Identification of RCC1- LCK as a novel fusion gene in pediatric erythroid sarcoma

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Abstract

Erythroid sarcoma is very rare form of pure erythroid leukemia with undetermined biological features. Here, we present an infant with a multifocal erythroid sarcoma, diagnosed because the tumor cells were positive for glycophorin A. After acute myeloid leukemia-oriented chemotherapy and surgical resection followed by cord blood transplantation, he has successfully maintained complete remission without any late effects. Total transcriptome analysis of the tumor identified a novel fusion gene, RCC1-LCK, and high LCK expression levels, suggesting that LCK overexpression was involved in leukemogenesis in this case.

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Abbreviations

AEL	acute erythroid leukemia
ALL	acute lymphoblastic leukemia
AML	acute myeloid leukemia
CBT	cord blood transplantation
ES	erythroid sarcoma
FCM	flow cytometry
FPKM	fragments per kilobase of exon per million mapped reads
GPA	glycophorin A
LCK	lymphocyte-specific protein tyrosine kinase
MRI	magnetic resonance imaging
PAS	Periodic acid-Schiff
PEL	pure erythroid leukemia
RCC1	regulator of chromosome condensation 1

ABSTRACT

Erythroid sarcoma is very rare form of pure erythroid leukemia with undetermined biological features. Here, we present an infant with a multifocal erythroid sarcoma, diagnosed because the tumor cells were positive for glycophorin A. After acute myeloid leukemia-oriented chemotherapy and surgical resection followed by cord blood transplantation, he has successfully maintained complete remission without any late effects. Total transcriptome analysis of the tumor identified a novel fusion gene, *RCC1-LCK* , and high *LCK*expression levels, suggesting that LCK overexpression was involved in leukemogenesis in this case.

INTRODUCTION

Pure erythroid leukemia (PEL) presenting as erythroid sarcoma (ES) is extremely rare in children.[1-6] Due to the rarity of PEL and ES in childhood, genetic studies of such cases are scarce.

In this report, we identified a novel kinase-related fusion gene,*RCC1-LCK* , in an infant case with PEL presenting as ES, which provides insight into the leukemogenic mechanism in this case.

CASE REPORT

A 6-month-old infant was referred to our hospital because humeral osteolysis was unexpectedly detected when he was examined for fever. Laboratory examination of peripheral blood showed the following: hemoglobin, 9.2 g/dL; platelet count, 310,000/μL; leucocyte count, 11,600/μL, without blasts; C-reactive protein, 15.82 mg/dL; lactate dehydrogenase, 892 U/L; and ferritin, 922 ng/mL. Computed tomography showed multiple tumors in the left humerus, intracranium, orbita, and chest, and beside his spinal cord. Bone marrow examination revealed infiltration of tumor cells with round coarse nuclei and plentiful basophilic cytoplasm, sometimes with cytoplasmic budding. Tumor cells also frequently contained several cytoplasmic vacuoles, but not Auer-bodies or cytoplasmic granules. Tumor cells were negative for myeloperoxidase, chloroacetate esterase, and Periodic acid-Schiff (PAS) on cytochemical staining. Flow cytometry (FCM) analysis showed that they expressed only CD36, without CD1a, CD14, CD45, CD56, HLA-DR, or glycophorin A (GPA), resulting in no definite diagnosis. Immunohistochemistry analysis of a bone marrow clot revealed negativity for CD3, TdT, CD20, CD138, synaptophysin, chromogranin A, CD99, CD30, and ALK. Karyotype analysis detected 46, XY in 20/20 metaphases and fluorescent in situ hybridization did not detect split signals for*ALK* , *KMT2A* , or *EWSR1* . Since acute symptomatic seizures caused by intracranial tumors were observed, we were obliged to administer a single dose of vincristine, resulting in rapid shrinkage of the intracranial tumors detected by brain magnetic resonance imaging (MRI). A month later, he had symptoms of acute flaccid paralysis of both lower extremities. Spine MRI revealed recurrence of a paraspinal tumor that compressed

the spinal cord at Th9-L1. Cerebrospinal fluid examination revealed infiltration of tumor cells, while no tumor cell infiltration was detected on bone marrow aspiration. FCM analysis of resected paraspinal tumor cells was positive for CD36 and GPA. Culture of excised tumor cells in liquid medium for several days led to marked formation of intracellular vacuoles (Fig. 1A), and some tumor cells showed granular to diffuse positive results on PAS staining (Fig. 1B). FCM analysis revealed that the cultured cells were positive for CD71 (transferrin receptor), CD99, CD36, and GPA (Fig. 1C), as well as blood group A antigen, consistent with the patient's blood type (Fig. 1C). Based on these findings, a diagnosis of PEL presenting as ES was finally made.

He received multi-agent chemotherapy, consisting of cytarabine, anthracyclines, and etoposide, including intrathecal injection of cytarabine, methotrexate, and hydrocortisone.[7] After induction therapy, the residual tumors shrank somewhat; however, ES relapsed with an epidural tumor of the spinal cord at L1-2 after the second consolidation therapy. No bone marrow relapse was detected. He underwent tumor resection, followed by allogeneic cord blood transplantation (CBT), with 5/6 HLA matching at the antigen level. The conditioning regimen consisted of fludarabine, busulfan, and melphalan. He has maintained complete remission for 20 months after CBT and currently has no late effects.

To investigate the underlying pathogenesis of this unique case, we performed whole-exome sequencing and total transcriptome analysis of the tumor. Although whole-exome sequencing did not detect any pathogenic genetic mutations, total transcriptome analysis identified a novel fusion gene, *RCC1-LCK* . Fusion of the 5' end of *RCC1*(NM_001048199.3) exon 2 and the 3' end of *LCK* (NM_001330468.2) exon 2 was confirmed by reverse transcriptase PCR and Sanger sequencing (Figs. 2A and 2B). In this fusion gene, the 5' untranslated region of*RCC1* was joined to the entire coding region of *LCK* , resulting in swapping of the *LCK* promoter, due to intrachromosomal deletion (Fig. 2C). Consistent with this promoter swapping, transcriptome analysis revealed a fragments per kilobase of exon per million mapped reads (FPKM) value for *LCK* of 23.2, which was much higher than that in hematological malignancies without*LCK* rearrangement (FPKM values ranging from approximately 0 to 1).

DISCUSSION

A previous comprehensive genomic analysis of pediatric acute erythroid leukemia (AEL) showed alterations of several genes; however, the majority were not common to more than one case, suggesting genetic heterogeneity in this leukemia subtype and no *LCK* alterations were reported in AEL, including PEL.[8] Only six cases of pediatric PEL have ever been reported to have rearrangements, which include *NFIA-CBFA2T3* , *NFIA-RUNX1T1* , and*ZMYND8-RELA* ;[5,6,9-12] however, the precise leukemogenic mechanisms underlying the fusion genes in these cases remain unknown.

Here, we present a case report of a very rare pediatric patient with ES carrying an *RCC1-LCK* fusion gene. Given the high FPKM value of*LCK* , overexpression of LCK may have contributed to leukemogenesis in this case. The molecular mechanism underlying overexpression of LCK is promoter swapping; as a result of the translocation, the constitutively active promoter of the partner gene drives ectopic expression of LCK.[13]

Regulator of chromosome condensation 1 (RCC1) is a known guanine nucleotide exchange factor of RAN, a nuclear RAS-like G protein, which is ubiquitously expressed in more than 25 organs and associated with the cell cycle, DNA damage, and oncogenesis. High expression of RCC1 acts as a pathogenic partner, promoting the development of some tumors, such as lung adenocarcinoma and cervical cancer.[14]

Lymphocyte-specific protein tyrosine kinase (LCK) is a member of the SRC family of protein tyrosine kinases and a key molecule in regulation of T-cell functions; for example, LCK regulates the initiation of TCR signaling, T-cell development, and T-cell homeostasis.[15] Further, LCK expression is reported to be high in hematological malignancies, such as chronic lymphocytic leukemia, B-cell acute lymphoblastic leukemia (ALL), and acute myeloid leukemia (AML), and even in non-hematological malignancies, such as breast, colorectal, and lung cancers.[16] In addition, LCK functions as both an important signaling molecule and a therapeutic target in ALL and AML.[17-20] Duque-Afonso et al. showed that suppression of LCK expression decreased the phosphorylation of PLCγ2 in a form of B-ALL with LCK overexpression, and that PLCγ2

had a pathogenic role in this form of leukemia, while inhibition of LCK reduced leukemic cell growth in vitro and in vivo.[17]In T-ALL, Gocho et al. reported that tumors with high LCK activity were more sensitive to dasatinib than those with low LCK activity.[18] Further, in AML, Rouer et al. showed that high expression of LCK was present in leukemic cells from patients with less differentiated AML, and that a normal LCK expression pattern was restored when complete remission was achieved.[19]Intriguingly, similar to T-ALL, Li et al. reported that LCK-overexpressing AML cells (CTV1) were strongly inhibited by several LCK inhibitors, including dasatinib.[20] Hence, although the dysregulation of LCK in PEL or ES has not been reported, LCK overexpression via promoter swapping is potentially a key factor in our case, and dasatinib may be an effective therapy option for such cases.

In conclusion, we present a diagnostically challenging and unique case of an infant with ES harboring a novel *RCC1-LCK* fusion gene. As the precise leukemogenic mechanism underlying LCK upregulation in PEL or ES is unknown, further studies are required to clarify the role of LCK overexpression in this case.

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Legends

Figure 1. Morphologic and immunophenotypic features of this case. (A) Wright-Giemsa-stained cultured tumor cells (× 1000). The tumor cells formed marked intracellular vacuoles. (B) PAS-stained cultured tumor cells (x 1000). Some tumor cells showed granular to diffuse positive staining. (C) Flow cytometry analysis of cultured tumor cells. Tumor cells were positive for CD71 (transferrin receptor), CD99, CD36, GPA, and blood group A antigen.

Figure 2. Detection of the *RCC1-LCK* fusion. (A) Gel image showing the amplified fragment. *RCC1-LCK* , reverse transcriptase PCR product obtained with the primer combination, RCC1-123F, and LCK-396R (band size, 239 bp). *GAPDH* , cDNA of the glyceraldehyde-3-phosphate dehydrogenase gene (band size, 142 bp) was amplified as a control. M, size marker; N, healthy volunteer as a negative control; P, patient leukemic cells from left humerus tumor samples. (B) Partial chromatogram showing the junction of the*RCC1* and *LCK* genes. cDNA sequences showing the fusion of*RCC1* exon 2 and *LCK* exon 2. (C) Schematic representation of fusion transcripts in this case. Chromosomal organization of the*RCC1* gene (exons, white boxes) and *LCK* (exons, black boxes). The positions of translation initiation sites (ATG) and stop codons (TGA) are indicated. Exons are not to scale. Arrows, primer positions. Below, schematic showing the composition of hybrid transcripts.