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Gene mutation patterns of Chinese acute myeloid leukemia patients by targeted next-generation sequencing and bioinformatic analysis

Xiaoyu Han, Wei Li, Na He, Panpan Feng, Yihua Pang, Chunyan Ji, Daoxin Ma*

Department of Hematology, Qilu Hospital of Shandong University, Jinan, Shandong 250012, PR China

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ABSTRACT

Purposes: The conventional risk stratification of acute myeloid leukemia (AML), based on cytogenetics, cannot meet the demand for accurate prognostic evaluations. In recent years, gene mutations are found to be potential markers for more accurate risk stratification, but reports on mutation screening of Chinese AML are limited. We aim to display the mutation patterns of Chinese AML patients, reveal the genotype-phenotype correlations and make a comparison with Caucasians patients.

Methods: Genome DNA from 78 patients' bone marrow were extracted for targeted gene mutation panel by next-generation sequencing (NGS) technology. Statistics and bioinformatics were used to analyze the correlations between gene mutations and clinical features, as well as the comparison of our results with the Cancer Genome Atlas Research Network (TCGA) public AML dataset.

Results: We found patients with mutations of FLT3 and TET2 had higher bone marrow blasts, peripheral blasts and white blood cell (WBC) count, mutations of SRSF2 were related with age, and mutations of FLT3-ITD, DNMT3A, IDH1, TET2 and SRSF2 were risk factors for overall survival. What's more, we discovered 15 novel mutations and difference of mutational incidence in 6 genes between Chinese and Caucasians AML. Bioinformatic analysis revealed some relationship between gene mutations and expressions as well as drug sensitivities.

Conclusions: We made an investigation on the mutation patterns of Chinese AML patients by NGS technique and revealed correlations between gene mutations and clinical features. Thus we recommend routine testing of suspected genes for better prognostic prediction and individualized treatment.

1. Introduction

Acute myeloid leukemia is a heterogenous clonal disease originated from myeloid hematopoietic progenitor cells, characterized by uncontrolled accumulation of myeloid blasts in the bone marrow and peripheral blood as well as inhibition of normal hematopoiesis. To date, chemotherapy remains the most important treatment for AML patients, without revolutionary advances showing up for decades. But the disease course and prognosis differ from person to person, and not every patient responds the same way to chemotherapeutics. As many studies revealing the potential role of molecular abnormalities in AML, precision medicine and individualized medicine were put forward [1]. And some variations on genes such as FLT3-internal tandem duplication (FLT3-ITD) have been incorporated into the evaluation system for risk stratification and prognosis. In the era of large-scale application of NGS technique, the study of gene mutations and their roles in disease can better integrate with precision medicine.

Previous studies reported many recurrent mutations in patients with AML [2–13], from which some were confirmed harmful or beneficial to the patients' prognosis or therapeutic effect. However, the correlations between most somatic mutations and clinical characteristics and the underlying mechanisms still needs further investigations. What's more, reports on mutational patterns of Chinese AML patients are limited.

To provide additional information on the above subjects and evidence to both diagnosis and treatment, we performed targeted NGS of 22 suspected genes on 78 patients. This gene panel consists of FLT3, NPM1, KIT, CEBPA, DNMT3A, IDH1, IDH2, TET2, EZH2, RUNX1, ASXL1, PHF6, TP53, SF3B1, SRSF2, U2AF1, ZRSR2, NRAS, CBL, SETBP1, ETV6 and JAK2, that have been suggested by NCCN guidelines and previous reports [14–18]. There is broad consensus that mutations of FLT3-ITD and DNMT3A are generally associated with an inferior outcome in AML, and biallelic mutation of CEBPA and mutations with NPM1 but without FLT3-ITD are favorable markers. However, the mutational impact of other genes such as TET2, SRSF2 and IDH on survival is controversial depending on different reports [19–30]. Here, we report our findings on the frequently mutated genes in Chinese AML

* Corresponding author.
E-mail addresses: jichunyan@sdu.edu.cn (C. Ji), daoxinma@sdu.edu.cn (D. Ma).

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population and their correlations with clinical features.

2. Materials and methods

2.1. Patients

A total of 78 newly diagnosed and follow-up AML patients who took NGS assays in Qilu Hospital of Shandong University from February 2016 to October 2017 were enrolled in this study. The patients included M1, M2, M3, M4, M5, M6 and not-specified types according to French-American-British (FAB) classification system. Complete remission (CR) was defined based on International Working Group Criteria. This study was approved by the Medical Ethical Committee of Qilu Hospital of Shandong University. All the patients have signed informed consent in accordance with the Declaration of Helsinki.

2.2. Sample preparations

Bone marrow aspirates of the 78 AML patients were collected and lysed by red blood cell lysis buffer to obtain mononuclear cells. Genomic DNA for further sequencing was isolated using TIANamp Genomic DNA Kit (Tiangen Biotech, Beijing, China) according to manufacturer's instructions.

2.3. Targeted next-generation sequencing

Targeted NGS of a 22-gene panel and subsequent BLAST analysis were performed by Yuanqi Biomedical Technology (Shanghai, China, Co., Ltd.). The panel consists of 84 amplicons captured by pairs of oligonucleotides designed to hybridize flanking targeted regions of interest. The library preparation was performed using at least 200ng of genomic DNA. The sequencing run was conducted on the HiSeq system (Illumina Inc., San Diego, CA, USA), with average read depth 2000×. Reads were aligned to human reference genome GRCh37.

2.4. Sanger sequencing

A subset of somatic mutations detected by NGS was independently confirmed through Sanger sequencing by BioSune Biotechnology (Shanghai, China, Co., Ltd.). Specific primers were designed for targeted mutated regions, followed by Polymerase Chain Reactions (PCR). Sanger sequencing of the PCR products were performed on an ABI PRISM 3730xl Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA) with the BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies Corporation, Carlsbad, CA, USA). Subsequent visualization of the sequence was carried out by SnapGene Viewer 3.2 for windows (GSL Biotech LLC, Chicago, USA).

2.5. Statistics

The data were analyzed by SPSS version 24.0 for Windows (SPSS Inc., Chicago, IL, USA). Continuous variables with non-normal distribution were present as median (interquartile range). Mean of two continuous variables with normal distribution were compared by independent samples Student's test. And non-normal variables were compared by nonparametric test. Fourfold table data were analyzed by chi-square test or Fisher's exact test. Linear regression and logistic regression analysis were used as univariate and multivariate analysis. Survival analysis were analyzed by Kaplan-Meier Log-rank test and Cox regression analysis. A value of $p < .05$ was considered significant [31].

2.6. Bioinformatic analysis

The impact prediction of a novel point mutation PHF6 H302R to protein or disease was performed through Mutationtaster [32], PolyPhen2 [33], Mutationassessor [34] and SIFT [35] tools followed by instructions on their websites. The figures of gene mutation profile and landscapes were made by cBioPortal [36]. The comprehensive analysis of TCGA public AML dataset were calculated and plotted by cBioPortal and UALCAN [37]. The correlation between gene mutations and drug sensitivity was integrated by the Genomics of Drug Sensitivity in Cancer (GDSC) database [38].

Table 1
Clinical and laboratory characteristics of the 78 AML cases.

Characteristic	Value
Male/female	44/34
Age at study entry, year, median (range)	53(14–77)
Bone marrow blasts at diagnosis, %, median (interquartile range)	73(41–90)
Peripheral blood blasts at diagnosis, %, median (interquartile range)	47.5(13–81)
Proportion of bone marrow abnormal cells by flowcytometry, %, median (interquartile range)	42.38(22.81–79.71)
WBC at diagnosis, $\times 10^9$/L, median (interquartile range)	5.91(2.46–32.34)
RBC at diagnosis, $\times 10^{12}$/L, median (interquartile range)	2.25(1.75–2.70)
HGB at diagnosis, g/L, median (interquartile range)	72(62–88)
PLT at diagnosis, $\times 10^9$/L, median (interquartile range)	40(23–71)
AML FAB subtype, no./total no. (%)	
AML with minimal maturation: M0	0/78
AML without maturation: M1	1/78
AML with maturation: M2	5/78
Acute promyelocytic leukemia: M3	9/78
Acute myelomonocytic leukemia: M4	8/78
Acute monoblastic or monocytic leukemia: M5	42/78
Acute erythroid leukemia: M6	4/78
Acute megakaryoblastic leukemia: M7	0/78
Other subtype	9/78
Cytogenetic risk group, no./total no. (%)	
Favorable	5/65
Intermediate	45/65
Unfavorable	15/65
Missing data	13/78
Status after the first course of chemotherapy, no./total no. (%)	
CR	30/72
Not CR	42/72
Unavailable data	6/78
Mutation, no./total no. (%)	
FLT3	20/78(26)
NRAS	13/78(17)
NPM1	12/78(15)
DNMT3A	11/78(14)
IDH2	10/78(14)
ASXL1	9/78(12)
KIT	7/78(9)
TET2	7/78(9)
IDH1	7/78(9)
U2AF1	7/78(9)
CEBPA	5/78(6)
SRSF2	5/78(6)
EZH2	3/78(4)
SF3B1	3/78(4)
TP53	2/78(3)
SETBP1	2/78(3)
ETV6	2/78(3)
PHF6	1/78(1)
RUNX1	1/78(1)
CBL	1/78(1)
JAK2	1/78(1)
ZRSR2	0/78(0)

WBC: white blood cell; RBC: red blood cell; HGB: hemoglobin; PLT: platelet; FAB: French-American-British; CR: complete remission.

3. Results

3.1. Gene mutation patterns and clinical characteristics

3.1.1. Mutation profile

78 newly diagnosed and follow-up AML patients with a median age