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Short communication

Cytogenetic biclonality in a child with hypocellular primary myelodysplastic syndrome

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Abstract A 13-year-old boy with hypocellular primary myelodysplastic syndrome, classified as refractory cytopenia, underwent umbilical cord blood transplantation. Cytogenetic analysis revealed two rare biclonal chromosomal aberrations, del(17)(p12) and del(11)(q23). Cytogenetic analysis was a valuable tool in diagnosis, in clinical decision-making, and in treatment and follow-up. To our knowledge, this is the first reported case of cytogenetic biclonality involving chromosomes 17 and 11.

1. Introduction

Myelodysplastic syndromes (MDS) comprise a heterogeneous group of clonal bone marrow disorders characterized by varying degrees of pancytopenia and morphological and functional abnormalities of hematopoietic cells, and an increased risk of transformation into acute myeloid leukemia [1]. MDS typically occurs in elderly people, and in the great majority of cases the bone marrow presents hypercellular or normocellular patterns. Hypocellular patterns occur in a lower frequency [2].

Cytogenetic studies have been extremely useful for identifying clonality in primary MDS and for defining specific clinicopathological entities [3]. Nevertheless, the prognostic and therapeutic significance of cytogenetically independent clones remains to be determined. The frequency of unrelated clones detected by cytogenetic analysis in MDS has been reported as between 4.3 and 6.5% [4]. Because of the relative rarity of MDS in children, the cytogenetic findings reported in childhood MDS are limited, compared with those reported in adults.

Here, we describe a rare case of biclonal chromosomal alterations in a child with hypocellular primary MDS, classified as refractory cytopenia (RC), who underwent umbilical cord blood transplantation. We consider also the value of cytogenetic analysis in diagnosis, prognosis, and guidelines for therapy.

2. Case report

A 13-year-old boy was referred in April 2005 to the National Cancer Institute Bone Marrow Transplant Center (CEMO–INCA), Rio de Janeiro, with suspicion of MDS. He presented with pancytopenia. Peripheral blood count values were hemoglobin 7.5g/dL, platelets 38×10^9/L, and leucocytes 3.0×10^9/L. Bone marrow examination showed hypocellularity with moderate megaloblastic features and no fibrosis; abnormal localization of immature precursors was absent, as were blast cells.

Treatment was initiated with supportive care only (blood transfusions), but the pancytopenia persisted. The choice of appropriate protocols of treatment was discussed, and the hypothesis of aplastic anemia was proposed. Cytogenetic analysis of bone marrow cells after GTG banding showed 46,XY,del(17)(p12)[9]/46,XY,del(17)(p12),del(12)(p12)[5]/46,XY,del(11)(q23)[3]/46,XY[34] (Fig. 1). Karyotype description is according to ISCN 1995 [5].

Fluorescence in situ hybridization (FISH) analysis was performed using a *TP53* gene probe (LSI p53, SpectrumOrange; Abbott Molecular/Vysis, Des Plaines, IL) to confirm the 17 chromosome short arm deletion and an *MLL* gene probe (LSI MLL dual-color, break-apart rearrangement

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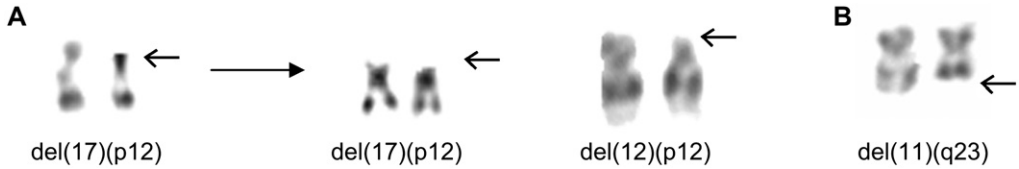

Fig. 1. Partial karyogram of the patient by G-banding. (A) del(17)(p12) as mainline, and del(17)(p12) and del(12)(p12) as clonal evolution. (B) Unrelated clone showing del(11)(q23).

probe; Abbott Molecular/Vysis) to confirm the deletion of the 11q23 region (Fig. 2). Immunophenotyping was performed with a panel of directly conjugated antibodies: CD45, CD4, CD8, CD2, CD3, CD19, CD10, CD33, CD34, CD61, CD7, and anti-HLA-Dr (BD Biosciences, San Jose, CA).

The immunophenotypic analysis revealed the following abnormalities: hypogranular neutrophils demonstrated by CD45 versus side light scatter, CD10− granulocytes and myeloid lineage expressing nonmyeloid antigens such as CD2. Based on morphological, immunophenotypic, and cytogenetic studies, the final diagnosis was MDS-RC (according to Hasle et al. [6]).

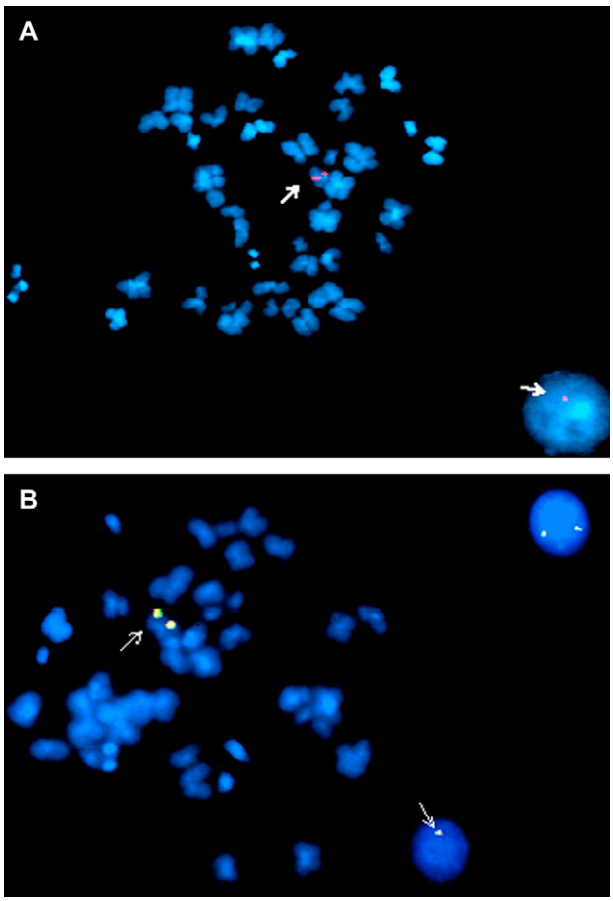

Fig. 2. Fluorescence in situ hybridization analysis of bone marrow cells. (A) *TP53* gene probe (LSI p53, SpectrumOrange), showing only one signal in metaphase and interphase nuclei, confirming the deletion of the *TP53* gene. (B) *MLL* gene probe (LSI MLL dual-color, break-apart rearrangement probe), showing one fusion signal in metaphase and interphase nuclei; the absence of the other fusion signal corresponds to the deletion of the *MLL* gene.

The patient was referred for bone marrow transplantation, but no histocompatible bone marrow donor was available. In November 2005, he underwent umbilical cord blood transplantation. Prior to the transplant he was transfused with more than 15 units (red blood cells). He underwent 4/6 umbilical cord blood transplantation from a female donor with 4.6×10^7/kg nucleated cells; cyclophosphamide, antithymocyte globulin, and total body irradiation were used as conditioning. Prophylaxis for graft-versus-host disease (GVHD) was cyclosporine, prednisolone, and human immunoglobulin.

By day 32, the patient had developed a grade II acute GVHD, with good partial response to prednisolone. At day 34, he was discharged from the hospital. The corticosteroid dose was gradually tapered, but skin GVHD flared and cyclosporine was replaced by tacrolimus. At this time, bone marrow examination showed normal morphology pattern, with 100% donor chimerism according to variable-number tandem repeats and cytogenetic analysis (46,XX[40]). As of writing, at 17 months after transplantation, the patient was in cytogenetic and molecular remission; he is still using the immunosuppression treatment because a moderate skin GVHD.

3. Discussion

Cytogenetically unrelated clones are uncommon findings in hematologic disorders. In MDS, the frequency is 4.3–6.5% [4]. Our group have been studying clinical and biological characteristics in MDS since 1991, and out of a cohort of 313 patients this is the first case showing biclonality chromosomal alterations, representing a frequency of 0.3%.

It is unclear if the cytogenetically unrelated clones indicate true biclonality of malignancy or represent the result of clonal evolution from a common precursor. We believe in the hypothesis that the karyotypically unrelated clones originate from the common malignant clone through submicroscopic molecular genetic changes and evolutionary process as suggested by some authors [4]. The present case showed two karyotypically unrelated clones, del(17)(p12) and del(11)(q23), with deletion of the *TP53* and *MLL* genes, respectively, confirmed by FISH analysis. The clone with

del(17p) acquired a second chromosomal abnormality, del(12p), suggesting the evolution of the disease.

The most frequent chromosome abnormalities involved in unrelated clones were del(5q), +8, del(20q), del(7q), +11, +21, and −22 [4]. From our review of the literature, this case represents the first case of pediatric MDS showing the involvement of these chromosomes alterations in a hypocellular MDS-RC. In some cases the hypocellular bone marrow makes the diagnosis between MDS and aplastic anemia a difficult process, and the cytogenetic and immunophenotypic studies may be considered valuable tools for differential diagnosis. We note that clinically aplastic anemia and MDS patients with hypocellular marrow show great similarities and, in some cases, cytogenetic analysis is considered the only way to distinguish between the two diseases [7,8].

In the present case, cytogenetic analysis was a valuable tool for diagnosis, in clinical decision-making, and in treatment and follow-up. In the International Prognostic Scoring System, alterations involving chromosomes 11, 12, and 17 are considered intermediate risk [9]. Nevertheless, some studies suggest that chromosomes 11 and 17 are involved with disease evolution [1,10]. Our patient was at initial phase of disease (refractory cytopenia), but already had a clonal evolution chromosomal pattern.

Based on cytogenetic and clinical studies, the child was referred for bone marrow transplantation; in the absence of a histocompatible bone marrow donor, the stem cell source used was the umbilical cord blood. Umbilical cord blood is a valuable alternative source of hematopoietic stem cell for transplantation of patients who need an allogeneic transplant but lack a suitable sibling donor [11], and it has been shown to be a curative therapy for several hematologic malignancies [12]. In the present case, the patient showed a favorable clinical response and was, at writing, in cytogenetic and molecular remission. Because stem cell transplantation is the only modality demonstrated to cure patients with MDS, a larger number of cases with these characteristics is necessary to establish the prognostic and therapeutic significance of finding cytogenetically independent clones.

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