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Primary myelodysplastic syndrome with two rare recurrent chromosome abnormalities [t(3q26.2;q22) and trisomy 13] associated with resistance to chemotherapy and hematopoietic stem cell transplantation

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Summary

We present a case of primary myelodysplastic syndrome (MDS) in a young male with two rare but recurrent chromosome abnormalities, i.e., t(3;21)(q26.2;q22) and trisomy 13. He obtained one Dacogen course at the BMT Center followed by sequential transplantation of allogeneic bone marrow and peripheral blood hematopoietic stem cells from an HLA-DQB1 mismatched donor. The rejection of the first graft was documented on day 29 after transplantation, whereas the 2nd allo-HSCT grafting was more successful. The article contains serial cytogenetic findings and time-dependent changes in donor chimerism. We discuss individual resistance to the therapy, in view of recently proposed molecular mechanisms of resistance which might be responsible for resistance of cells in this case with complex chromosomal pathology.

Keywords

Myelodysplastic syndrome, primary translocation t(3;21)(q26.2;q22), trisomy 13, EVI1/RUNX1 gene, allogeneic hematopoietic stem cell transplantation, therapy resistance.

Introduction

The t(3;21)(q26.2;q22) translocation, as well as trisomy 13 are rare but recurrent chromosome abnormalities which, to our best knowledge, have not yet encountered simultaneously in MDS/AML patients and, hence, have not been treated by means of allo-HSCT. The t(3;21) translocation is not rarely found in patients with therapy-related MDS/AML [1-7]. This disorder is, generally, characterized by aggressive clinical course [8, 9] and short event-free survival [10].

The t(3;21)(q26.2;q22) translocation results into a fusion between the RUNT domain of *RUNX1*, and *EVI1* gene [11]. The *EVI1* gene is known to encode a dual domain of the zinc-finger transcription factor which exhibits DNA binding activity acting together with a histone methyltransferase (SET) domain [12-14]. This molecule promotes self-renewal in hematopoietic stem cells. The t(3;21) translocation has been reported to occur in approximately 1% of AML or MDS cases [15]. Its clinical significance in MDS has been poorly determined, though poor prognosis appears to be proven in CML patients with t(3;21) [16-18]. It has been recently shown that t-MDS and t-AML positive for t(3;21)

(q26.2;q22) are resistant both to cytostatic chemotherapy, and to allo-HSCT treatment [19].

Moreover, according to multicentric cytogenetic studies, trisomy 13 was observed in 0.2% patients, detectable as an single aberration, or combined with an additional abnormality [20]. A sufficient gender imbalance was reported for this condition (21 males of 27 cases). The median age of patients was 73 years. Isolated trisomy 13 was revealed in 96% of the cases. Of note, these patients with higher-risk MDS did not respond to therapy with hypomethylating agents. In general, trisomy 13 rarely occurs in MDS (0.2 to 0.8% of total), mostly as an isolated event, being something more frequent in AML (1-2%). Most of these patients are males over 70 years old.

Typically, MDS proceeds with blast excess and moderate pancytopenia having poor prognosis, with median survival ranging between <6 months and 1 year. The patients with AML and trisomy 13 do not respond to standard intensive chemotherapy. For instance, treatment with hypomethylating agents is inefficient in this MDS type, though high-dose lenolidomide could be an option here. It should be also kept in mind, that trisomy 13 is associated with overexpression of FLT3 gene located at the extra chromosome. Besides, a close association between trisomy 13 and mutations of RUNX1 have been recently found [21, 22].

Materials and methods

Cytogenetic and molecular techniques

Serial cytogenetic investigations were carried out by a standard G-banding technique which has been recently published [23]. Identification of chromosomes and chromosomal aberrations was carried out according to International System Human Cytogenetic Nomenclature (ISCN) [24]. A serial molecular biological testing of donor chimerism was done on peripheral blood or bone marrow samples at the time of hematologic engraftment, as well as every 2 weeks between 1 and 3 months and every month between 3 and 12 months after transplantation were obtained and processed. Shortly, the evaluation was done by means of PCR-based Chimerix FA Kit (Inogene, Russia), by analyzing selected polymorphic short tandem repeat (STR) loci: D11S488, HUMVWFA31, D13S317, D8S639, D19S246, D4S2366, D12S1064, D16S539 and the sex determination marker Amelogenin. The amplified sample was analyzed by capillary electrophoresis using an ABI Prism 3500xl Genetic Analyser (Applied Biosystems). Quantification of the mixed chimerism (calculated as percent of recipient DNA) was performed by analyzing the proportion of the fluorescent peak areas corresponding to donor and patient genotypes.

Clinical case description

The patient was a 22-year-old male, initially presented in August 2006 with complaints for moderate fatigue and fever (37.4°C). Peripheral blood examination showed pancytopenia. Bone marrow aspirate was hypocellular with features of aplastic anemia. Cyclosporin A (CSA) was given, but it was taken by the patient only for two weeks due to hepatic discomfort. During the following three years, the patient did not visit a hematologist, and his condition was stable. In January of 2009 he noted exacerbation of fatigue and skin hemorrhages which required hospitalization to the Hematology Department. Peripheral blood counts were as follows: Hb, 55 g/L; WBC, $1.5x10^9$/L; platelets, $4x10^9$/L. The bone marrow aspirate was hypocellular, contained 15.5% blasts with diffuse-type periodic acid Schiff (PAS) staining, and 30% ring sideroblasts, whereas most neutrophilic cells were peroxidase-deficient. Immunophenotyping of the blast cells showed positivity for CD34, CD13, CD33, and HLA-DRA. The results of serial cytogenetic investigations and chimerism testing using molecular biology approaches are presented in Table 1.

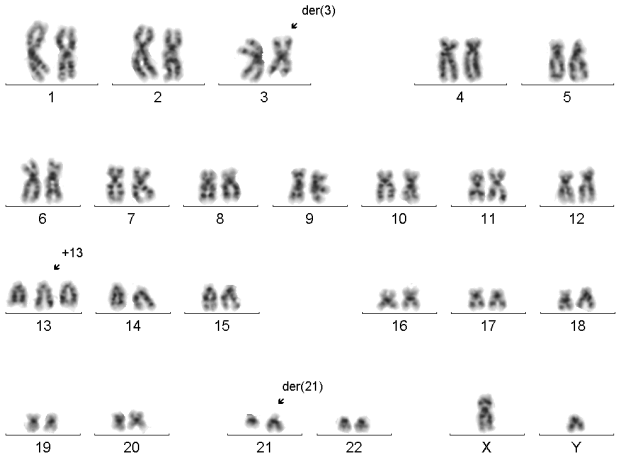

Figure 1. Karyogram of bone marrow cell 47, XY, t(3;21)(q26;q22),+13 from patient with primary myelodysplastic syndrome (RAEB-II)

Initial marrow karyotype was as following: 47, XY, t(3;21)(q26.2;q22),+13, (Fig. 1) with involvement into arrangement *EVI1* gene (Fig. 2), whereas 10% metaphases had normal chromosome complement. Meanwhile, a standard molecular investigation with primers for common chromosome translocations did not show any oncogene anomalies. Therefore, a diagnosis of MDS RAEB -II IPSS 3.0 was established. Since the patient was transfusion-dependent for a long time, his ferritine level reached 2500 mg/mm^3.

Table 1. The results of HLA-immunophenotyping in the recipient and donor

HLA-locuses	A	B	Cw	DRB1	DQB1
Donor	02,03	35,51	01,04	1101	0301,0501
Recipient	02,03	35,51	01,04	1101	0301

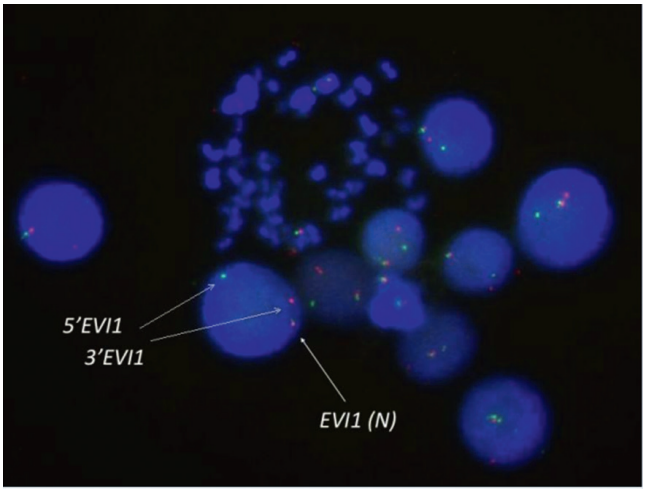

Figure 2. Fluorescence in situ hybridization (FISH) with BreakApart EVI1 probe (CytoCell, UK) showing one normal EVI1 gene (one fusion yellow signal) and one abnormal EVI1 gene (one green and one red split signals on interphase cells) in the patient with translocation t(3;21)(q26.2;q22) RUNX1/EVI1

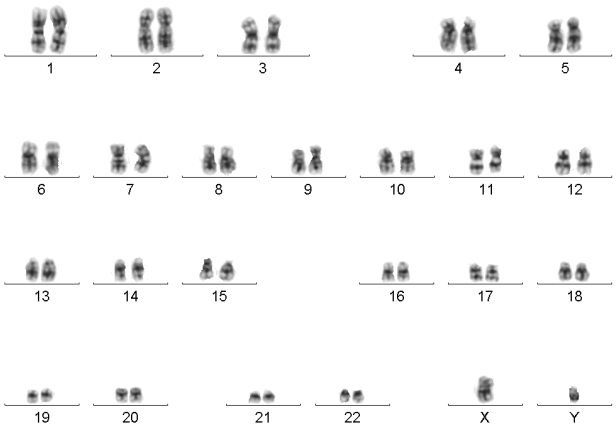

Figure 3. Karyotype of donor's bone marrow cell 46,XY from a patient with primary myelodysplastic syndrome after allogeneic bone marrow transplantation prepared during a short-term hematological and cytogenetic remission

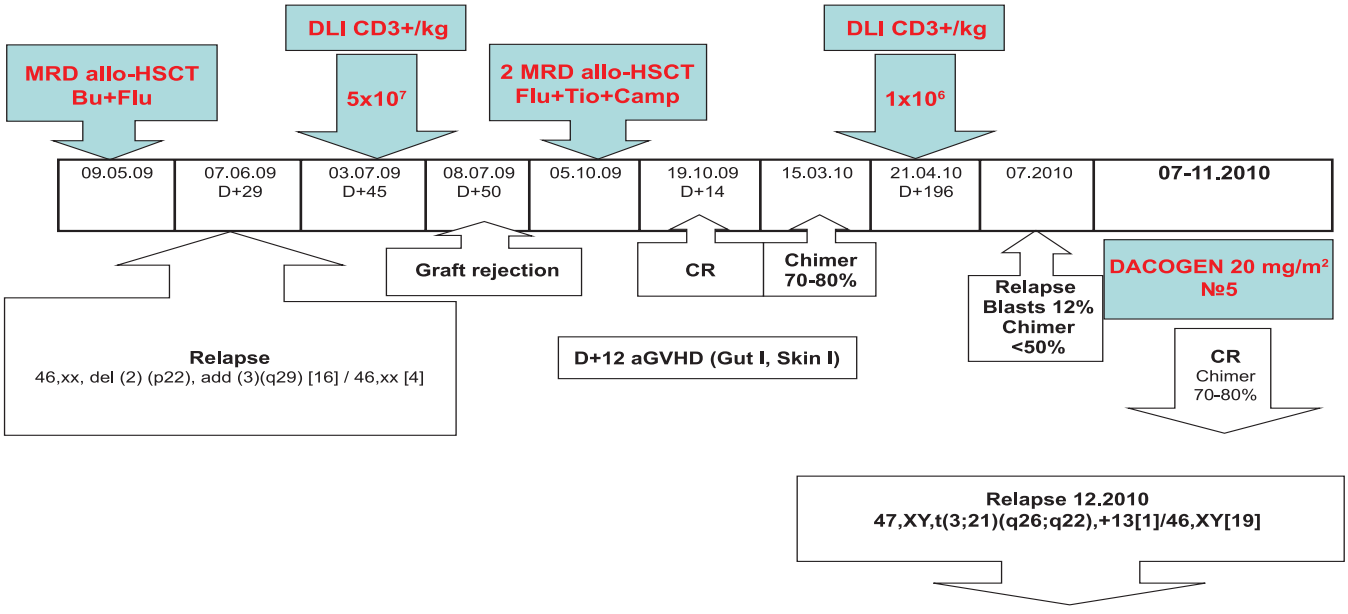

Figure 4. Schematic presentation of clinical course in a patient with primary myelodysplastic syndrome with karyotype 47,XY,t(3;21)(q26.2;q22),+13 associated with resistance to standard chemotherapy, hypomethylating agents and allo-HSCT

Before bone marrow transplantation, the patient obtained one 5-day course of Dacogen (Decitabine) as demethylating therapy. It did not change common blood parameters, but allowed to reduce his transfusion dependence. Since the patient had an HLA-DQB1-mismatched related donor (Table 1), allo-HSCT was considered. The patient was enrolled into the RIC/MAC study protocol, and randomized to RIC branch (Busulfan + Fludarabine – 800 and 300 mg, respectively). Graft-versus-host-disease (GvHD) prophylaxis included CSA and Methotrexate (MTX). Following the allo-HSCT, stem cell engraftment was detected on day +15, when mixed chimerism was 45-55%. By this time, bone marrow contained only 2.4% of blasts, and the previously detected chromosomal abnormalities were not found (Fig. 3, Table 1). GvHD symptoms were absent, but a cytogenetic relapse was documented two weeks later (on day +29), due to appearance of the marker chromosomal aberrations and decreasing donor chimerism which could be caused by small number of CD34+ stem cells in the graft. Therefore, cyclosporine A was stopped on day +38, and, a week later, a donor lymphocyte infusion (DLI) was performed at a dose of $5.0x10^7$ cells/kg, however, without any clinical effect. The graft rejection was diagnosed on day D+50 (Fig. 4). By that time, the patient had hemoglobin levels of 57 g/L; WBC, $1.7x10^9$/L; and platelets, l$3.0x10^9$/L. Bone marrow aspirate was hypocellular with 3.4% blasts, and the donor chimerism faded away (<5%). Because of allo-BMT failure, a second allo-HSCT was administered, using peripheral blood stem cells from the same donor. It was performed after RIC preparative regimen containing Fludarabine 350 mg, Thiotepa 960 mg, and Campath 40 mg. GvHD prophylaxis included Tacrolymus and CellCept (Mycophenolate Mofetyl). Engraftment was registered since day D+10. Two days later, acute GvHD of gut and skin (grade I) was diagnosed. Steroids were added to the treatment schedule which resulted into a significant improvement of GvHD. Peripheral blood findings on the day +16 after 2nd transplant were as follows: Hb, 92.0 g/L; WBC, $4.3x10^9$/L, and platelets, $35.0x10^9$/L. Bone marrow aspirate was hypocellular without blast excess, and full donor chimerism was achieved. Cytogenetic study has shown a repeated karyotype normalization. Due to high risk of relapse, another DLI procedure was performed. Despite the immunotherapy, a new cytogenetic relapse, along with reduced donor chimerism levels, was diagnosed in July 2010. An attempt to achieve another remission with hypomethylating agents (5 courses of Dacogen) was not successful. A repeated cytogenetic relapse has been diagnosed in December 2010, causing his death at the BMT Center two months later.

Discussion

To our knowledge, it is the first clinical description of primary MDS with t(3;21)(q26.2;q22), combined with trisomy 13, and treated by two subsequent allo-HSCTs from a partially HLA-mismatched sibling (brother). The first earliest relapse occurred on the day 29, which could be, in part, explained by low cellularity of transplant. Since a second transplant was required, a reduced-intensity conditioning was chosen again, but peripheral blood stem cells were used for the 2nd transplant. The second engraftment was longer. A new cytological relapse was diagnosed 2 months later as evidenced by increased blast counts in bone marrow up to 12% accompanied by recurrence of the above cytogenetic aberrations, as well as decreasing donor chimerism. Moreover, the patient did not respond to hypomethylating agents (5 courses of Dacogen), aiming to correct the hematopoietic disorder.

Recent studies have shown that distinct portions of *RUNX1* gene may fuse in variable manner to *MDS1* and *EVI1* genes located within the 3q26 region. On the other hand, these different fusion products are capable of blocking myeloid differentiation interfering with normal transcriptional regulatory functions of RUNX1 [14].

As a result, our MDS case with combined t(3;21) chromosome anomalies and trisomy 13, showing poor clinical prognosis, appears to be related to imbalance of *GATA, ERG, MEIS1, HOXA9* and *FLT3* genes at the stem cell level which could be responsible for leukemia cells resistance to both chemotherapy and allo-HSCT.

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Conflict of interest

No conflicts of interests are declared.

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Первичный миелодиспластический синдром с двумя редкими повторяющимися прогностически неблагоприятными цитогенетическими нарушениями [транслокацией t(3q26.2;q22) и трисомией 13], ассоциированными с резистентностью к химиотерапии и к трансплантации гемопоэтических стволовых клеток

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Резюме

Представлено наблюдение миелодиспластического синдрома (МДС) с двумя редкими, повторяющимися и прогностически неблагоприятными нарушениями хромосом: транслокацией t(3;21)(q26.2;q22) и трисомией 13, которые обеспечили патологическим элементам резистентность к химиотерапии и трансплантации гемопоэтических стволовых клеток от сиблинга. На основании полученных данных и обсуждения в свете недавно открытых молекулярных механизмов резистентности к терапии при данном виде хромосомной патологии сделано заключение об ответственности данных нарушений хромосом за развитие резистентности к терапии, включая алло-ТГСК у больных МДС.

Ключевые слова

Первичный миелодиспластический синдром, t(3;21)(q26.2;q22), трисомия 13, ген *EVI1/RUNX1*, аллогенная трансплантация гемопоэтических клеток, резистентность к терапии.