A case of KMT2A–SEPT9 fusion–associated acute megakaryoblastic leukemia

Christopher J. Forlenza,1 Yanming Zhang,2 JinJuan Yao,2 Ryma Benayed,2 Peter Steinherz,1 Kavitha Ramaswamy,1 Rachel Kessel,3 Mikhail Roshal,2 and Neerav Shukla1

1Department of Pediatrics, Memorial Sloan Kettering Cancer Center, New York, New York 10065, USA; 2Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, New York 10065, USA; 3Division of Hematology/Oncology and Stem Cell Transplantation, Cohen Children’s Medical Center of New York, New Hyde Park, New York 11042, USA

Abstract

Acute megakaryoblastic leukemia (AMKL) constitutes ~5%–15% of cases of non-Down syndrome AML in children, and in the majority of cases, chimeric oncogenes resulting from recurrent gene rearrangements are identified. Based on these rearrangements, several molecular subsets have been characterized providing important prognostic information. One such subset includes a group of patients with translocations involving the KMT2A gene, which has been associated with various fusion partners in patients with AMKL. Here we report the molecular findings of a 2-yr-old girl with AMKL and t(11;17)(q23;25) found to have a KMT2A–SEPT9 fusion identified through targeted RNA sequencing. A KMT2A–SEPT9 fusion in this subset of patients has not previously been reported.

CASE PRESENTATION

A previously healthy 2-yr-old female was initially seen for recurrent fevers and decreased appetite. She was treated for suspected otitis media but continued to experience a poor appetite and fevers. A complete blood count showed anemia (Hb 7.2 g/dl) and thrombocytopenia (plt 60 k/µl), with a white blood cell count in the normal range (6.54 k/µl, ANC 2500/ml). A bone marrow biopsy and aspiration were performed that demonstrated a normocellular marrow with left-shifted granulopoiesis, progressive erythroid maturation, and atypical, hypolobulated megakaryocytes. Reticulin staining demonstrated variable increase in reticulin fibrosis from mild to marked (1–3+). The specimen contained an expanded blast population (30%), with blasts that were variable in size and had round nuclei, fine chromatin, variable nucleoli, and agranular cytoplasm. Some blasts showed cytoplasmic blebs with some of the larger blasts having vacuolated cytoplasm. Flow-cytometric analysis showed the blast population had expression of CD4, CD33, CD38, CD41, CD45, CD61, CD71, CD117, and CD123. The findings were consistent with a diagnosis of AMKL.

Cytogenetic analysis showed 46, XX, t(11;17)(q23;q25) in nine of 20 metaphase cells and the presence of a rearrangement involving KMT2A was confirmed by FISH in 8% of 300 cells. Targeted RNA sequencing identified a corresponding KMT2A–SEPT9 fusion transcript.

The patient received induction chemotherapy consisting of daunorubicin, cytarabine, and etoposide (ADE) in combination with gemtuzumab ozogamicin. Repeat bone marrow
analysis at the end of induction demonstrated an MRD-negative complete remission. Because of the poor outcomes associated with KMT2A rearrangements in pediatric patients with AMKL, the decision was made to proceed to bone marrow transplantation in first complete remission. After completing two additional cycles of consolidative therapy, she received an allogeneic bone marrow transplant from an HLA-matched unrelated donor and has no evidence of disease more than 2 months after her transplant.

**TECHNICAL ANALYSIS**

The presence of the translocation involving Chromosomes 11 and 17 was identified by standard karyotype analysis and confirmed by a break-apart FISH probe (Abbott Molecular) (Fig. 1A,B). Targeted RNA sequencing using a customized 199-gene Archer FusionPlex panel identified the KMT2A–SEPT9 transcript involving exon 7 and exon 2 of KMT2A and SEPT9, respectively (Table 1; Fig. 1C).

**SUMMARY**

KMT2A–SEPT9 fusions are rare events that have been most commonly described in various myeloid leukemias exhibiting monocytic differentiation (Taki et al. 1999; Yamamoto et al. 2002; Shih et al. 2006; Strehl et al. 2006; Kurosu et al. 2008). They have infrequently been described in M0/M1/M2 AML, t-AML, and de novo myelodysplastic syndrome (Supplemental Table 1; Osaka et al. 1999; Strehl et al. 2006; Kreuziger et al. 2007; Saito et al. 2010;
Santos et al. 2010). To our knowledge, this is the first report of KMT2A–SEPT9 fusion–associated AMKL, as well as the first reported occurrence of any KMT2A–SEPTIN fusion occurring in AMKL (Cerveira et al. 2011). In this case the fusion is located at the intron 7 breakpoint. KMT2A–SEPT9 fusions may have a propensity to involve the intron 7 or 8 breakpoint, as the majority of reported cases involve this region. This contrasts with more common KMT2A fusion partner genes, which most frequently involve the region between exon 9 and intron 11 (Meyer et al. 2018). However, the limited number of cases prevents any definitive conclusions.

AMKL is a subtype of AML with bimodal age distribution, with peaks occurring in early childhood before the age of 3 and later in adulthood (Tallman et al. 2000; Athale et al. 2001). In patients with Down syndrome (DS), AMKL is the most frequently occurring form of AML and is characterized by the presence of mutations involving GATA1 (Wechsler et al. 2002). In patients with non-DS pediatric AMKL, several molecular subsets have recently been characterized and provide valuable prognostic information (de Rooij et al. 2016, 2017; Hara et al. 2017). Commonly reoccurring rearrangements include RBM15–MKL1, CBF2T3–GLIS2, NUP98–KDM5A, and KMT2A (de Rooij et al. 2016, 2017; Hara et al. 2017). Patients with fusions involving KMT2A make up 7%–17.4% of pediatric patients with non-DS AMKL (de Rooij et al. 2016, 2017; Hara et al. 2017). Numerous KMT2A fusion partners have been identified in children with AMKL such as MLLT1, MLLT3, MLLT6, MLLT9, and MLLT10 (de Rooij et al. 2016, 2017; Hara et al. 2017). Although little is known about the prognostic implications of the various KMT2A fusion partners, collectively it appears the presence of these rearrangements is a high-risk feature associated with a greater risk of relapse and worse overall survival, indicating a role for allogeneic transplantation in first remission, which was recommended for the patient discussed in this case (de Rooij et al. 2016, 2017).

The KMT2A gene located on Chromosome 11 band q23 is a frequent target of translocation events with more than 100 recurrent rearrangements having been identified (Meyer et al. 2018). KMT2A rearrangements are commonly seen in both adult and pediatric acute leukemias but have particularly strong associations with infant ALL (Meyer et al. 2018), M4/M5 AML (Cimino et al. 1995; Schoch et al. 2003; Meyer et al. 2018), and therapy-related AML (t-AML) (Smith et al. 1994; Meyer et al. 2018), where it typically is found in patients exposed to topoisomerase II inhibitors. The KMT2A gene product is a DNA-binding protein capable of positively regulating gene expression, including the Hox family of genes, which play an important role in hematopoiesis and lymphoid cell development (Caslini et al. 2000; Milne et al. 2002). Chimeric proteins resulting from KMT2A rearrangements can efficiently transform hematopoietic precursors into leukemic stem cells (Krivtsov and Armstrong 2007). However, the fusion partner appears to play an important role in transformation because simply enhancing KMT2A promoter activity is not sufficient to induce leukemogenesis (Corral et al. 1996).

The septin family of genes is an evolutionarily conserved GTP-binding, filament-forming protein believed to be involved in polarity determination, cytoskeletal reorganization,
membrane dynamics, vesicle trafficking, and exocytosis (Kartmann and Roth 2001). Aside from SEPT9, several human septin genes have been identified as partners for translocation events with KMT2A including SEPT5, SEPT6, and SEPT11 (Hall and Russell 2004). The role of SEPT9 in leukemogenesis has not been clearly elucidated. However, studies have shown that variants of SEPT9 interact with both α and γ tubulin, and cells with enhanced expression of SEPT9 experienced defects in both cytokinesis and mitotic spindle defects, contributing to genomic instability (Peterson et al. 2011). The role of SEPT9 in malignant transformation may not be restricted to AML/MDS, as alterations in expression or deletion of SEPT9 are frequently observed in breast and ovarian cancer, indicating its potential role as a tumor suppressor (Kalikin et al. 2000; Burrows et al. 2003).

The mechanism by which the KMT2A–SEPT9 fusion drives leukemogenesis has not been firmly established. For many KMT2A fusion partner genes, it is believed that rearrangement events result in the fusion of transcriptional activation domains to KMT2A and are capable of driving leukemogenesis (So and Cleary 2003; Zeisig et al. 2003). However, as is the case with SEPT9, several KMT2A partners are localized to the cytoplasm and unlikely to have nuclear function. In a number of these partners dimerization of fusion oncoproteins has been identified as an alternative mechanism of transcriptional activation of KMT2A (Martin et al. 2003; So et al. 2003). Likewise, homo-oligomerization of KMT2A–SEPT6 fusion products proved to be capable of immortalizing stem cell progenitors (Ono et al. 2005). Drawing from sequence homology across the septin family of proteins and the ability of SEPT9 to form homodimers, it is reasonable to hypothesize that KMT2A–SEPT9 fusion protein dimerization is a key step in leukemic transformation (Abbey et al. 2016).

In summary, this report describes the first documented case of KMT2A–SEPT9 fusion–associated AMKL and is also the first report of any KMT2A–SEPTIN fusion occurring in AMKL. This rearrangement was first detected by conventional cytogenetics and confirmed by targeted RNA sequencing. Despite the numerous documented cases of the KMT2A–SEPT9 fusion, the mechanism of its role in leukemic transformation and its prognostic impact are unclear.

**ADDITIONAL INFORMATION**

**Data Deposition and Access**
The variant described in this manuscript was deposited in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and assigned the accession number SCV000852004.

**Ethics Statement**
Informed and signed consent was obtained for the research performed and publication of the results. The patient was enrolled in the Memorial Sloan Kettering Cancer Center (MSKCC) targeted gene sequencing research study (Genomic profiling in cancer patients; NCT01775072) with approval from the MSKCC Institutional Review Board under protocol IRB# 12-245.

**Acknowledgments**
We thank Joseph Olechnowicz for editorial assistance.

**Author Contributions**
C.J.F., N.S., Y.Z., and M.R. conceived the study. Y.Z., J.Y., and R.B. provided figures and associated legends. All authors reviewed and drafted the manuscript.

---

The authors have declared no competing interest.
Funding
We acknowledge support of the National Cancer Institute (NCI) Cancer Center Support Grant (P30 CA008748)

REFERENCES

Abby M, Hakim C, Anand R, Lafera J, Schambach A, Kispert A, Taft MH, Kaever V, Kotlyarov A, Gaestel M, et al. 2016. GTPase domain driven dimerization of SEPT7 is dispensable for the critical role of septins in fibroblast cytokinesis. Sci Rep 6: 20007.

Athale UH, Razzouk BI, Raimondi SC, Tong X, Behm FG, Head DR, Srivastava DK, Rubnitz JE, Bowman L, Pui CH, et al. 2001. Biology and outcome of childhood acute megakaryoblastic leukemia: a single institution’s experience. Blood 97: 3727–3732.

Burrows JF, Chanduloy S, McIlhatton MA, Nagar H, Yeates K, Donaghy P, Price J, Godwin AK, Johnston PG, Russell SE. 2003. Altered expression of the septin gene, SEPT9, in ovarian neoplasia. J Pathol 201: 581–588.

Caslini C, Shilitafid A, Yang L, Hess JL. 2000. The amino terminus of the mixed lineage leukemia protein (MLL) promotes cell cycle arrest and monocytic differentiation. Proc Natl Acad Sci 97: 2797–2802.

Cerveira N, Bizarro S, Teixeira MR. 2011. MLL-SEPTIN gene fusions in hematological malignancies. Biol Chem 392: 713–724.

Cimino G, Rapanotti MC, Elia L, Biondi A, Fizzotti M, Testi AM, Testi S, Croce CM, Canaani E, Mandelli F, et al. 1995. ALL-1 gene rearrangements in acute myeloid leukemia: association with M4–M5 French–American–British classification subtypes and young age. Cancer Res 55: 1625–1628.

Corral J, Lavenir I, Impey H, Warren AJ, Forster A, Larson TA, Bell S, McKenzie AN, King G, Rabbits TH. 1996. An Mll–Af9 fusion gene made by homologous recombination causes alternative fusion events in chimeric mice: a method to create fusion oncogenes. Cell 85: 853–861.

de Rooij JD, Masetti R, van den Heuvel-Eibrink MM, Cayuela JM, Trij K, Reinhardt D, Rasche M, Sonneveld E, Alonzo TA, Fornerod M, et al. 2016. Recurrent abnormalities can be used for risk group stratification in pediatric AMKL: a retrospective intergroup study. Blood 127: 3424–3430.

de Rooij JDE, Branstetter C, Ma J, Li YJ, Walsh MP, Cheng JJ, Obulkasim A, Dang JJ, Easton J, Verboon LJ, et al. 2017. Pediatric non–Down syndrome acute megakaryoblastic leukemia is characterized by distinct genomic subsets with varying outcomes. Nat Genet 49: 451–456.

Hall PA, Russell SEH. 2004. The pathobiology of the septin gene family. J Pathol 204: 489–505.

Hara Y, Shiba N, Ohki K, Tabuchi K, Yamato G, Park MJ, Tomizawa D, Kinoshita A, Shimada A, Arakawa H, et al. 2017. Prognostic impact of specific molecular profiles in pediatric acute megakaryoblastic leukemia in non–Down syndrome. Genes Chromosomes Cancer 56: 394–404.

Kalikin LM, Sims HL, Petty EM. 2000. Genomic and expression analyses of alternatively spliced transcripts of the MLL septin-like fusion gene (MSF) that map to a 17q25 region of loss in breast and ovarian tumors. Genomics 63: 165–172.

Kartmann B, Roth D. 2001. Novel roles for mammalian septins: from vesicle trafficking to oncogenesis. J Cell Sci 114(Pt 5): 839–844.

Kreuziger LM, Porcher JC, Ketterling RP, Steensma DP. 2007. An MLL-SEPT9 fusion and t(11;17)(q23;q25) associated with de novo myelodysplastic syndrome. Leuk Res 31: 1145–1148.

Krivtsov AV, Armstrong SA. 2007. MLL translocations, histone modifications and leukaemia stem-cell development. Nat Rev Cancer 7: 823–833.

Kurosu T, Tsuji K, Ohki M, Miki T, Yamamoto M, Kikihana K, Koyama T, Taniguchi S, Miura O. 2008. A variant-type MLL/SEPT9 fusion transcript in adult de novo acute monocytic leukemia (MSb) with t(11;17)(q23;q25). Int J Hematol 88: 192–196.

Martin ME, Milne TA, Bloyer S, Galoian K, Shen W, Gibbs D, Brock HW, Slany R, Hess JL. 2003. Dimerization of MLL fusion proteins immortalizes hematopoietic cells. Cancer Cell 4: 197–207.

Meyer C, Burmeister T, Groger D, Tsaur G, Fechina L, Renneville A, Sutton R, Venn NC, Emerenciano M, Pombod-Oliveira MS, et al. 2018. The MLL recombinome of acute leukemias in 2017. Leukemia 32: 273–284.

Milne TA, Briggs SD, Brock HW, Martin ME, Gibbs D, Allis CD, Hess JL. 2002. MLL targets SET domain histone methyltransferase activity to Hox gene promoters. Mol Cell 10: 1107–1117.

Ono R, Nakajima H, Ozaki K, Kumagai H, Kawashima T, Taki T, Kitamura T, Hayashi Y, Nosaka T. 2005. Dimerization of MLL fusion proteins and FLT3 activation synergize to induce multiple-lineage leukemogenesis. J Clin Invest 115: 919–929.
Osaka M, Rowley JD, Zeleznik-Le NJ. 1999. MSF (MLL septin-like fusion), a fusion partner gene of MLL, in a therapy-related acute myeloid leukemia with a t(11;17)(q23;q25). Proc Natl Acad Sci 96: 6428–6433.

Peterson EA, Stanbery L, Li C, Kocak H, Makarova O, Petty EM. 2011. SEPT9,11 and genomic instability: mechanistic insights and relevance to tumorigenesis. Genes Chromosomes Cancer 50: 940–949.

Saito H, Otsubo K, Kakimoto A, Komatsu N, Ohsaka A. 2010. Emergence of two unrelated clones in acute myeloid leukemia with MLL-SEPT9 fusion transcript. Cancer Genet Cytogenet 201: 111–115.

Santos J, Cerveira N, Correia C, Lisboa S, Pinheiro M, Torres L, Bizarro S, Vieira J, Viterbo L, Mariz JM, et al. 2010. Coexistence of alternative MLL-SEPT9 fusion transcripts in an acute myeloid leukemia with t(11;17)(q23;q25). Cancer Genet Cytogenet 197: 60–64.

Schoch C, Schnittger S, Klaus M, Kern W, Hiddemann W, Haferlach T. 2003. AML with 11q23/MLL abnormalities as defined by the WHO classification: incidence, partner chromosomes, FAB subtype, age distribution, and prognostic impact in an unselected series of 1897 cytogenetically analyzed AML cases. Blood 102: 2395–2402.

Shih LY, Liang DC, Fu JF, Wu JH, Wang PN, Lin TL, Dunn P, Kuo MC, Tang TC, Lin TH, et al. 2006. Characterization of fusion partner genes in 114 patients with de novo acute myeloid leukemia and MLL rearrangement. Leukemia 20: 218–223.

Smith MA, Rubinstein L, Ungerleider RS. 1994. Therapy-related acute myeloid leukemia following treatment with epipodophyllotoxins: estimating the risks. Med Pediatr Oncol 23: 86–98.

So CW, Cleary ML. 2003. Common mechanism for oncogenic activation of MLL by forkhead family proteins. Blood 101: 633–639.

So CW, Lin M, Ayton PM, Chen EH, Cleary ML. 2003. Dimerization contributes to oncogenic activation of MLL chimeras in acute leukemias. Cancer Cell 4: 99–110.

Strehl S, Konig M, Meyer C, Schneider B, Harbott J, Jager U, von Bergh AR, Loncarevic I, Jarosova M, Schmidt HH, et al. 2006. Molecular dissection of t(11;17) in acute myeloid leukemia reveals a variety of gene fusions with heterogeneous fusion transcripts and multiple splice variants. Genes Chromosomes Cancer 45: 1041–1049.

Taki T, Ohnishi H, Shinohara K, Sako M, Bessho F, Yanagisawa M, Hayashi Y. 1999. AF17q25, a putative septin family gene, fuses the MLL gene in acute myeloid leukemia with t(11;17)(q23;q25). Cancer Res 59: 4261–4265.

Tallman MS, Neuberg D, Bennett JM, Francois CJ, Paietta E, Wiernik PH, Dewald G, Cassileth PA, Oken MM, Rowe JM. 2000. Acute megakaryocytic leukemia: the Eastern Cooperative Oncology Group experience. Blood 96: 2405–2411.

Wechsler J, Greene M, McDevitt MA, Anastasi J, Karp JE, Le Beau MM, Crispino JD. 2002. Acquired mutations in GATA1 in the megakaryoblastic leukemia of Down syndrome. Nat Genet 32: 148–152.

Yamamoto K, Shibata F, Yamaguchi M, Miura O. 2002. Fusion of MLL and MSF in adult de novo acute myelomonocytic leukemia (M4) with t(11;17)(q23;q25). Int J Hematol 75: 503–507.

Zeisig BB, Schreiner S, Garcia-Cuellar MP, Slany RK. 2003. Transcriptional activation is a key function encoded by MLL fusion partners. Leukemia 17: 359–365.