An activating ALK gene mutation in ALK IHC-positive/FISH-negative nonsmall-cell lung cancer

ALK inhibitors (i.e. crizotinib and alectinib) exhibit marked antitumor activity against nonsmall-cell lung cancer (NSCLC) with ALK rearrangements [1, 2]. The detection of ALK rearrangements is mainly carried out using fluorescence in situ hybridization (FISH). However, such analyses can yield false-positive and false-negative results [3, 4]. Other ALK diagnostic techniques have been developed, including immunohistochemistry (IHC) for the detection of the ALK protein [5]. Several studies have shown that IHC is sensitive and specific for the determination of ALK protein expression and is an accessible, cost-effective, and rapid alternative to the ALK FISH assay. In addition, some authors have reported significant clinical improvement with crizotinib in patients with tumors that were designated as ALK-negative using FISH but were found to be ALK-positive using IHC, raising the possibility that the FISH assay may miss cases that could benefit from ALK inhibitors [4]. Recently, Ilie et al. reported that the discrepancies observed between IHC and FISH data can reflect unexpected biological events, rather than technical issues, which could potentially have a major impact on therapeutic strategies involving ALK inhibitors [3]. In our present study, we discovered an ALK kinase domain mutation (R1192G) in an ALK IHC-positive but FISH-negative NSCLC specimen (Figure 1A). ALK was phosphorylated when this mutation was overexpressed in the HEK293 cell line (Figure 1B). A focus formation assay using the NIH-3T3 cell line showed that the ALK R1192G mutation had transformational abilities, compared with the controls (Figure 1B). The ALK mutation-overexpressed Ba/F3 cell line showed IL-3-independent growth and was sensitive to ALK inhibitors (crizotinib and alectinib) (Figure 1C). The sensitivity of the cell lines to drugs was consistent with the suppression of phospho-ALK (Figure 1C). In vivo, the growth of tumors from the Ba/F3-ALK R1192G cell line was reduced by alectinib treatment in a dose-dependent manner (Figure 1D). These findings indicate that ALK inhibitors are effective against NSCLC cells carrying the ALK R1192G mutation.

In conclusion, we identified an ALK-activating mutation in an NSCLC clinical sample with ALK IHC-positive/FISH-negative findings and showed that ALK inhibitors can be effective against NSCLC cells carrying this mutation. Because clinical data are lacking, further clinical testing to validate the use of ALK inhibitors for patients with NSCLC carrying this mutation should be carried out. To ensure that candidates for treatment with ALK inhibitors are not missed, further comprehensive analyses, such as NGS, should be introduced into clinical practice.

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Figure 1. (A) Immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), and direct sequencing for ALK in clinical samples. Case 1 (63-year-old man, past smoker) was focally positive, case 2 (65-year-old man, past smoker) was negative, and case 3 (68-year-old woman, never smoker) was positive for ALK IHC. The ALK FISH split assay showed merged signals in cases 1 and 2, suggesting no rearrangement of ALK, but showed split 5'-side (green) and 3'-side (red) signals in case 3, suggesting an ALK rearrangement. ALK exon 23 sequencing revealed an ALK R1192G mutation (C > G) in case 1. We used case 2 as a wild-type control. Scale bars, 20 µm. (B) Phosphorylation of ALK in the transfected HEK293 cell lines and transformational ability of each ALK mutation. The expressions of ALK in the transfected cell lines were confirmed using western blot analyses. ALK was phosphorylated in the HEK293-ALK F1174L (positive control) and HEK293-ALK R1192G cell lines, compared with the HEK293, HEK293-EGFP, and HEK293-ALK wild-type cell lines. β-Actin was used as an internal control. Based on a focus formation assay and the NIH-3T3 cell lines, both ALK mutations exhibited transformational abilities. A cell line overexpressing the KRAS G12D mutation was used as a positive control. Scale bars, 50 µm. (C) Sensitivities of the transfected Ba/F3 cell lines to ALK inhibitors and phosphorylation level of ALK after exposure to ALK inhibitors. Both ALK inhibitors (crizotinib and alectinib) were effective against each mutant-driven cell lines, while the Ba/F3-KRAS G12D cell line was not sensitive to the inhibitors. The respective 50% inhibitory concentrations (IC50) of each Ba/F3 cell line to crizotinib and alectinib were 0.039 and 0.0062 µM for F1174L and 0.042 and 0.0078 µM for R1192G. In contrast, the IC50 values of the Ba/F3-KRAS G12D cell line to crizotinib and alectinib were 0.87 and 1.38 µM, respectively. The phosphorylation levels were measured in samples collected 3 h after exposure to the ALK inhibitors (0, 0.003, 0.01, and 0.03 µM). The phosphorylation levels were measured in samples collected 3 h after exposure to the ALK inhibitors (0, 0.003, 0.01, and 0.03 µM) using western blot analyses. Both inhibitors induced a significant decrease in the phosphorylation levels of ALK in the transfected cell lines. β-Actin was used as an internal control. Line, mean of independent triplicate experiments. (D) Xenograft study. We used the Ba/F3-ALK R1192G cell line in the xenograft study. In the treatment groups, alectinib (20 or 60 mg/kg) was administered by oral gavage daily for 10 days; the control animals received 0.5% methylcellulose as the vehicle. Tumor growth from the Ba/F3-ALK R1192G cell line was reduced by treatment with alectinib in a dose-dependent manner [vehicle: 1345.49 ± 269.84 mm3 versus alectinib (20 mg/kg): 838.1 ± 127.43 mm3, P = 0.0052* or versus alectinib (60 mg/kg): 550.0 ± 158.6 mm3, P = 0.0004*]; alectinib (20 mg/kg) versus alectinib (60 mg/kg), P = 0.013*]. Lines, mean of values for five mice; error bars, SD; *P < 0.05.

disclosure

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references


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