Case Report

The Partial Duplication of the 5′ Segment of KMT2A Revealed KMT2A-MLLT10 Rearrangement in a Boy with Acute Myeloid Leukemia

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The duplication of 5′ segment of KMT2A is a rare molecular event in childhood leukemia, and the influence on prognosis is unknown. Here, we report on a boy who developed acute monocytic leukemia. Fluorescence in situ hybridization revealed the duplication of the 5′ segment with 2 normal alleles at KMT2A which was eventually found to be fused with MLLT10. Chemotherapy promptly induced the first complete remission in the patient at our facility, and the patient remained in first complete remission with negative minimal residual disease at 3.5 years from diagnosis. Our case is similar to two previously reported patients who had partial duplication of the 5′ segment of KMT2A with a KMT2A-MLLT10 rearrangement. Further studies and experience with this cryptic translocation may shed more light on the management of acute myeloid leukemia.

1. Introduction

The histone-lysine N-methyltransferase 2A enzyme (MLL1), encoded by the KMT2A gene, is an upregulator of global, hematopoietic gene transcription, and translocation rearrangement within KMT2A causes variable risk stratification in acute leukemia based on the final genetic outcome.

Patients with MLL rearrangement other than t(9;11) and t(11;19) have an inferior outcome [1], and there are additional aberrations in KMT2A rearrangement, such as fusion with preferential partner gene MLLT10, that also carry prognostic significance [2].

One of these aberrations is the duplication/amplification of the 5′ segment of KMT2A, which is a very rare molecular event, and the influence of this on patient prognosis is unknown. However, in general, KMT2A amplification as an acquired genetic aberration has been reported to result in a poor prognosis [3]. With this deleterious effect of KMT2A overexpression in acute leukemia in mind, it is possible that even partial amplification could affect the patient’s prognosis. In the literature, only 2 cases have been reported: one pediatric case of AML-M5b (FAB: French-American-British Classification) with the duplicated 5′ segment of KMT2A relapsed 16 months after diagnosis during maintenance therapy and was later salvaged by allogeneic transplantation [4] and an adult case of AML-M5a (FAB) with an amplified 5′ part of 11q23.3 where KMT2A was located eventually needed transplantation [5]. In both literature cases, this partial duplication was paired with KMT2A-MLLT10 rearrangement.

Although preferential fusion with MLLT10 has been well documented, the isolated prognostic importance of the partial amplification of KMT2A remains unknown. Here, we
present the case of a pediatric male patient with AML who was successfully treated by multiagent chemotherapy alone. The 5′ duplication of KMT2A was identified by fluorescence in situ hybridization (FISH) before treatment, but fusion to MLLT10 was discovered by RNA sequencing after completion of the treatment even though reverse transcription-PCR at the diagnosis did not detect any fusion partners.

2. Case Description

A 6-year-old boy was admitted with complaints of low-grade fever, multiple joint pain, skin rash, and neutropenia. A complete blood count was conducted; leukocytes were $1.6 \times 10^9/L$, hemoglobin was 9.1 g/dl, and platelet count was $222 \times 10^9/L$. The bone marrow was replaced by 90% monoblasts. Flow cytometry was conducted on the leukemic cells. HLA-DR, CD58, CD99, CD56, CD38, cy-MPO, CD11b, CD13, CD33, CD65, CD64, CD117, CD36, CD61, CD4, and 7.1 were positive, and CD14, CD15, CD19, CD20, CD3, and CD7 were negative. He was diagnosed with AML (FAB M5a). FISH analysis using a KMT2A locus-specific dual-color DNA probe (Vysis LSI MLL Dual Color, Break Apart Rearrangement Probe, Abbott Laboratories, IL, USA) was used to characterize the partial 5′ duplication and 2 other normal KMT2A alleles (Figure 1). Cell culture for chromosomal analysis failed. The search for KMT2A-MLLT10 fusion was performed according to a publication previously reported [6]. Reverse transcription-polymerase chain reaction of major fusion partners to KMT2A (including MLLT10) was conducted and none were amplified. The primer for KMT2A was designed in exon 8.

Final diagnosis, in this case, was AML with 11q23/KMT2A abnormalities (FAB M5a) without any confirmation of partner genes. As he presented no abnormalities such as t(8;21), inv(16), -7, 5q-, t(16;21)(p11;q22), Ph1, and FLT3-ITD, he underwent multimodal chemotherapy for the intermediate risk group according to JPLSG AML05, which consists of induction 1 (ECM: etoposide 150 mg/m²/day on days 1–5, cytarabine 200 mg/m²/day on days 6–12, mitoxantrone 5mg/m²/day on days 6–10, and intrathecal chemotherapy on day 6), induction 2 (HCEI: cytarabine 3g/m² every 12 hours on days 1–3, etoposide 100mg/m²/day on days 1–5, idarubicin 10mg/m²/day on days 1–5, and intrathecal chemotherapy on day 1), and 3 intensification therapy (HCM: cytarabine 2g/m² every 12 hours on days 1–5, mitoxantrone 5mg/m²/day on days 1–3, and intrathecal chemotherapy on day 1; HCEI; and HCM) [7]. The chemotherapy promptly induced a first complete remission in the patient which has persisted 3.5 years from diagnosis without hematopoietic stem cell transplantation.

RNA sequencing on NextSeq500 (illumina, Inc., CA, USA) was then used to screen for fusion partners, revealing a KMT2A-MLLT10 rearrangement. The RNA analysis was conducted as follows: RNA was purified from the patient’s bone marrow at diagnosis using ISOGEN (Nippon Gene Co., Ltd., Tokyo, Japan) according to the manufacturer’s instructions. A sequencing library was then generated from 500ng of total RNA using a TrueSeq Stranded mRNA Library Prep Kit v2 (illumina) according to the manufacturer’s instructions. Next, sequencing was conducted at i-Laboratory LLP (Ibaraki, Japan). Obtained reads were aligned

Table 1: All sequencing data for breakpoint.

<table>
<thead>
<tr>
<th>Sequencing Data</th>
<th>Breakpoint</th>
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<tbody>
<tr>
<td>Underlined</td>
<td>KMT2A</td>
</tr>
<tr>
<td>Nonunderlined</td>
<td>MLLT10</td>
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Figure 1: FISH and RT-PCR of the KMT2A rearrangement. (a) FISH of the KMT2A break apart signal: the green signal shows the 5′ segment, and the red signal shows the 3′ segment of KMT2A. FISH revealed 2 normal signals (arrowhead) and 1 additional 5′ segment (arrow). Positive cells with duplication of the 5′ segment of KMT2A were observed in 686 out of 1000 cells. (b) The characterization of KMT2A-MLLT10 fusion transcript at bone marrow by RT-PCR. 1: ladder; 2: normal control; 3: at diagnosis; 4: after induction therapy 1; 5: after induction therapy 2; 6: after intensification 1; 7: after intensification 2; 8 and 9: after intensification 3.
toward human genome assembly hg19, and fusion gene analysis was conducted with CLC Genomics Workbench Ver. 7.5.1 software (Qiagen, Venlo, Netherlands). Four sequencing tags supporting KMT2A-MLLT10 rearrangement were obtained, which were then confirmed by Sanger sequencing. All sequencing data are shown in Table 1. Designed primers were 5'-TCAATTGCTGGCTAGAAAGA-3' (KMT2A exon 5) and 5'-CIGAGCTTAAAGAGCTTGCAATT-3' (MLLT10 exon 16). The KMT2A breakpoint was located on intron 6, which was the upstream region of the sequencing primer for screening at diagnosis, and the MLLT10 breakpoint was located on exon 14. Minimal residual disease (MRD) by reverse transcription-polymerase chain reaction (RT-PCR) was located on exon 14. Minimal residual disease (MRD) by primer for screening at diagnosis, and the intron 6, which was the upstream region of the sequencing primer for screening at diagnosis, and the MLLT10 breakpoint was located on exon 14. Minimal residual disease (MRD) by reverse transcription-polymerase chain reaction (RT-PCR) was assessed from diagnosis as shown in Figure 1. Although MRD was positive at diagnosis, it changed to negative after induction therapy 1.

3. Discussion

Pediatric acute myeloid leukemia is classified by chromosomal and/or genetic abnormalities according to the World Health Organization Classification published in 2008. Chimeric genes including KMT2A rearrangements are used to predict disease outcome. KMT2A rearrangements are seen in about 20% of pediatric AML and are associated with poor outcome, while the disease outcome depends on its partner gene [1]. KMT2A has many partner genes, and each chimeric gene has a different prognosis. It is difficult to quantify the effect of the partial duplication/amplification of KMT2A as the two patients previously reported had the same rearrangement of KMT2A-MLLT10 with different treatment outcomes. A single abnormality within amplification of KMT2A is reported to have a gain-of-function effect for leukemogenesis [3]; however, the exact role of this “partial” duplication of KMT2A in acute leukemia is still unclear.

It is interesting to note that both previously reported and the current cases have the same rearrangement along with this partial duplication/amplification and diagnosis of FAB M5. Hence, we hypothesize that the leukemic cells that partially duplicate KMT2A tend to undergo KMT2A-MLLT10 fusion and may act more similarly to KMT2A-MLLT10 rearrangements caused by the insertion of KMT2A in chromosome 10p or an unbalanced translocation. This points to different causes producing the same prognostic effect. Similar rearrangement and the duplication of the 5' segment of KMT2A might result in cell culture difficulties in other cases. Therefore, we can recommend using FISH assays to detect partial KMT2A duplication, and RNA sequencing may be useful to specify the fusion partner in such cases.

4. Conclusion

Partial duplication of the 5' segment of KMT2A can be easily detected by FISH, but the crucial details of the KMT2A-MLLT10 rearrangement may remain hidden from standard PCR testing, which might result in poor prognosis.

Ethical Approval

This study was approved by the ethics committee of the University of Tsukuba Hospital (H23-128) following the Ethical Guidelines for Medical and Health Research Involving Human Subjects of the Ministry of Health, Labor and Welfare of Japan and the Declaration of Helsinki.

Consent

Written informed consent was obtained from the patient's parents.

Conflicts of Interest

The authors have no conflicts of interest to declare with regard to this work.

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