A novel variant translocation (1;9)(p22;q34) resulting in a DEK/NUP214 fusion gene in a patient with acute myeloid leukemia: A case report

QISHAN HAO1,2, QI ZHANG1,3, CHENGWEN LI1, SHUNING WEI1, QINGHUA LI1, YANG SONG1 and YINGCHANG MI1,3

1Department of Leukemia, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin 300020; 2Zibo Central Hospital, Zibo, Shandong 255000; 3State Key Laboratory of Experimental Hematology, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin 300020, P.R. China

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Abstract. The present case report describes a 46-year-old female patient diagnosed with M4 acute myeloid leukemia (AML), accompanied with a t(1;9)(p22;q34) chromosomal abnormality. Transcriptome sequencing identified a DEK proto-oncogene (DEK)/nucleoporin (NUP)214 fusion gene, which results from the t(6;9)(p23;q34) chromosomal translocation. Polymerase chain reaction analysis and fluorescence in situ hybridization were used to verify the existence of the DEK/NUP214 fusion gene. Few patients with AML with the t(6;9)(p23;q34) chromosomal translocation have been reported to have other chromosomal or karyotype changes. To our knowledge, no AML patient with the DEK/NUP214 fusion gene but without the classic t(6;9)(p23;q34) translocations had been reported until now. The prognosis of AML cases with the DEK/NUP214 fusion gene is poor. The rate of complete remission is ~65% (71% in children, 58% in adult patients), while the estimated 5-year survival rate is 28% for children and 9% for adults. The 2008 revision of World Health Organization classification have defined the DEK/NUP214 mutation as a recurrent genetic abnormality of AML. The overall survival of the patient in the current report was ~29 months, and they relapsed twice. To the best of our knowledge, this is the first report of at(1;9)(p22;q34) variant translocation that results in expression of the DEK/NUP214 fusion gene.

Introduction

The chromosomal abnormality t(6;9)(p23;q34) present in ~0.5 - 4% of patients with acute myeloid leukemia (AML) (1), primarily in those with M2 and M4 AML (2-4). AML is a disease that has unique characteristics and patients typically have a poor prognosis. Currently, ~400 cases of AML with t(6;9)(p23;q34) have been reported since it was first identified in 1976, and most of these are reports of small numbers of cases (1,4-12). This chromosomal abnormality has also been demonstrated in patients with myelodysplastic syndromes (MDS) and myeloproliferative neoplasms. The DEK proto-oncogene/nucleoporin (DEK/NUP214) fusion gene results from t(6;9)(p23;q34) in AML as reported (1,3,11,13).

Case report

Initial patient details. The patient was a 46-year-old female who presented with a fever on June 26, 2013 and admitted to Institute of Hematology and Blood Diseases Hospital (Tianjin, China). The patient had a white blood cell (WBC) count of 22.8x10^9/l, a hemoglobin level of 71 g/l and a platelet count of 101x10^9/l. Bone marrow smears with Wright's staining, as described previously (14), revealed that bone marrow hyperplasia, myeloblasts and immature monocytes comprised 59.5% of the bone marrow, with visible Auer rods. The immunophenotype was analyzed using multi-color flow cytometry, as described previously (15), and the results showed blasts highly expressed CD13, CD33 and CD117, and dim expressed CD34, CD64, myeloperoxidase (MPO) and human leukocyte antigen D related (HLA-DR).

Chromosomal analysis. A chromosomal analysis was performed using the R-banding technique as described previously (16), and the samples were karyotyped according to the International System for Human Cytogenetic Nomenclature (17). The results of the chromosomal analysis were as follows (Fig. 1): 46, XX, t(1;9)(p22;q34)[9]/46; XX, t(1;9)(p22;q34)[-2,+22][1]/45, XX, t(1;9)(p22;q34)-21[1]/46, XX[9]. Somatic mutations were
detected by targeted next-generation sequencing (18,19): The patient was negative for FLT3/ITD, FLT3/TKD, C-KIT Exon 8, C-KIT D816, CEBPα/TAD, CEBPα/BZIP, DNMT3A/ZNF and DNMT3A/Mtase mutations. In order to identify the specific fusion gene formed by the chromosomal abnormalities in the patient, transcriptome sequencing (RNA-seq) was performed on the patient's bone marrow mononuclear cells and peripheral blood cells in the complete remission (CR) state. Expression of DEK/NUP214 fusion gene was demonstrated in the bone marrow and peripheral blood mononuclear cells, which suggested that the patient had a variant translocation of t(6;9)(p23;q34) (Fig. 2). Fluorescent in situ hybridization (FISH) using the DEK/NUP214 probes, nested polymerase chain reaction (PCR) and agarose gel electrophoresis were performed to confirm the existence of the DEK/NUP214 fusion gene (Figs. 3 and 4). The diagnosis of the patient was AML M4 with the DEK-NUP14 fusion gene.

Chemotherapy and outcome. Daunorubicin (DNR; 60 mg/day, days 1-3) and cytosine arabinoside (Ara-C, 200 mg/day, days 1-7) were administered to the patient for induction chemotherapy from June 29, 2013. The patient did not achieve CR according to response criteria of International Working Group (20). Mitoxantrone (MTZ), Ara-C, and cyclophosphamide (CTX) were administered for re-induction chemotherapy from July 24, 2013, which contained MTZ 15 mg/day (day 1) and 10 mg/day (days 2-3), Ara-C 200 mg/day (days 1-7) and CTX 800 mg/day (days 1 and 4). From September 25, 2013, a third course of chemotherapy was administered, which included 3 mg/day homoharringtonine (days 1-7), 200 mg/day Ara-C (days 1-7) and 20 mg/day aclacinobin (days 1-7). The patient did not achieve CR. The patient was offered the FLAG regimen [Fludarabine (Flu;45 mg/day, days 1-5), Ara-C (1.5 g/day, days 1-5) and granulocyte colony-stimulating factor (G-CSF 300 µg/day, days 0-5)], from November 16, 2013. Bone marrow examination revealed that the patient achieved CR on December 16, 2013. From December 17, 2013, the patient received a second FLAG regimen at the same dose as a consolidation treatment. The patient suffered from a severe pulmonary infection in the second FLAG chemotherapy regimen and refused further treatment. Instead, the patient chose outpatient follow-up only. The patient was re-admitted on December 24, 2014 because of fever; a bone marrow smear revealed that the percentage of myeloblasts was 6.5% and immature monocytes accounted for 13.5%. From December 26, 2014, the re-induction chemotherapy CAG regimen (100 mg/day Ara-C, days 1-7; 20 mg/day Acla, days 1-6; and 300 µg/day G-CSF, days 1-7) was used. The patient achieved CR2. Two cycles of Ara-C (150 mg/day, days 1-5) and Acla (20 mg/day, day 1-5) chemotherapy were administered, with the last treatment administered in March 2015. Bone marrow examination on November 12, 2015 demonstrated that
the patient relapsed again and succumbed in June 2016. The overall survival was ~36 months from diagnosis.

Materials and methods

RNA-seq. Total RNA was extracted from frozen mononuclear cells as described previously (21). A total of 5 µg of RNA was used for RNA-seq, which was performed using an IlluminaHiSeq 2000 Sequencer (Illumina, Inc., San Diego, CA, USA). The RNA-seq was performed by BGITech Solutions Co., Ltd. (Shenzhen, China).

Detection of the DEK/NUP214 fusion transcript. To analyze the expression of DEK/NUP214, the total RNA of frozen mononuclear cells was isolated using the RNeasy mini kit according to the manufacturer's protocol (Takara Biotechnology Co., Ltd., Dalian, China). Reverse transcription was carried out using TaqMan reverse transcriptase reagents (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's instructions (21). Primer pairs were purchased from Invitrogen (Thermo Fisher Scientific, Inc.) and the obtained products were subjected to direct sequencing (Invitrogen; Thermo Fisher Scientific, Inc.). The primer sequences for the first step were as follows: DEK/NUP214 forward, 5'-TGCCAATGTAAAGAAGCAGATAG-3' and reverse, 5'-GGCAAGATTTGGTGTAAGAT-3'. The primer sequences for the second step were as follows: DEK/NUP214 forward, 5'-AGCAGCACCACCAGAAGAAT-3' and reverse, 5'-GTCTCTCGCTCTGGCACAAG-3'. DEK/NUP214 fusion transcripts were amplified using the ABI Prism 7500 Real Time PCR system (Thermo Fisher Scientific, Inc.). cDNA was subjected to 40 cycles of denaturing (94°C, 15 sec) and annealing (60°C, 60 sec) using the Leukemia Related Fusion Gene Detection kit, (Shanghai Yuanqi Bio-Pharmaceutical Co., Ltd., Shanghai, China). PCR products were run on a 1% agarose gel.

FISH. FISH was performed according to the manufacturer's protocol. Kreatech FISH probes targeting DEK/NUP214 (cat. no. KBI-10306) were obtained from Leica Biosystems (Amsterdam, Netherlands). Automated in situ hybridization staining was performed using ThermoBrite, an automatic instrument (Leica Biosystems). Data analysis was performed using ISIS software (version 5.4.6; MetaSystems GmbH, Altlussheim, Germany).

Discussion

AML with t(6;9)(p23;q34) is a rare type of AML where the bone marrow morphology is frequently accompanied by increased levels of basophils. Auer rods and fine particles are also visible in certain blast cells. The immunophenotypic analysis from a previous study demonstrated that CD9, CD13, CD33, HLA-DR, CD38, CD45, CD34, CD15 and terminal deoxynucleotidyl transferase (TdT) were expressed in the
patient with this translocation (22). AML with t(6;9) translo-
cation, which accounts for ≤70% more cases compared with
other types of AML, is associated with the FLT3/ITD mutation
(22,23). The 2008 revision of World Health Organization
classification have defined AML with t(6;9) translocation as a
recurrent genetic abnormality of AML (24).

Few AML patients with t(6;9)(p23;q34) have been
reported to have other chromosomal or karyotype changes.
Furthermore, no cases of DEK/NUP214-positive AML
without t(6;9)(p23;q34) have been reported until now, to
the best of our knowledge. Slovak et al (4) reported the cases of
69 patients with AML or MDS with t(6;9)(p23;q34) in 2006.
These patients were identified among 7690 patients with eval-
uable karyotypes, accounting for 0.9%. The t(6;9) translocation
was the sole clonal karyotypic aberration in 61/69 patients
(88%) in this study; only 4 pediatric and 4 adult cases (12%)
had secondary anomalies. A total of 3 patients (5%) had
complex translocations involving 3 or 4 chromosomes. The
typical translocation of t(6;9)(p23;q34) was not found in the
chromosomal karyotype analysis of the patient in the present
study; however, the t(1;9)(p22;q34) translocation was revealed
to form the DEK/NUP214 fusion gene.

The prognosis of patients with AML who have t(6;9)
(p23;q34) is poor. The CR rate is ~65% (71% in children, 58%
in adult patients), while the observed 5-year survival rate is
28% for children and 9% for adults based on the cases reported
by Slovak et al (4).

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