed effects model was used to calculate the pooled OR/HR. Otherwise, random-effects model was used [61]. Sensitivity analysis was also conducted to evaluate stabilities of pooling results by omitting studies that brought heterogeneity or publication bias. Potential publication bias was checked by Begg’s funnel plots and Egger’s test [62, 63].

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Authors’ contributions

Designed the study: Q. L., F. L., and X. M. Searched databases and collected full-text papers: Q. L. and F. L. Extracted and analyzed the data: Y. Z. and L. F. Statistical analyses: Q. L., C. W., X. C., and S. G. Wrote the main manuscript text: Q. L. and X. M. All authors reviewed the manuscript.

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