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Comparison of Rearranged During Transfection (*RET*) Gene Rearrangements in Primary *Versus* Metastatic Non-Small Cell Lung Cancer (NSCLC)

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Background: *RET* rearrangements have been reported in 30% of papillary thyroid carcinomas and 1–2% of non-small cell lung cancer (NSCLC). In these tumors, *RET* gene fusion product provides a constitutively active tyrosine kinase (TKR), leading to uncontrolled cellular proliferation, differentiation, and migration. In this investigation we assessed the positivity rate of *RET* gene rearrangement in primary and metastatic non-small cell lung cancer and explored their relationships.

Material/Methods: Between January 2013 and May 2015, we collected 384 cases of primary metastatic non-small cell lung cancer, which included 246 matched metastatic tumors cases from multiple centers. The *RET* rearrangement uniformity in metastatic lymph nodes and tumor specimens were contrasted and the relationships between *RET* rearrangement and patients' clinical features were investigated.

Results: For those 384 cases, 7 (1.82%) cases had tumors with identified *RET* rearrangement. Among the 246 paired cases, 3 (1.22%) cases of primary tumor had identified *RET* rearrangement and 2 (0.81%) cases of metastases had identified *RET* rearrangement. The sensitivity was 66.67% (2/3) and the specificity was 100% (243/243).

Conclusions: The results of this research indicate that the metastases of non-small cell lung cancer can predict *RET* rearrangement of the primary tumor tissue in the majority of cases. Testing for *RET* rearrangement in metastases can be used as an alternative to testing of primary tumor tissue if it is inaccessible.

MeSH Keywords: Genetic Heterogeneity • Lung Neoplasms • Transfection

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Background

Lung cancer is a major cause of cancer-related mortality worldwide, and it is classified into small cell lung cancer and non-small cell lung cancer. Non-small cell lung cancer represents about 80% of all lung cancers, which includes adenocarcinoma, squamous cell carcinoma, and large cell carcinoma [1]. Recent progress in sequencing technology has facilitated the detection of gene rearrangements in the cancer genome and transcriptome, and chromosomal rearrangements involving receptor tyrosine kinases (RTKs) are considered as drivers of cancer progression.

Rearrangements in the rearranged during transfection (*RET*) gene, including inversions on chromosome 10 or translocations with other chromosomes involving different gene partners, have been reported in 30% of papillary thyroid carcinomas and in 1–2% of NSCLC [2]. The *RET* gene rearrangement product provides a constitutively active RTK, leading to uncontrolled cellular proliferation, differentiation, and migration [3–6]. Targeted therapy has reshaped the therapeutic landscape for patients with lung cancers [7]. *RET* rearrangements have been associated with clinical benefit from multikinase inhibitors such as cabozantinib and vandetanib [8]. At least 12 forms of *RET* rearrangements have been identified in NSCLC, including *KIF5B-RET*, *CCDC6-RET*, *NCOA4-RET*, *MYO5C-RET*, *EPHA5-RET*, *CLIP1-RET*, *ERC1-RET*, *PICALM-RET*, *FRMD4A-RET*, *RUFY2-RET*, *TRIM24-RET*, and *TRIM33-RET* gene fusions. Kinesin family member 5B (*KIF5B*) has been identified as the most common partner combined with *RET* (72%) to date [9]. However, further molecular screening for *RET* fusions is warranted. *RET* rearrangements have mainly been discovered in younger patients aged <60 years, who are former light smokers or never smokers [10]. In this investigation, the aim was to assess the positivity rate of *RET* gene rearrangement in primary and metastatic non-small cell lung cancer and their relationships with clinical characteristics.

Material and Methods

Sample collection

Between January 2013 and May 2015, we collected patients with pathologically confirmed non-small cell lung cancer from multiple centers. Eligible patients were enrolled from Fujian Cancer Hospital, Zhejiang Rongjun Hospital, and Zhejiang Cancer Hospital, China. The diagnosis of non-small cell lung cancer was determined according to pathological examination of the lesion, and the histological type was based on the World Health Organization (WHO) standards [11]. The stage of tumor was determined according to the 7th version of the Tumor, Node, and Metastasis (TNM) Classification of Lung Cancer [12]. All Ethics Committees of the 3 institutions evaluated and authorized the study. All the patients provided informed consent to take part in this research and agreed to use of their pathological specimens. None of the patients received any neoadjuvant treatment before the study. Surgery and biopsy samples from 384 NSCLC patients were examined for *RET* rearrangements, including matched primary and metastatic samples from 246 patients.

RET detection

Paraffin-embedded tissues (4–8 slices) were cut into 4-μm slices and dewaxed. *RET* was detected using a *RET* Detection Kit (Amoy Diagnostics, Xiamen, China) based on reverse transcriptase-polymerase chain reaction. Genomic RNA was extracted based on the kit instructions, using EB solution as a blank control, and 1 μl of the RNA sample was amplified using an ABI7500 real-time fluorescence quantitative PCR instrument (Applied Biosystems Life Technologies, Foster City, CA, USA) according to the methods provided in the 9 *RET* fusion detection kits for lung cancer. Positive and negative controls were established as described in the kit instructions. *RET* rearrangements were detected using a method previously described. *RET* gene rearrangements were detected and compared between primary and metastatic tissue samples. The relationships between *RET* rearrangements and clinical data were also analyzed statistically.

Statistical and database analyses

The prevalence of *RET* rearrangements was compared between primary and metastatic tissues using the χ^2 test. For clinical characteristics, categorical variables were evaluated with the Fisher's exact test. *P* values of less than 0.05 were statistically significant; $\kappa>0.75$ was considered remarkable consistency, $0.4\leq\kappa\leq0.75$ represented good consistency, and $\kappa<0.4$ represented inconsistency. All analyses were completed using SPSS software (version 19.0 for Windows, IBM Corp., Armonk, NY, USA).

Results

RET rearrangements in primary and metastatic tissue samples

Altogether, 384 NSCLC patients participated in this study. *RET* rearrangements were detected in 1.82% (7/384) of primary tumors. Among the 246 paired primary and metastatic samples, *RET* rearrangements were detected in 1.22% (3/246) of primary tumors and 0.81% (2/246) of metastases. All patients who had *RET* rearrangement in the metastatic sample had *RET* rearrangement in the primary sample, but 1 patient had rearrangement in the primary but not the metastatic sample

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Table 1. *RET* rearrangement in advanced primary NSCLC tissues and matched metastatic samples.

M	Cases (n=246)	P		P value	κ value
		+	–		
+	2	2	0	<0.001	0.798
–	244	1	243		
Cases	246	3	243		

M – metastatic samples; NSCLC – non-small cell lung cancer; P – primary cancerous tissue.

Table 2. Correlation of *RET* rearrangement in advanced primary NSCLC tissue and basic patient characteristics.

Characteristics	Cases (n=384)	*RET* rearrangement	*RET* non-rearrangement	%	χ^2 and P values
Sex					
Male	181	3	178	1.66%	χ^2=0.000 P=1.000
Female	203	4	199	1.97%	
Age (year)					
≥60	189	2	187	1.06%	χ^2=0.520 P=0.471
<60	195	5	190	2.56%	
Smoking status					
Smoker	177	2	175	1.13%	χ^2=0.309 P=0.578
Non-smoker	207	5	202	2.42%	
Pathological type					
Adenocarcinoma	242	7	235	2.89%	χ^2=2.724 P=0.099
Non-adenocarcinoma	142	0	142	0	

NSCLC – non-small cell lung cancer.

(Table 1). The prevalence of *RET* rearrangements was significantly higher in primary lesions compared with metastases (χ^2=91.117, P<0.001). *RET* rearrangement in the primary tumor were predicted by rearrangement in the corresponding metastasis (κ=0.798, P<0.001), with a sensitivity of 66.67% (2/3) and specificity of 100% (243/243).

RET rearrangement and clinical characteristics

Among the 384 cancerous tissue specimens, active *RET* rearrangement was detected in 7, giving a rearrangement rate of 1.82%. The frequency of *RET* rearrangement in primary cancerous tissue was not significantly related to patient age, sex, pathological type, or smoking history (P>0.05) (Table 2). There was also no relationship between *RET* rearrangement in the primary or metastatic samples and clinical details among the 246 patients with paired primary and metastatic samples (Tables 3, 4).

Discussion

Recent progress in sequencing technology has enabled the extensive detection of gene rearrangements in the cancer genome and transcriptome. Chromosomal rearrangements involving RTKs are an important class of cancer-related somatic variation and have emerged as oncogenic drivers in solid tumors and hematologic malignancies [13,14]. The main potentially targetable gene fusions in NSCLC involve the *ALK*, *ROS1*, *NTRK*, and *RET* genes. Although these represent a small fraction of NSCLC patients (3–7%, 3.3%, 1–2%, and 0.7–2%, respectively) [15–19], the significance of treating these rare chromosome rearrangements is profound, given that about 1.8 million new cases of lung cancer per year are reported worldwide [20].

RET gene fusion accounts for about 1% to 2% of all NSCLC [2], with a high rate of *KIF5B-RET* gene fusion (72%) [8,9]. *RET* gene fusion rarely occurs simultaneously with other driver

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Table 3. Correlation of *RET* rearrangement in primary and metastasis tissue paired with advanced primary NSCLC tissue and basic patient characteristics.

Characteristics	Cases (n=384)	*RET* rearrangement	*RET* non-rearrangement	%	χ^2 and P values
Sex					
Male	121	1	120	0.83%	χ^2=0.000 P=1.000
Female	125	2	123	1.60%	
Age (year)					
≥60	112	1	111	0.89%	χ^2=0.000 P=1.000
<60	134	2	132	1.49%	
Smoking status					
Smoker	113	1	112	0.88%	χ^2=0.000 P=1.000
Non-smoker	133	2	131	1.50%	
Pathological type					
Adenocarcinoma	145	3	142	2.07%	χ^2=0.747 P=0.388
Non-adenocarcinoma	101	0	101	0	

NSCLC – non-small cell lung cancer.

Table 4. Correlation of *RET* rearrangement in primary and metastasis tissue paired with advanced metastatic NSCLC tissue and basic patient characteristics.

Characteristics	Cases (n=384)	*RET* rearrangement	*RET* non-rearrangement	%	χ^2 and P values
Sex					
Male	121	1	120	0.83%	χ^2=0.000 P=1.000
Female	125	1	124	0.80%	
Age (year)					
≥60	112	1	111	0.89%	χ^2=0.000 P=1.000
<60	134	1	133	0.75%	
Smoking status					
Smoker	113	1	112	0.88%	χ^2=0.000 P=1.000
Non-smoker	133	1	132	0.75%	
Pathological type					
Adenocarcinoma	145	2	143	1.38%	χ^2=0.215 P=0.643
Non-adenocarcinoma	101	0	101	0	

NSCLC – non-small cell lung cancer.

genes, such as *EGFR*, *ALK*, or *KRAS* [21], suggesting that *RET* fusion genes are an independent driver in NSCLC. Patients with NSCLCs harboring *RET* rearrangements can be sensitive to cytotoxic chemotherapies, including pemetrexed-based regimens, which have an objective response rate (ORR) of 45% and median progression-free survival 19 months, similar to

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ALK- or *ROS1*-rearranged lung cancers [22]. However, although the effects of multi-targeted tyrosine kinase inhibitors have been studied in *RET* fusion cell lines, randomized clinical trials and retrospective clinical trials assessing the activity of these drugs in *RET*-positive NSCLC patients are still rare. No RET-selective inhibitors have been approved to date, but several multi-targeted drugs with anti-RET activity, including vandetanib, cabozantinib, sunitinib, and sorafenib, have been evaluated in preclinical models and clinical trials [9]. Furthermore, a new targeted kinase inhibitor, BLU-667, has recently emerged, which acts as a potent and selective RET inhibitor and induced tumor regression in cancer models with *RET* mutations and fusions. BLU-667 showed an efficacy rate of 46% and a control rate >90% in patients with *RET* mutations or gene fusions [23], and it may thus revolutionize the treatment of *RET*-altered tumors.

In the present study, we detected *RET* gene rearrangements in 384 patients with advanced primary NSCLC by RT-PCR and found a *RET* gene rearrangement rate of 1.82%, which was consistent with the results of Takeuchi et al. [18]. We also found a higher prevalence of *RET* gene rearrangements in primary compared with metastatic lesions (χ^2=91.117, P<0.001). The presence of *RET* rearrangement in the primary tumor could be predicted by rearrangement in the corresponding metastatic lesion (κ=0.798, P<0.001) with a sensitivity of 66.67% (2/3) and specificity of 100% (243/243). Furthermore, Wang et al. found that patients with *RET* rearrangements had small primary lesions (<3 cm) but were more likely to have N2 disease compared with other LADCs with small lesions (54% *vs.* 23%) [16], meaning that primary samples are more difficult to obtain than metastatic specimens. However, our results suggest that metastatic samples may be used as a surrogate to predict *RET* gene rearrangement in the primary tumor when it is difficult to acquire the primary tumor tissue.

Wang et al. and Gautschi et al. found that *RET* gene fusion was more common in patients who had never smoked or who had lung adenocarcinomas (LADCs) [8,16]. However, our results showed no significant relationship between the presence of *RET* gene rearrangements and any of the tested clinical characteristics, based on either primary or metastatic tumor samples. No *RET* rearrangements were identified in non-adenocarcinomas, but the overall sample size was too small to demonstrate a statistically significant difference in *RET* rearrangement prevalence between adenocarcinomas and non-adenocarcinomas. It is possible that our sample size was too small to demonstrate a significant difference. It is also possible that the apparent discrepancy with previous studies was due to dissimilarities in sample size, race, and/or the rate of lung adenocarcinoma and squamous cell cancer.

Conclusions

Our results show that *RET* gene fusion status differed between metastatic and primary tumors. For most molecular alterations, there is no good evidence to favor testing of either primary or metastatic tumors. It is therefore important to establish, for each driver alteration in NSCLC, whether the alteration is homogeneous or heterogeneous between primary and metastatic tumor. In conclusion, there is growing evidence to suggest that testing for *RET* gene rearrangements will be important in personalizing treatment of NSCLC in the future. We showed that *RET* rearrangement in NSCLC metastases could predict rearrangement in the primary lesion in the majority of cases, and it could thus be used as an alternative means of detecting *RET* rearrangements in cases where it is difficult to obtain a primary specimen. Nevertheless, molecular targeted therapy should consider the possible heterogeneity of gene rearrangements between primary and metastatic samples. Furthermore, although we found no significant association between *RET* gene rearrangements in either primary or metastatic samples and clinical characteristics, this may have been due to the small sample size, and further studies with larger samples are needed to verify our results.

Conflict of interest

None.

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