Transformation of Severe Aplastic Anemia into Donor Cell Leukemia after Allogeneic Hematopoietic Stem Cell Transplantation: A Rare Case Report

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Conflict of interest: None declared

Patient: Female, 51-year-old

Final Diagnosis: Donor cell leukemia

Symptoms: Petechiae and fatigue

Clinical Procedure: —

Specialty: Hematology

Objective: Rare disease

Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an important treatment for severe aplastic anemia (SAA). It is known that SAA can evolve into malignant clonal diseases, such as acute myeloblastic leukemia (AML) or myelodysplastic syndrome. However, the transformation of SAA into AML after allo-HSCT is a rare phenomenon. Here, we report a case of SAA transformed into AML after patient received human leukocyte antigen (HLA)-matched sibling peripheral blood stem cell transplantation.

Case Report: A 51-year-old female patient presented with petechiae and fatigue and received a diagnosis of idiopathic SAA. The immunosuppressive therapy combined with umbilical cord blood transplantation failed for this patient. Then, she received HLA-matched sibling allogeneic peripheral blood stem cell transplantation (allo-PBSCT). However, 445 days after allo-PBSCT, the patient had a diagnosis of AML by bone marrow puncture. Donor-recipient chimerism monitoring and cytogenetic analysis confirmed that the leukemia was donor cell origin. Notably, a new HOXA11 mutation was detected in the peripheral blood of the patient after transplantation by whole-exome sequencing, which was the same gene mutation detected in the donor. The patient received 1 cycle of induction chemotherapy with azacytidine and achieved complete remission. However, the leukemia relapsed after 2 cycles of consolidation chemotherapy. Unfortunately, the patient died of leukemia progression 575 days after allo-HSCT.

Conclusions: The mechanism of how normal donor hematopoietic cells transform to leukemia in the host remains unclear. Donor cell leukemia provides a unique opportunity to examine genetic variations in donors and hosts with regards to the progression to malignancy.

Keywords: Aplastic Anemia, Idiopathic • Hematopoietic Stem Cell Transplantation • Leukemia

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**Background**

Aplastic anemia (AA) is an immune-mediated bone marrow failure disease. Previous studies have reported that AA can evolve into malignant clonal diseases, such as myelodysplastic syndrome (MDS) or acute myeloblastic leukemia (AML), with a transformation rate of 10% to 20% [1]. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the recommended treatment for patients with severe aplastic anemia (SAA) who have suitable donors and are younger than 40 years old or have failed in immunosuppressive therapy, including antithymocyte globulin (ATG) and cyclosporine A [2]. The development of secondary malignancies is one of the long-term complications after transplantation. Donor cell leukemia (DCL) is defined as leukemia that develops in engrafted cells of donor origin. Studies have found that DCL can account for more than 2% of leukemia relapses after transplantation [3]. However, only 5 cases of DCL transformed from SAA after allo-HSCT have been reported to date [4-8]. With the development of whole-exome and genome sequencing, some candidate genes that may contribute to the occurrence of DCL have been detected [3]. Here, we present a case of DCL transformed from SAA 445 days after human leucocyte antigen (HLA)-matched sibling peripheral blood stem cell transplantation. In addition, we found a new HOXA11 mutation in the peripheral blood of the patient after transplantation by whole-exome sequencing, which was the same gene mutation detected in the donor.

**Case Report**

A 51-year-old female patient was admitted to our hospital with petechiae and fatigue in April 2021. The complete blood count (CBC) showed pancytopenia, with a white blood cell count of 2.13×10^9 cells/L, hemoglobin level of 79 g/L, and platelet count of 25×10^9 cells/L. The neutrophil count was 0.38×10^9 cells/L, and the reticulocyte count was 0.02×10^12 cells/L. Tests for viral infections, including hepatitis virus, HIV, syphilis, cytomegalovirus, and Epstein-Barr virus, were negative. Autoimmune diseases were excluded by serological tests. There was no indication of hemolytic anemia, nutritional anemia, or paroxysmal nocturnal hemoglobinuria. Bone marrow smear showed marked hypoplasia and contained mainly lymphocytes (Figure 1A, 1B). Bone marrow biopsy showed about 10% of cellularity, and most of the hematopoietic tissue was replaced by adipose tissue (Figure 1C, 1D). Furthermore, no megakaryocytes or blast cells were identified on bone marrow smears or biopsy specimens. Acute leukemia and MDS were further excluded by immunophenotype analysis. Conventional cytogenetic analysis showed a normal 46, XX karyotype in 24 metaphases. Mitomycin C induced peripheral blood chromosome breakage detection, and chromosome aberration tests were normal. Comet assay was used to detect the DNA damage in peripheral blood lymphocytes. Comet cells were formed when fragmented DNA migrated out of the nucleoid (also known as the “comet head”) and formed a DNA stain in the agarose gel (also known as the “comet tail”). The percentage of comet cells was 16%. There was no history of exposure to drugs, chemicals, or radiation. The patient’s family members were all healthy and none of them had similar blood disorders. There was no history of consanguineous marriage in her family. The patient had no symptoms of infection. Physical examination revealed pallor and petechiae. The patient did not have any developmental abnormalities. Therefore, idiopathic SAA was diagnosed.

The patient received immunosuppressive therapy combined with umbilical cord blood transplantation in April 2021. A total of 31.8 mL autologous frozen umbilical cord blood was infused. The immunosuppressive therapy regimen consisted of rabbit ATG (3.5 mg/kg/d×5 days, intravenously) and cyclosporine A (3-5 mg/kg, orally, dosage adjusted according to drug concentration). The patient was treated with granulocyte colony stimulating factor (G-CSF) for 14 days. The granulocytes were engrafted at day 14 (neutrophils >0.5×10^9 cells/L for 3 consecutive days). Donor-recipient chimerism analysis by short tandem repeat was 96.97% at day 36. However, the hemoglobin level and the platelet count did not return to normal. Bone marrow smear and biopsy at day 104 showed marked hypoplasia, and no megakaryocytes were seen. The immunosuppressive therapy combined with umbilical cord blood transplantation failed for this patient.

In November 2021, the diagnosis of idiopathic SAA was confirmed by a bone marrow puncture performed at People’s Hospital of Peking University. The patient received HLA-matched sibling allogeneic peripheral blood stem cell transplantation (allo-PBSCT). The donor was her 49-year-old younger brother. Before transplantation, the donor had comprehensive examinations, including CBC, coagulation routine test, liver and kidney function, viral infection tests, urinalysis, stool routine test, electrocardiogram, echocardiography, chest X-ray, and abdominal ultrasound. All these examinations were normal. The results of bone marrow morphology, biopsy, immunophenotype analysis, and chromosome karyotype analysis confirmed that the hematopoietic function of the donor was normal. Psychological assessment was performed to exclude psychological disorders, such as schizophrenia and depression. Physical examination of the donor was normal. The donor had no vascular complications or other diseases. The donor was demonstrated to be clinically healthy.

Before allo-PBSCT, whole-exome sequencing was performed on the patient to further exclude congenital bone marrow failure. Only a heterozygous missense mutation was detected in RTEL1 (p.K472N) in patient’s peripheral blood, with the variant allele frequency of 34.32%. The conditioning regimen consisted of...
cyclophosphamide/fludarabine/ATG (Cy 50 mg/kg/d×3 days, Flu 30 mg/m²/d×5 days, ATG 2.5 mg/kg/d×5 days). A total of 7.67×10⁸ mononuclear cell/kg containing 2.48×10⁶/kg CD34+ cells were infused within 2 days. Cyclosporine A, mycophenolate mofetil, and short-term methotrexate were used to prevent graft-vs-host disease. The granulocytes were engrafted at day 14. Bone marrow smear and biopsy showed normal hyperplasia and megakaryocytes counts. The patient developed cytomegaloviremia at day 27. The chimerism was complete donor chimerism in the first 6 months after transplantation. However, the short tandem repeat indicated mixed donor chimerism at months 9, 10, and 11 after transplantation, with donor cells proportions of 92.66%, 88.35%, and 89.6%, respectively. The CBC result was still close to normal. No blast cells were identified on bone marrow smears or biopsy specimens. The patient did not develop acute graft-vs-host disease, chronic graft-vs-host disease, or other complications after allo-PBSCT.

However, 445 days after allo-PBSCT, the CBC showed anemia and thrombocytopenia with leukocytosis. The white blood cell count was 65.79×10⁹ cells/L, hemoglobin level was 71 g/L, and platelet count was 10×10⁹ cells/L. Bone marrow smear and biopsy were consistent with the diagnosis of AML (Figure 2A, 2B). The immunophenotype analysis showed that 45.2% of the blast cells had monocytic features (AML-M5). The conventional cytogenetic analysis showed a normal donor karyotype of 46, XY in 3 metaphases. Short tandem repeat remained complete donor chimerism. KRAS, NF1, and FLT3 mutations were detected among the 55-gene screening profile, with mutation frequencies of 18.9%, 92.1%, and 0.9%, respectively. These 3 gene mutations were not detected by whole-exome sequencing before allo-PBSCT. AML-M5 was diagnosed. At the time of AML diagnosis, we performed whole-exome sequencing again using peripheral blood samples from the patient and donor to determine whether there were leukemia-related gene mutations. Three gene mutations were detected in the donor, including HOXA11, RTEL1, and THBD. RTEL1 (p.K472N) and THBD (p.R85H) were heterozygous missense mutations with the variant allele frequencies of 52.34% and 50%, respectively. HOXA11 (p.P97_A99del) was heterozygous non-shift code missing, with
the variant allele frequency of 40.64%. Interestingly, only the \textit{HOXA11} (p.P97_A99del) mutation (variant allele frequency of 34.82%) was detected in the patient, while the \textit{RTEL1} mutation disappeared after transplantation. The percentage of myeloblasts in the tested blood sample was 43%. This percentage was compatible with the frequency of the mutated \textit{HOXA11} allele. A third transplantation was one of the treatment options for this patient. However, the patient did not have any other HLA-matched sibling donors or HLA-matched unrelated donors. Haploidentical hematopoietic stem cell transplantation was the only alternative transplantation method for this patient. Considering the transplant-related mortality, the patient and her family preferred chemotherapy. The patient received 1 cycle of induction chemotherapy with azacytidine and achieved complete remission. The minimal residual disease was negative (<0.01%) by flow cytometric immunophenotype analysis. However, the leukemia relapsed after 2 cycles of consolidation chemotherapy. Immunophenotype analysis showed that abnormal myeloid blasts accounted for 77.95%. Considering that the patient had severe pulmonary infection during myelosuppression after induction chemotherapy, the patient then received 2 cycles of demethylation therapy combined with venetoclax, and 1 cycle of low-dose chemotherapy with homoharringtonine and cytarabine. Unfortunately, the leukemia was not controlled, and the patient died of leukemia progression 575 days after allo-PBSCT. The donor remained healthy until the manuscript was finished.

### Discussion

The mechanism of how normal donor hematopoietic cells transform to leukemia in the host remains unclear. Hypotheses include occult leukemia or preleukemic potential in the donor, leukemic transformation of engrafted cells, transfer of oncogenic material from host cells to donor cells, residual effects of cytotoxic chemotherapy or radiotherapy, defective marrow microenvironment, inadequate immune surveillance result from post-transplant immunosuppression, viral transfection/integration, and telomere shortening and replicative stress [9]. In addition, prolonged use of G-CSF has been reported as a risk factor for the malignant evolution of SAA [10]. However, G-CSF use is usually associated with poor granulocytes reconstruction after immunosuppressive therapy or transplantation; therefore, the impact of G-CSF cannot be accurately assessed. Monosomy 7 arises as a recurrent chromosome aberration in DCL [6] and has been previously described in DCL after bone marrow transplantation for SAA. In the setting of decreased hematopoietic stem progenitor cells pools, accelerated telomere attrition is considered a possible mechanism for early myeloid oncogenesis and aneuploidy development in MDS/AML after SAA [10].

DCL is a rare but severe post-transplant complication. Most cases of DCL are primary AML, acute lymphoblastic leukemia, or MDS, accounting for 93% of all reported cases [11]. Only 5 cases of DCL transformed from SAA after allo-HSCT have been reported. Generally, the prognosis of DCL is poor, and there is no recommended standard treatment strategy at present. Standard AML- or acute lymphoblastic leukemia-specific regimens can induce complete remission in DCL; however, the treatment-related mortality is high. Considering the graft-vs-leukemia effect, secondary hematopoietic stem cell transplantation from an alternative donor remains a more logical treatment strategy [9]. For DCL transformed from SAA, treatment experience was obtained from case report studies. Among the 5 reported cases, 1 patient died of refractory septic shock after receiving a second allo-HSCT from the same donor [6]. Another 1 patient died of leukemia progression after receiving intensive chemotherapy consisting of idarubicin (10 mg/m²/day for 3 days) and cytarabine (300 mg/m²/day for 7 days) [5].

![Figure 2. (A) Bone marrow smear showed marked hyperplasia and increased blasts with monocytic morphology (Jenner-Giemsa stain, 400×). (B) Bone marrow biopsy showed infiltration by immature cells (hematoxylin-eosin stain, 400×).](image-url)
The other 3 patients are alive. One patient was treated with standard 3+7 induction chemotherapy with cytarabine and daunorubicin and then received a second allo-HSCT from an alternative donor [7]. One patient obtained remission with chemotherapy [4]. Another patient remained molecular remission for more than 3 years with chemotherapy and IL-2 maintenance therapy [8]. A brief comparison of the similarities and differences between our case and the other 5 cases is summarized in Table 1.

In our case, the patient received immunosuppressive therapy combined with umbilical cord blood transplantation and allo-PBSCT successively, leading to long-term immunosuppression and impaired immune surveillance, which may have contributed to the development of leukemia. The G-CSF was not used for a long time, and no chromosomal aberrations were found in this patient. RTEL1, HOXA11, and THBD are genes associated with inherited diseases in the blood system. Based on the American College of Medical Genetics and Genomics criteria, these genetic changes themselves are considered of uncertain significance. The limitation is that we did not further check these mutations in the patient’s tissue. RTEL1 mutation was detected in both the patient and donor, which was associated with telomere erosion and dyskeratosis congenita. Their parents did not have the genome sequencing, so the possibility of familial inheritance could not be completely ruled out. The family did not have any telomere biology disorder-related manifestations, such as pulmonary fibrosis, liver cirrhosis, and premature greying. The telomere length was not detected, so it was difficult to determine whether this family had telomere biology disorder. However, based on the diagnostic criteria reported by Kam et al [12], we could preliminarily exclude telomere biology disorder in this family to some extent. The panels used in whole-exome sequencing before and after transplantation were the same. Therefore, we think that the loss of the RTEL1 mutations after transplantation was a true

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<td>208 days</td>
<td>AML-M0</td>
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<tr>
<td>15-year-old male/ Manivannan et al, 2014 [7]</td>
<td>Allo-PBSCT</td>
<td>MSD</td>
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<td>Complex karyotype [46,XY (80%)/45,XY,del(7q),-20 (10%)/hyperdiploidy (10%)]</td>
<td>Chemotherapy and followed by a second allo-PBSCT from another donor</td>
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<tr>
<td>25-year-old male/ Ma and Liu, 2016 [8]</td>
<td>Allo-PBSCT</td>
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<td>51-year-old female/ Present paper</td>
<td>Allo-PBSCT</td>
<td>MSD</td>
<td>445 days</td>
<td>AML-M5</td>
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DCL – donor cell leukemia; allo-BMT – allogeneic bone marrow transplantation; MSD – matched sibling donor; AML – acute myeloid leukemia; CR – complete remission; allo-PBSCT – allogeneic peripheral blood stem cell transplantation.
finding, which had not been reported to date. RTEL1 variants are associated with AA, idiopathic cytopenia, and hypolymphocytic myelodysplastic syndrome. The RTEL1 mutation disappeared in the patient after allo-PBSCT; therefore, we inferred that RTEL1 may not have been involved in leukemogenesis in this case. The possible explanation for the disappearance of RTEL1 may have been a natural revertant following allo-PBSCT. Somatic reversion of a mutant phenotype was first identified in Lesch-Nyhan syndrome in 1988 [13]. This phenomenon has been reported in other hematological conditions, such as primary immunodeficiency diseases and Fanconi anemia [14]. During transplantation, the patient was exposed to several types of stress, such as replicative stress, or increased inflammatory cytokines. In these circumstances, the natural revertant might have occurred and led to the disappearance of the RTEL1 mutation. The THBD mutation was associated with the susceptibility to atypical hemolytic uremic syndrome and thrombosis, and it was detected only in the donor. At present, the correlation between THBD and the pathogenesis of AML remains unknown. Recently, Zhang et al reported a case that an AML patient with STK11 and THBD mutations had a family aggregation of hematological malignancies [15]. HOXA11 is involved in upper limb development in early embryogenesis as well as in normal hematopoiesis and leukemogenesis. Interestingly, HOXA11 mutation was 1 of 3 gene mutations in the donor that occurred in the patient after allo-PBSCT, suggesting that the bone marrow microenvironment of the patient might play a unique role in leukemia development and gene clonal evolution. In this case, we found the HOXA11 mutation in DCL for the first time. HOXA11 is not a well-known myeloid tumor-related gene, and few studies have reported HOXA11 mutation in AML. In chronic myeloid leukemia, Juvenile myelomonocytic leukemia and MDS cases, chromosomal translocation t (7; 11) (p15; p15) encoding NUP98/HOXA11 fusion has been recurrently detected [16], indicating that HOXA11 is involved in leukemogenesis. Li et al reported that increased expression of the 4-homeoboxgene signature composed of HOXA7, HOXA9, HOXA11, and PBX3 was an independent predictor of poor prognosis in AML patients with cytogenetic abnormalities [17]. Fu et al found that HOXA11 expression increased cell apoptosis and predicted improved response to cytarabine in AML. HOXA11 upregulation is associated with AML harboring MLL-t and RAS signaling mutations [18]. According to current studies, HOXA11 can participate in the process of leukemogenesis. However, the donor with HOXA11 mutation remains healthy. Although the role of HOXA11 mutation in DCL is unclear, this finding is still important. Some studies have shown that donor clonal hematopoiesis and donor genetic susceptibility variations can affect the outcome of allo-HSCT. With the development of whole exome and genome sequencing, the candidate genes that can contribute to the occurrence of DCL have been detected [3]. Whether HOXA11 is one of the candidate genes involved in DCL needs to be confirmed by additional similar cases. More studies are needed to elucidate the function of HOXA11 in the development of DCL.

Clonal hematopoiesis is a strong risk factor for subsequent hematologic cancer. Donor clonal hematopoiesis is closely related to the clinical outcomes of transplant recipients, with differential impact on graft alloimmune function and potential for leukemic transformation related to mutated gene and somatic clonal abundance [19]. DNMT3A, TET2, ASXL1, PPM1D, JAK2, SF3B1, SRSF2, and TP53 are the most common somatic mutation genes detected by whole-exome sequencing [20]. The risk of DCL in allo-HSCT is driven by somatic MDS-associated mutations or germline predisposition in donors. However, the role of clonal hematopoiesis with RTEL1, HOXA11, and THBD mutations in hematological malignancy remains unknown. More studies are needed to explore the potential function of RTEL1, HOXA11, and THBD mutations in the future. Further accumulation of such cases is necessary to clarify the genetic characteristics of DCL.

There are some other limitations in our study. First, due to the scattered and heterogeneous nature of cases, the mechanisms and treatment strategies of DCL transformed from SAA need to be further explored. Second, there was a lack of functional experiment to elucidate the function of HOXA11 in the development of DCL.

Conclusions

In conclusion, the precise mechanism of SAA transformed into DCL after allo-HSCT remains unclear. In the present case, the unique status of bone marrow microenvironment may have played a crucial role in the development of DCL. Leukemogenesis is more than a 1-step process, and DCL provides a unique opportunity to examine genetic variations in donors and hosts with regards to the progression to malignancy.

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Ethics Statement

This study was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University.

Declaration of Figures’ Authenticity

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