The formation of an aberrant PAX5 transcript in a patient with mixed phenotype acute leukemia harboring der(9)t(7;9)(q11.2;p13)

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ABSTRACT

We experienced the case of a 56-year-old male with B-lymphoid/myeloid lineage mixed phenotype acute leukemia (MPAL). A cytogenetic analysis of the patient's bone marrow revealed a complex karyotype, including der(9)t(7;9)(q11.2;p13). We identified an aberrant PAX5 transcript, including the exons 1A to 5 and the contiguous intron 5/6 sequence using the 3′ rapid amplification of cDNA ends-polymerase chain reaction method, and confirmed their expression in the leukemic cells. Our case suggests that der(9)t(7;9)(q11.2;p13) can cause the truncation of the PAX5 transcript, which is supposed to contribute to the generation of MPAL, in addition to three previously reported types of PAX5 fusion.

1. Introduction

Mixed phenotype acute leukemia (MPAL) is a rare hematological malignancy which is characterized by the generation of leukemic blasts with multilineage potential. Genetic analyses revealed that the BCR-ABL1 and KMT2A (MLL1) gene rearrangement in B-lymphoid/Myeloid MPAL are frequently detected, resulting in the formation of each subtype of MPAL. In addition, deletion 6 and 12p11.2 abnormalities have been observed in multiple cases [1,2]. On the other hand, the frequency and significance of PAX5 gene alteration located on 9p13.2, which have been reported in B-progenitor acute lymphoblastic leukemia (B-ALL), remains unknown in MPAL.

We herein report an MPAL patient with der(9)t(7;9)(q11.2;p13) which generated a truncated PAX5 transcript.

2. Case report

A 56-year-old male was referred to our hospital for evaluation of leukocytosis ($29,800 \times 10^9$/L). A bone marrow examination showed a marked proliferation of blasts (88.3%) that were negative for myeloperoxidase (MPO) staining. These blasts were uniformly positive for CD19, CD10, TdT, CD34, MPO and HLA-DR based on the findings of flow cytometry (Fig. 1A), thus suggesting the presence of biphenotypic acute leukemia. A chromosomal analysis of the bone marrow cells revealed a complex karyotype including the derivative chromosome der(9)t(7;9)(q11.23;p13) (Fig. 1B). Transcripts of BCR-ABL1, ETV6-RUNX1, E2A-PBX1, MLL-AF4, MLL-AF6, MLL-AF9 and MLL-ENL were not detected. The patient was therefore diagnosed with MPAL of B-lymphoid/myeloid lineage (not otherwise specified).

3. Result and discussion

Three types of PAX5 fusion in B-ALL with t(7;9)(q11.2;p13) and der(9)t(7;9)(q11.2;p13), including PAX5-ELN, PAX5-AUTS2 and PAX5-POM12, have been reported thus far [3]. In order to analyze the alteration of the PAX5 gene in the present case, we utilized the 3′ RACE-PCR method. We detected an aberrant PAX5 transcript, including exons 1A to 5 and the contiguous intron 5/6 sequence. The presumably truncated PAX5 protein was composed of 256 amino acids. It preserved the paired domain for DNA binding at the N-terminus and acquired an aberrant C-terminus, instead of the transactivation and inhibitory domains for transcription regulation. The expression of this transcript and wild-type transcript (derived from a wild-type allele) in the leukemic blasts was confirmed by reverse transcription (RT)-PCR (Fig. 2).

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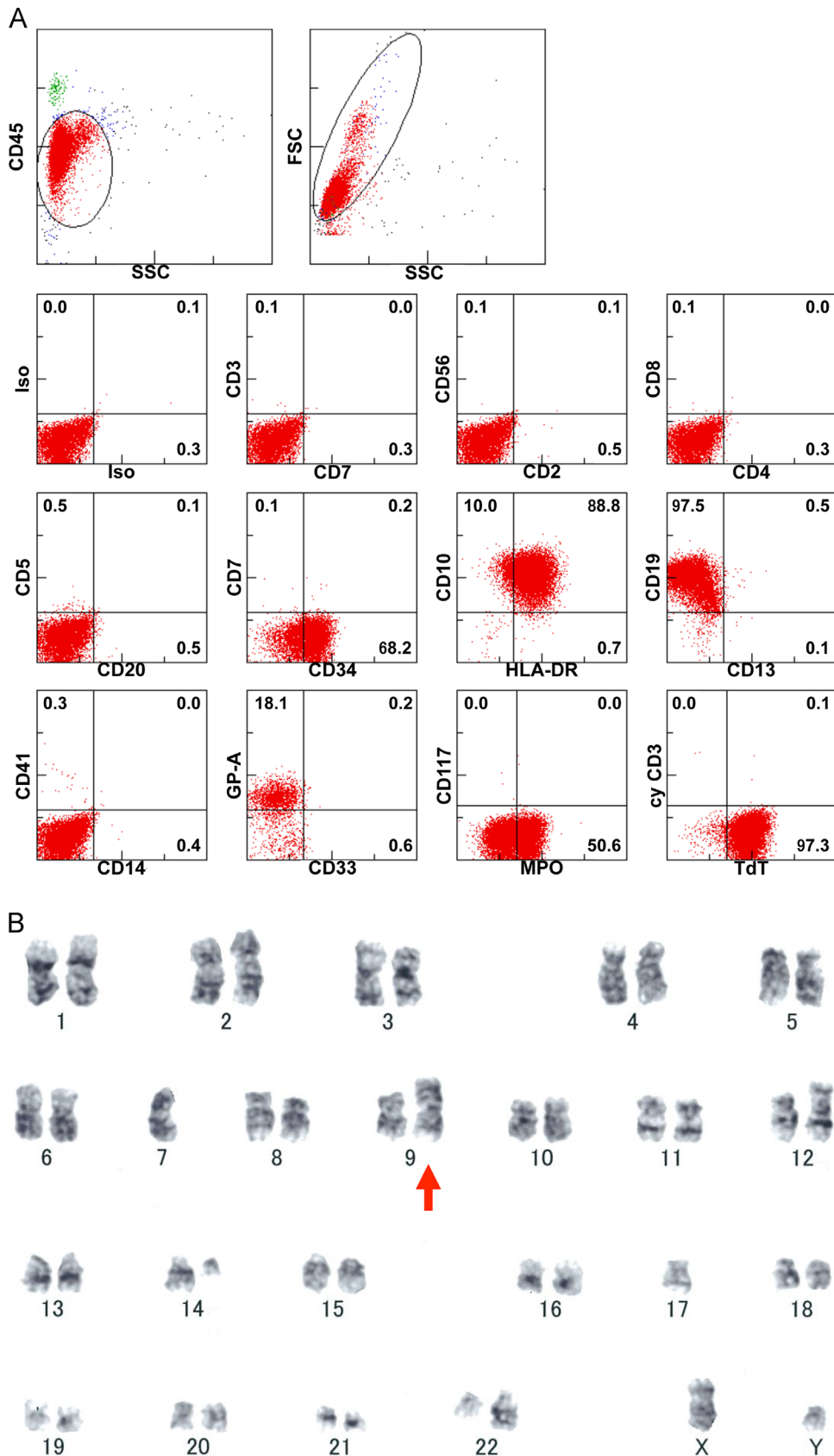E-mail address: hmatsu@is.icc.u-tokai.ac.jp (H. Matsushita).

Fig. 1. The characterization of leukemic cells. A. A flow cytometry analysis of the cellular surface markers. The blasts were found to be positive for CD19, CD10, TdT, CD34, MPO and HLA-DR, while they were negative for CD13, CD33 and CD3. B. The cytogenetic analysis of our case. The karyotype panel shows 44, XY, -7, der(9)t(7;9)(q11.2;p13), dic(12;17)(p11.2;p11.2), ?t(14;22)(q13,q13). The red arrow indicates the derivative chromosome der(9)t(7;9)(q11.23;p13). (For interpretation of the references to color in this figure legend,the reader is referred to the web version of this article.)
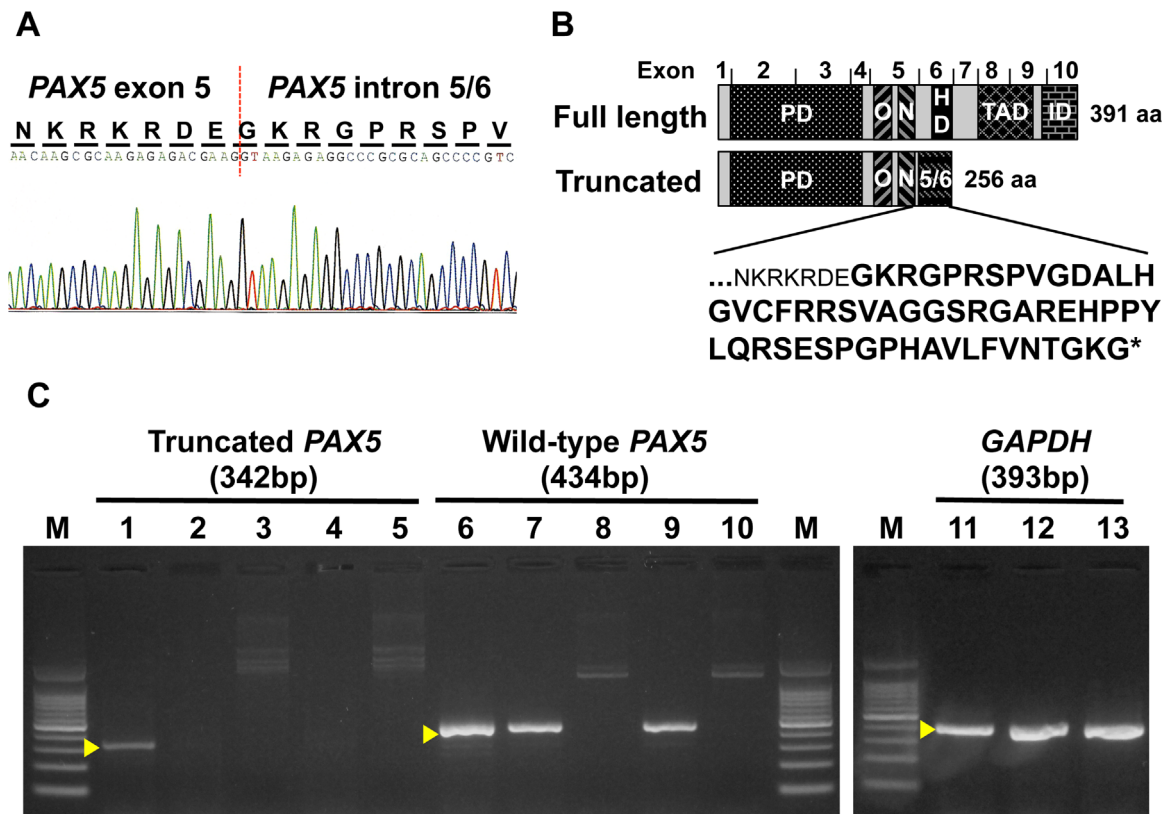
Fig. 2. The detection of the aberrant *PAX5* transcript. A. A sequence chromatogram of the aberrant *PAX5* transcript. B. The presumed structure of the truncated PAX5 protein. The amino acid sequence derived from the intron 5/6 sequence (5/6) is indicated at the bottom of the panel. PD, paired domain; O, octapeptide; N, nuclear localization sequence; HD, partial homeodomain; TAD, transactivation domain; ID, inhibitory domain. **C.** The expression of the truncated and wild-type *PAX5*, and *GAPDH* transcripts. The arrowheads indicate the specific amplified bands. Burkitt cell lines, Daudi and Akata cells, were used as negative controls. Lanes 1, 6 and 11, the cDNA from the patient; lanes 2, 7 and 12, the cDNA from the Akata cells; lanes 3 and 8, the genomic DNA from the Akata cells; lanes 4, 9 and 13, the cDNA from the Daudi cells; lanes 5 and 10, the genomic DNA from the Daudi cells. M, DNA size markers.

The addition of the intron 5/6 sequence at the 3′ end was supposed to be generated by chromosomal translocation without the fusion partner gene at 9q13; The *PAX* gene was simply broken at intron 5/6 without the supply of a splice acceptor site at the 3′ end of the intron, resulting in the in-frame transcription of the unremoved intron sequence. An identical truncated transcript was also detected in 3 B-ALL cases including a case with dic(9;16)(p13; q11), but not der(9)t(7;9)(q11.2;p13) [4], suggesting that this genetic alteration can be generated by genomic instability that is not related to a specific rearrangement of *PAX5*.

The *PAX5* gene encodes a transcriptional factor, which is specifically expressed at the early stages of B-cell differentiation and it is required for B-cell development. Its dysregulation is involved in the leukemogenesis of B-ALL. The *PAX5* gene rearrangements account for approximately 2.5% of pediatric B-ALL cases [5]. A genome-wide analysis revealed that one-third of pediatric B-ALL cases had somatic mutations in PAX5, which resulted in the generation of a hypomorphic allele of the *PAX5* gene [6]. Chemical and retroviral mutagenesis significantly increases the penetrance of B-ALL in mice with a heterozygous loss-of-function mutation of *PAX5* [7], suggesting that the reduced expression and dysregulation of PAX5 function are closely related to the development of B-ALL.

However, the PAX5 function in bi-phenotypic B-lymphoid/ myeloid cells remains obscure; one study showed that ectopic *PAX5* expression was required for their maintenance [8]. Another demonstrated that low-level *PAX5* expression was required for MPAL development [9]. To the best of the authors' knowledge, there are no other case reports of *PAX5* gene or 9p13.2 abnormalities in MPAL patients. The truncated PAX5 that was observed in the present study behaves as a competitive inhibitor of wild-type

PAX5 transcriptional activity [6], suggesting that the truncated PAX5 may also contribute to the development of MPAL by inhibiting the function of wild-type PAX5.

The leukemogenesis of MPAL could be heterogeneous and even complicated in some cases, because the development of MPAL requires the inhibition of multilineage differentiation in leukemic blasts. The complex karyotype in the present case indicates the possible combined involvement of PAX5 inhibition derived from truncated PAX5 and myeloid dysregulation provided by other genetic abnormalities such as deletion 6 and 12p11.2 cytogenetic aberrations resulting in the development of MPAL.

Regarding the technical effectiveness of RACE, RACE is not always successful at identifying chimeric transcripts even if PAX5 fusions are observed. As MPAL cases associated with 9p13 translocation are quite rare, a molecular analysis using these individual cases are thus considered to be important to understand the expression and function of PAX fusion in MPAL leukemogenesis. Therefore, comprehensive molecular techniques, such as RNA sequencing, may be helpful and informative for this purpose.

In conclusion, this is the first case of MPAL with der(9)t(7;9) (q11.2;p13), where an aberrant PAX5 transcript was detected.

Authors' contributions

JA collected the clinical information and drafted the manuscript. HMa designed the study and drafted the manuscript. YK carried out the molecular analyses. RN carried out the histopathological analyses. HMu collected the clinical information and a bone marrow sample. MK supervised the study. KA supervised the

study and drafted the manuscript. All authors read, discussed and approved the final version of the manuscript.

Competing interests

The authors declare no competing interests in association with the present study.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.lrr.2016.04.001.

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