Aggressive Transformation of Juvenile Myelomonocytic Leukemia Associated with Duplication of Oncogenic *KRAS* due to Acquired Uniparental Disomy

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A small fraction of cases of juvenile myelomonocytic leukemia (JMML) develop massive disease activation. Through genomic analysis of JMML, which developed in an individual with mosaicism for oncogenic *KRAS* mutation with rapid progression, we identified acquired uniparental disomy at 12p. We demonstrated that duplication of oncogenic *KRAS* is associated with rapid JMML progression. *(J Pediatr 2013;162:1285-8).*

Juvenile myelomonocytic leukemia (JMML) is a rare pediatric myeloproliferative disorder, characterized by malignant transformation in the stem cell compartment with clonal proliferation of progeny that variably retain the capacity to differentiate. The clinical course of JMML is heterogeneous. Some patients require prompt allogeneic hematopoietic stem cell transplantation, whereas some demonstrate a milder clinical course, and some of them eventually exhibit spontaneous improvement.[1] Recent investigations into the molecular pathogenesis of JMML revealed that approximately 80% of patients harbored mutually exclusive mutations in genes regulating the Ras-mitogen-activated protein kinase (MAPK) pathway, including *RAS*, *PTPN11*, *NF1*, and *CBL*, leading to aberrant activation of the Ras-MAPK pathway.[1,2] The spectrum of mutations described thus far in JMML provides potential new opportunities for both diagnosis and therapy.

Previous studies reported that a small fraction of patients with JMML develop rapid and massive disease activation after an indolent clinical course. A report showed the incidence of progression to blastic phase to be 13%.[3] The etiology of the aggressive transformation remains unelucidated, however. We present a patient with JMML with a *KRAS* mutation who developed aggressive transformation and died. We performed genomic analysis to investigate the molecular pathology of this rapid and fatal progression.

Methods

A 1-year-old boy presented with leukocytosis (white blood cell count 46 800/mm^3, 20% monocytes, no blast cells) and hepatosplenomegaly. Bone marrow aspiration revealed hypercellular marrow, with 0.5% blast cells. Karyotyping was normal, and reverse-transcription polymerase chain reaction detected no *BCR-ABL* fusion. Fetal hemoglobin concentration was elevated (22%). Spontaneous growth of colony-forming unit granulocyte macrophages and hypersensitivity to granulocyte macrophage colony-stimulating factor were demonstrated, and mutation analysis revealed a heterozygous *KRAS* mutation (G12D: GGT>GAT) in peripheral blood mononuclear cells (PBMCs), all of which were consistent with JMML.[4] By 2 months after diagnosis, leukocytosis and hepatosplenomegaly had progressed. Oral 6-mercaptopurine (6-MP) therapy was started, and the patient remained stable for the next 10 months.

At 1 year after diagnosis, the patient suddenly developed tachypnea, impaired consciousness, and massive hepatosplenomegaly. Laboratory data revealed a white blood cell count of 124 400/mm^3 (38% monocytes, 5% blast cells; **Figure 1**, A). The patient's condition deteriorated rapidly, and he died from respiratory dysfunction. Autopsy revealed dysplastic cells infiltrating the bone marrow, lymph nodes, central nervous system, lungs, liver, spleen, pancreas, and kidneys (**Figures 1**, B and **2**; **Figure 2** available at www.jpeds.com).

Our genomic analysis was approved by the Ethics Board of the University of Tokyo, and informed consent was obtained from the child's guardian. Direct sequencing of the *KRAS* gene was performed for his normal muscle, heart, and lung (obtained at autopsy) and for PBMCs at diagnosis and at progression. Genome-wide analysis for genetic lesions was performed by single nucleotide polymorphism (SNP) array analysis. DNA

6-MP	6-mercaptopurine
JMML	Juvenile myelomonocytic leukemia
MAPK	Mitogen-activated protein kinase
PBMC	Peripheral blood mononuclear cell
SNP	Single nucleotide polymorphism
UPD	Uniparental disomy

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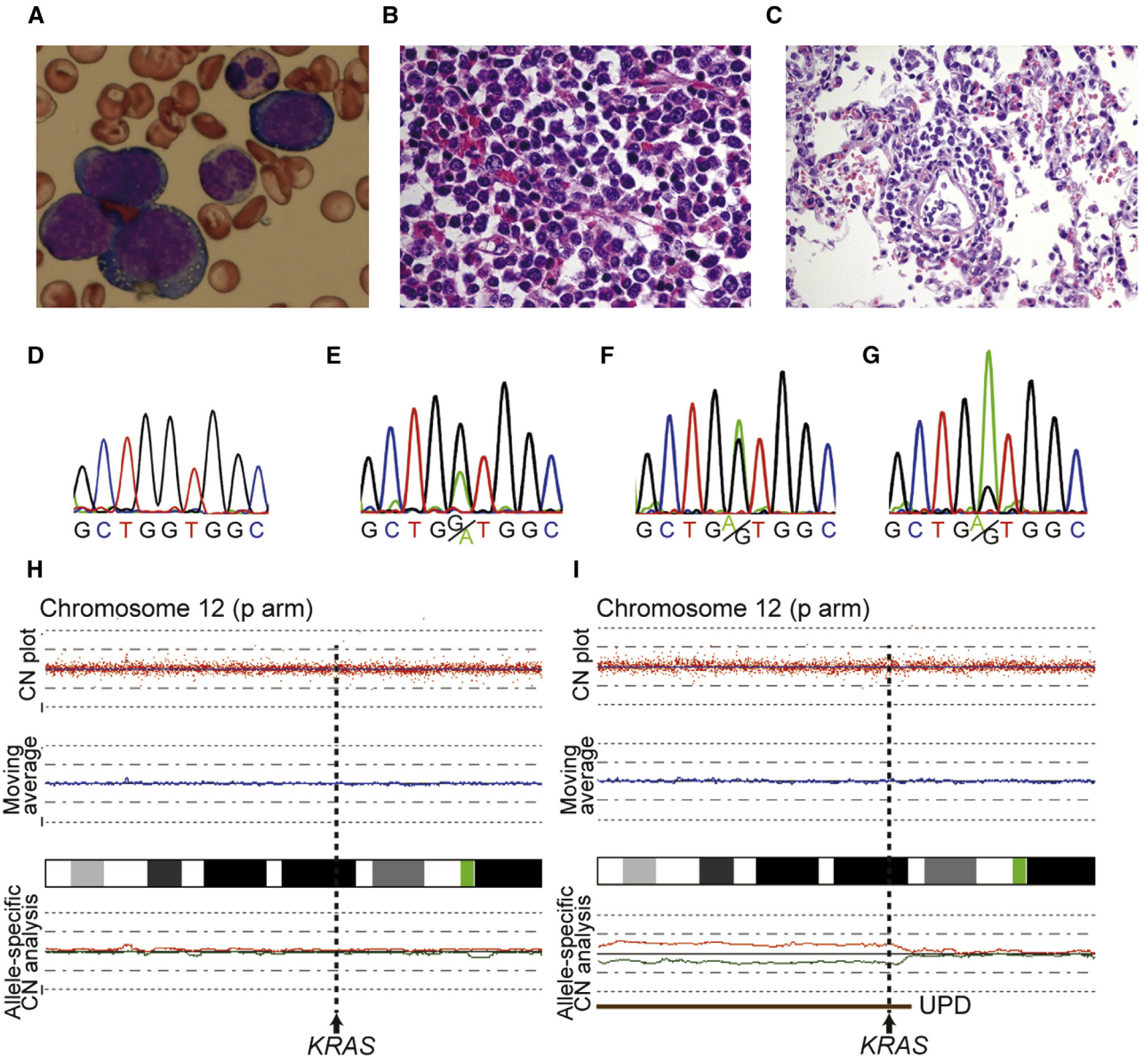

Figure 1. Blood smear, autopsy, and genomic analysis results. **A,** May-Giemsa staining of peripheral blood at progression. Hematoxylin & eosin staining of **B,** bone marrow and **C,** lung. Results of direct sequencing for the *KRAS* gene in **D,** heart, **E,** muscle, **F,** peripheral blood at diagnosis, and **G,** peripheral blood at progression. Results of SNP array analysis for PBMCs at **H,** diagnosis and **I,** progression. UPD at the 12p locus was detected at progression (*brown line*), whereas UPD was absent at diagnosis. Total copy number plots from each probe (*red points*) and moving average (n = 20; *blue line*) are shown *above* the cytoband. Results from allele-specific analyses are given *below* the cytoband. The larger allele is presented in *red*; the smaller allele, in *blue*. *CN*, copy number.

extracted from samples was analyzed using the GeneChip Human Mapping 250K *NspI* array (Affymetrix, Santa Clara, California). The data thus obtained were processed using CNAG/AsCNAR software (http://www.genome.umin.jp).[5,6]

Results and Discussion

This case shows that JMML can progress rapidly during an indolent clinical course, with invasion into multiple organs. The aggressive transformation of JMML is similar to that of blast crisis in chronic myelogenous leukemia but is rare,[2,3] and the definition and molecular biology of the blast crisis–like aggressive transformation of JMML remain unclear.

Direct sequencing of the *KRAS* gene revealed a mutation in normal muscle. The same mutation was found in the lung, but not in the heart. The mutation was also detected in PBMCs at diagnosis, and the mutation became homozygous in PBMCs at progression (Figure 1, D-G). SNP array analysis