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

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Received April 18, 2016; Accepted February 13, 2017

DOI: 10.3892/ol.2017.7133

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/NUP214fusion 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.

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Key words: acute myeloid leukemia, t(1,9), DEK/NUP214 fusion gene

Introduction

The chromosomal abnormality t(6;9)(p23;q34) presentsin~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, CD38, 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

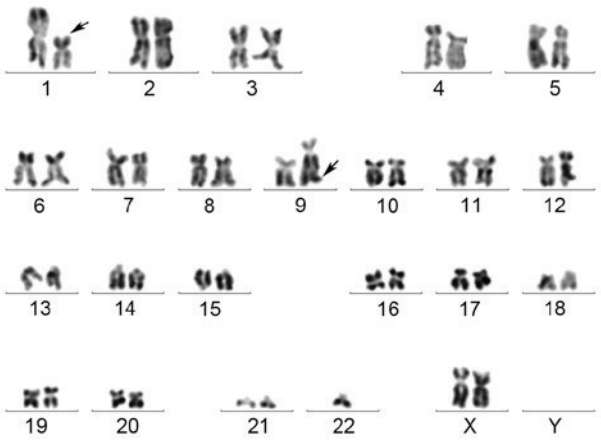

Figure 1. R-banded karyotype of the patient. Arrows indicate t(1;9)(p22;q34) chromosomal abnormalities.

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 aclarubicin (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

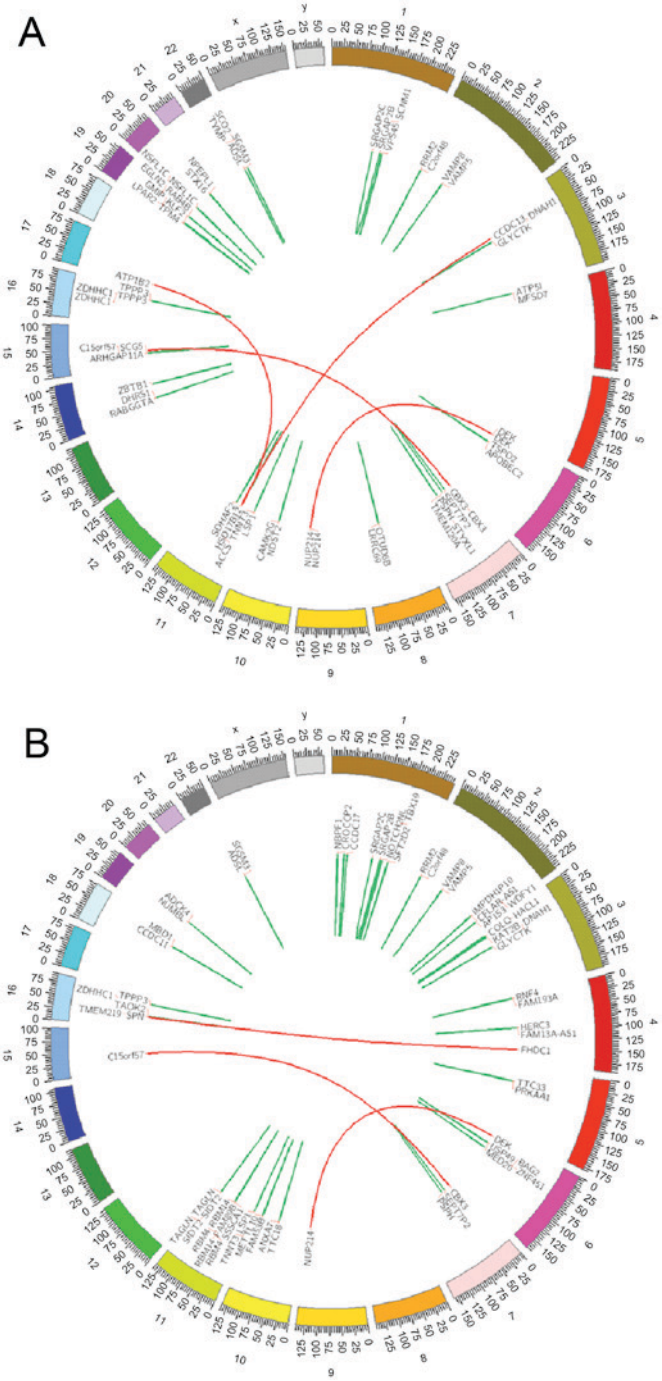

Figure 2. DEK/NUP214 fusion gene expression in the bone marrow and peripheral blood mononuclear cells of the patient. (A) Transcriptome sequencing results of bone marrow mononuclear cells. (B) Transcriptome sequencing results of peripheral blood mononuclear cells. DEK, DEK proto-oncogene; NUP214, nucleoporin 214.

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

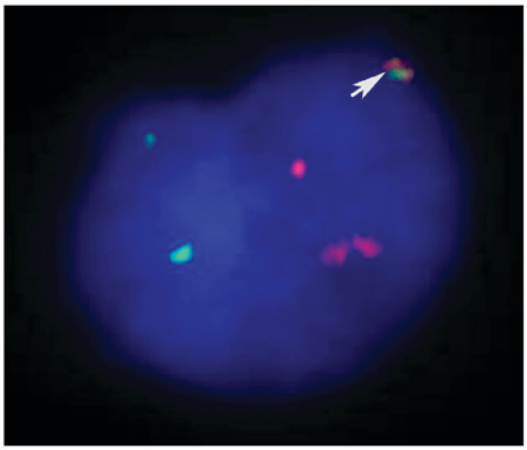

Figure 3. Fluorescence *in situ* hybridization detection of the DEK/NUP214 fusion gene. Arrow indicates the fusion probe signal, where red indicates the NUP214 probe and green indicates the DEK probe. DEK, DEK proto-oncogene; NUP214, nucleoporin 214.

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'-TGCCAATGTTAAGAAAGC AGATAG-3' and reverse, 5'-GGCAAGGATTTGGTGTGA GAT-3'. The primer sequences for the second step were as follows: DEK/NUP214 forward, 5'-AGCAGCACCACC AAGAAGAAT-3' and reverse, 5'-GTCTCTCGCTCTGGC ACAAG-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).

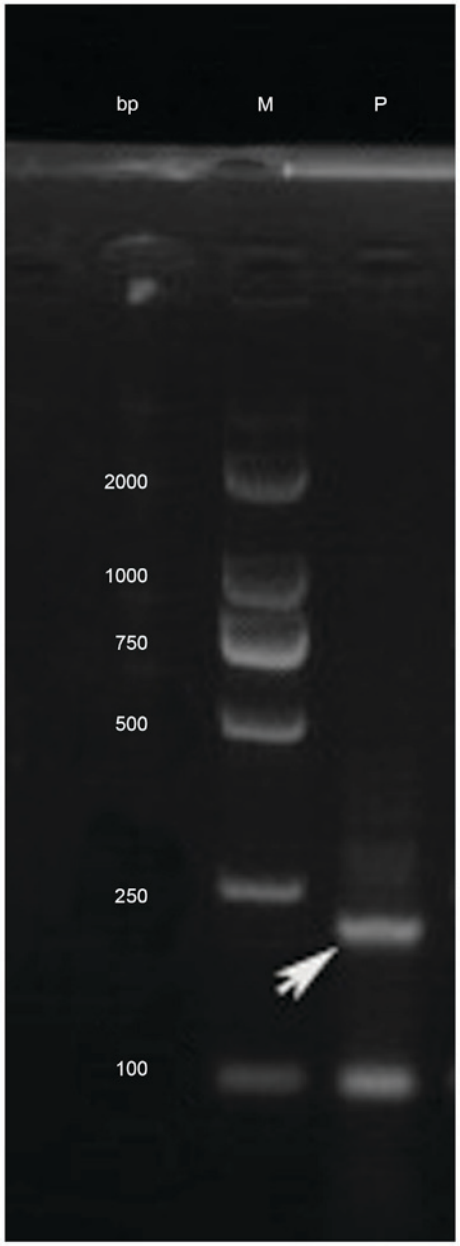

Figure 4. Agarose gel electrophoresis of the DEK/NUP214 fusion gene nested polymerase chain reaction products. Arrow indicates the DEK/NUP214 gene. M, molecular weight standard; P, patient standard; bp, base pairs; DEK, DEK proto-oncogene; NUP214, nucleoporin 214.

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) translocation, 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 at(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 evaluable 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 of3 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 estimated 5-year survival rate is 28% for children and 9% for adults based on the cases reported by Slovak *et al* (4).

Acknowledgements

The present study was supported by the National Science and Technology Pillar Program (grant no. 2014BAI09B12) and the Tianjin Research Program of Application Foundation and Advanced Technology (grant no. 15JCZDJC36400).

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