Acta Med. Okayama, 2014
Vol. 68, No. 2, pp. 119–123

Acta Medica Okayama

http://escholarship.lib.okayama-u.ac.jp/amo/

Case Report

Detection of *RBM15-MKL1* Fusion Was Useful for Diagnosis and Monitoring of Minimal Residual Disease in Infant Acute Megakaryoblastic Leukemia

Akiko Takeda[a], Akira Shimada[a]*, Kazuko Hamamoto[b], Syuuji Yoshino[b], Tomoko Nagai[c], Yousuke Fujii[a], Mutsuko Yamada[a], Yoshimi Nakamura[d], Toshiyuki Watanabe[d], Yuki Watanabe[a], Yuko Yamamoto[a], Kanae Sakakibara[e], Megumi Oda[f], and Tsuneo Morishima[a]

Departments of [a]Pediatrics, [f]Pediatric Hematology/Oncology, [d]Division of Medical Support, Okayama University Hospital, Okayama 700–8558, Japan, [b]Department of Pediatrics, Hiroshima Red Cross Hospital & Atomic-bomb Survivors Hospital, Hiroshima 730–8619, Japan, [c]Department of Pharmacology, Meijo University, Nagoya 468–8503, Japan, [e]Department of Laboratory Medicine, National Hospital Organization Okayama Medical Center, Okayama 701–1154, Japan

Acute megakaryocytic leukemia (AMKL) with t(1 ; 22)(p13 ; q13) is a distinct category of myeloid leukemia by WHO classification and mainly reported in infants and young children. Accurate diagnosis of this type of AMKL can be difficult, because a subset of patients have a bone marrow (BM) blast percentage of less than 20% due to BM fibrosis. Therefore, it is possible that past studies have underestimated this type of AMKL. We present here the case of a 4-month-old female AMKL patient who was diagnosed by presence of the *RBM15-MKL1* (*OTT-MAL*) fusion transcript by RT-PCR. In addition, we monitored *RBM15-MKL1* fusion at several time points as a marker of minimal residual disease (MRD), and found that it was continuously negative after the first induction chemotherapy even by nested RT-PCR. Detection of the *RBM15-MKL1* fusion transcript thus seems to be useful for accurate diagnosis of AMKL with t(1 ; 22)(p13 ; q13). We recommend that the *RBM15-MKL1* fusion transcript be analyzed for all suspected AMKL in infants and young children. Furthermore, monitoring of MRD using this fusion transcript would be useful in treatment of AMKL with t(1 ; 22)(p13 ; q13).

Key words: AMKL, infant, *RBM15-MKL1*, *OTT-MAL*

Infant acute megakaryoblastic leukemia (AMKL) with t(1 ; 22)(p13 ; q13) is a distinctive myeloid malignancy by WHO classification, representing < 1% of all acute myeloid leukemia (AML) cases [1]. The prognosis of all AMKL is usually worse than that of other AML types, except for Down syndrome (DS)-related AMKL with *GATA1* mutation, but AMKL with t(1 ; 22)(p13 ; q13) appears to be uncertain. Some studies have found that AMKL patients with t(1 ; 22)(p13 ; q13) respond well to intensive AML chemotherapy and achieve long disease-free survival [1, 2]. Furthermore, the disease is mainly reported in infants and young children, and most cases occur in the first 6 months of life (median, 4 months) [1]. However, the diagnosis of AMKL can be difficult, because a subset of patients have a bone marrow (BM) blast percentage of less than 20% due to difficulties with BM aspiration secondary to fibrosis.

Received July 25, 2013; accepted November 12, 2013.
*Corresponding author. Phone:+81–86–235–7249; Fax:+81–86–221–4745
E-mail:pajj236e@okayama-u.ac.jp (A. Shimada)

On the other hand, this translocation also generates the fusion of the RNA-binding motif protein-15 (*RBM15*) (also known as *OTT*) and megakaryocyte leukemia-1 (*MKL1*) (also known as *MAL*) genes [3]. The resulting fusion gene modulates HOX-induced differentiation and extracellular signaling pathways [4].

In addition, the detection of minimal residual disease (MRD) is important for leukemia treatment, and is generally achieved using either flowcytometry [5] or detection of the *RBM15-MKL1* fusion transcript by reverse transcription polymerase chain reaction (RT-PCR) [6].

We present here the case of a 4-month-old female with a low blast count in BM aspirate because of fibrosis. *RBM15-MKL1* fusion was only detected by RT-PCR in the first diagnostic BM sample, it was not detected after the first induction chemotherapy even by the nested RT-PCR method. Further, the fusion transcript was not detected at about 2 years after the first induction chemotherapy. We will discuss the clinical outcome of this rare case of infant AMKL and the usefulness of this fusion gene as a marker of MRD.

Materials and Methods

Total RNA was extracted from BM mononucleated cells at the first diagnosis, after the first induction chemotherapy, after the second chemotherapy, at the end of chemotherapy and at 12 months after the end of chemotherapy. RNAs were reverse-transcribed to cDNA with a cDNA Synthesis Kit (Amersham Bioscience, Tokyo, Japan). Polymerase chain reaction (PCR) was performed using the primer pair of RBM15-S1, which was located in *RBM15* exon 1 (GenBank accession no. NM_022768.4), and MKL-AS2, which was located in *MKL1* exon 5 (GenBank accession no. NM_020831.3) as described previously [7]. We amplified a control gene (*B2M*) to check the mRNA integrity [7]. The PCR product was directly sequenced using an ABI 310 sequencer (Applied Biosystems, Tokyo, Japan). As a further analysis, we also performed a nested RT-PCR using the inner primer pair of OTT-MAL inner F (5'-cttcatgccttcccacctt-3') and OTT-MAL inner R (5'-tccaagctccttctctgctc-3').

Patient. A 4-month-old female baby was admitted to the hospital because of insufficient gain of body weight and poor sucking with hepatosplenomegaly. She was delivered at 39 weeks gestation, and her birth weight and height were 3,026 g and 47.0 cm. There were no complications at birth and no typical family history. Laboratory data showed anemia and thrombocytopenia with a low blast count (Table 1). A total of 4 BM aspirations were performed because of "dry tap" aspirate. BM biopsy revealed severe BM fibrosis with small round blasts resembling lymphoblasts (Fig. 1A, 1B). There was no evidence of cytomegalovirus infection. The tentative diagnosis was infant AML (FAB classification, M7). She received a first induction chemotherapy based on the Japanese AML05 protocol (VP16, cytarabine, and antracycline) with a 2/3 reduced dose and achieved a complete remission (CR). After the CR, she received an additional 4 courses of chemotherapy and was discharged from the hospital. She was healthy at 1.5 year after the end of chemotherapy.

Table 1 Laboratory data at admission to the hospital

WBC (×106/ml)	8,200	T.Bil (mg/dl)	0.6
Myelo (%)	1	AST (IU/L)	31
St (%)	3	ALT (IU/L)	15
Seg (%)	6	LDH (IU/L)	519
Eos (%)	2	BUN (mg/dl)	6.4
Bas (%)	1	Cr (mg/dl)	0.21
Mon (%)	3	CRP (mg/dl)	0.05
Lymph (%)	75		
Blast (%)	9		
RBC (×109/ml)	164	BM aspiration	
Hb (g/dl)	4.5	NCC (×106/ml)	38
Hct (%)	14.2	Mgk (×103/ml)	15
Plt (×106/ml)	9	Blast (%)	7.2
Ret (%)	2.2		

Results

RBM15-MKL1 fusion was detected in only the first diagnostic sample by RT-PCR (Fig. 2A). The fusion of exon 1 of *RBMK15* to exon 5 of *NKL1* was confirmed by direct sequencing (Fig. 2B). This fusion gene was not completely detected after the first induction chemotherapy, even by a nested RT-PCR at 18 months after the end of chemotherapy.

Discussion

AMKL with t(1 ; 22)(p13 ; q13) is rarely reported, represented $<1\%$ of all AML but occurred with

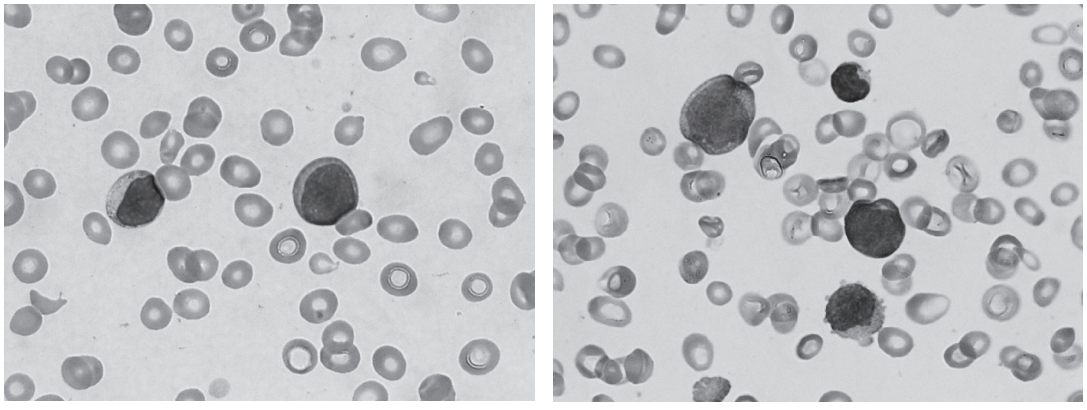

Fig. 1 Images of blasts in the peripheral blood (**A**) and bone marrow (**B**) at the first diagnosis. The blasts are round and resemble lymphoblasts.

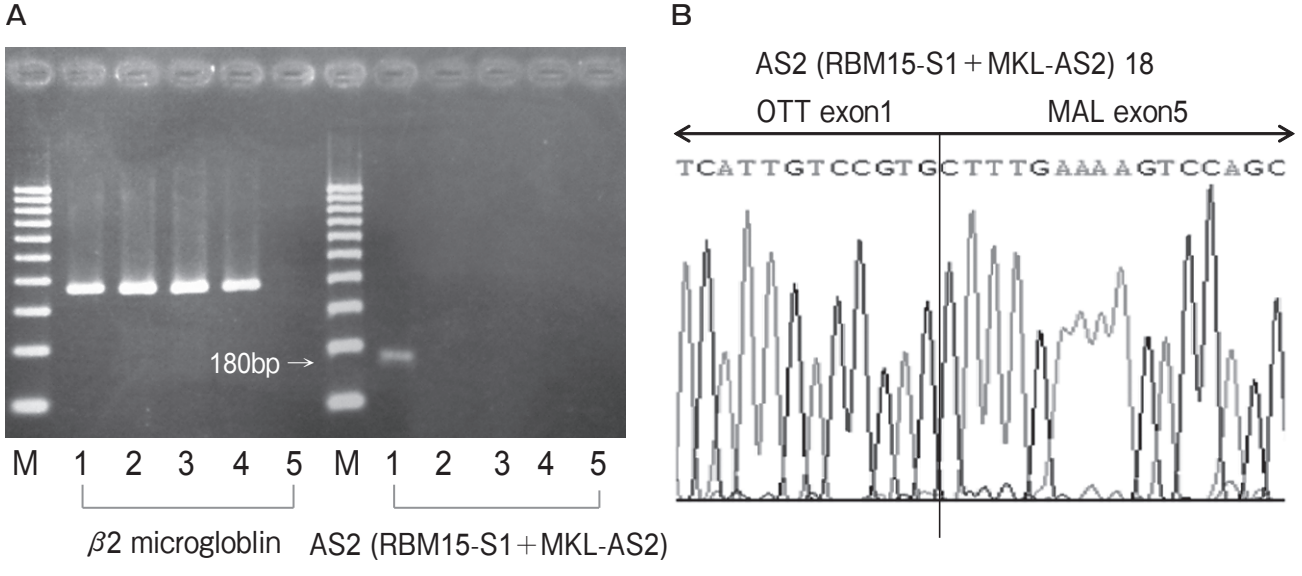

Fig. 2 *RBM15-MKL1* fusion transcript detected by RT-PCR. **A**, The *RBM15-MKL1* fusion transcript was detected only in the first diagnostic sample by RT-PCR. Lane M: molecular marker (100 bp); lanes 1–5: samples at the diagnosis (1), after 1 course of induction chemotherapy (2), after 2 courses of chemotherapy (3), at the end of chemotherapy (4), and a negative control (5) (the right side of each panel shows the result for PCR product of β2 microglobulin and the left side shows the result for the AS2 product). The fusion product at the diagnostic sample was completely negative after starting chemotherapy and even by the nested RT-PCR method (data not shown); **B**, Results of the direct sequencing of the fusion product from the diagnostic sample. The fusing of exon 1 of *RBMK15* to exon 5 of *NKL1* was confirmed by direct sequencing.

higher incidence in the pediatric population and accounts for about 70% of AMKL in infants [2]. However, in the AML99 study performed in Japan from 2000–2002, only 3 patients were diagnosed as having AMKL with t(1 ; 22)(p13 ; q13) among 11 non-Down AMKL patients. Accurate diagnosis appeared to be difficult because of the difficulty of accurately detecting this karyotypic abnormality. Ballerini *et al.* also reported that the real frequency of *OTT-MAL* (*RBM15-MKL1*) recombination events may be underestimated at least in children with AMKL [13]. Therefore, we strongly recommend that an RT-PCR

assay for the *RBM15-MKL1* fusion transcript be performed for all infants and young children with AMKL. However, the unavailability of BM mononucleated cells remains a problem due to BM fibrosis in AMKL. Therefore, if we could perform this analysis using RNA extracted from paraffin-embedded specimens, that would be a major step forward. However, we estimate that the RNA extracted from paraffin-embedded specimens would be shortened and show degradation. We will investigate possible solutions in a future study.

In addition, we do not know the exact prognosis of this rare type of AMKL due to the relative dearth of previous reports [8–11]. Older studies have reported a poor prognosis [9, 10], but more recent reports have described a relatively good prognosis compared to other types of AMKL [1, 2, 11]. In the AML99 study, 2 of 3 previous cases also survived more than 5 years with complete remission. A further large study including accurate diagnosis of this type of AMKL using the *RBM15-MKL1* fusion transcript will be needed.

In addition, it remains unknown what other molecular events may be associated with the *RBM15-MKL1* fusion transcript in AMKL with t(1 ; 22)(p13 ; q13). Hama *et al.* reported that there was no mutation in several established AML-related genes, such as *GATA1, JAK3, JAK2, TP53, NRAS, ASXL1, FLT3, IDH1/2, DNMT3A, RUNX1* and *CBL*, in 3 cases of AMKL with t(1 ; 22)(p13 ; q13) [8]. They also reported that there was no mutation in other types of nonDS-AMKL, except for one *GATA1* mutation, and thus it remains uncertain what other molecular events may take place in these AMKL patients.

In general, hematological CR means that the bone marrow blast percentage is $< 5\%$. On the other hand, molecular CR means that a single blast having a specific fusion transcript is present in less than one of 10^5 or 10^6 normal cells. Therefore, patients sometimes show hematological CR but can not achieve molecular CR after induction chemotherapy. Naturally, molecular CR is a better response to chemotherapy than a hematological response. Recently, MRD detection has become very important in AML therapy, including hematopoietic stem cell transplantation (HSCT). To detect an early relapse after HSCT, MRD monitoring is needed. However about 40% of AML patients do not have a specific MRD fusion transcript, such as *RBM15-MKL1, RUNX1-RUNXT1* (*AML1-MTG8*), *CBF β-MYH11, etc.* The *Wilms' tumor 1* (WT1) is overexpressed in > 70–80% of patients with AML and thus can be useful for minimal residual disease (MRD) monitoring [12]. In the recent AML99 study, the residual level of MRD after the first induction chemotherapy was correlated with the prognosis of AML patients [12]. In this case, the molecular remission seems to be quite deep, because the nested RT-PCR could not detect *RBM15-MKL1* fusion after the first induction chemotherapy. We therefore recommend that screening for this fusion transcript be conducted for all infants and young children with AMKL upon first diagnosis. In addition, a future study in which MRD is monitored in bone marrow and peripheral blood to compare the clinical outcomes is warranted. We conclude that detection of the *RBM15-MKL1* fusion transcript is effective for the accurate diagnosis of AMKL as well as a useful marker for MRD.

Acknowledgments. We thank for all the medical staff who participated in the patient care. This work was supported in part by a Grant-in-Aid for Cancer Research and a grant for Clinical Cancer Research and Research on Children and Families from the Ministry of Health, Labor and Welfare of Japan.

References

1. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, Harris NL, Le Beau MM, Hellström-Lindberg E, Tefferi A and Bloomfield CD: The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood (2009) 114: 937–951.
2. Orazi A: Histopathology in the diagnosis and classification of acute myeloid leukemia, myelodysplastic syndromes, and myelodysplastic/myeloproliferative diseases. Pathobiology (2007) 74: 97–114.
3. Ma Z, Morris SW, Valentine V, Li M, Herbrick JA, Cui X, Bouman D, Li Y, Mehta PK, Nizetic D, Kaneko Y, Chan GC, Chan LC, Squire J, Scherer SW and Hitzler JK: Fusion of two novel genes, RBM15 and MKL1, in the t(1 ; 22)(p13 ; q13) of acute megakaryoblastic leukemia. Nat Genet (2001) 28: 220–221.
4. Mercher T, Coniat MB, Monni R, Mauchauffe M, Nguyen Khac F, Gressin L, Mugneret F, Leblanc T, Dastugue N, Berger R and Bernard OA: Involvement of a human gene related to the Drosophila spen gene in the recurrent t(1 ; 22) translocation of acute megakaryocytic leukemia. Proc Natl Acad Sci USA (2001) 98: 5776–5779.
5. Feller N, van der Pol MA, van Stijn A, Weijers GW, Westra AH, Evertse BW, Ossenkoppele GJ and Schuurhuis GJ: MRD parameters using immunophenotypic detection methods are highly reliable in predicting survival in acute myeloid leukaemia. Leukemia (2004) 18: 1380–1390.
6. Doubek M, Palasek I, Pospisil Z, Borsky M, Klabusay M,

Brychtova Y, Jurcek T, Jeziskova I, Krejci M, Dvorakova D and Mayer J: Detection and treatment of molecular relapse in acute myeloid leukemia with RUNX1 (AML1), CBFB, or MLL gene translocations: frequent quantitative monitoring of molecular markers in different compartments and correlation with WT1 gene expression. Exp Hematol (2009) 37: 659–672.
7. Torres L, Lisboa S, Vieira J, Cerveira N, Santos J, Pinheiro M, Correia C, Bizarro S, Almeida M and Teixeira MR: Acute megakaryoblastic leukemia with a four-way variant translocation originating the RBM15-MKL1 fusion gene. Pediatr Blood Cancer (2011) 56: 846–849.
8. Hama A, Muramatsu H, Makishima H, Sugimoto Y, Szpurka H, Jasek M, O'Keefe C, Takahashi Y, Sakaguchi H, Doisaki S, Shimada A, Watanabe N, Kato K, Kiyoi H, Naoe T, Kojima S and Maciejewski JP: Molecular lesions in childhood and adult acute megakaryoblastic leukaemia. Br J Haematol (2012) 156: 316–325.
9. Bernstein J, Dastugue N, Haas OA, Harbott J, Heerema NA, Huret JL, Landman-Parker J, LeBeau MM, Leonard C, Mann G, Pages MP, Perot C, Pirc-Danoewinata H, Roitzheim B, Rubin CM, Slociak M and Viguie F: Nineteen cases of the t(1 ; 22) (p13 ; q13) acute megakaryoblastic leukemia of infants/children and a review of 39 cases: report from a t(1 ; 22) study group. Leukemia (2000) 14: 216–218.
10. Carroll A, Civin C, Schneider N, Dahl G, Pappo A, Bowman P, Emami A, Gross S, Alvarado C and Phillips C: The t(1 ; 22) (p13 ; q13) is nonrandom and restricted to infants with acute megakaryoblastic leukemia: a Pediatric Oncology Group Study. Blood (1991) 78: 748–752.
11. Duchayne E, Fenneteau O, Pages MP, Sainty D, Arnoulet C, Dastugue N, Garand R and Flandrin G; Groupe Français d'Hématologie Cellulaire: Acute megakaryoblastic leukemia: a national clinical and biological study of 53 adult and childhood cases by the Group Francais d'Hematologie Cellulaire (GFHC). Leuk Lymphoma (2003) 44: 49–58.
12. Shimada A, Taki T, Koga D, Tabuchi K, Tawa A, Hanada R, Tsuchida M, Horibe K, Tsukimoto I, Adachi S, Kojima S and Hayashi Y: High WT1 mRNA expression after induction chemotherapy and FLT3-ITD have prognostic impact in pediatric acute myeloid leukemia: a study of the Japanese Childhood AML Cooperative Study Group. Int J Hematol (2012) 96: 469–476.
13. Ballerini P, Blaise A, Mercher T, Pellegrino B, Perot C, van den Akker J, Gatbois E, Adam M, Douay L, Berger R, Bernard O and Landman-Parker J: A novel real-time RT-PCR assay for quantification of OTT-MAL fusion transcript reliable for diagnosis of t(1 ; 22) and minimal residual disease (MRD) detection. Leukemia (2003) 17: 1193–1196.
14. Kwon M, Martínez-Laperche C, Infante M, Carretero F, Balsalobre P, Serrano D, Gayoso J, Pérez-Corral A, Anguita J, Díez-Martín JL and Buño I: Evaluation of minimal residual disease by real-time quantitative PCR of Wilms' tumor 1 expression in patients with acute myelogenous leukemia after allogeneic stem cell transplantation: correlation with flow cytometry and chimerism. Biol Blood Marrow Transplant (2012) 18: 1235–1242.