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LETTER TO THE EDITOR

Identification of *RET* gene fusion by exon array analyses in "pan-negative" lung cancer from never smokers

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Dear Editor,

The incidence of lung cancer from never smokers has increased dramatically in China nowadays. Strikingly, approximately 30% of the lung cancer patients in East Asian population are never smokers [1, 2]. The majority of these patients are females with lung adenocarcinomas [2]. Identification of oncogenic drivers, which the tumors are "addicted to" and rely on for survival, has significantly reformed the current strategies for lung cancer treatment in clinic and initiated the era of personalized therapy [3]. Therapeutics specifically targeting EGFR mutations, frequently observed in never smoker patients with lung cancer, have been very helpful in improving the clinical symptoms as well as the progression-free survival [4-6]. Similarly, patients with lung tumors positive for *ALK* fusions also benefit from ALK-targeted therapy [7, 8].

Our previous efforts have constructed a quite comprehensive map of those essential oncogenic drivers in 52 lung adenocarcinomas from never smokers [9]. We have uncovered the oncognic drivers in about 90% of these lung tumors including mutations of *EGFR*, *HER*2, *KRAS*, as well as *EML4-ALK* fusion [9], thus providing a strong clinical guidance for molecular-targeted therapy for this subset of disease. However, there is still about 10% (5/52) of these never smoker patients were "pan-negative" for all known oncogenic driver mutations and could not benefit from the effective targeted therapy in clinic.

Similar to oncogenic gene mutations, gene fusions such as *EML4-ALK* or *CD74-ROS1* are also essential for lung cancer development [8, 10], and serve as effective therapeutic targets. Since great efforts have been paid in searching gene mutations, we instead focus on our efforts in identification of novel oncogenic gene fusions. Previous studies have demonstrated that exon array analyses are capable of detecting gene fusions based on the differential expression of the exons located at either side of the breakpoint, which is frequently resulted from genomic translocation [7, 11, 12]. For example, in the case of *ALK* fusion, the expression levels of *ALK* exon 1-20 and exon 21-29 flanking the breakpoint are significantly different and can be readily detected by exon array analyses [7, 11, 12]. Therefore, we performed exon array (Affymetrix Exon 1.0) using all the five "pan-negative" samples plus another 12 samples with known oncogenic drivers to search novel oncogenic gene fusions. We initially identified about 1 000 potential gene fusions from exon array analyses. Since most of known oncogenic drivers are kinases, we manually went through all the heatmaps of those potential kinase fusions. Interestingly, we identified one potential *RET* fusion, with an obvious expressional change between exon 11 and exon 12, in the "pan-negative" lung cancer sample 181LC (Figure 1A). We then preformed the 5′ RACE assay to detect the partner of this potential *RET* fusion. We found that the DNA band obtained from 5′ RACE (about 1.4 kb) is actually the fusion of *RET* exon 12 to *CCDC6* exon 1 (Figure 1B-1C), which has been previously reported in human thyroid carcinomas [13]. This *RET* fusion is derived from somatic genetic alteration since it is undetectable in paired normal lung tissue 181NL (data not shown). To clone the genomic breakpoint, we further designed a series of primers (22 forward primers at *CCDC6* intron 1 with 1-3 kb intervals, and a reverse primer at *RET* exon 12) and performed long-range PCR using genomic DNA from the "pan-negative" lung cancer sample 181LC. Interestingly, we found that the intron 1 of *CCDC6* is fused to a part of *RET* exon 11 at genomic DNA level (Figure 1D-1E), which results in the expression of *CCDC6-RET* fusion (*CCDC6* exon 1 fused to *RET* exon 12) after RNA splicing. Previously studies have shown that RET signaling pathway promotes cell survival and cell proliferation through RAS-ERK pathway and PI3K-AKT pathway [14]. *RET* fusions, mainly found in papillary thyroid carcinomas [15], are oncogenic drivers and capable of transforming thyroid epithelial cells *in vitro* as well as inducing papillary thyroid carcinoma in transgenic mice [16, 17, 18]. We found that the *CCDC6-RET* fusion from lung cancer sample 181LC is undetectable in the rest of 4 "pan-negative" samples as well as those with known oncogenic driver mutations from never smokers (data not

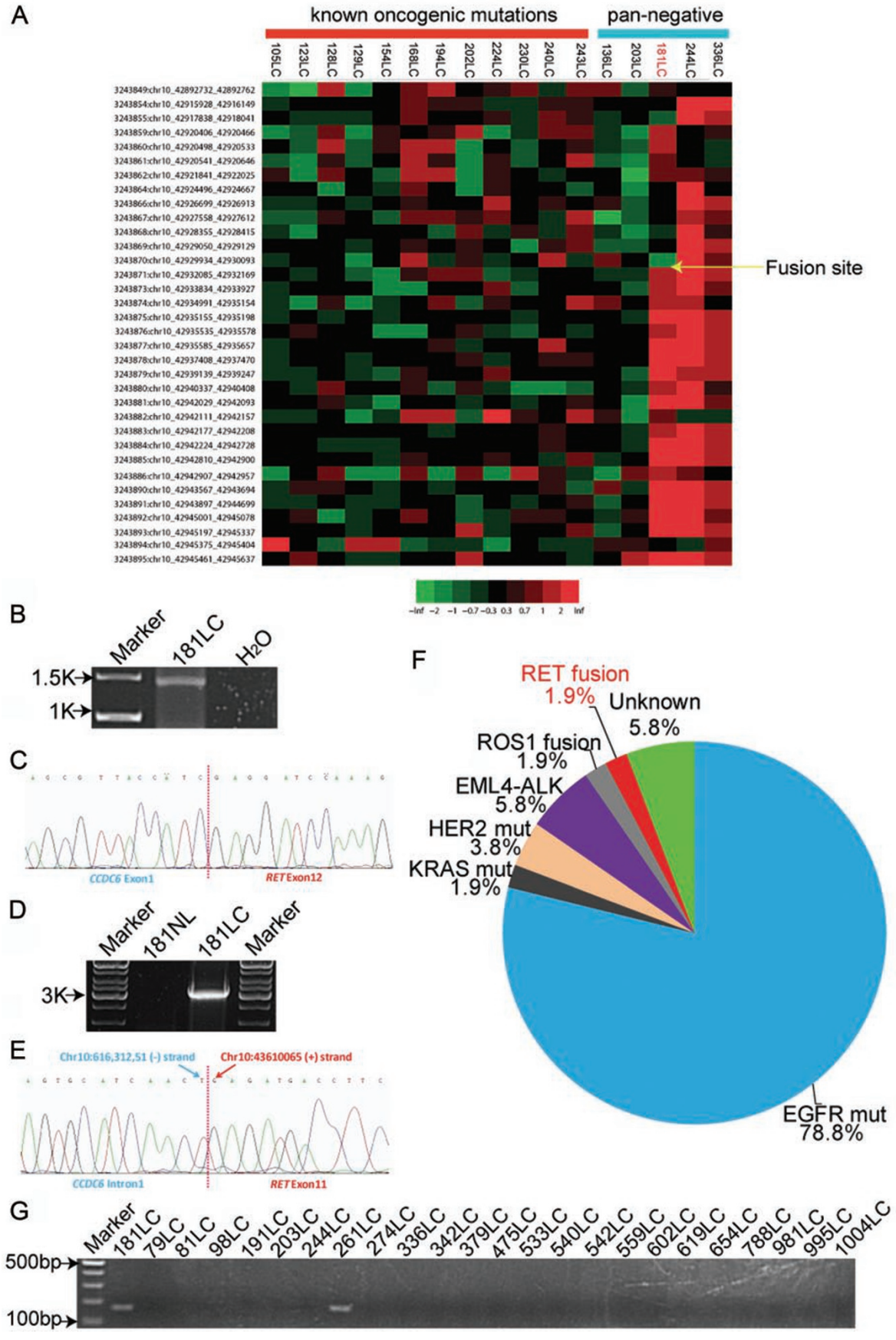
A
known oncogenic mutations
pan-negative
105LC 123LC 128LC 129LC 154LC 168LC 194LC 202LC 224LC 230LC 240LC 243LC 136LC 203LC 181LC 244LC 336LC
Fusion site
−Inf −2 −1 −0.7 −0.3 0.3 0.7 1 2 Inf
B
Marker
181LC
H2O
1.5K
1K
C
CCDC6 Exon1
RET Exon12
D
Marker
181NL
181LC
Marker
3K
E
Chr10:616,312,51 (−) strand
Chr10:43610065 (+) strand
CCDC6 Intron1
RET Exon11
F
RET fusion 1.9%
Unknown 5.8%
ROS1 fusion 1.9%
EML4-ALK 5.8%
HER2 mut 3.8%
KRAS mut 1.9%
EGFR mut 78.8%
G
Marker
181LC
79LC
81LC
98LC
191LC
203LC
244LC
261LC
274LC
336LC
342LC
379LC
475LC
533LC
540LC
542LC
559LC
602LC
619LC
654LC
788LC
981LC
995LC
1004LC
500bp
100bp

Figure 1 Identification of *CCDC6-RET* fusion in the "pan-negative" lung adenocarcinomas from never smokers and the construction of a more comprehensive spectrum of oncogenic drivers in this subset of lung cancer. **(A)** Exon array analyses of 5 samples "pan-negative" for oncogenic drivers and 12 samples with known oncogenic drivers have identified a potential *RET* fusion in lung cancer sample 181LC. The potential fusion point is indicated by yellow arrow. **(B)** 5′ RACE analyses of the "pan-negative" lung cancer sample 181LC showed a PCR band about 1.4 kb, which is obviously different from the wild-type *RET* band (about 960 bp). **(C)** Sequencing result confirmed the *CCDC6-RET* fusion in lung cancer sample 181LC. **(D)** Long-range PCR detection of the genomic breakpoint of *CCDC6-RET* fusion in lung cancer sample 181LC. **(E)** Sequencing result showed the detailed genomic breakpoint of *CCDC6-RET* fusion in lung cancer sample 181LC. **(F)** A more comprehensive spectrum of oncogenic drivers is constructed for the cohort containing 52 lung adenocarcinomas from never smokers. **(G)** Detection of *CCDC6-RET* fusion in 24 "pan-negative" samples identified from the 202 lung adenocarcinomas from never smokers. Except for lung cancer sample 181LC, 261LC also harbors the *CCDC6-RET* fusion. LC: lung cancer. NL: normal lung.

shown), consistent with the mutual exclusive pattern of oncogenic drivers. Together with our recent identification of the *CD74-ROS1* fusion in another "pan-negative" sample [9, 19], we have further improved our original work and uncovered the oncogenic drivers in about 94% (49/52) of these lung adenocarcinoma from never smokers [10]: *EGFR* mutations (78.8%), *HER2* mutations (3.8%), *KRAS* mutations (1.9%), *EML4-ALK* fusions (5.8%), *CD74-ROS1* fusion (1.9%) and *CCDC6-RET* fusion (1.9%) (Figure 1F).

Recently we have expanded the study of oncogenic mutation spectrum from the original 52 sample set to a large cohort with additional 150 samples and identified a total of 24 "pan-negative" lung adenocarcinomas from never smokers [19]. In an effort to detect the *RET* fusion in these "pan-negative" lung cancer samples, we found another lung cancer sample 261LC positive for *CCDC6-RET* fusion (Figure 1G). No other *RET* fusions including the recently reported *KIF5B-RET* fusion were found in these "pan-negative" samples [20] (data not shown). Interestingly, both patients with *RET* fusion are females; one is 46 years old with lung adenocarcinoma and the other is 61 years old with bronchioloalveolar carcinoma. Collectively, about 1% (2/202) of lung adenocarcinomas from never smokers harbors the *CCDC6-RET* fusion. Our data have not only provided a method for detection of novel oncogenic gene fusions but also constructed a more comprehensive map for oncogenic drivers in this subset of disease, which potentially helps develop the strategies for molecular-targeted therapies in clinic.

Experimental materials and methods are depicted in the Supplementary information, Data S1.

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(**Supplementary information** is linked to the online version of the paper on the *Cell Research* website.)