PAX5 alterations in an infant case of KMT2A-rearranged leukemia with lineage switch

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Funding information
Japan Society for the Promotion of Science KAKENHI, Grant/Award Number: JP19J11112, JP17H04224, JP18K19467, JP20H00528, JP21K19405; Project for Cancer Research and Therapeutic Evolution, Grant/Award Number: JP20cm0106509h9905; Practical Research for Innovative Cancer Control, Grant/Award Number: Japan Agency for Medical Research and Development, Grant/Award Number: JP19ck010646h0001, JP21ck0106531; Princess Takamatsu Cancer Research Fund; The Mother and Child Health Foundation; Japan Leukemia Research Fund.

Abstract
Lineage switch is a rare event at leukemic relapse. While mostly known to occur in KMT2A-rearranged infant leukemia, the underlying mechanism is yet to be depicted. This case report describes a female infant who achieved remission of KMT2A-MLLT3-rearranged acute monocytic leukemia, but 6 months thereafter, relapsed as KMT2A-MLLT3-rearranged acute lymphocytic leukemia. Whole exome sequencing of the bone marrow obtained pre-post lineage switch revealed two somatic mutations of PAX5 in the relapse sample. These two PAX5 alterations were suggested to be loss of function, thus to have played the driver role in the lineage switch from acute monocytic leukemia to acute lymphocytic leukemia.

KEYWORDS
infant leukemia, KMT2A rearrangement, lineage switch, PAX5, whole exome sequencing

Abbreviations: ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; BCP-ALL, B cell precursor lymphoblastic leukemia; KMT2A, histone-lysine N-methyltransferase 2A; MLLT3, super elongation complex subunit; MRI, magnetic resonance imaging; PAX5, paired box 5; PAX-alt, PAX-altered; RNA-seq, RNA sequencing; SIFT, sorting intolerant from tolerant; VAF, variant allele frequency.

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**1 | INTRODUCTION**

Lineage switch is defined as conversion of leukemic cell lineage on relapse (i.e., AML to ALL, or vice versa), while retaining the same clonal origin. It is a rare phenomenon, occurring in approximately 0.6% of pediatric leukemia cases. Most cases of lineage switch occur in KMT2A-rearranged infant leukemia and result in an extremely poor prognosis. However, the underlying mechanism is unknown.

Here, we describe a female infant who presented initially with KMT2A-MLLT3-rearranged AML; however, at 6 months post-remission, she relapsed with KMT2A-MLLT3-rearranged ALL. The patient was treated with chemotherapy and hematopoietic stem cell transplantation, by which she achieved complete remission. Whole exome sequencing of the relapse bone marrow sample identified two somatic mutations in PAX5, which provides insight into the pathogenesis underlying lineage switch.

The study was approved by the Ethics Committee of Kyoto University and carried out in accordance with the Declaration of Helsinki. The patient’s parents provided written informed consent.

**2 | METHODS AND RESULTS**

**2.1 | Case presentation**

A female infant born at 38 weeks had a blueberry muffin baby appearance. As previously reported, extramedullary blast infiltrates were seen in the skin and cerebrospinal fluid. Bone marrow examination led to an initial diagnosis of AML-M5a (Figure 1A,C). KMT2A-MLLT3 gene rearrangement was confirmed by FISH and RT-PCR. The patient was treated according to the standard regimen for AML and achieved complete molecular remission. However, at 6 months post-treatment she was readmitted with fever and rapid enlargement of the cervical lymph nodes.

On admission, laboratory blood analyses indicated the following: white blood cell count, 509,640/μl; blast cell count, 98.5%; hemoglobin level, 9.8 g/dl; and platelet count, 1.5 × 10⁹/μl. Head MRI and cerebral fluid analysis showed no evidence of central nervous system involvement. Bone marrow examination showed monotonous proliferation of small and immature lymphoblasts (Figure 1B). Immunophenotyping by flow cytometry showed that these cells were CD19- and cyCD79-positive, and CD10-partially positive. Morphologic and flow cytometry examination of blast cells suggested a diagnosis of BCP-ALL (Figure 1D). However, KMT2A-MLLT3 rearrangement was also seen in the relapse sample, suggesting that the leukemic blasts had undergone a lineage switch from AML to ALL.

The patient was treated according to the Japanese Pediatric Leukemia/Lymphoma Study Group mixed lineage leukemia (MLL)-10 protocol. She achieved complete remission after early consolidation therapy, but relapsed again within 1 month. Therefore, she received salvage therapy consisting of clofarabine, etoposide, and cyclophosphamide. After one course, the patient reached complete molecular remission. Taking into account the favorable response to clofarabine, she underwent cord blood transplantation using a preconditioning regimen consisting of clofarabine (40 mg/m²/day, days −7 to −3) and busulfan (once daily with an area under the receiver operating characteristic curve target of 4000–5000 μmol*min/L, days −6 to −3). Post-transplant recovery was uneventful, and neutrophil engraftment was achieved on day 19. At 12 months post-transplant, the patient remains in complete molecular remission, with no signs of graft-versus-host disease.

**2.2 | Whole exome sequencing**

Whole exome sequencing was undertaken on bone marrow samples obtained at initial diagnosis, remission, and the time of lineage switch relapse. The DNA sample obtained at remission was used as a germline control. Genomic DNA was extracted from bone marrow with NucleoSpin Blood (Macherey-Nagel). Sequence alignment to the hg19 human genome and mutation calling were carried out using our in-house pipelines, with minor modifications. The results revealed nine and 27 exonic variants in the diagnosis and relapse samples, respectively, but no mutations were shared (Table S1). All of these variants were located in the copy number neutral and loss of heterozygosity free portions of the genome. To infer the clonal structures, we clustered each variant by VAF and identified three mutation clusters (Figure 2A) through sciClone (https://github.com/genom e/sciclone). While the primary samples harbored only subclonal clusters with a paucity of mutations (Cluster 1; VAF range 5.1%–7.0%), the relapse sample harbored a high-VAF cluster (Cluster 2; VAF range 26.9%–54.7%) corresponding to its founding clone, as well as a subclonal cluster (Cluster 3; VAF range 5.3%–19.4%). Of note, Cluster 2 contained two mutations in PAX5: a nonsense variant (p.Ser285X) and a missense variant (p.Gly30Lys).

**2.3 | RNA sequencing**

To investigate the effects of these PAX5 alterations, we compared the RNA-seq gene expression profiles between our samples and the publicly available RNA-seq profiles of 579 childhood ALL and AML cases from the St. Jude Cloud dataset. RNA was extracted from bone marrow with an RNaseq kit (Qiagen). Sequencing libraries were prepared using NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (New England Biolabs) according to the manufacturer’s protocol, and prepared libraries were sequenced with an MGI DNSEQ platform. The read count of each annotated gene was calculated using the HTSeq package and passed on to the interactive T-distributed stochastic neighbor embedding workflow of St. Jude Cloud (https://github.com/stjudedcloud/expression-classification). The relapse samples showed a gene expression profile closer to the PAX5-alt subtype of BCP-ALL rather
than the KMT2A-rearranged subtype (Figure 2B). This suggests that the two PAX5 alterations consistent with biallelic targeting (p.Ser285X and p.Gly30Lys) are driver mutations of equal importance to those found in the PAX5-alt BCP-ALL subtype. In addition to DNA mutations, RNA sequencing of the primary sample identified two previously reported fusion transcripts (SS18L1–ADRM1 and USP15–MON2) in addition to KMT2A–MLLT3, but only KMT2A–MLLT3 was detected in the relapsed samples. Additionally, RNA-seq confirmed that the fusion breakpoints at the exon level of KMT2A–MLLT3 were identical between primary AML and relapse BCP-ALL cells. Thus, the primary and relapsed samples shared only one founder fusion gene, KMT2A–MLLT3, indicating that the disease relapsed from a preleukemic ancestral clone. The clonal evolution from initial diagnosis to the time of relapse is shown in Figure 2C. Regarding PAX5 alterations at relapse, we speculate from the VAF values that PAX5 p.Ser285X was the initial mutation and PAX5 p.Gly30Lys was acquired subsequently.

3 | DISCUSSION

Lineage switch is a rare event. Indeed, a large cohort of 1482 pediatric leukemia patients contained only nine such cases (0.6%), and only two of the nine were a lineage switch from AML to ALL. Interestingly, eight out of the nine cases were infant leukemia, while seven harbored KMT2A gene rearrangement. Given the frequency of lineage switch to KMT2A-rearranged infant leukemia, past studies suggest several hypotheses to explain the underlying mechanism. These include the existence of bipotential progenitors, cell reprogramming, dedifferentiation, and clonal selection; however, the precise mechanism has not been identified. The present case study is the first to identify a mutation in PAX5 in a relapsed clone, suggesting that mutation of the PAX5 gene could play a driver role in the lineage switch from AML to ALL in KMT2A-rearranged infant leukemia.

The PAX5 gene is a master regulator of B cell development, and its monoallelic mutation is seen frequently in pediatric BCP-ALL.
A recent report identified a subtype of BCP-ALL in which biallelic PAX5 alterations causing loss of WT PAX5 expression were suggested to be the driver of acute lymphoblastic leukemogenesis. The two PAX5 variants observed in our case were a nonsense variant (p.Ser285X) and a missense variant (p.Gly30Lys). The latter resides in the DNA binding domain of PAX5, which is a mutation hotspot associated with loss of PAX5 function. Also, the SIFT score was 0.0 for p.Gly30Lys and considered deleterious. Therefore, both PAX5 alterations in our case are thought to result in biallelic inactivation of PAX5. Moreover, the fact that heterozygous deletion of PAX5 often coincides with KMT2A-MLLT3 ALL whereas KMT2A-MLLT3 itself is more frequently associated with AML than with ALL supports the notion that PAX5 is an important contributor to lineage determination in infant KMT2A-MLLT3 rearranged leukemia.

KMT2A rearrangement in AML is thought to occur in hematopoietic stem cells or myeloid progenitor cells; the former is considered to initiate leukemia more rapidly and is less responsive to chemotherapy. The clinical course of the patient described herein suggests that KMT2A rearrangement occurred at the stem cell level, as discussed in previous reports. Thus, we suggest that KMT2A-MLLT3-rearranged leukemia initiating cells, which survived after chemotherapy for primary AML, acquired PAX5 mutations, lead to PAX5 inactivation, which then induced full-blown BCP-ALL.
In conclusion, the case presented herein provides insight into the mechanism underlying lineage switch in KMT2A-rearranged infant leukemia.

ACKNOWLEDGMENTS
The authors would like to thank the patient and families for their cooperation in this study. This work was supported by JSPS KAKENHI Grant Numbers JP19J11112, JP17H04224, JP18K19467, JP20H00528, and JP21K19405; Project for Cancer Research and Therapeutic Evolution (P-CREATE; grant no. JP20cm0106509h9905), and Practical Research for Innovative Cancer Control (grant no. JP19ck0106468h0001and JP21ck0106531) from Japan Agency for Medical Research and Development (AMED); Princess Takamatsu Cancer Research Fund; The Mother and Child Health Foundation; Japan Leukemia Research Fund.

DISCLOSURE
The authors have no conflict of interest.

APPROVAL OF THE RESEARCH PROTOCOL BY AN INSTITUTIONAL REVIEW BOARD
N/A.

INFORMED CONSENT
The patient’s parents provided written informed consent.

REGISTRY AND REGISTRATION NO. OF THE STUDY/TRIAL
N/A.

ANIMAL STUDIES
N/A.

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