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BRIEF COMMUNICATION

An ider(17)(q10)t(15;17) with spliced long-type *PML-RARA* fusion transcripts in a case of acute promyelocytic leukemia

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The ider(17)(q10)t(15;17) is a relatively rare chromosomal rearrangement in acute promyelocytic leukemia patients. We describe herein a case of APL with a poor prognosis and ider(17)(q10)t(15;17)(q22;q12), which was confirmed by fluorescence in situ hybridization. Reverse transcription polymerase chain reaction (RT-PCR) and sequencing of PCR products were used to detect the *PML-RARA* fusion gene and delineate the sequence of the fusion transcripts. We found that the *PML-RARA* fusion gene of this patient was the long isoform, which only generated transcripts of a splice variant lacking *PML* exon 5 and a splice variant lacking *PML* exons 5 and 6. Although the clinical and prognostic significance of patients with an ider(17)(q10)t(15;17) remains unclear, a combination of cytogenetics and molecular biology analysis should be performed to obtain further information about this chromosomal abnormality.

Keywords Acute promyelocytic leukemia, ider(17)(q10)t(15;17), long-type *PML-RARA*, splice variant

Acute promyelocytic leukemia (APL), a distinct subtype of acute myeloid leukemia (AML), is characterized by the specific chromosomal rearrangement, the t(15;17)(q22;q21), resulting in the fusion of the retinoic acid alpha gene (*RARA*) on 17q21 to the promyelocytic leukemia gene (*PML*) on 15q22 (1,2). The *PML-RARA* fusion transcripts are supposed to play a pivotal role in the leukemogenesis of APL and the sensitivity of APL to all-trans retinoic acid (ATRA) (3). The ider(17)(q10)t(15;17)(q22;q21) is an infrequent variant cytogenetic abnormality among APL patients. The clinical features and prognosis of patients with this chromosomal abnormality are currently unclear (4–10).

In the *PML* gene, three breakpoint cluster regions are primarily found: intron 6 (bcr1), exon 6 (bcr2), and intron 3 (bcr3). Depending on the different breakpoint positions, three main *PML-RARA* isoforms usually result, and are referred to as long (bcr1), variant (bcr2), and short isoform (bcr3). The *PML* gene consists of nine exons and several alternatively spliced *PML* transcripts. In APL patients with the bcr1-type isoform, the *PML-RARA* fusion gene usually generates three different transcripts, including a full-length variant without *PML* exon splicing, a splice variant that lacks *PML* exon 5, and a splice variant that lacks *PML* exons 5 and 6 (11,12). We report herein a rare APL case with an ider(17)(q10)t(15;17). Reverse transcription-polymerase chain reaction (RT-PCR) and sequencing detected a long-type *PML-RARA* fusion isoform, which did not transcribe a full-length variant, but only a variant splicing *PML* exon 5 and *PML* exons 5-6.

Materials and methods

Case report

A 46-year-old man was admitted to our hospital because of gingival bleeding and dark stools for 1 week. He had neither previous history of chemotherapy or radiotherapy, nor exposure to known leukemogens. His initial peripheral blood examination showed a hemoglobin level of 57 g/L, platelet count of 12 $\times$ 10^9/L, and white blood cell count of 7.54 $\times$ 10^9/L, with 61% promyelocytes, 14% neutrophils, 3% monocytes, and 22% lymphocytes. Coagulation tests revealed the prothrombin time and activated partial thromboplastin time was

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within normal ranges, but fibrinogen degraded to 1.6 g/L. The bone marrow aspirate showed hypercellular marrow with 93.5% of promyelocytes that had numerous granules, increased Auer rods, and strong myeloperoxidase activity. In immunophenotypic analysis, the blasts were positive for CD13 (88.5%), CD33 (96.6%), CD117 (71.4%), and CD64 (47.3%), and negative for CD34, HLA-DR, CD3, CD7, CD19, TdT, CD14, CD41, CD61, and CD56. A PCR mutational analysis of the *FLT3* gene yielded negative findings. Karyotypic analysis, fluorescence in situ hybridization (FISH), RT-PCR for the *PML-RARA* fusion gene, and sequence analysis of *PML-RARA* were also conducted. The patient was subsequently diagnosed with APL. He was treated with ATRA at a dose of 20 mg/m^2 per day and arsenic trioxide (As_2O_3) at a dose of 0.16 mg/kg per day, as soon as the diagnosis was suspected based on cytological criteria. Despite combined therapy of ATRA and As_2O_3 and positive supportive therapy, he did not achieve remission and finally died of a cerebral hemorrhage and severe infection after 4 weeks of the initial treatment.

Karyotypic analysis and FISH

The patient's bone marrow cells were cultured for 24 hours, exposed to colcemid for 1 hour followed by hypotonic treatment for 40 minutes. Cytogenetic analyses were performed on G-banded chromosome preparations in 20 metaphase cells. Karyotypes were described according to ISCN 2005 (13).

The FISH analysis was performed using a Vysis LSI *PML-RARA* dual-color, dual-fusion translocation probe (Abbott Molecular, Des Plaines, IL), according to the manufacturer's instructions. Signal patterns of 500 interphase cells were scored using a fluorescence microscope.

Molecular genetics

An RT-PCR assay was performed to detect the *PML-RARA* fusion gene. Total RNA was extracted from the patient's bone marrow cells and then reverse transcribed to cDNA with oligo(dT) primers. Nested PCR was used to detect the specific transcripts. In the first round of PCR reactions, cDNA was amplified by forward primers from *PML* exon 3 and reverse primers from *RARA* exon 3. The PCR products were then subjected to a second amplification in nested PCR reactions. To minimize the chance of a false-negative result, two different primer sets were used for detection of *PML-RARA* fusion transcripts. Forward primers from *PML* exon 3 and reverse primers from *RARA* exon 3 were used to identify all *PML-RARA* transcripts as different bands, while forward primers from *PML* exon 6 and reverse primers from *RARA* exon 3 were used to identify all transcripts as a single band (11). The PCR products were purified, directly sequenced on an ABI 3730 automated DNA sequencer (Applied Biosystems), and blasted against GenBank (http://www.ncbi.nlm.nih.gov/genbank/).

Results

The patient's karyotype was interpreted as 46,XY,t(15;17)(q22;q21)[4]/46,XY,der(15)t(15;17) (q22;q21),ider(17)(q10)t(15;17)(q22;q21)[16] (Figure 1). FISH signals from *PML-RARA* probes indicated nuc ish (PML × 4), (RARA × 4), (PML con RARA × 3)[417/500]/(PML × 3), (RARA × 3), (PML con RARA × 2)[64/500] (Figure 1). The variant fusion pattern with three fusion signals in 83.4% of the examined cells corresponded to the clone with the ider(17)(q10)t(15;17), while the typical fusion pattern with two fusion signals in 12.8% of the examined cells represented the clone with the t(15;17).

In this study, the RT-PCR products of the bcr1 isoform positive control in lane 1 (Figure 2) displayed three bands (697 bp PCR products containing *PML* exons 3-6, 553 bp products lacking *PML* exon 5, and 294 bp products lacking *PML* exons 5-6, respectively), all of which were amplified by forward primers from *PML* exon 3, and one band (374 bp products) that was amplified by forward primers from *PML* exon 6. The patient's RT-PCR products showed three bands in lane 2 (553 bp, 374 bp, and 294 bp products, respectively), which meant that this patient expressed the long-type *PML-RARA* transcripts, but only those that consisted of the splice variant lacking *PML* exon 5 and the splice variant lacking *PML* exons 5-6 (Figure 2). Sequencing of the PCR products confirmed the results of the RT-PCR and showed the detailed composition of the fusion transcripts. The results identified *PML-RARA* transcripts of this patient, with fusion between *PML* intron 6 and *RARA* intron 2. Exon 5 or exons 5-6 of the *PML* gene was spliced out of the *PML-RARA* fusion products (Figure 2).

Discussion

APL is a well-defined subtype of AML that is characterized by abnormal promyelocytes. Although the specific t(15;17)(q22;q21) is observed in 70–90% of APL cases, some rare chromosome translocations have been reported (14–16). The ider(17)(q10)t(15;17) is a relatively rare variant cytogenetic abnormality among APL patients and has been reported in only 71 APL cases world-wide (4–10,17–23). This study describes a rare APL case with a poor prognosis and ider(17)(q10)t(15;17)(q22;q12). The molecular genetics studies demonstrated that the *PML-RARA* fusion transcripts of this patient were spliced long-type isoforms.

In all 72 cases with the ider(17)(q10)t(15;17), including the case in this study, clinical course and follow-up data were available in only 36 cases. The complete remission (CR) rate was 77.8% (28 of 36). Almost all the adult patients with an ider(17q)(q10)t(15;17) who did not die during induction therapy had favorable responses to ATRA. In the follow-up periods, one patient developed a therapy-related AML 1 year after attaining CR. A total of 17 patients died: 9 relapsed after achieving CR and 8 were non-remission cases. Because of the low incidence of ider(17)(q10)t(15;17) cytogenetic abnormalities, the prognostic significance of patients with an ider(17)(q10) t(15;17) is still controversial.

The ider(17)(q10)t(15;17) is an isochromosomal abnormality that might occur due to loss of the short arm and duplication of the long arm of a der(17)t(15;17) after initial reciprocal translocation of the t(15;17), resulting in three *PML-RARA* gene fusions. Alternatively, both events might take place simultaneously. Thus, the clone with the ider(17)(q10)t(15;17) could be the subclone evolved from