Abstract. The present study described an extremely rare case of acute promyelocytic leukemia (APL) characterized by a complex three-way (15;15;17)(q24;q14;q21) translocation. It was identified in a 59-year-old male through karyotype, molecular, and fluorescence in situ hybridization (FISH) analyses. The third translocation breakpoint 15q14 was identified on the same chromosome 15 that also contained the classical t(15;17)(q24;q21) and may have evolved from the classical t(15;17) clone, as indicated by interphase FISH analysis. A complex translocation involving two breakpoints on the same chromosome is extremely rare, such that this case can provide insights into complex translocations in APL.

Introduction

The translocation t(15;17)(q24;q21) is the genetic hallmark of acute promyelocytic leukemia (APL) and leads to the fusion of the promyelocytic leukemia (PML) gene on chromosome 15 with the retinoic acid receptor α (RARα) gene on chromosome 17 (1). Although in most patients with APL this PML::RARα fusion occurs through a classical translocation t(15;17)(q24;q21), in ~10% of patients, an APL variant characterized by three-way or more complex translocations is detected (2,3). The fusion gene PML::RARα is created on derivative chromosome 15 and the fusion transcript are hypothesized to play a serve role in the pathogenesis of APL (4). Third or fourth breakpoints in three-way or more complex translocations have been reported, but they are likely to occur recurrently in particular chromosome bands, such as 1q36, 11q3, 2q21, 3p21, 4q21, 6q23, 19p13, 20p13, and Xq13 (5). However, a third breakpoint on the same chromosome 15 involved in the classical t(15;17)(q24;q21) defect is extremely rare, with only one case reported to date, to the best of the author's knowledge (6).

The present study presented a case of a complex three-way translocation (15;15;17)(q24;q14;q21). The results of fluorescence in situ hybridization (FISH), molecular studies, and chromosome analysis suggested that it evolved sequentially from a typical t(15;17)(q24;q21) translocation.

Case report

A 59-year-old man visited Keimyung University Dongsan Hospital (Daegu, Republic of Korea) due to massive epistaxis and dizziness for a day. The results of his complete blood count (CBC) were as follows: white blood cells (WBCs), 0.82x10^9/l; hemoglobin, 75  g/l; and platelets, 36x10^9/l. In coagulation tests he had a prothrombin time of 13.5 sec (reference range, 10.0‑14.0 sec), a normal prothrombin international ratio of 1.18, a prothrombin activity of 73.3% (reference range, 80‑150%), an activated partial thromboplastin time of 31.6 sec (reference range, 20.0‑33.5 sec), a fibrinogen level of 297.8 mg/dl (reference range, 200.0‑400.0 mg/dl) and a D‑dimer of 4.26 µg/ml (reference range, 0‑0.4 µg/ml). The patient did not have hepatosplenomegaly. He was therefore referred to the hematology department and hospitalized for a bone marrow (BM) evaluation. In a BM aspiration smear, 95.0% of all nucleated cells were abnormal promyelocytes, with bilobed or folded nuclei, indistinct nucleoli, clumped chromatin, and many azurophilic granules. Multiple faggot cells were also found (Fig. 1A). BM immunophenotyping using a Navios flow cytometer (Beckman Coulter, Inc.) showed an abnormal population with the phenotype CD13+, CD33+, CD117+, cMPO+, CD3‑, CD5‑, CD7‑, CD19‑, CD79a‑, CD34‑ and HLA‑DR‑.

A typical PML::RARα fusion transcript was detected in multiplex reverse transcription-polymerase chain reaction (RT-PCR; HemaVision®, DNA Technology A/S). The G-banding karyotype showed a translocation between chromosomes 15 and 17 in all metaphase cells, but it was not consistent with typical t(15;17)(q14;q21) findings. Fig. 1 indicates the presence of two clones: One carrying the typical t(15;17)(q24;q21) and the other with a complex three-way translocation involving...
I is added on slides and fusion transcripts. The present study was performed using the QIAamp RNA Blood Mini kit (Qiagen GmbH) assay. The isolation of total RNA was performed according to the manufacturer's instructions. Next, 1 µg of non-degraded RNA was used as a template for synthesis of cDNA in RT reaction. HemaVision® RT-PCR assay used cDNA as a template for multiplex PCR amplification reactions, followed by nested PCR reactions. In the first master PCR amplification, the samples were initially heated at 95°C for 15 min, followed by 25 cycles of amplification each at 95°C for 30 sec, 58°C for 30 sec and 72°C for 1 min 30 sec. In nested master PCR amplification, the samples were initially heated at 95°C for 15 min, followed by 20 cycles of amplification each at 95°C for 30 sec, 58°C for 30 sec and 72°C for 1 min 30 sec, and culminating with a final cycle at 72°C for 10 min. In the nested split-out PCR amplification, the samples were initially heated at 95°C for 15 min, followed by 20 cycles of amplification each at 95°C for 30 sec, 58°C for 30 sec and 72°C for 1 min 30 sec, and culminating with a final cycle at 72°C for 10 min. All PCR reactions were performed with primer mixes from the HemaVision® HV01-28N kit. The final PCR products were analyzed by 2% agarose gel electrophoresis and visualized with ethidium bromide. The detailed information about 28 leukemia-causing translocations detected by HemaVision®-28N is presented in Table S1.

Flow cytometry. A total of 3 ml EDTA-anti-coagulated bone marrow sample was collected. Sample (1 ml) was centrifuged at 1,428 x g for 3 min at room temperature and the buffy coat was separated. Then the buffy coat was washed by PBS. During cell washing, the cells were centrifuged at 1,428 x g for 3 min at room temperature and resuspended in PBS three times. The cell suspension concentration was adjusted to 15.0x10⁶/ml. A total of 50 µl cell suspension was added to each tube, and then the fluorochrome-combined antibodies (without dilution) were added to cell suspension for incubation at room temperature for 20 min. Finally, 500 µl VersaLyse Lysing Solution (cat. no. A07800; Beckman Coulter, Inc.) was added to the cells with and incubated for 10 min at room temperature. The fluorochrome-combined monoclonal antibodies were as follows: Anti-CD45 (PC5; cat. no. A07785), anti-CD10 (FITC; cat. no. A07759), anti-CD19 (PE; cat. no. A07769), anti-CD20 (FITC; cat. no. A07772), anti-CD5 (PE; cat. no. A07753), anti-CD3 (FITC; cat. no. A07746), anti-CD22 (PE; cat. no. IM1835), anti-HLA-DR (FITC; cat. no. IM1638U), anti-CD13 (PE; cat. no. A07762), anti-CD34 (FITC; cat. no. IM1870), anti-CD41 (PE; cat. no. A07781), anti-CD117 (PE; cat. no. IM2732), anti-CD64 (FITC; cat. no. IM1604U), anti-CD7 (FITC; cat. no. A07755), anti-CD33 (PE; cat. no. A07775), anti-CD14 (PE; cat. no. A07764), anti-cMPO (FITC; cat. no. IM1874), anti-cTDT (FITC; cat. no. IM3524) and anti-cCD79a (PE; cat. no. IM2221). All of the

15q14, 15q24 and 17q21. The metaphase karyotype and the corresponding metaphase FISH using a PML/RARA dual-color dual fusion probe (Abbott Laboratories) revealed one fusion signal on der(17) and an additional green signal on the proximal region of the same der(17). On der(15), only one orange signal was detected but a fusion signal was not observed. Orange and green signals were also detected on normal chromosomes 15 and 17, respectively. An evaluation of the interphase FISH results revealed two green and two orange signals and one fusion signal (2G2O1F) in 159 (79.5%) out of 200 cells, consistent with the metaphase findings. However, one green, one orange, and two fusions (1G1O2F) were detected in 31 (15.5%) out of 200 cells, which suggested the typical t(15;17)(q24;q21) although it was not seen on the metaphase karyotype or in the metaphase FISH findings. Taken together, these results indicate the presence of two clones: One carrying the typical t(15;17)(q24q21) and the other with a complex three-way translocation involving 15q14, 15q24, and 17q21.

Next-generation sequencing was performed using a custom panel targeting 49 genes associated with AML (MiSeqDX®; Illumina, Inc.). A somatic variant of FLT3 (NM_004119.2) was detected within a variant allele frequency of 14%. The patient was started on all-trans retinoic acid (ATRA) treatment followed 1 week later by induction chemotherapy with ATRA and anthracycline. The follow-up CBC performed at 3 weeks showed a WBC count of 0.37x10⁹/l. The BM aspirate cytology revealed blasts (21% 2G2O1F and 4% 1G1O2F). At the time of evaluation at 3 weeks showed hypocellularity and evidence of maturation about 28 leukemia-causing translocations detected by interphase FISH analysis. A fusion signal was detected in 25% of nucleated cells (21% 2G2O1F and 4% 1G1O2F). At the time of writing the patient has a stable disease condition.

FISH analysis. Interphase and metaphase FISH analysis was performed according to the standards of the International System for Human Cytogenetic Nomenclature 2020. The 0.5 ml of bone marrow aspirate sample was mixed with 7.5 ml RPMI 1640 medium (cat. no. 31800-022; Gibco; Thermo Fisher Scientific, Inc.), 1.5 ml FBS (cat. no. 16000-044; Gibco; Thermo Fisher Scientific, Inc.) and 50 µl colcemide (cat. no. 15212-012; Gibco; Thermo Fisher Scientific, Inc.), then incubated for 30 min at 37°C in a CO₂ incubator. The cells were then centrifuged at 282 x g at 25°C for 8 min, then incubated for 30 min at 37°C in a CO₂ incubator. The fluorochrome-combined monoclonal antibodies were as follows: Anti-CD45 (PC5; cat. no. A07785), anti-CD10 (FITC; cat. no. A07759), anti-CD19 (PE; cat. no. A07769), anti-CD20 (FITC; cat. no. A07772), anti-CD5 (PE; cat. no. A07753), anti-CD3 (FITC; cat. no. A07746), anti-CD22 (PE; cat. no. IM1835), anti-HLA-DR (FITC; cat. no. IM1638U), anti-CD13 (PE; cat. no. A07762), anti-CD34 (FITC; cat. no. IM1870), anti-CD41 (PE; cat. no. A07781), anti-CD117 (PE; cat. no. IM2732), anti-CD64 (FITC; cat. no. IM1604U), anti-CD7 (FITC; cat. no. A07755), anti-CD33 (PE; cat. no. A07775), anti-CD14 (PE; cat. no. A07764), anti-cMPO (FITC; cat. no. IM1874), anti-cTDT (FITC; cat. no. IM3524) and anti-cCD79a (PE; cat. no. IM2221). All of the
fluorochromes and antibodies were acquired from Beckman Coulter, Inc. Flow cytometry was performed by Navios (Beckman Coulter, Inc.) and data were analyzed with Kaluza software (v2.1; Beckman Coulter, Inc.).

**Next-generation sequencing (NGS) and bioinformatics.** NGS service consisting 49 genes was provided by GC Genome. Genomic DNA (gDNA) and RNA were isolated from the patient’s BM specimen using the QIAamp DNA Blood Mini kit (Qiagen GmbH). For mutation analysis, gDNA of an adequate quantity and quality was fragmented to a size ranging from 200–400 bp, followed by adaptor ligation. Adaptor-ligated DNA underwent hybrid capture using a HEMA panel that contained 49 genes related to hematological malignancies. The entire capture process was performed according to the manufacturer’s protocol using reagents supplied by Integrated DNA Technologies. The captured libraries were sequenced with a NextSeq (Illumina, Inc.).

The sequence data were aligned to the reference human genome (GRCh37) and subjected to adaptor trimming and sequencing quality control. Single nucleotide variants with a variant allele fraction >1%, as well as small insertions and deletions <50 bp in size were detected using Varscan v2.3.9 (http://varscan.sourceforge.net) (7). Possible germline polymorphisms were filtered out if the allele frequency was >0.1% in the Genome Aggregation Database (http://gnomad.broadinstitute.org/). It was not possible to submit the NGS data to a public database because the NGS service was provided by the commercial laboratory.

**Discussion**

To date, ~50 cases with three-way or more complex translocation variants of t(15;17)(q24;q21) have been reported (8–13). Third or fourth breakpoints in complex translocations involving several chromosomes have been described, but a third breakpoint on the same chromosome 15 involved in the typical t(15;17)(q24;q21) is extremely rare (6).

Previous studies that have examined the possible mechanisms underlying complex translocations (14,15) have proposed either two-step evolution from the classical t(15;17), in which case this clone is usually detected by karyotype or FISH analysis, or the simultaneous occurrence of all translocations by multiple simultaneous breakage of several chromosomes following mismatch joining. In the patient of the present study, a clone with the
classical t(15;17) was detected, although only in the interphase FISH study and at a low percentage (15.5%). The second, main clone had a complex translocation. These findings suggested the following mechanism. First, the typical t(15;17)(q24;q21) occurred and the PML::RARa fusion was created on der(15). Second, 15q14 of der(15) was translocated to 17q21 of der(17), resulting in the separation of the RARa::PML fusion. Third, the 3' PML region was re-translocated to 15q14 and the 15q region containing PML::RARa fusion was translocated to der(17). Finally, der(17) became contained within the PML::RARa fusion, creating an additional green signal (remaining 5' RARa region), with the change in der(15) resulting in only one orange signal (3' PML region) (Fig. 2). The absence of the classical clone in metaphase FISH analyses and in karyotyping can be attributed to the low mitotic index of the classical t(15;17) clone in the patient.

Molecular studies are needed to precisely identify the third breakpoint, but these could not be performed at Keimyung University Dongsan Hospital; instead, it was diagnosed by conventional karyotyping as 15q14. A previous study (16) has reported a 15q14 region that includes the genes encoding kinetochore scaffold protein (KNL1) or the zinc finger five-domain-containing protein 19 (ZFYVE19) as one of the recurrent translocation points in leukemia. The involvement of those genes and the molecular effects remain to be elucidated (17).

In t(15;17)(q24;q21), the fusion PML::RARa is created on chromosome 15, and the fusion RARa::PML is on chromosome 17 (1,4). The former plays a vital role in the pathogenesis of APL and is usually preserved even in variant complex translocations. RT-PCR can distinguish between PML::RARa and RARa::PML fusion transcripts and in the patient revealed the typical PML::RARa fusion transcript. The detected FISH fusion signal was therefore the PML::RARa fusion, not the RARa::PML fusion.

In a case reported by Tirado et al (6), the translocation was very similar to that identified in the present study, as it consisted of t(15;17;15) and the typical t(15;17) was not identified by conventional karyotype analyses although its signal was detected in 5% of the cells in the interphase FISH analysis. The proposed mechanism in that case consisted of two steps and the patient had a good response to ATRA treatment.

The prognostic course of patients with a complex variant of t(15;17) does not differ from that of patients with the typical t(15;17) as long as the PML::RARa fusion is intact. According to the literature (5,6,12,13,18), almost all patients with APL involving variant translocations have a good response to ATRA treatment. The patient in the present study has thus far also had a good response to ATRA. However, the prognostic impact of a complex translocation in APL and that of the FLT3 mutation is unclear and long-term observation is needed.

In conclusion, the present study reported an extremely rare case of a patient with APL characterized by a complex three-way translocation (15;15;17)(q24;q14;q21) identified by conventional karyotyping, RT-PCR, and FISH analysis and hypothesized to have evolved from the classical t(15;17). The complex translocation consisted of 15q14 on the same chromosome as the classical t(15;17) and was present as a third translocation partner. Based on the preserved PML::RARa, this patient is expected to have a good prognosis, similar to that of patients with the classical t(15;17) translocation, but long-term observation is needed.
Acknowledgements

The author would like to thank Mr. SK Lee and Mrs. JS Kim (Keimyung University Dongsan Hospital, Daegu, Republic of Korea) for their support in performing the chromosome and FISH analyses.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the current study (except NGS data) are available from the corresponding author on reasonable request. It was not possible to submit NGS data to a public database because the NGS service was provided by a commercial laboratory, therefore an accession number to a public NGS database is not available.

Authors' contributions

DK carried out the BM aspiration, karyotyping, FISH analysis, wrote the manuscript and approved the final version of the manuscript. DK confirms the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of Keimyung University Dongsan Hospital (IRB File No 2023-02-053). Informed consent was obtained from the subject involved in the present study.

Patient consent for publication

The patient provided written informed consent for the publication of any data.

Competing interests

The author declares that he has no competing interests.

References


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