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CBFA2T3::GLIS2 유전자재배열을 동반한 소아 급성거핵모구백혈병의 국내 첫 증례 보고

First Korean Case of Pediatric Acute Megakaryoblastic Leukemia with *CBFA2T3::GLIS2* Fusion

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Acute megakaryoblastic leukemia (AMKL) is a rare type of acute myeloid leukemia that evolved from primitive megakaryoblasts. We report a case of non-Down syndrome AMKL with *CBFA2T3::GLIS2* fusion that has not been described before in Korea. A 20-month-old girl presented with a fever and was diagnosed with AMKL after a bone marrow study and flow cytometry analysis. An RNA fusion test observed a fusion transcript between exons 9 and 2 of the *CBFA2T3* and *GLIS2* genes, respectively. The patient achieved complete morphological remission after induction chemotherapy. However, her prognosis was poor as measurable residual disease remained, detected using reverse transcription PCR. RNA fusion analysis could be a helpful tool to identify clinically actionable genomic markers for patient risk stratification and measurable residual disease monitoring.

Key Words: Acute megakaryoblastic leukemia, *CBFA2T3*, GLIS2, Gene rearrangement

INTRODUCTION

Acute megakaryoblastic leukemia (AMKL) is a rare type of acute myeloid leukemia (AML) that evolved from primitive megakaryoblasts and is classified in the French-American-British Classification of AML as M7 [1]. AMKL is classified mainly as pediatric and adult cases, and pediatric cases are classified as Down syndrome AMKL (DS-AMKL) and non-Down syndrome AMKL (non-DS-AMKL).

The *CBFA2T3::GLIS2* fusion, resulting from a cryptic inversion of chromosome 16, is identified in up to 30% of non-DS-AMKL cases and reported as a poor prognostic factor in pediatric AMKL [2]. In AML, patients with *CBFA2T3::GLIS2* fusion have specific gene expression signatures that cluster them independently from other patients with non-DS-AMKL [3]. AML with *CBFA2T3::GLIS2* fusion has recently been classified as AML with other rare recurring translocations in the International Consensus Classification of Myeloid Neoplasms and Acute Leukemias [4], and as AML with other defined genetic alterations in the 5th edition of the World Health Organization Classification of Haematolymphoid tumours [5].

We report the first Korean case of AMKL with *CBFA2T3::GLIS2* fusion. The Institutional Review Board of Severance Hospital, Seoul, Korea, approved this study (4-2022-1294) and waived the need for informed consent.

CASE REPORT

A 20-month-old girl with no significant past medical history presented with a fever and elevated C-reactive protein levels and was hospitalized at a tertiary hospital in October 2021. The patient's fever persisted in wax and wane patterns, and a workup for a fe-

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ver of unknown origin was done. Diffuse osteolytic lesions in the whole axial skeleton, pelvic bone, proximal femurs, skull base, and facial bone were observed in computed tomography. The patient was transferred to the hemato-oncology division, and a bone marrow test was performed. The complete blood count was as follows: white blood cell (WBC) count 5.13×10^9/L; hemoglobin 9.4 g/dL; and platelet count 208×10^9/L. Blasts were 1 and 23.4% in peripheral blood and bone marrow (BM), respectively, negative in peroxidase and nonspecific esterase stains and positive in the periodic acid-Schiff stain. Flow cytometry analysis showed that the blasts were positive for CD33, CD117, CD41, CD34, and CD45 and negative for CD3, CD5, CD7, CD10, CD19, CD20, CD22, CD13, CD14, CD64, HLA-DR, cCD3, cCD22, cCD79a, cMPO, and TdT, indicating the presence of AMKL. Chromosome analysis showed the following karyotype: 46,XX,t(5,16)(q22;q23),inc[5]/46,XX[15] (Fig. 1A). Fluorescence *in situ* hybridization (FISH) was negative for *CBFB::MYH11, RUNX1::RUNX1T1, PML::RARA*, and *KMT2A* rearrangements. Multiplex reverse transcription PCR using a HemaVision kit (DNA Technology, Aarhus, Denmark) revealed no recurrent fusions. The patient received induction chemotherapy for three days. However, there was extravasation after chemoport insertion. The patient was referred to Severance Hospital for Hickmann catheterization and continued chemotherapy.

Subsequently, a second BM biopsy was performed. The BM contained blasts at 12.9% (Fig. 1B), and flow cytometry analysis revealed blasts with megakaryocytic differentiation and positive for CD33, CD117, CD41, CD61, and CD34 (Fig. 2). This was a rare case of AMKL that expressed CD34, as most AMKL cases are negative for CD34. No significant somatic variants were observed in a next-generation sequencing panel targeting 497 genes related to hematologic neoplasms. A targeted RNA fusion panel (FusionPlex Pan-Heme Panel; ArcherDX, CO, USA) detected 67 fusion reads

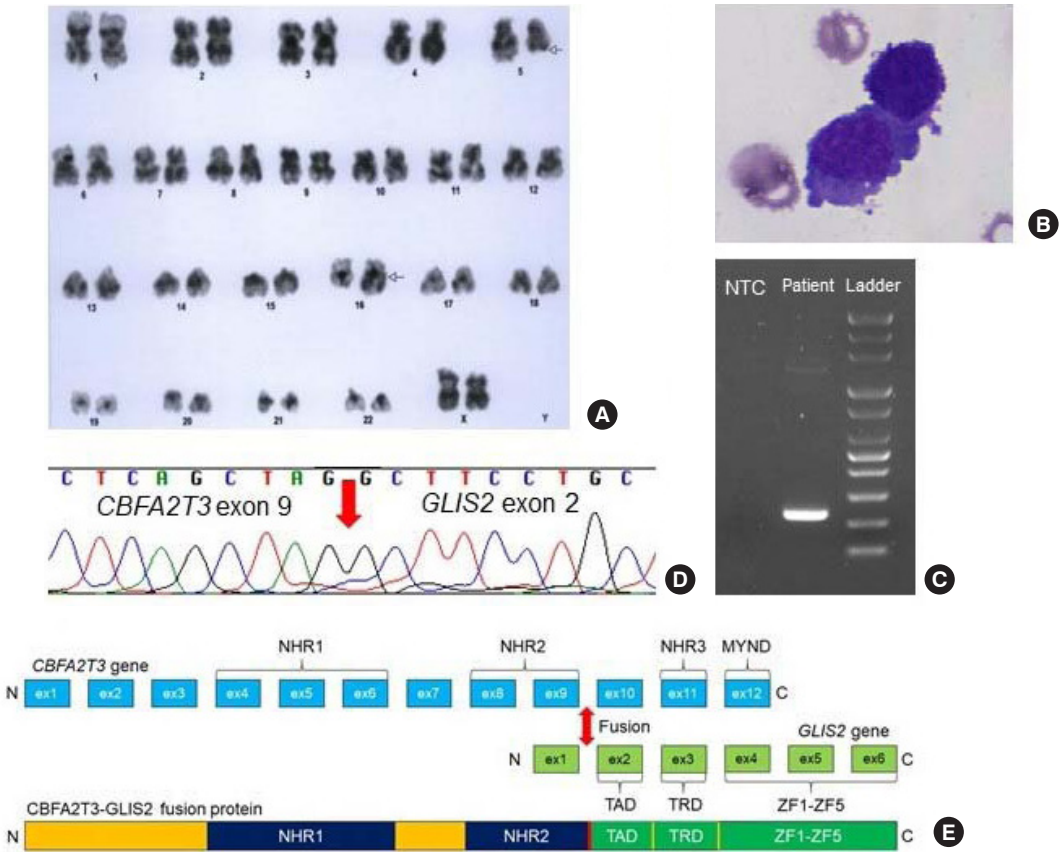

Fig. 1. Morphological and genetic analysis. (A) G-banding karyotyping revealed 46,XX,t(5,16)(q22;q23),inc[5]/46,XX[15]. (B) Blasts on a bone marrow aspiration smear (Wright–Giemsa stain, 1,000×). (C) In-house reverse transcription (RT)-PCR using cDNA and (D) subsequent Sanger sequencing confirmed breakpoints in exons 9 and 2 of *CBFA2T3* and *GLIS2*, respectively. (E) Schematic representation of the *CBFA2T3::GLIS2* fusion.
Abbreviations: NTC, non-template control; NHR, nervy homology regions; MYND, myeloid, nervy, and DEAF-1; ex, exon; TAD, topologically associating domain; TRD, trans-repression domain; ZF, zinc finger.

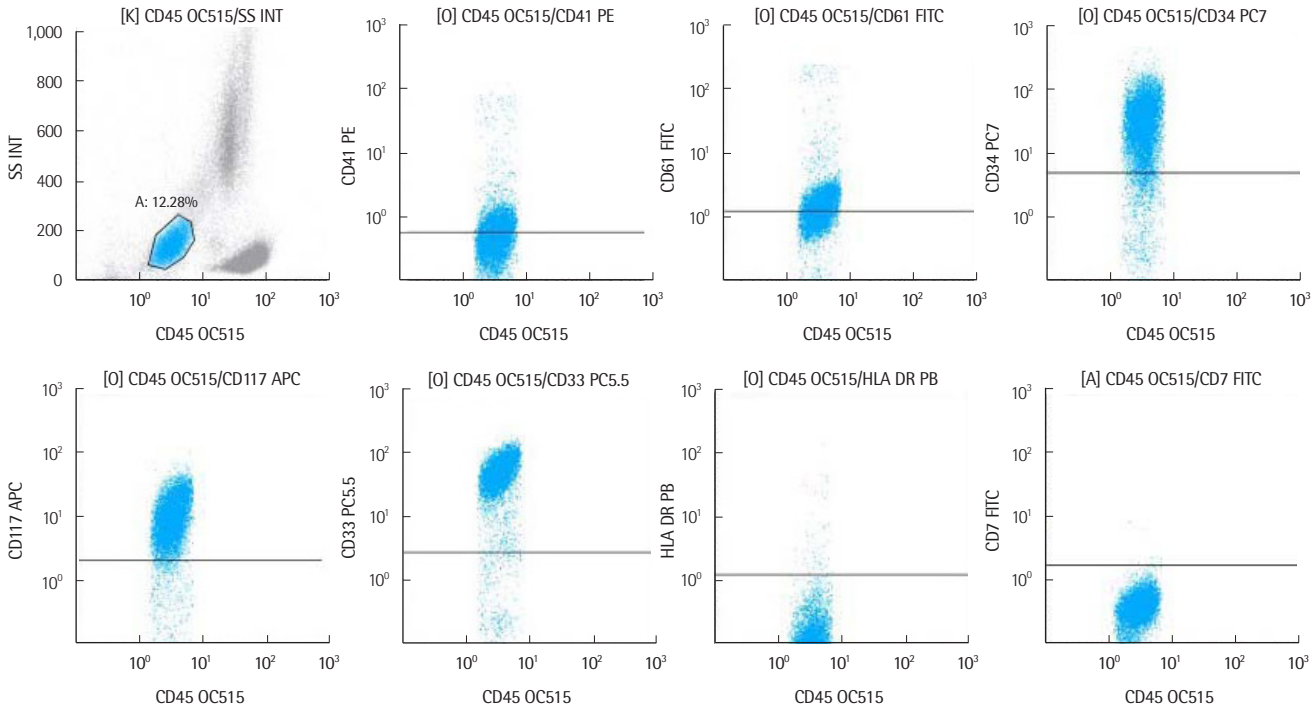

Fig. 2. Flow cytometry findings. Abnormal leukemic cells highlighted in blue (12.28% of total cells) exhibited a CD33+, CD117+, CD41+dim, CD61+dim, and CD34+ phenotype.

between exon 9 of *CBFA2T3* and exon 2 of *GLIS2* (97.1% of total reads). The identified fusion transcript was confirmed using in-house RT-PCR and direct sequencing (Figs. 1C and 1D). A schematic representation of the *CBFA2T3::GLIS2* fusion is provided in Fig. 1E.

Induction chemotherapy with cytarabine and idarubicin was administered for ten days. The patient suffered from *Klebsiella pneumoniae* sepsis, *K. pneumoniae* cellulitis, and *Clostridium difficile* infection during this time. The symptoms resolved, and cytarabine and mitoxantrone were administered for a second induction therapy. After one month of chemotherapy, the patient reached morphological complete remission (CR) with blasts reduced to 4.5%. Then only 0.9% and 2.9% of blasts were observed after two and four months of chemotherapy, respectively. Measurable residual disease (MRD) was assessed using flow cytometry and in-house RT-PCR. In the flow cytometry analysis, residual blasts were reported up to two months after chemotherapy and were not observed at four months. Because of these residual blasts, the regimen of the patient was changed to fludarabine, cytarabine, and idarubicin in the fourth month of chemotherapy. With the RT-PCR, the *CBFA2T3::GLIS2* fusion transcript was detected up to four months after chemotherapy; therefore, molecular CR was not reached. The patient suffered neutropenic fever in the fifth month after treatment. *K. pneumoniae* was identified in the blood culture, and the patient expired from septic shock.

DISCUSSION

The *CBFA2T3* gene is located at 16q24.3 and encodes a member of the myeloid translocation gene family, which interacts with transcription factors and functions as a transcriptional repressor via interaction with corepressor complexes [3]. The *GLIS2* gene is located in 16p13.3 and encodes a nuclear transcription factor that has a role in Hedgehog pathway signaling [3]. *CBFA2T3::GLIS2* fusion between two transcriptional regulators results in aberrant expression of the genes controlled by *CBFA2T3* or *GLIS2*, which plays a role in megakaryoblastic leukemia development [6]. Patients with *CBFA2T3::GLIS2* fusion usually have few somatic mutations and a poor prognosis [7, 8], as in our case. Overall survival for five years with *CBFA2T3::GLIS2* is 22.0% versus 63.0% in fusion-negative cases [9]. Clinical data and test results between our case and previous reports of patients with non-Down syndrome pediatric AMKL in Korea were compared in Table 1.

There was a disparity between the chromosome result and the

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Table 1. Summary of reported non-Down syndromic pediatric acute megakaryocytic leukemia in Korea

Characteristics	Present case	Cho, et al. [11]	Seo, et al. [12]
Sex	Female	Female	Male
Age	1Y8M	4Y	10M
Complete blood count	WBC count: 5.13×10^9/L with 1% blasts Hb: 9.4 g/dL Platelet count: 208×10^9/L	WBC count: 4.630×10^9/L with a few blasts Hb: 8.4 g/dL Platelet count: 132×10^9/L	WBC count: 46.41×10^9/L
Bone marrow	23.4% of blasts with irregular cytoplasmic blebs	Dry-tapped marrow	68% of blasts with irregular cytoplasmic blebs
Flow cytometry	Positive for CD33, CD117, CD41, CD34, and CD45	Positive for CD13, CD33, CD34, HLA-DR, and CD61	Positive for CD41 and HLA-DR
Chromosome	46,XX,t(5,16)(q22;q23),inc[5]/46,XX[15]	48,XX,t(1;22)(p13;q13),+der(1) t(1;22),+2 [1]/46,XX [19]	45,XY,-22,t(2;11)(q32;q23),der(14)t(14;22) [6]/46,XY,t(2;11)(q32;q23)[8]
Gene rearrangement	*CBFA2T3::GLIS2* fusion	Not tested	Not tested
Somatic mutation	None	Not tested	Not tested
Treatment and outcome	Died from septic shock five months after diagnosis	Complete remission for 8 months after induction chemotherapy using enocitabine, idarubicin, cytarabin, and thiguanine followed by consolidation chemotherapy using low-dose cytosine	Complete remission after allogenic bone marrow transplantation

RNA fusion panel test. The t(5;16)(q22;q23) was the chromosome result obtained by another institution where the patient first visited. Due to poor chromosome quality, "inc" (i.e., an incomplete karyotype) was used in the nomenclature. No related fusion transcript was detected by the RNA fusion panel. The possible explanations are that t(5,16)(q22;q23) does not generate fusion transcripts or that the targeted RNA fusion panel does not include fusion genes of t(5,16)(q22;q23) in their targets. The t(5;16)(q22;q23) was not previously reported as related to hematologic malignancy. Therefore, the relationship of t(5;16)(q22;q23) with leukemogenesis cannot be ruled out. Detecting *CBFA2T3::GLIS2* fusion using conventional cytogenetics, FISH, or commercial multiplex RT-PCR (e.g., the HemaVision test) is challenging. The inv(16)(p13.3q24.3), accompanied by *CBFA2T3::GLIS2* fusion, is cytogenetically cryptic [2, 3]. Although FISH probes targeting *CBFA2T3* and *GLIS2* can detect the fusion, it is inefficient to use many probes to detect recurrent fusions observed in AML. Additionally, the HemaVision test can only detect 28 recurrent fusions and not *CBFA2T3::GLIS2*.

There was a significant difference between the percentage of fusion transcript reads (97.1% of total reads) and the bone marrow blast percentage (12.6% of all nucleated cells). Regarding RNA sequencing, reads are obtained from transcriptionally active regions [10]. Therefore, the percentage of the *CBFA2T3::GLIS2* fusion transcript detected in RNA sequencing might differ from that of bone marrow blasts because normal blood cells have low *CBFA2T3* and *GLIS2* expressions.

In conclusion, we describe the first Korean report of non-DS-AMKL with *CBFA2T3::GLIS2* fusion. Although a multiplex RT-PCR panel did not detect recurrent fusion, a targeted RNA fusion panel detected *CBFA2T3::GLIS2* fusion. Additionally, in MRD assessed using in-house RT-PCR, *CBFA2T3::GLIS2* fusion was observed to be more sensitive than flow cytometry. Thus, RNA fusion analysis could be a helpful tool to identify actionable genomic markers for patient risk stratification and MRD monitoring.

요 약

급성거핵모구백혈병(AMKL)은 원시 거핵모구에서 유래한 드문 유형의 급성골수백혈병이다. 연구자들은 한국에서 한번도 보고되지 않은 *CBFA2T3::GLIS2* 유전자 융합을 가진 다운증후군이 아닌 AMKL의 사례를 보고하고자 한다. 발열을 주소로 내원한 20개월 여아가 골수 검사와 유세포분석을 통해서 AMKL로 진단되었다. RNA fusion panel 검사를 시행하여 *CBFA2T3*의 9번 엑손과 *GLIS2*의 2번 엑손 사이의 융합 전사체를 발견했다. 환자는 유도 화학요법을 투여 받은 후 형태학적 완전 관해를 보였지만, 역전사 중합효소연쇄반응으로 측정한 미세잔존질환은 양성을 보였고 나쁜 예후를 보였다. RNA fusion 검사는 환자의 위험도를 계층화하고 미세잔존질환을 추적관찰하는 등 임상적인 의미를 갖는 유전적 표지자를 검출하기 위한 유용한 도구로 사용될 수 있을 것으로 보인다.

Conflicts of Interest

None declared.

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