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ORIGINAL ARTICLE

Prevalence of ROS1 fusion in Chinese patients with non-small cell lung cancer

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Abstract

Background: The study was conducted to investigate the clinicopathological features and prevalence of *ROS1* gene fusion in Chinese patients with non-small cell lung cancer (NSCLC).

Methods: The presence of *ROS1* fusion was assessed by quantitative real-time PCR. Associations between *ROS1* fusion and clinical characteristics were analyzed.

Results: In total, 6066 patients with pathologically confirmed NSCLC and *ROS1* fusion test results were enrolled. The average age was 60.89 ± 10.60 years and fusion was detected in 157 (2.59%) patients. Fusion frequency was significantly correlated with age, gender, smoking status (all $P < 0.001$), pathology type ($P = 0.017$), and lymph node metastasis stage ($P = 0.027$). *ROS1* fusion-positive patients were significantly younger (55.68 ± 11.34 vs. negative 61.02 ± 10.44 years; $P < 0.01$). Fusion frequency was higher in women (3.71% vs. men 1.81%), never-smokers (3.33% vs. smokers 1.21%), and patients with adenocarcinoma (2.77% vs. squamous lung cancer 0.93%) and at advanced node stages (1.31%, 1.40%, 2.07%, and 3.23% for N0, N1, N2, and N3, respectively). No significant correlation between *ROS1* fusion status and pathological stage was found in subgroups classified by pathological, tumor, or metastasis stage ($P > 0.05$). Age, smoking status, and lymph node stage were statistically significantly correlated with *ROS1* fusion frequency (all $P < 0.05$); gender and pathology type were not significantly correlated with *ROS1* fusion status after adjusting for smoking status.

Conclusion: An overall *ROS1* fusion frequency of 2.59% was confirmed in this study. *ROS1* fusion was more prevalent among younger patients, never-smokers, and those at advanced node stages.

Introduction

Lung cancer remains the leading cause of cancer death worldwide, and non-small cell lung cancer (NSCLC) accounts for more than 80% of lung cancer cases.[1,2] During the last decade, the identification of key driver genes in NSCLC, such as *EGFR* and *ALK*, and the promising results obtained with the use of tyrosine kinase inhibitors (TKIs) that target these driver genes to treat NSCLC have rapidly facilitated the development of targeted therapy and precision medicine.[3–12] In this era of precision medicine, molecular testing has become extremely important for both the classification and treatment of lung cancer.[13]

ROS1 is a receptor tyrosine kinase of the insulin receptor family. It was first discovered in NSCLC in 2007.[14] The *ROS1* fusion partners identified in lung cancer to date include *CD74*, *SLC34A2*, *GOPC*, *CCDC6*, *SDC4*, *TPM3*, *EZR*, *LRIG3*, *KDELR2*, *LIMA1*, *MSN*, *CLTC*, *TPD52L1*, *FIG*, *TMEM106B*, *FAM135B*, and *SLC6A17*, with an overall prevalence of 0.9–2.6% in NSCLC[15–25] and up to 3% in lung adenocarcinoma,[19,26] representing a novel molecular subgroup of NSCLC. Several important clinical studies have shown that crizotinib, an *ALK* inhibitor, has high activity when treating NSCLC patients harboring *ROS1* fusion, with a response rate of 72–80%.[27,28] Based on these promising results, the American, Japanese, and Chinese authorities have approved crizotinib for the treatment of *ROS1* fusion-positive NSCLC patients. This development has highlighted the need for thorough investigations of *ROS1* fusions in patients with NSCLC.

Similar to patients with *ALK* fusions, *ROS1* fusion-positive patients tend to be younger, never-smokers, with adenocarcinoma histology.[9–11,21,22] However, the clinical features of patients harboring a *ROS1* fusion gene are not fully understood; the vast majority of studies have had small to modest sample sizes,[17,29–32] which compromised the detection power of each individual study. Therefore, in this study, we performed a large-scale, retrospective analysis to determine the prevalence and clinicopathological features of *ROS1* fusion in Chinese patients with NSCLC.

Methods

Study design

This investigation was a real-world, retrospective, multicenter, epidemiological study of *ROS1* fusion prevalence in patients with NSCLC from 10 hospitals across China. The primary objective of the study was to assess the frequency of *ROS1* gene fusion. The secondary objective was to investigate the correlations between *ROS1* fusion status and demographic and clinical factors.

Patients

Eligible patients had pathologically confirmed NSCLC with *ROS1* fusion detection results. The following data were collected: age, gender, smoking status, pathological type and stage, and tumor node metastasis (TNM) stage. Pathological types and stages were determined according to the 2015 World Health Organization classification.[33] The TNM stage was classified according to 7th edition of the Union for International Cancer Control (UICC)/American Joint Committee on Cancer (AJCC) TNM staging.[34] The Institutional Review Board of Shanghai Chest Hospital approved the study. All patients provided written informed consent before enrollment.

Detection of *ROS1* fusion by quantitative real-time PCR

Total RNAs isolated from formalin-fixed paraffin-embedded (FFPE) tissue from each patient were used to detect *ROS1* fusion with the quantitative real-time (qRT)-PCR based ADx-ARMS ROS1 Gene Fusion Detection Kit, ADx-ARMS ALK/ROS1 Gene Fusion Joint Detection Kit, or ADx-ARMS EGFR/ALK/ROS1 Gene Joint Detection Kit (Amoy Diagnostics Co., Ltd., Xiamen, China), according to the manufacturer's instructions (Table S1). In brief, the qRT-PCR conditions for complementary DNA were as follows: one cycle of 95 °C for 5 minutes; 15 cycles of denaturation at 95 °C for 25 seconds, annealing at 64 °C for 20 seconds, and elongation at 72 °C for 20 seconds to ensure specificity; and up to 31 cycles of 93 °C for 25 seconds, 60 °C for 35 seconds (data collection), and 72 °C for 20 seconds. An external control for each sample and an internal control for each tube were used to check the effects of DNA insufficiency or PCR inhibitors.

Statistical analyses

A two-tailed Student's *t*-test was used to compare the ages of the *ROS1* fusion positive and negative groups. Chi-square or Fisher's exact tests were used to analyze the relationship between *ROS1* fusion and other characteristics of NSCLC, including gender, smoking status, and pathological type and stage. All statistical calculations were performed using R 3.4.1 (R Foundation for Statistical Computing, Vienna, Austria), and $P < 0.05$ was defined as significant with a two-sided test. To better predict *ROS1* fusion frequency, multivariate logistic regression was performed for factors with a P value < 0.05 in the univariate analysis, and the significance level was set at 1% because of the large data set.

Results

Patients

The 6066 patients eligible for this study comprised 3584 men and 2482 women, at an average age of 60.89 ± 10.60 years. The sample types for these 6066 patients were 2011 (33.15%) postoperative pathologic specimens, 181 (2.98%) cytology specimens, and 3874 (63.86%) biopsies.

Positive rate of *ROS1* fusion in non-small cell lung cancer (NSCLC) patients

ROS1 fusions were detected in 157 of the 6066 patients with NSCLC, for a 2.59% positive rate. In the subgroup with known *EGFR* gene and *ALK* fusion status, the positive rate of *ROS1* was 4.36% (68/1559) in patients with *EGFR* wild-type and *ALK* fusion-negative status (Fig 1a).

Correlation analysis of *ROS1* fusion status and characteristics in NSCLC patients

We compared age, gender, smoking history, and pathological types and stages between *ROS1* fusion positive and negative patients. *ROS1* fusion correlated significantly with age, gender, smoking history, pathological type, and N stage, as shown in Table 1.

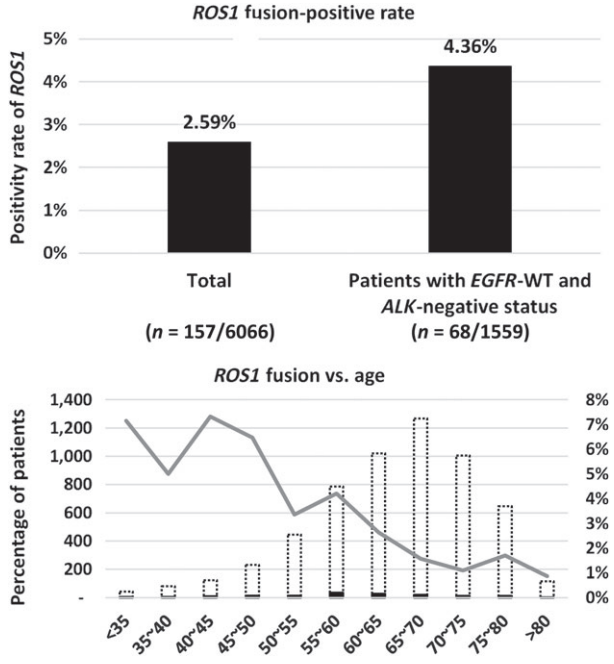

Figure 1 (a) The *ROS1* fusion positive rate among all patients and patients with wild-type *EGFR* and *ALK* negative status. (b) Different age groups in relation to *ROS1* fusion status. (■) *ROS1* positive, (⋯) *ROS1* negative, and (——) *ROS1* positive %.

There was a significant difference in age between *ROS1* fusion positive (56.09 ± 11.38 years) and negative patients (61.23 ± 10.55 years; $P < 0.001$). The positive rate of *ROS1* fusion was higher in women (3.71%, 92/2482) than in men (1.81%, 65/3584; $P < 0.001$) and in patients without a smoking history (3.33%, 111/3329) than in patients with a smoking history (1.21%, 23/1903) ($P < 0.001$). The Fisher's exact test revealed a significant difference in *ROS1* fusion positivity among subgroups classified by pathological type ($P < 0.001$). The positive rate of *ROS1* fusion in patients with adenocarcinoma was higher (2.77%, 136/4912) than in patients with squamous carcinoma (0.93%, 4/430).

Correlation analysis of *ROS1* fusion status with pathological stage showed no significant difference between subgroups classified by P, T, or M stage ($P > 0.05$), whereas the *ROS1* fusion positive rate in patients increased with N stage (1.31%, 1.40%, 2.07%, and 3.23% for N0, N1, N2, and N3, respectively, $P < 0.05$). Distant metastasis did not correlate with *ROS1* fusion status (M0 vs. M1; $P > 0.05$).

Multivariate logistic regression (at the 5% significance level) identified age, smoking status, and N stage (all $P < 0.05$) as independent predictive factors for *ROS1* fusion status (Table 2). Gender and pathology type were no longer significant when stratified by smoking status (Fig 2).

With increasing age, the positive rate of *ROS1* exhibited a decreasing trend. With respect to different age groups, the highest expression of *ROS1* was in the age range of 55–60 years, while the *ROS1* negative population was concentrated in the age range of 65–70 years. Therefore, patients with positive *ROS1* fusion status are younger than those with negative *ROS1* fusion status (Fig 1b).

Discussion

This study is the first real-world, multicenter, retrospective study to investigate the prevalence and clinicopathological characteristics of *ROS1* fusion in Chinese patients with NSCLC. In this study, we found that the *ROS1* fusion positive rate was higher than that reported previously.[15,17,26] We confirmed that *ROS1* fusion was more prevalent in younger patients, women, never-smokers, patients with adenocarcinoma, and patients at more advanced stages (stage III–IV). Patient age, smoking status, and N stage were independent predictive factors for *ROS1* fusion status. Gender and pathology type were not significantly correlated with tumor *ROS1* fusion status when the results were stratified by smoking status.

Our study provides evidence to guide prescreening in NSCLC patients to select a more enriched population who are more likely to harbor this specific fusion. *ROS1* fusion is rare in patients with NSCLC. In 2012, Bergethon *et al.*

Table 1 Summary of *ROS1* fusion prevalence and statistical analysis of subgroups classified by clinicopathological characteristics

	All NSCLC patients			
Features	*ROS1* positive	*ROS1* negative	Total	*P*
Age (years, Mean ± SD)	56.09 ± 11.38	61.23 ± 10.55	61.11 ± 10.60	<0.001†
Gender (*n*, %)				<0.001‡
Female	92 (3.71%)	2390 (96.29%)	2482	
Male	65 (1.81%)	3519 (98.19%)	3584	
Smoking history (*n*, %)				<0.001‡
Non-smoker	111 (3.33%)	3218 (96.67%)	3329	
Smoker	23 (1.21%)	1880 (98.79%)	1903	
NA	23 (2.76%)	811 (97.24%)	834	
Pathological types (*n*, %)				0.01742‡
Adenocarcinoma	136 (2.77%)	4776 (97.23%)	4912	
Squamous carcinoma	4 (0.93%)	426 (99.07%)	430	
Others	17 (2.35%)	707 (97.65%)	724	
Pathological stage (*n*, %)				0.6826§
0	0.00%	16 (100.00%)	16	
I	13 (2.19%)	580 (97.81%)	593	
II	6 (2.18%)	269 (97.82%)	275	
III	34 (3.27%)	1006 (96.73%)	1040	
IV	75 (2.59%)	2824 (97.41%)	2899	
NA	29 (2.33%)	1214 (97.67%)	1243	
T stage (*n*, %)				0.1567§
T1	12 (3.20%)	363 (96.80%)	375	
T2	17 (2.66%)	623 (97.34%)	640	
T3	3 (1.05%)	283 (98.95%)	286	
T4	23 (2.04%)	1102 (97.96%)	1125	
NA	102 (2.80%)	3538 (97.20%)	3640	
N stage (*n*, %)				0.0171§
N0	6 (1.31%)	451 (98.69%)	457	
N1	4 (1.40%)	282 (98.60%)	286	
N2	18 (2.07%)	853 (97.93%)	871	
N3	26 (3.23%)	779 (96.77%)	805	
NA	103 (2.82%)	3544 (97.18%)	3647	
M stage (*n*, %)				1‡
M0	20 (2.29%)	854 (97.71%)	874	
M1	34 (2.29%)	1448 (97.71%)	1482	
NA	103 (2.78%)	3607 (97.22%)	3710	

†Two-tailed Student's *t*-test. ‡Fisher's exact test. §Chi-square test for trend. NA, not available; NSCLC, non-small cell lung cancer; SD, standard deviation.

reported that 18 of 1073 (1.67%) NSCLC tumors had a *ROS1* rearrangement, and all 18 *ROS1* positive tumors were adenocarcinomas (2.59%, 18/694).[15] Our study showed a similar trend, with a *ROS1* fusion prevalence of 2.77% in Chinese patients with adenocarcinoma and extremely rare *ROS1* fusion positive results in patients with non-adenocarcinoma. In our study, patients that were younger, female, without a smoking history, with adenocarcinoma, and at an advanced clinical stage were more likely to harbor a *ROS1* fusion, and such patients should be genetically tested. The recent National Comprehensive Cancer Network Guidelines for NSCLC recommend testing for *ROS1* fusion in all patients with advanced-stage NSCLC regardless of gender, race, smoking history, or other clinical risk factors to guide patient selection for first-line therapy with crizotinib.[35]

Testing methodology also plays a very important role in accurately reflecting the *ROS1* fusion prevalence. In this study, *ROS1* fusions were detected with qRT-PCR kits approved by the China Food and Drug Administration (CFDA) for clinical use. Compared to qRT-PCR, the traditional immunohistochemistry (IHC) assay is simple, inexpensive, and is routinely conducted in pathology laboratories. However, most previous studies have revealed that the IHC assay for *ROS1* expression detection has significant false-positive results because of aneuploidy leading to aberrant expression.[36–38] Fluorescence in situ hybridization (FISH) can be performed even if the concrete fusion partner is unknown and has the potential to discover all *ROS1* fusions in NSCLC. In the PROFILE 1001 clinical trial, FISH was used as a standard method to detect *ROS1*

Table 2 Multivariate logistic regression analysis for *ROS1* fusion status

Comparison	Variable	Regression coefficient estimate	Standard error	Odds ratio estimate (95% CI)	*P*
Smoking vs. age	Intercept	−1.1999	0.4312		
	Smoking	−0.9195	0.2330	0.3987 (0.2525–0.6295)	0.0001
	Age	−0.0378	0.0076	0.9629 (0.9487–0.9774)	0.0000
Age vs. N stage	Intercept	−2.5851	0.8198		
	Age	−0.0311	0.0125	0.9693 (0.9459–0.9933)	0.0126
	N stage	0.3233	0.1465	1.3817 (1.0369–1.8412)	0.0273
Smoking vs. N stage	Intercept	−4.2088	0.3497		
	Smoking	−1.0476	0.3421	0.3508 (0.1794–0.6859)	0.0022
	N stage	0.3826	0.1467	1.4661 (1.0998–1.9545)	0.0091
Smoking vs. gender	Intercept	−3.3045	0.1146		
	Smoking	−0.9080	0.2695	0.4033 (0.2378–0.6840)	0.0008
	Gender	−0.1972	0.2078	0.8210 (0.5463–1.2338)	0.3426
Smoking vs. pathology type	Intercept	−3.6562	0.5932		
	Smoking	−1.0200	0.2480	0.3606 (0.2218–0.5863)	0.0000
	Pathology type	0.3115	0.5944	1.3654 (0.4259–4.3771)	0.6003

CI, confidence interval; SE, standard error.

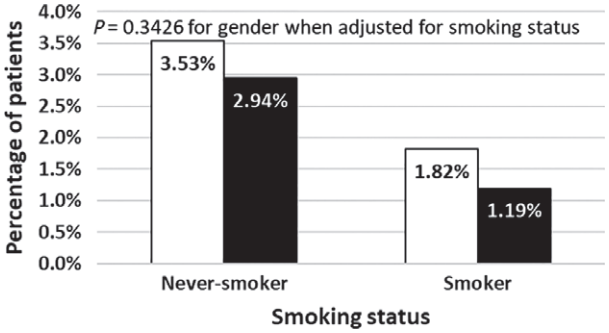

Figure 2 Combined effect of gender and smoking status on the frequency of *ROS1* fusion. (□) Women with *ROS1* fusion positive tumors, and (■) Men with *ROS1* fusion positive tumors.

rearrangement.[28] The qRT-PCR assay is easy to perform, highly sensitive, and relatively inexpensive. In addition, qRT-PCR can identify concrete fusion partners, which can be confirmed by subsequent sequencing if necessary. qRT-PCR cannot discover novel fusion partners other than the known and designed partners. In terms of data interpretation, qRT-PCR is more objective than IHC. For the current real world study, the qRT-PCR method was the only option to detect *ROS1* fusion as there are no CFDA-approved ROS1 IHC or FISH assays for routine clinical practice in China.

Some previous studies have reported that NSCLC patients with *ROS1* fusion share many clinicopathological features with patients harboring *ALK* fusions.[39,40] Similar routes of pathogenesis might exist in these two subtypes of NSCLC, and this possibility is supported by both structural and functional evidence: the *ALK* and *ROS1* kinase domains share 77% sequence homology;[17,40] and *ROS1* signaling and cell viability are substantially inhibited by crizotinib, an *ALK* inhibitor, in cell lines expressing *ROS1* fusions.[15,41] Crizotinib was the first targeted agent approved by the United States Food and Drug Administration for the treatment of advanced *ROS1*-rearranged NSCLC, based on a phase II crizotinib trial. That trial demonstrated an objective response rate of 72% and median progression-free survival of 19.2 months in advanced *ROS1*-rearranged NSCLC patients.[28] The Asian OO12-01 clinical trial, the first and largest prospective phase II trial in East Asian patients with *ROS1* positive advanced NSCLC, reported an overall response rate of 71.7% and median progression-free survival of 15.9 months in *ROS1* fusion patients treated with crizotinib.[42] Based on these data, the Japanese Ministry of Health, Labour and Welfare approved crizotinib for the treatment of metastatic NSCLC with *ROS1* fusion in early 2017, and the AmoyDx ROS1 Fusion Kit was approved simultaneously as the companion diagnostic reagent for crizotinib. This kit was the first officially approved *ROS1* companion diagnostic reagent in the world. Based on evidence from the OO12-01 clinical trial, crizotinib was then approved by the CFDA as a *ROS1* TKI in late 2017. Our findings could facilitate the patient selection process for targeted therapy with *ROS1* inhibitors.

Whether *ROS1* gene alterations influence patient survival remains controversial. In our study, we found that the *ROS1* fusion positive rate was higher in patients with nodal metastasis. Jin *et al.* reported that *ROS1* fusion positive status was highly associated with micropapillary component and aerogenous spread, which has been identified as a marker of aggressive tumor biology.[43] In addition, our study also found that distant metastasis did not correlate with *ROS1* fusion status. However, because of the limited prognostic information, we could not evaluate the clinical implications of *ROS1* rearrangement. Further study is required to evaluate the clinical significance of *ROS1* fusion.

Rare cases of double-positive lung cancer have been reported. In 2017, two patients harboring concomitant *ROS1* and *ALK* fusions were reported in the literature.[44,45] In our study, we found only one patient with co-occurring *ROS1* and *ALK* fusions, suggesting that the co-occurrence is rare in Chinese NSCLC patients. Currently, there is no consensus on standard therapy for tumors with double-positive mutations or fusions. If concurrent driver mutations are identified, molecular diagnosis should be confirmed before proceeding with targeted therapy. *ROS1* fusion was more prevalent in *EGFR* negative and *ALK* negative patients (4.36%), indicating that combined detection of *EGFR* mutations and *ALK* and *ROS1* fusions would increase patient benefits from targeted therapy. With the 15 wide use of *ROS1* inhibitors expected in the near future, 16 accurate and extensive diagnosis of *ROS1* fusions in NSCLC is essential for clinical practice.

In summary, the positive rate of *ROS1* fusion in Chinese patients with NSCLC was 2.59%, whereas in *EGFR* wild-type and *ALK* negative patients, the positive rate of *ROS1* fusion was 4.36%. Our results showed that *ROS1* fusion was more prevalent in patients that were younger, female, without a smoking history, with adenocarcinoma, and at advanced stages. The prevalence of *ROS1* gene fusion was 2.77% in patients with adenocarcinoma and was significantly lower (0.93%) in patients with squamous carcinoma. The observed frequency of tumor *ROS1* fusion in demographic and clinical subgroups of Chinese patients suggests that *ROS1* fusion testing should be considered for all NSCLC patients with stage IIIB/IV adenocarcinoma. Such an approach will help ensure the optimal identification and treatment of patients whose tumors harbor a *ROS1* fusion.

Disclosure

No authors report any conflict of interest.

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Supporting Information

Additional Supporting Informationmay be found in the online version of this article at the publisher's website:

Table S1. *ROS1* fusion genes detectable by the AmoyDx assay.

