Rare Occurrence of the JAK1 Mutation in Acute Promyelocytic Leukemia Patients

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Acute promyelocytic leukemia (APL) is a distinct subtype of acute myeloid leukemia characterized by a chromosome translocation of t(15;17)(q24;q21) which involves fusion of the retinoic acid receptor α (*RAR α*) gene in chromosome 17 and the promyelocytic leukemia (*PML*) gene in chromosome 15. The fusion protein encoded by the *PML/RAR α* gene makes APL sensitive to all-*trans* retinoic acid, a derivative of vitamin A. With the introduction of all-*trans* retinoic acid and arsenic trioxide in the past several decades, the complete remission (CR) rate and 5-year disease-free survival for APL were elevated to more than 90% [1].

The Janus kinase (JAK) is a nonreceptor tyrosine kinase, including 4 family members, i.e. JAK1, JAK2, JAK3, and TYK2. The *JAK1* gene is organized into 25 exons, including 24 coding exons, and spans about 133.28 Kb in the chromosome 1p31.3 region, which is responsible for transducing cytokine-induced signals via JAK-STAT pathway aberrations; JAK1 signal activation is partially responsible for leukemogenesis. Recently, Wartman et al. [2] found Jak1 (V657F) in a murine APL model via whole genomic sequencing. This Jak1 (V657F) was identified in 6 other mice that were not closely related to the proband [2]. After analyzing 186 acute adulthood leukemias, 30 multiple myelomas, and 278 common solid cancers, Jeong et al. [3] discovered 4 *JAK1* mutations in patients with acute leukemia. Among them, the *JAK1* mutations

V658F and L783F occurred in 4 leukemia patients, while the p.V658F mutation was found in 1 patient with APL [3]. In our previous report, the *JAK1* mutation was found in 3 out of 49 in acute T-lymphocytic leukemia patients [4]. To explore the frequency of the *JAK1* mutation in APL, we performed *JAK1* gene sequencing in a group of APL patients in our center. This study was approved by the Ethics Committee of the First Affiliated Hospital of Soochow University. Informed consent was provided following the Declaration of Helsinki.

A total of 86 patients with de novo APL were screened in all 24 exons of *JAK1* using genomic DNA-PCR amplification sequencing. The clinical characteristics of these patients are listed in table 1. Bone marrow was aspirated and cultured with colcemid overnight at 37°C and harvested according to standard procedures [5]. Karyotypic analysis was performed on R-banded metaphases and described according to the recommendations of the ISCN. Metaphases were analyzed by R banding and karyotype showed that 82 out of 84 patients were t(15;17) with a positive *PML/RAR α* fusion gene, while 1 patient displayed t(11;17) with the *PLZF/RAR α* fusion gene. Total DNA was extracted from bone marrow using a DNA

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Fig. 1. Sequencing diagram of a representative specimen in the V658F area where mutations have been reported by earlier studies. The coding sequence for residue V658 is underlined.

Table 1. Clinical characteristic of patients with APL

Characteristics	
Patients, n	85
Age, years	34 (7–79)
Male/female ratio	45/40
White blood cells, $\times 10^9/l$	7.50 (0.95–194.7)
Hemoglobin, g/l	94 (21–132)
Platelets, $\times 10^9/l$	24.5 (3–145)
Bone marrow blasts, %	87.7 (56–98.4)
Cytogenetics	
t(15;17) with PML/RARa	84
t(11;17) with PLZF/RARa	1

Values are presented as medians (range) unless otherwise stated.

Table 2. Characteristics of APL patients with JAK1 new SNPs and silent mutation

Case	Sex	Age, years	Change(s)	Exon	Type of change	Protein level	Status
1	female	60	c.277C>T	4	SNP	Arg93Cys	novel
2	male	24	c.677A>G	7	SNP	Asn226Ser	novel
3	male	50	c.655A>G	7	SNP	Asn252Ser	novel
4	female	16	c.1659C>T	12	synonymous	Asn553Asn	novel

Mini Kit (PureLink; Invitrogen, USA) according to the manufacturer's instructions. PCRs were performed in a volume of 25 μ l using 23 pairs of primers for all 24 *JAK1* coding exons. The PCR products were separated on 2% agarose and visualized with GelRed. Direct sequencing was carried out with the Big Dye Terminator v3.1 Cycle Sequencing Reaction (Applied Biosystems) and was run on an ABI 3730xL DNA analyzer.

DNA sequencing analysis of the *JAK1* gene found 4 novel heterozygous single-nucleotide variations which were not included in the NCBI SNP database. c.1659C>T (Asn553Asn) was a synonymous mutation and the other three were nonsynonymous mutations. All four mutations were genomic variants rather than somatic mutations since they were identified in the CR samples (table 2).

The genetic variation of the *JAK1* gene in APL patients is unknown. By sequencing a large cohort of patients, we

found 4 novel SNPs in patients with CR. Thus, our results have shown that *JAK1* mutation is rare in newly diagnosed APL patients, suggesting that these mutations in *JAK1* were unlikely to be involved in the pathogenesis of APL. Earlier studies identified the mutations in the same amino acid (or homologous amino acids) of V658F; however, we did not find these mutations in any of the 84 specimens (fig. 1). Because of the low frequency of the *JAK1* mutation in APL, larger patient cohorts are needed for further study.

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