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RAB29-NUCKS1 유전자재배열을 동반한 공격성NK세포백혈병: 증례보고

Aggressive NK Cell Leukemia with *RAB29-NUCKS1* Gene Rearrangement: A Case Report

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Aggressive natural killer (NK) cell leukemia (ANKL) is a rare form of leukemia that may be accompanied by various chromosomal abnormalities such as del(6)(q21q25) or del(11q). Here, we describe a case of a patient with ANKL and an *RAB29-NUCKS1* rearrangement that has never been described before. An RNA fusion panel test found a gene fusion between exon 5 of *RAB29* and exon 2 of *NUCKS1* at chromosome 1q32.1. Additionally, reverse-transcription polymerase chain reaction and Sanger sequencing confirmed cryptic *RAB29-NUCKS1* fusion. *RAB29* encodes a protein that regulates exocytic and endocytic pathways. *NUCKS1* encodes a chromatin-associated protein involved in DNA damage responses. Further studies will be necessary to understand the molecular pathogenesis of ANKL related to an *RAB29-NUCKS1* rearrangement.

Key Words: Aggressive NK cell leukemia, *RAB29, NUCKS1,* Gene rearrangement

INTRODUCTION

Aggressive natural killer (NK) cell leukemia (ANKL) is a rare type of leukemia that occurs due to systemic neoplastic proliferation of NK cells and mainly involves peripheral blood, bone marrow (BM), the liver, and the spleen. It is rare but more prevalent in Asians than in other ethnic populations [1]. Epstein-Barr virus (EBV) is commonly found in leukemic cells in patients with ANKL, suggesting an association between EBV and the pathogenesis of ANKL [2]. In patients with ANKL, various chromosomal abnormalities such as del(6)(q21q25) or del(11q) can be detected [3]. Here, we report a case of a patient with ANKL and a novel *RAB29-NUCKS1* rearrangement. This work was approved by the institutional review board of Yongin Severance Hospital, Yongin, Korea (IRB no. 9-2021-0189).

CASE REPORT

A 62-year-old man with a history of asthma was scheduled to undergo excision for a mass on his back. He visited the outpatient clinic in the plastic surgery department at Yongin Severance Hospital in November 2021 for a preoperative evaluation. His complete blood counts were as follows: white blood cell count, 206.94 $\times 10^9$/L (abnormal cells: 84%, segmented neutrophils: 5%, lym-

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phocytes: 9%, monocytes: 2%, nucleated RBC: 1/100 white blood cells); hemoglobin level, 126 g/L; and platelet count, 68×10^9/L (Fig. 1A). Based on his results, he was referred to the hematology department and hospitalized for a BM biopsy and further evaluations. He complained of a weight loss of 5 kg over the last 6 months; however, there were no signs of coagulopathy, hemophagocytic syndrome, and multi-organ failure. His serum lactate dehydrogenase level had increased to 381 IU/L; nevertheless, the findings of other blood test results were not remarkable. EBV polymerase chain reaction (PCR) of whole blood was negative. Computed tomography of the abdomen and pelvis revealed splenomegaly (length: 24 cm). Computed tomography scans did not show any abnormal lymph node enlargement or neoplasms in the chest and neck. A BM aspiration smear showed 56.0% abnormal cells with atypical nuclei, distinct nucleoli, clumped chromatin, and basophilic cytoplasm containing azurophilic granules (Fig. 1B). BM biopsy showed approximately 50% interstitially scattered CD56+ and granzyme B focal+ atypical NK cell proliferation (Fig. 1C, D). Flow cytometry (Beckman Coulter, Miami, FL, USA) using a BM specimen showed an abnormal population (85.3% of total nucleated cells) with the following phenotype: CD3-, diminished cytoplasmic CD3 expression (cytoplasmic CD3 dim+), CD5-, CD7+, CD8+, CD16+, CD38+, and CD56+ (Fig. 2).

Next-generation sequencing and karyotype analysis were performed using the BM aspirate. A custom next-generation sequencing panel targeting 497 genes associated with hematologic neoplasms was performed using the NextSeq 550Dx platform (Illumina, San Diego, CA, USA), and a matched skin biopsy sample from the patient was used to differentiate somatic and germline variants. A somatic variant of *DNMT3A* p.Arg882His (variant allele frequency: 46.8%) was detected. Mutations of *DNMT3A* have been reported in hematologic malignancies such as myelodysplastic syndrome, acute myeloid leukemia, and angioimmunoblastic T-cell lymphoma [4]. An RNA fusion panel (FusionPlex Pan-Heme Panel, ArcherDX, Boulder, Colorado, USA) detected a gene fusion between exon 5 of *RAB29* and exon 2 of *NUCKS1* at chromosome 1q32.1 (Fig. 1F). The fusion reads of *RAB29-NUCKS1* were 907 (25.69% of total reads). The G-banding karyotype was 46,XY,t(5;7)(p15.3;q11.2)[10]/46,XY[10] (Fig. 1E), which was not identified in the RNA fusion panel test. The t(5;7)(p15.3;q11.2) karyotype has been reported in a patient with monoclonal gammopathy [5]. However, the association or clinical implications in specific blood cancer of the t(5;7)(p15.3;q11.2) type have not been revealed. Cryptic *RAB29-NUCKS1* fusion was confirmed using reverse-transcrip-

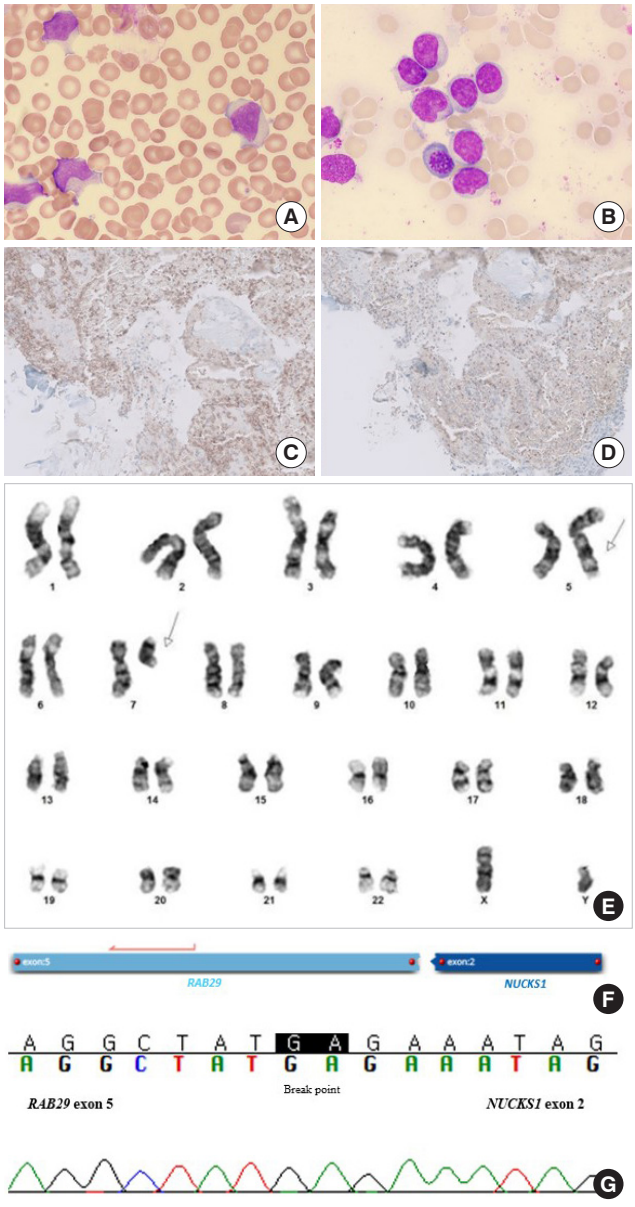

Fig. 1. Peripheral blood smear and bone marrow findings and genetic test results of the patient. (A) Abnormal cells resembling large granular lymphocytes (Wright-Giemsa stain, ×1,000). (B) Abnormal cells with irregular nuclear folding, clumped chromatin, distinct nucleoli, and lightly basophilic cytoplasm containing azurophilic granules in the bone marrow aspiration smear (Wright-Giemsa stain, ×1,000). Proliferation of neoplastic cells showing positive staining for (C) CD56 (×200) and (D) granzyme B (×200) in the bone marrow biopsy section. (E) G-banded karyogram showing 46,XY,t(5;7)(p15.3;q11.2). (F) *RAB29-NUCKS1* gene rearrangement detected in the RNA fusion panel test. (G) Identification of the gene fusion breakpoint between exon 5 of the *RAB29* gene (reference transcript: NM_003929.2) and exon 2 of the *NUCKS1* gene (NM_022731.4) by using Sanger sequencing.

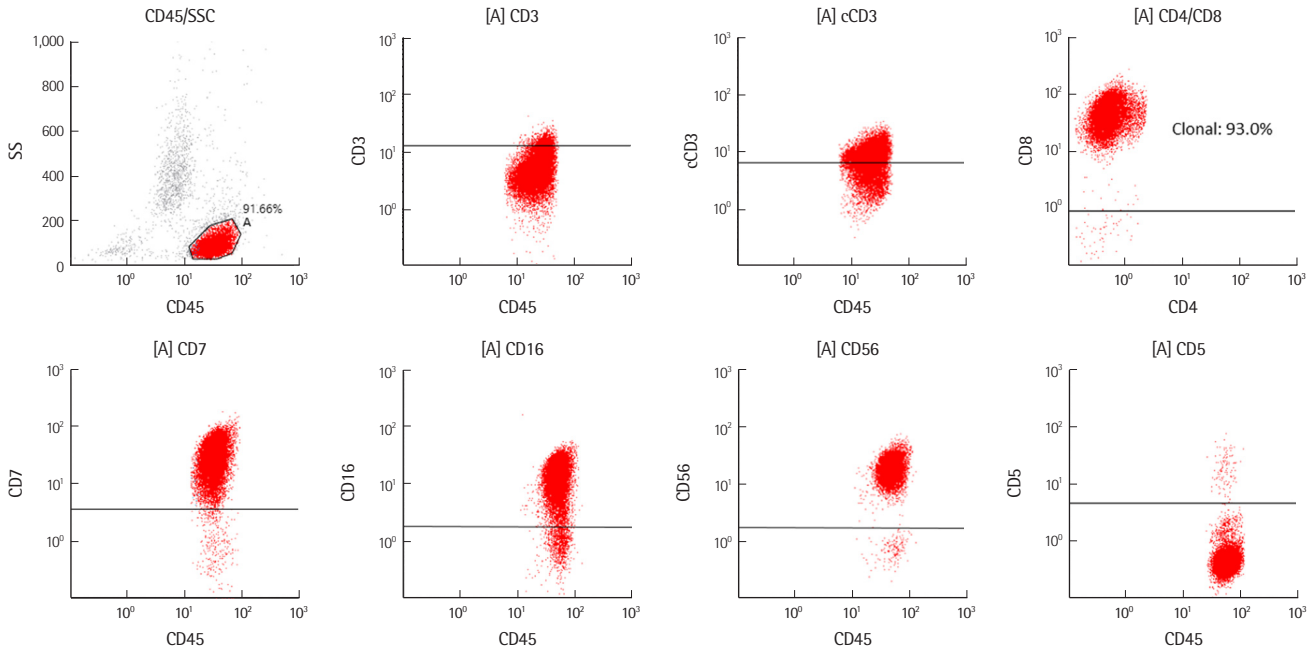

Fig. 2. Flow cytometry findings of the patient. Abnormal lymphoid cells highlighted in red exhibited the CD3-, cCD3 dim+, CD5-, CD7+, CD8+, CD16+, and CD56+ phenotypes at diagnosis.
Abbreviations: cCD3, cytoplasmic CD3; SSC, side scatter.

tion PCR and Sanger sequencing (Fig. 1G) with forward primer (5′-TCAGCTGTGGGATATTGCAG-3′) and reverse primer (5′-GTTGTTGGCGCACATTTTTA-3′).

DISCUSSION

There were three possible diagnoses associated with NK cells: ANKL, extranodal NK/T-cell lymphoma, nasal type, and chronic lymphoproliferative disorder of NK cells. The neoplastic cells of ANKL typically show the following phenotype: CD2(+), surface CD3(-), CD3-epsilon(+), CD5(-), and CD56(+) and positivity for cytotoxic molecules. CD16 is frequently positive (75%) in ANKL but usually negative in extranodal NK/T-cell lymphoma [6]. In our patient, flow cytometry of the BM specimen showed CD3(-), CD5(-), CD16(+), and CD56(+). There was no definite involvement of the upper aerodigestive tract (the nasal cavity, nasopharynx, paranasal sinuses, and palate). Furthermore, given the clinical presentation and laboratory findings, chronic lymphoproliferative disorder of NK cells, which is clinically indolent and usually associated with diminished CD56 expression, could be ruled out. Therefore, he was diagnosed with ANKL and transferred to another hospital for a second opinion. Then, he began receiving chemotherapy consisting of vincristine, cyclophosphamide, and prednisolone.

Compared with classical cytogenetics, a target RNA panel allows accurate and more comprehensive identification of rare gene fusions [7]. However, it is important to exclude analytical errors and the possibility of chimeric RNAs in normal tissues to interpret the RNA fusion panel results [8]. We confirmed *RAB29-NUCKS1* fusion using reverse-transcription PCR and Sanger sequencing to exclude false positivity. In addition, several studies have reported that RNA fusion such as *BCR-ABL1* exists in normal individuals [9, 10]. However, in normal individuals, *BCR-ABL1* fusion usually exists at a very low level [9]. However, the *RAB29-NUCKS1* fusion detected in the case of our patient was detected with a high read count at the time of ANKL diagnosis. Since *RAB29-NUCKS1* fusion was not previously observed in normal individuals or normal tissues, the possibility that this fusion gene is pathological cannot be completely excluded.

There are no recurrent cytogenetic abnormalities including leukemic fusion in ANKL. *RAB29* and *NUCKS1* have been reported to be associated with Parkinson's disease [11]. *RAB29* encodes Ras-related protein Rab-7L1, which belongs to the small GTPase superfamily. Rab proteins play important roles in the regulation of exocytic and endocytic pathways [12]. *RAB29* maintains the in-

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tegrity of the trans-Golgi network and is associated with the morphogenesis and retrograde trafficking of mannose-6-phosphate receptor [13]. Cation-independent mannose-6-phosphate receptor plays a role in binding and internalizing leukemia inhibitory factor, which stimulates the proliferation of some hematopoietic cells and has a direct inhibitory action on NK cells [14, 15]. *NUCKS1* encodes nuclear ubiquitous casein and cyclin-dependent kinase substrate 1, a chromatin-associated protein involved in DNA damage response [16]. It has been reported to be associated with various types of diseases and is regarded as a potential biomarker [17]. *NUCKS1* showed low expression in adult T-cell leukemia/lymphoma and pediatric acute lymphoblastic leukemia in an *in vitro* study [17]. A *NUCKS1-PM20D1* fusion was previously identified in a glioblastoma sample [18]. How *NUCKS1* contributes to the development of several cancers and other diseases including hematologic malignancies is currently unclear. However, based on the findings of our patient and the characteristics of *RAB29* and *NUCKS1*, it is possible that the *RAB29-NUCKS1* rearrangement contributes to the pathogenesis of ANKL in a way that is currently unknown.

In conclusion, to our knowledge, this is the first report of a case of ANKL with a *RAB29-NUCKS1* fusion. Further studies will be needed to elucidate the molecular pathogenesis of ANKL related to a *RAB29-NUCKS1* rearrangement. If the *RAB29-NUCKS1* fusion is found to be involved in the molecular pathogenesis of ANKL, it may be a potential novel biomarker for the diagnosis and treatment of ANKL.

요 약

공격성NK세포백혈병(ANKL)은 del(6)(q21q25) 또는 del(11q)과 같은 다양한 염색체 이상을 동반할 수 있는 드문 형태의 백혈병이다. 본 연구진은 이전에 보고된 바 없는 *RAB29-NUCKS1* 재배열을 가진 ANKL 환자의 증례를 보고하고자 한다. RNA fusion panel 검사를 시행하여 염색체 1q32.1에서 *RAB29*의 5번 엑손과 *NUCKS1*의 2번 엑손 사이의 유전자 융합을 발견했다. 또한 역전사 PCR과 Sanger 염기서열분석을 통해 암호화된 *RAB29-NUCKS1* 융합을 확인하였다. *RAB29*는 세포내이입과 세포외유출 경로를 조절하는 단백질을 암호화한다. *NUCKS1*은 DNA 손상 반응에 관여하는 염색질 관련 단백질을 암호화한다. *RAB29-NUKS1* 재배열과 관련된 ANKL의 분자생물학적 발생 기전을 이해하기 위해서는 추가적인 연구가 필요할 것으로 보인다.

Conflicts of Interest

None declared.

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