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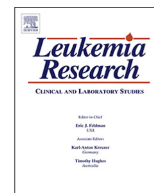

Research paper

Retrospective analysis of 36 fusion genes in 2479 Chinese patients of *de novo* acute lymphoblastic leukemia

Xue Chen[a], Fang Wang[a], Yang Zhang[a], Mangju Wang[b], Wenjun Tian[c], Wen Teng[a], Xiaoli Ma[a], Lei Guo[a], Jiancheng Fang[a], Ying Zhang[b], Ping Zhu[b], Hongxing Liu[a,d,*]

[a] Pathology & Laboratory Medicine Division, Hebei Yanda Lu Daopei Hospital, Langfang, 065201, China
[b] Department of Hematology, Peking University First Hospital, Beijing, 100034, China
[c] Department of Clinical Laboratory, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, 250021, China
[d] Beijing Lu Daopei Institute of Hematology, Beijing, 100176, China

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ABSTRACT

Fusion genes are major molecular biological abnormalities in hematological malignancies. To depict the common recurrent gene-fusion landscape in acute lymphoblastic leukemia (ALL), 36 recurrent fusion genes in hematologic malignancies were assessed using multiplex-nested RT-PCR in 2479 patients with *de novo* ALL. 17 kinds of distinct fusion genes were detected in 712 (28.72%) cases. Co-occurrence of different fusion genes was observed in 6 (0.24%) patients. Incidence of fusion genes in B-ALL was significantly higher than in T-ALL (31.40% vs. 14.50%, $P < 0.001$). Pediatric ALL had higher prevalence of *ETV6-RUNX1*, *TCF3-PBX1,* and *STIL-TAL1,* while *BCR-ABL1* and *SET-NUP214* were more common in adult ALL. *BCR-ABL1*, *TCF3-PBX1*, *KMT2A-AFF1* and *ETV6-RUNX1* were more frequent in B-ALL. On the contrary, *KMT2A-MLLT4*, *SET-NUP214* and *STIL-TAL1* were of higher incidence in T-ALL. In comparison with Western cohorts, the incidence of *BCR-ABL1* (5.94%) was much higher in our series, while the occurrence of *ETV6-RUNX1* (13.19%) was significantly lower in pediatric B-ALL patients in our study than in Western reports. This study provides a genetic landscape of common fusion genes in ALL patients and may serve as a foundation for further improvement of a fusion gene screening panel for clinical applications.

1. Introduction

Fusion genes are the main molecular biological abnormalities of hematological malignancies. They exist stably with tumor cells and are well established as diagnostic and prognostic markers for leukemia. They can also be used as molecular markers for monitoring minimal residual disease (MRD) with high sensitivity [1–4]. Based on their essential role in the mechanisms of tumorigenesis, the WHO classification of neoplastic diseases of the hematopoietic and lymphoid tissues has incorporated some fusion genes into the diagnostic criteria for leukemia since 2000 [5].

Over the past several decades, a growing number of fusion genes have been identified, dozens of which are common in hematological malignancies. Of note, most patients are positive of only one kind of fusion gene. The laboratory of hematology of Peking University First Hospital began to carry out fusion genes screening using multiplex-nested reverse transcription-PCR (RT-PCR) method according to Pallisgaard et al. [6] with modification in clinical practice since September 2002 and this screening method has been applied in many hospitals nationwide ever since then. Nowadays, screening multiple fusion genes simultaneously and then quantitatively monitoring the positive ones has become a routine clinical application. Hebei Yanda Lu Daopei Hospital carried out fusion genes screening service in leukemia since its establishment.

Both the two hospitals accumulated a large number of cases and data on fusion genes. Hence, we performed this study to analyze the panoramic incidence of 36 common fusion genes in 2479 patients of *de novo* acute lymphoblastic leukemia during a 15-year period. The differences in spectrum of these fusion genes between different age groups and different immunophenotypes were systematically compared. Differences in both incidence and spectrum of these fusion genes between Chinese and Western cohorts were also compared.

* Corresponding author at: Pathology & Laboratory Medicine Division, Hebei Yanda Lu Daopei Hospital, 6 Sipulan Road, Langfang, 065201, China.
E-mail address: starliu@pku.edu.cn (H. Liu).

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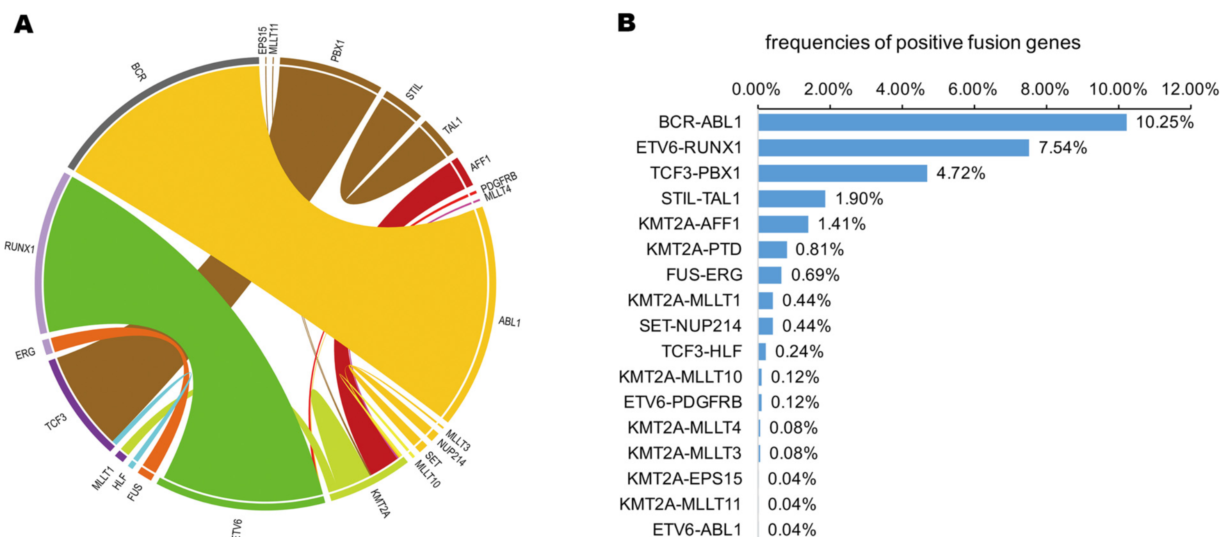

Fig. 1. Fusion genes detected in the cohort of 2479 patients. (A) 17 distinct fusion genes detected in 2479 patients. The plot was created using Circos software. Ribbon widths are proportional to the frequency of fusion genes. Chromosomes were individually colored and are arranged clockwise from chromosome 1 to 22, starting with chromosome 1 at 12 o'clock. (B) Positive rates of each fusion gene detected in 2479 patients.

2. Materials and methods

2.1. Case selection

From September 2002 to April 2017, a total of 2479 patients diagnosed with *de novo* ALL in Peking University First Hospital and Hebei Yanda Lu Daopei Hospital were included in this study. Among them, 1636 (65.99%) were children (< 19 years, 1003 males and 633 females, median age 6 years, range 2 months-18 years) and 843 (34.01%) were adults (≥19 years, 514 males and 329 females, median age 49 years, range 19–91 years). 2086 (84.15%) were B-ALL (1380 children and 706 adults) and 393 (15.85%) were T-ALL (256 children and 137 adults). The diagnosis of ALL was according to the WHO 2016 classification of tumors of hematopoietic and lymphoid tissues [7].

The studies were approved by the ethics committee at the two hospitals. Written informed consents for medical record review were obtained from all patients or their guardians in accordance with the Declaration of Helsinki.

2.2. RNA extraction and cDNA synthesis

Total RNA was isolated from peripheral blood or bone marrow of patients by the guanidinium thiocyanate-phenol chloroform method using a TRIZOL reagent according to the manufacturer's recommendations (Invitrogen Corporation, Carlsbad, CA, USA). Complementary DNA (cDNA) was synthesized using M-MLV Reverse Transcriptase (Promega Corporation, Madison, WI, USA) or a Maxima First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocol.

2.3. Multiplex-nested RT-PCR

A multiplex-nested RT-PCR strategy was designed to detect 36 fusion genes which were commonly found in leukemia patients according to Pallisgaard et al. [6] with modification of some of the primers. About 4–8 bases which were complementary to the 3' end of the corresponding primer were added to the 5' end of each primer so as to reduce nonspecific amplification before the PCR cycles and improve sensitivity and specificity of the reactions. Details of the fusion transcripts and the number of splice variants of them were listed in Table S1. All primers used were listed in Tables S2–S3. The specific fusion gene and splice variant was identified by comparing the size of the PCR segment with the interpretation table provided in Table S4.

2.4. Statistical analysis

The χ^2 and Fisher's exact tests were used to compare differences of frequencies of fusion genes between different age groups, different ALL subtypes, and different populations using SPSS statistics (version 20, IBM Corp., Armonk, NY). Two-sides $P < 0.05$ were considered to be statistically significant.

3. Results

3.1. Spectrum and incidence of fusion genes in ALL patients

Here, 17 kinds of distinct fusion genes were detected in 712 (28.72%) of the 2479 patients. Patients who were positive of *BCR-ABL1*, *TCF3-PBX1*, *KMT2A-AFF1*, *FUS-ERG*, *KMT2A-MLLT1*, *KMT2A-MLLT4*, *KMT2A-MLLT3*, *KMT2A-EPS15*, *KMT2A-MLLT11*, *TCF3-HLF*, and *ETV6-PDGFRB* all showed corresponding chromosomal abnormalities. On the contrary, patients who were positive of *ETV6-RUNX1*, *STIL-TAL1*, *KMT2A-PTD*, *SET-NUP214*, and *ETV6-ABL1* could not be diagnosed by routine cytogenetics analysis. Two of the three patients who were positive of KMT2A-MLLT10 had t(10;11)(p12;q23), while no karyotype abnormality was found in the other patient.

Five fusion gens with relative high incidences were: *BCR-ABL1* (10.25%), *ETV6-RUNX1* (7.54%), *TCF3-PBX1* (4.72%), *STIL-TAL1* (1.90%) and *KMT2A-AFF1* (1.41%). Positive rates of the remaining fusion genes were all below 1.00% (Fig. 1).

Six cases with co-existence of two different fusion genes were identified (three adults and three children), accounting for 0.24% of all cases enrolled in this study and 0.84% of all positive cases. Four of them were B-ALL and were positive for *BCR-ABL1* (e1a2) + *KMT2A-PTD*, *BCR-ABL1* (e1a2) + *FUS-ERG*, *FUS-ERG* + *ETV6-RUNX1*, and *FUS-ERG* + *SET-NUP214*, respectively. The other two cases were T-ALL and were positive for *STIL-TAL1* + *BCR-ABL1* (e1a2) and *STIL-TAL1* + *ETV6-PDGFRB*, separately. The experimental procedures performed on these patients were carefully examined and repeated in different laboratories to eliminate false positive due to PCR product contaminations.

3.2. Difference of gene-fusion spectrum between pediatric and adult cases

In the group of 843 adult ALL, 11 kinds of distinct fusion genes were

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