Case Report

Mosaic Trisomy 21 and Trisomy 14 as Acquired Cytogenetic Abnormalities without *GATA1* Mutation in A Pediatric Non-Down Syndrome Acute Megakaryoblastic Leukemia

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ABSTRACT

One case of acute megakaryoblastic leukemia (AMKL) with trisomy 21, trisomy 14 and unmutated *GATA1* gene in a phenotypically normal girl was reported. The patient experienced transient myelodysplasia before the onset of AMKL. The bone marrow blasts manifested typical morphology of megakaryoblast both by the May-Giemsa staining and under the electronic microscopy. Leukemic cells were positive for CD13, CD33, CD117, CD56, CD38, CD41 and CD61 in flow cytometry analysis. Cytogenetic study showed karyotype of 48, XX, +14, +21 in 40% metaphases. Known mutations of *GATA1* gene in Down syndrome or acquired trisomy 21 were not detected in this case.

Key words: Acute megakaryoblastic leukemia, Myelodysplasia, Cytogenetics, *GATA1*

INTRODUCTION

Acute megakaryoblastic leukemia (AMKL) is the most frequent type of acute myeloid leukemia (AML) in children with Down syndrome (DS)[1]. There are no specific cytogenetic abnormalities for AMKL. Constitutional or acquired abnormalities of chromosome 21 are generally regarded as one of the most frequent abnormalities occurring in AMKL[2, 3]. Acquired trisomy 21 and trisomy 14, however, is a very rare karyotypic abnormality in hematological malignancies and has never been reported in patients with non-DS AMKL. We reported here one case of pediatric non-DS-AMKL patient who presented with myelodysplasia as the initial clinical manifestation. The detailed cytogenetics and the sequence of the *GATA1* gene were analyzed to provide better understanding of leukemogenesis in this rare case.

CASE REPORT

A one–and-half-year old girl was admitted to our department with petechia on the lower extremities for three months. No fever or documented infection was recorded. No lymph node enlargement and hepatosplenomegaly can be palpated on physical examination. Complete blood count indicated thrombocytopenia (blood platelet count: 13×10^9/L) and moderate anemia (hemoglobin: 65 g/L) with normal white blood cell (WBC) counts. Bone marrow aspirate cytology showed intermediately differentiated blasts (14%) with deep blue cytoplasm and cytoplasmic blebs (Figure 1A). Marked dysplasias of trilineage blood cells, especially dysmegakaryocytopoiesis including the presence of micro-megakaryocytes, were easily found in her bone marrow smear. On cytochemical staining, these blasts were negative for myeloperoxide stain, periodic acid-Schiff (PAS) stain, naphthol-AS-D-Chloroacetate esterase (AS-D-CE) and acid α-naphthyl acetate esterase (ANAE) stain. Abnormal localization of immature precursor (AILP) can be observed in her bone marrow biopsy. The cytogenetic analysis at that time indicated normal karyotype (46, XX) in 20 metaphases. She received supportive care and intermittent transfusion of platelets. To alleviate hemorrhage, she was also prescribed with immunoglobulin and a moderate dose of corticosteroid (prednisone, 30 mg/d). After that, her hemoglobin level gradually recovered while the platelet count fluctuated between 20×10^9/L and 50×10^9/L. On the 90th day after the first hospitalization, the patient presented a high fever (39.2℃ at peak) and extreme weakness. Her bone marrow smear then showed 22.5% megakaryoblasts with round nuclei of dense chromatin (Figure 1B). Cytoplasm was deep blue with cloudy-shaped rim. On cytochemical staining, leukemic blasts were negative for peroxidase (POX), ANAE, and Sudan black B; PAS was weakly positive. The ultrastructure of the leukemic blasts was indicative of megakaryoblasts with irregular morphology and bony prominence. The nuclei were irregular with obvious nucleoli. Abundant mitochondria can be observed in its cytoplasm; rough endoplasmic reticulum was in long and cord shape (Figure 2). Cytogenetics study demonstrated karyotype of 48, XX, +14, +21 in 40% (8 of 20) of metaphases (Figure 3). A

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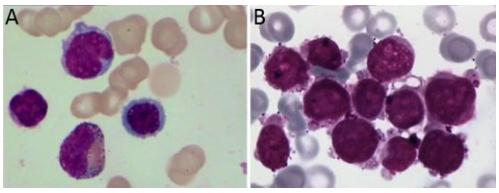

Figure 1. May-Giemsa staining of bone marrow smear. A: Myelodysplasia with irregular concave, folding or twisting nucleus can be observed preceding AMKL, (×400). B: Megakaryoblasts with deep blue cytoplasm and cloudy-shaped rim after transforming from myelodysplasia into AMKL, (×400).

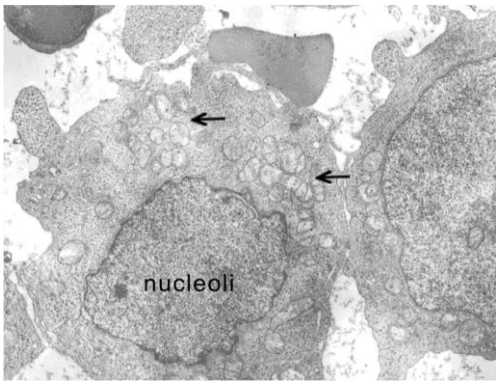

Figure 2. Ultrastructure of AMKL blast. An AMKL blast from non-DS AMKL with irregular cytomorphology and bony prominence can be observed under transmission electronic microscope. The morphology of nuclei was irregular with obvious nucleoli. Abundant mitochondria (black arrows) existed in its cytoplasm. Rough endoplasmic reticulum was in long and cord shape, (×12000).

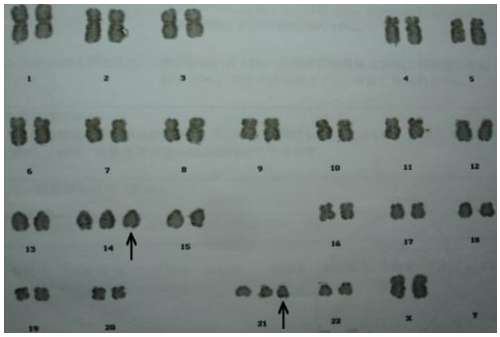

Figure 3. Cytogenetics of AMKL bone marrow. Cytogenetic analysis showed 48, XX, +14, +21 (40%)/46, XX (60%). The black arrows indicate trisomy14 and trisomy 21.

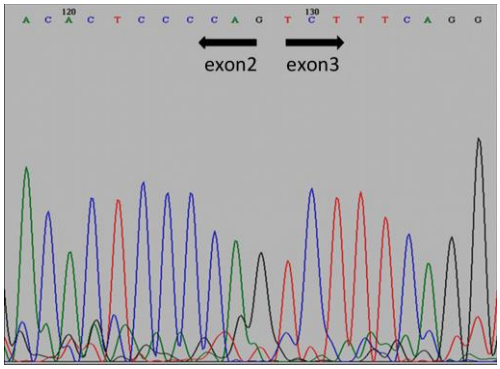

Figure 4. Sequencing of *GATA*1 gene in non-DS AMKL. Direct sequence of the RT-PCR product from bone marrow cells in this non-DS AMKL patient. Direct data showed no finding of a deletion of AG at 128-129 bp.

cluster of abnormal cells which occupied 23% of nucleated cells at CD45/SSC gating expressed CD33 (58.22%), CD117 (12.29%), CD56 (16.02%), CD38 (39.85%), CD41 (65.5%) and CD61 (87.5%), but did not express CD2, CD3, CD5, CD10, CD19 and CD20. RNA was extracted from separated bone marrow mononuclear cells and screened for mutations in *GATA1* gene by RT-PCR and direct DNA sequencing. PCR conditions were initial denaturation for 5min at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s at 56°C and 45 s at 72°C, using a PCR Thermal Cycler (Applied Biosystem, USA). PCR amplification products of all coding exons were prepared using a Qiaquick PCR purification kit (Qiagen, Germany) to remove unincorporated nucleotides and were subjected to automated nucleotide sequencing by an ABI PRISM 3130XL Genetic Analyzer (PerkinElmer/Applied Biosystems, Foster City, USA). All of the six exons of the *GATA1* gene were directly sequenced. No mutations were detected in known sites of *GATA1* gene that is involved in the transient myeloproliferative disorder (TMD) and AMKL of DS (Figure 4). The patient was diagnosed as non-DS AMKL. Two courses of standard dose cytosine arabinoside were given to the patient but she failed the induction and died of severe pneumonia and acute respiratory distress syndrome 102 days after the diagnosis.

DISCUSSION

DS is one of the most frequently acquired human genetic disorders, resulting from the presence of an extra copy of chromosome 21. Children with constitutional trisomy 21 have an approximately 500-fold increased risk of developing AMKL[4]. The cytogenetic profile of AMKL in children is complex, which reflects the heterogeneity of the disease. In the analysis of 45 AMKL children, cytogenetic abnormalities of leukemic cells were classified into seven categories[5]: normal karyotype or constitutional trisomy 21 in DS-AMKL, other numerical abnormalities only, t(1;22)(p13;q13), 3q21q26 abnormalities, t(16;21)(p11;q22), -5/del(5q) and/or -7/del(7q), and other structural changes. To our best knowledge, mosaic trisomy 21 and trisomy 14 as acquired cytogenetic abnormalities in non-DS AMKL has not been reported in literature. In our case, myelodysplasia (three months) preceded AMKL. Although the exact mechanism of how trisomy 21 and trisomy 14 contribute to the leukemogenesis is unknown, it may play a key role in the transforming from myelodysplasia to AMKL. Only limited reports with regard to AMKL after myelodysplastic syndrome (MDS) are available[6,7]. From a retrospective study of 37 cases of AMKL treated in M.D. Anderson Cancer

Center, 27% patients presented myelodysplasia before diagnosis with median time of 4 months (2-160 months)[8].

GATA1, an important transcription factor for the differentiation of the erythroid and megakaryocytic cell lineages through cooperative regulation of key molecules, is tightly associated with AMKL in children with DS[9, 10]. Acquired mutations in *GATA1*, preventing synthesis of full length *GATA1*, have been identified in constitutional trisomy 21 DS-AMKL, suggesting that *GATA1* plays a critical role in trisomy 21 megakaryoblastic leukemogenesis[4,11-14]. Mutations in exon 2 of the *GATA1* gene present in almost all cases of DS-associated AMKL[7, 14]; in contrast to DS-AMKL, they were rarely found in patients with non-DS AMKL[15]. Therefore, all of the exons of the *GATA1* gene were evaluated to confirm that karyotype in this case was not associated with DS.

In conclusion, a phenotypically normal female case of non-DS AMKL showing mosaic trisomy 21 and trisomy 14 as acquired cytogenetic abnormalities without *GATA1* mutation was reported.

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