

Letter to the Editor

Detection of *EML4-ALK* and Other *ALK* Fusion Genes in Lung Cancer: A Lesson from the Leukemia Fusion Gene Analysis and Future Application

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We read with interest the article “*EML4-ALK* Fusion Gene in Korean Non-Small Cell Lung Cancer” in a recent issue of the Journal of Korean Medical Science by Jin et al. (1). In this study, *EML4-ALK* fusion gene was detected in 10 of 167 non-small cell lung cancer (NSCLC) patients by using reverse transcriptase-polymerase chain reaction (RT-PCR) as a basic screening technique instead of fluorescence in situ hybridization (FISH) method. The frequency of *EML4-ALK* fusion gene in this study (6.0%) was not largely different from a previous study that used the FISH method on Korean NSCLC patients (4.2%) (2), while it did not diverge from the results in previous studies on a general NSCLC patient population that yielded 3%-7% ratios as well. Based on the experience of detecting leukemia fusion genes with several new molecular methods during the diagnosis of leukemia (3-6), we would like to mention the pros and cons of such methods in the analysis of *EML4-ALK* fusion genes and introduce the usefulness of long distance- (LD-) or long distance inverse-polymerase chain reaction (LDI-PCR) on solid tumors including lung cancer.

As correctly mentioned by Jin et al. (1), the shortcomings of *ALK* breakpoint FISH test include its inability to identify accurate subtypes of the *EML4-ALK* fusion gene and discriminate the changes in *ALK* partner genes, which is due to the characteristics of the breakpoint FISH probe. To confirm the presence of a partner gene or the fusion gene subtype, additional methods such as PCR would be always necessary. In that sense, the fact that Jin et al. (1) used RT-PCR as a molecular screening method was a good approach to overcome the above mentioned limitations of FISH. However, it should be noted that previous studies have indicated a number of variant *EML4-ALK* fusion gene subtypes which could be missed if not designing a complete set of multiplex oligonucleotides (7). This is one of the important limitations of RT-PCR in fusion gene analysis that Jin et al. (1) have overlooked. Furthermore, *ALK* gene has additional fusion

partners such as *TGF*, *KIF5B*, and *KLC1* in lung cancer (8).

We believe that the established methodologies concerning *MLL* rearrangements in leukemia patients could be a prototypic example of similar technical pitfalls. As widely known, *MLL* rearrangements have more than 70 partner genes while each *MLL* rearrangement can also show various types of fusion genes due to the involvement of different introns of the *MLL*- and the corresponding partner gene (3). As mentioned in our recent paper, “Diagnostic standardization of leukemia fusion gene detection system using multiplex reverse transcriptase-polymerase chain reaction in Korea,” (6) these characteristics limit a complete detection of all *MLL* rearrangements in any multiplex RT-PCR method that has been developed so far. This is also pertinent to the reason why FISH has been used as a screening method in various leukemia fusion gene analyses, despite its aforementioned limitations. Moreover, another important limitation of the RT-PCR method is that it uses RNA and a subsequent complementary DNA (cDNA) as clinical specimens that are relatively unstable (compared to genomic DNA). In fusion gene analyses on hematologic malignancies, it is not uncommon to encounter such a situation where an alternative method is required because of the unstable nature of RNA samples as well as the unavailability of the sample in the first place. Recently, we also have been trying to resolve such limitations of FISH and RT-PCR methods in our molecular analyses on leukemia patients that showed fusion genes other than *MLL* rearrangements by using a LD-/LDI-PCR method (9-11).

The principles of LD-/LDI-PCR were explained in our previous papers in detail (3, 4). To briefly elaborate, LD-/LDI-PCR is a fusion breakpoint analysis method that uses stable genomic DNA and incorporates both the sensitivity of the FISH method that can screen all types of fusion genes and the specificity of the RT-PCR method that can confirm specific subtypes of a fusion gene. Fusion gene analysis using such LD-/LDI-PCR method has not only been successfully implemented in the diagnostic schemes for leu-

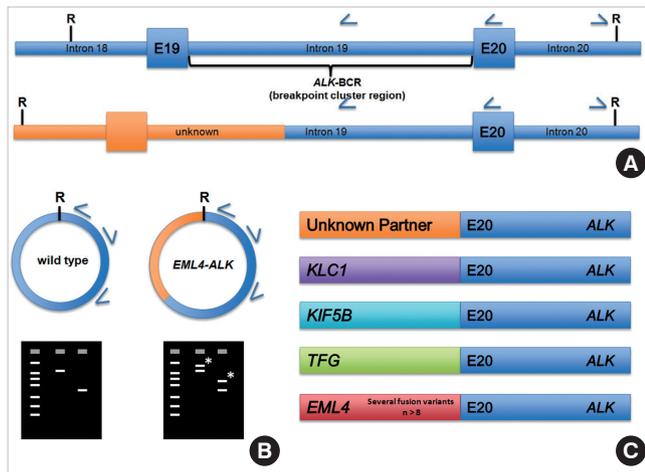


Fig. 1. Schematic representation of long distance inverse-polymerase chain reaction (LDI-PCR) for analyzing *ALK* rearrangements. LDI-PCR can analyze any kind of *EML4-ALK* fusion variant and other (or unknown) *ALK* partner genes. (A) One *ALK* wildtype allele and one rearranged *ALK* allele are presented. Genomic breakpoint cluster region (BCR) of *ALK* in non-small cell lung cancer (NSCLC) is located in 19th intron of *ALK* gene. (R: restriction enzyme) (B) General principle of LDI-PCR for the detection of *ALK* fusion gene analysis. Two asterisks show the derivative (target) bands by LDI-PCR in *ALK* fusion gene analysis. (C) Demonstration of known (*EML4*, *TFG*, *KIF5B*, *KLC1*) or unknown partner genes in *ALK* rearrangements.

kemia patients but has turned out to be informative also on solid tumors, like e.g. pilocytic astrocytoma or Lynch syndrome patients (5, 12, 13).

Intron 19 of the *ALK* gene resembles the breakpoint cluster region and has a size of about 1,932 bp. Thus, the LD-/LDI-PCR seems to be a highly feasible method, as it is for *MLL*, *PML-RARA* or *FGFR1* rearrangement analyses (3, 4, 9-11). LDI-PCR could be expected to be a powerful method to analyze all *EML4-ALK* variants and also any other *ALK* rearrangements having known or even unknown fusion partner genes (Fig. 1). Therefore, we believe that the implementation of such a novel technology for the molecular diagnoses of lung cancer patients would contribute to a more accurate identification of *ALK* fusion genes in NSCLC as well as to the molecular characterization of *EML4-ALK* fusion gene in Korean or other ethnic lung cancer patients that can be used as a more precise founding information for molecular targeted therapy. We hope this new LD-/LDI-PCR method can be used in a greater variety of molecular diagnosis of solid tumors in the near future.

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