
**DONOR-CELL-DERIVED MYELOYDYSPLASTIC SYNDROME INVOLVING U2AF1
MUTATION DEVELOPING 8 YEARS AFTER MATCHED UNRELATED BONE
MARROW TRANSPLANTATION FOR ACUTE LEUKEMIA AND LITERATURE
REVIEW**

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ABSTRACT: *We report the case of a man with acute erythroleukemia (AML-M6), who developed myelodysplastic syndrome (MDS) involving U2AF1 mutation 8 years after HLA-matched unrelated bone marrow transplantation. It's donor cell origin which proved by molecular analysis using Short Tandem Repeat (STR) sequences. To our knowledge, this is the first case that usage of demethylation drug in DCL patient. The recurrence of leukemia is mainly receptor-cell-derived leukemia after Allo-HSCT, and the incidence of DCL was proved to be extremely low by means of cytogenetic, molecular biology, or both. There are only a few sporadic reports at home and abroad, in this paper we report the case of a donor-cell-derived myelodysplastic syndrome involving U2AF1 mutation developing 8 years after matched unrelated bone marrow transplantation for acute leukemia and review of the literature.*

KEYWORDS: Donor-cell-derived, Myelodysplastic Syndrome, U2AF1, Bone Marrow Transplantation, Acute Leukemia

CASE

Patient, male, 50-year-old, due to the abnormal blood routine examination for about 9 years, being hypodynamic and leptystic for 2 months, he had been hospitalized in September 4, 2014. His smoking history is about 30 years, 5-6 a cigarettes per day. In September 2005, he had a fever, cough, stuffy nose symptoms after caught cold, coughed a little yellow sputum, and went to Qingdao Fuxin hospital. The blood routine test showed: WBC $2.8 \times 10^9/L$, Hb 98 g/L, MCV99fL, PLT $70 \times 10^9/L$, the proportion of lymphocytes accounted for 49%, the effect of anti-infection symptomatic treatment was still poor. he still had a fever, and his body temperature was about 38 °C. Myelodysplastic syndrome was diagnosed in local hospital after bone marrow examination, and in order to confirm the diagnosis he had a blood routine test in the Blood Disease Hospital of Tianjin Medical Academy of Sciences in October 24, 2006: WBC $2.1 \times 10^9/L$, Hb 87 g/L, MCV 100fL, PLT $76 \times 10^9/L$.

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Bone marrow cytology showed that bone marrow grew actively, granulocytic cells ratio decreased, among these cells, the original accounted for 2.5%, granulocytic cells accounted for 4%, promyelocyte cells accounted for 7%, late promyelocyte cells accounted for 7.5%, band neutrophils accounted for 3.5%, erythroid series ratio was significantly increased, polychromatic erythroblast and orthochromatic normoblast are the main cells, megaloblastic-like cells can be seen, The mature erythrocytes showed different size, megakaryocyte had abnormal-shaped accompanied with poor platelet formation; The positive rate of sideroblast was 79%; The score of AKP was 320 points; It can be observed of 22 large mononuclear micromegakaryocytes, 12 mononuclear micromegakaryocytes in one glass sheet and diagnosed with myelodysplastic syndrome, Thalidomide was given to treatment by half a month, and the decision to stop taking medication was due to drug rash, the patient used traditional Chinese medicine for half a month afterwards.

Because neither the patient's brother nor sister was his HLA-identical donor, and didn't find a proper donor in China Marrow Donor Program, he went to Chinese PLA No. 304 hospital for Allo-HSCT in April 4, 2006. Bone marrow cytology examination in June 5, 2006 showed his bone marrow grew hyperactively, M/E=0.4/1, the ratio of granulocytic cells was 27%, which accounted for 18% of myeloblastic cells (proportion of myeloblast accounted for 53%), cell count in other stages was low, with no morphologic abnormality, the ratio of erythroid series which are proliferous and active were 66.5%, proportion of polychromatic erythroblast increased significantly, the cells varied in size and shape. The early stage cells were larger and the dual nuclei were visible, the size of mature erythrocytes was different, the proportion of lymphocytes was 5%, the count of megakaryocyte was more than 20, the thromcytogenic megakaryocytes and the platelet were rare. The diagnosis was acute myeloid leukemia (M6).

His blood type was A, Rh (D) positive at that time. With the preparative regimen of Bu+Cy+Ara-C+ATG, he received unrelated donor hematopoietic stem cell suspension infusion for two consecutive days in June 19 to 20, 2006, the counts of mononuclear cells were calculated as $7.3 \times 10^8/\text{kg}$ of body weight, and CSA+MMF+MTX to prevent GVHD after transplantation, the patient recovered well. Multiple review results of routine blood test and bone marrow cytology were normal. Two months ago, the patient had fatigue without obvious incentive, accompanied with weight loss of about 5kg, chest tightness and shortness of breath, no fever, no lower limb edema, and he visited our hospital. The routine blood test results showed: WBC $2.2 \times 10^9/\text{L}$ 、 NEU $0.46 \times 10^9/\text{L}$ 、 RBC $1.91 \times 10^{12}/\text{L}$ 、 Hb 67g/L、 MCV 107.9fL、 MCH 35.1pg、 PLT $52 \times 10^{12}/\text{L}$.

Physical examination: A middle-aged man, anemic appearance, without haemorrhage dot, no enlarged lymph node was noted; palpebral conjunctiva was pale and no yellow sclera, the pupils had same size and normal light reflection, ear nose and throat had no abnormalities; respiratory rate was 19 / min, his lung auscultation was normal, heart rate was 74 beats /min, regular without pathological murmur; abdomen was soft, no tenderness and rebound tenderness, liver and spleen were impalpable, Murphy sign was negative, shifting dullness (-); there was no more positive signs.

After admission, his blood routine examination showed: WBC $2.26 \times 10^9/L$, NEU $0.55 \times 10^9/L$, RBC $1.81 \times 10^{12}/L$, Hb 63g/L, MCV 109.4fL, MCH 34.8 pg, PLT $43 \times 10^9/L$, erythrocyte sedimentation rate was 34mm/h. His blood type was AB, Rh (D) positive. In September 5, 2014, bone marrow cytology showed that the hyperplasia of bone marrow was still active:

- 1) The primitive cells accounted for 3%.
- 2) Granulocytic series: hyperplasia was still active, each stage of granulocytes were seen, the ratio was relatively reduced, the shape was normal.
- 3) Erythroid series: hyperplasia was still active, each stage of erythroblastic cells were seen, the ratio was relatively high, a small number of nuclear of erythroblastic cells were deformed, RBC size was different, part of the center of shallow dyeing.
- 4) The lymphocytic series: ratio and shape were normal.
- 5) Tissue eosinophilic granulocyte distributed as group at the end of the glass sheet, and its ratio increased, accounting for 8%. MPO (-) PAS (+). 6) There were 25 megakaryocytes in the slide, PLT was rare. Blood slide: WBC was low, and the ratio and morphology were normal, RBC morphology was the same as bone marrow slide, PLT was rare.

Opinion 1: Posttreatment of M6 (the primitive cells accounted for 3%, the end of the slide can be seen as a group of eosinophils, and its ratio increased, accounting for 8%). Bone marrow pathology showed: HE and PAS staining showed the bone marrow hyperplasia was extremely active (80%), granulocyte series and erythroid series ratio decreased, granulocytic cells at each stage was visible, and mainly included promyelocyte cells and the following stage of cells, erythroid series cells can be seen in each stage, mainly included orthochromatic normoblast, the count of megakaryocytes is normal, lobate nucleus was common, and some of the cells were small, with few segment, and the histiocyte were easy to see, which distributed evenly. The deposition of hemosiderin was visible. Reticular fiber staining (+).

Diagnosis: The hyperplasia of bone marrow was active, the proportion of erythroid series increased with the abnormal morphology of megakaryocyte, the histiocyte were easy to see, no significant increase of primitive cells and fibrosis. Flow cytometry showed that: mature lymphocyte group 37.3%, myelogenous primitive cell group 1%, immature and mature granulocyte group 37.6%, mature mononuclear cell group 4.9%, immature erythrocyte group 11.7%, B progenitor cell group 2.9%, eosinophil group 2.5%, each group showed no abnormal phenotype. DNA sequencing showed that U2AF1 was positive, the patient of myelodysplastic syndrome with U2AF1 mutation was prone to be secondary acute myeloid leukemia. DNMT3A-ZNF、SRSF2 (Exon1)、IDH1-EXON4 were all negative for DNA sequencing.

Chromosome karyotype description: 46,XY,?Der(19)t(1;19)(q23;p13)[9]/46,XY[11]. According to the Myelodysplastic syndromes (MDS) 2008 WHO revised classification, peripheral blood cells decreased, primitive cell $\leq 1\%$, one or multi series of bone marrow abnormal cell $< 10\%$

accompanied with cytogenetic abnormalities, primitive cell <5%. Diagnosis of this patient: 1, Myelodysplastic syndrome – unclassified (MDS-U) 2, Post-HLA identical allogeneic hematopoietic stem cell transplantation. Based on Myelodysplastic syndrome WHO WPSS 2011 assessment, the patient had complex karyotype, Moderate anemia, WPSS was 3 points, which is belonged to high risk group. It used oral mucosal epithelium and peripheral blood cell to test bone marrow transplantation donor cell DNA chimerism, the results appeared as shown in Table 1.

Table 1

STR Site	Pre Transplant Site		Post Transplant Site	
D8S1179	13	16	11	13
D21S11	30	32.2	29	30
D7S820	8	10	11	11
CSF1P0	10	13	10	10
D3S1358	15	17	17	17
TH01	6	9	7	9
D13S317	12	12	11	12
D16S539	9	12	11	11
D2S1338	23	25	16	17
D19S433	12	13	13	14
vWA	16	16	14	16
TPOX	8	11	8	8
D18S51	14	17	14	15
Amelogenin	X	Y	X	Y
D5S818	9	12	12	12
FGA	23	23.2	19	21

The patient was treated with erythropoietin 10000U QOD subcutaneous injection to treat anemia and took oral medication like Compound Zaofan pills, his condition improved and discharged. After discharge, he was treated with intermittent blood transfusion as maintenance therapy without chemotherapy or second bone marrow hematopoietic stem cell transplantation. In March 2, 2017, the patient was admitted to hospital again because of fatigue.

In March 6, 2017, the results of bone marrow cytology showed that the hyperplasia of marrow diminished.

- 1) Primitive cells accounted for 1%.
- 2) Granulocytic series: proliferation diminished, only promyelocyte cells and the following period granulocyte can be seen, which were mainly mature neutrophilic lobate nucleus granulocytes, part of granulocytes cytoplasm particles increased and thick, large segmented neutrophils can be seen.
- 3) Erythroid series: Hyperplasia diminished, basophilic normoblast cells and the following stage

normoblast can be seen, the ratio was reduced, the shape was roughly normal, RBC size was different, some of the central hypochromic area expanded.

- 4) Lymphocytic series: the ratio accounted for 56%, the morphology was normal.
- 5) There were two megakaryocytes in the marrow slide, it's multinuclear megakaryocyte, PLT was rare. Blood slide: WBC was low, the primitive cells accounted for 1%, the ratio was approximately normal, we could see that there were many large segmented neutrophils, and the appearance of RBC was the same as myelogenous, PLT was rare.

Opinion 2: Post-MDS treatment, the hypoplasia of bone marrow diminished, the primitive cells accounted for 1%, granulocyte series, erythroid series and megakaryocytic series diminished, the granulocytic cells and megakaryocyte were abnormal, PLT was rare. Flow cytometry showed that mature lymphocyte group 48.98%, immature and mature granulocyte group 14.29%, mature mononuclear cell group 21.92%, immature erythrocyte group 4.05%, the abnormal cell population accounted for about 4.43% of the nuclear cells, and expression of CD34, HLA-DR, CD33, CD13, CD36, weak expression of CD117, and no expression of CD38, CD11b, CD56 CD15, CD7, CD64, CD16, CD14, it's an abnormal immunophenotype of granulocyte series. The results indicated: in this sample, the abnormal myelogenous primitive cells accounted for 4.43%, the immunophenotype as above: the proportion of mononuclear cells significantly increased, no expression of HLA-DR, weak expression of CD33 and CD14; granulocytic series expression decreased in late promyelocyte and the following stages; part of erythroid series showed weak expression of CD36 and CD71.

Test of MDS related mutation genes showed that U2AF1 Exon2 mutation was positive, mutation frequency was 22.35%, the patient of myelodysplastic syndrome with U2AF1 mutation was prone to secondary acute myeloid leukemia. The other mutations were as follows: LYST Exon34 mutation(+), mutation frequency is 19.79%, DIS3 Exon15 mutation(+), mutation frequency is 25.00%. Chromosome karyotype : 46, XY[1] (this sample has fewer nuclear cells). Patients were treated with platelet transfusion for several times, and the platelet count was always lower than $20 \times 10^9/L$, and there was a tendency of bleeding. In March 28, 2017, the 5-day decitabine chemotherapy ($20mg \times 5day$) was given to the patient, the patient tolerated well and we'll keep report the follow-up treatment and results.

DISCUSSION AND LITERATURE REVIEW

Donor-cell-derived leukemia is extremely rare in clinic, and the incidence rate is about 5%^[1]. Fialkow et al. reported the first case of DCL^[2] in 1971. With the extensive application of cytogenetics and molecular biology, more and more DCL cases have been reported at home and abroad^[3-10]. In this case, the patient developed myelodysplastic syndrome (MDS) involving U2AF1 mutation 8 years after HLA-matched unrelated bone marrow transplantation which was confirmed by STR sequences.

It was reported that DCL occurred vary from 2 months to 11 years after transplantation^[3], and the median time between the diagnosis of DCL and transplantation was about 17 months^[4]. In

this case, the patient developed donor-cell-derived leukemia in 8 years after transplantation, The U2AF1 gene mutation of this patient was positive, it's a hotspot mutation, mainly in MDS and AML. U2AF1 mutation in patients with MDS tends to develop secondary acute myelogenous leukemia, but it's no effect on overall survival rate. The P635S mutation was found in patient's DIS3 gene encoding sequence, it mainly found in multiple myeloma, and DIS3 is a tumor suppressor gene, DIS3 gene mutation leads to the loss of tumor suppressor function, so patients with DIS3 mutation have poor prognosis. The R2875H mutation was found in LYST gene encoding sequence, 1000Genomes database shows that the mutation is less than 0.01 in the normal population, this mutation is likely to affect the protein function, mainly in familial hemophagocytic syndrome, Shetty Ark East syndrome (CHS), Chediak-Higashi syndrome and macrophage activation syndrome, LYST mutation is associated with a decrease of NK cell lysis. The mechanism of DCL is unknown; so far there is no report of the hematopoietic stem cell donor for DCL patients developing hematologic malignancies. The median follow-up time was 9 years and the follow-up period ranged from 6 to 30 years^[4], it may be related to the abnormality of bone marrow microenvironment and the instability of donor genotype^[3, 6]. In addition, with the use of large doses of immunosuppressor after the bone marrow transplantation resulted in decreased immunologic function, may cause secondary leukemia. The patient in this report has used CSA+MMF+MTX to prevent GVHD after transplantation. This patient's primary disease is MDS ,developing into of donor-cell derived MDS/AML after allo-HSCT, and has the chromosome abnormality and U2AF1 gene mutation. All of the above characteristics support that the immunosuppressive agents may increase the incidence of malignant diseases .Abnormal hematopoietic microenvironment, and the possible of the donor's malignant transformation of hematopoietic stem cells after induction of bone marrow stromal cells associated with leukemia clone^[5, 14].

The previous literature have less mention of the treatment DCL patients, sometimes serious infection occurs in the chemotherapy and patients died^[3, 12], some patients have received secondary bone marrow hematopoietic stem cell transplantation^[4, 12, 13], one patient of these cases had about 11 months remission time^[12]. In our report, this patient diagnosed of DCL live up to 3 years and didn't receive chemotherapy or a second bone marrow hematopoietic stem cell transplantation, only maintaining by blood transfusion treatment. So far this is the first report of patient used demethylation drugs for the treatment of DCL, with the progress of treatment, it will has a deeper understanding on the pathogenesis of DCL, and provide more treatment to those patients who have lost the chance of secondary hematopoietic stem cell transplantation.

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