LETTERS TO THE EDITOR

Novel splicing-factor mutations in juvenile myelomonocytic leukemia

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Myelodysplastic syndromes (MDS) and myelodysplastic/myeloproliferative neoplasms (MDS/MPN) are heterogeneous groups of chronic myeloid neoplasms characterized by clonal hematopoiesis, varying degrees of cytopenia or myeloproliferative features with evidence of myelodysplasia and a propensity to acute myeloid leukemia (AML).1 In recent years, a number of novel gene mutations, involving TET2, ASXL1, DNMT3A, EZH2, IDH1/2, and c-CBL, have been identified in adult cases of chronic myeloid neoplasms, which have contributed to our understanding of disease pathogenesis.2–7 However, these mutations are rare in neoplasms, which have contributed to our understanding of c-CBL pathogenesis of myelodysplasia.10 To investigate the role of the splicing-pathway mutations in the pathogenesis of pediatric myeloid malignancies, we have examined 165 pediatric cases, with the exception of germline or somatic TET2 mutations, involving four major splicing factors, U2AF35, ZRSR2, SRSF2, and SF3B1, commonly mutated in adult cases.

Bone marrow or peripheral blood tumor specimens were obtained from 165 pediatric patients with various myeloid malignancies, including de novo AML (n = 93), MDS (n = 28), CML (n = 17) and JMML (n = 27), and the genomic DNA (gDNA) was subjected to mutation analysis (Supplementary Table 1). The status of the RAS pathway mutations for the current JMML series has been reported previously (Supplementary Table 2).11,12 Nineteen leukemia cell lines derived from AML (YNH-1, ML-1, KASUMI-3, KG-1, HL60, inv-3, SN-1, NB4 and HEL), acute monocytic leukemia (THP-1, SCC-3, J-111, CTS, P31/FUJ, MOLM-13, IMS/MI and KOCL-48) and acute megakaryoblastic leukemia (CMS and CMY) were also analyzed for mutations. Peripheral blood gDNA from 60 healthy adult volunteers was used as controls. Informed consent was obtained from the patients and/or their parents and from the healthy volunteers. We previously showed that for U2AF35, SRSF2 and SF3B1, most of the mutations in adult cases were observed in exons 2 and 7, exon 1, and exons 14 and 15, respectively.10 Therefore, we confirmed mutation screening to these ‘hot-spot’ exons. In contrast, all the coding exons were examined for ZRSR2, because no mutational hot spots have been detected. Briefly, the relevant exons were amplified using PCR and mutations were examined by Sanger sequencing, as previously described.10 The Fisher’s exact test was used to evaluate the statistical significance of frequencies of mutations for U2AF35, SF3B1, ZRSR2 or SRSF2 in adult cases and pediatric cases. This study was approved by the Ethics Committee of the University of Tokyo (Approval number 948-7).

Figure 1. Novel U2AF35 and SRSF2 mutations detected in JMML cases. (a) Left panel: sequence chromatogram of a heterozygous mutation at R156 in N-terminal zinc-finger motifs of U2AF35 detected in a JMML case (JMML 4) is shown. Mutated nucleotides are indicated by arrows. Right panel: illustration of functional domains and mutations of U2AF35. Red arrow heads indicate hot-spot mutations at S34 and Q157 detected in the adult cases.10 Blue arrow head indicates the missense mutation at R156. (b) Left panel: sequence chromatogram of a 6-bp in-frame deletion (c.518-523delAAGTCC) in SRSF2 detected in JMML 17 is shown. Mutated nucleotides are indicated by arrows. Right panel: illustration of functional domains and mutations of SRSF2. Red arrow head indicates hot-spot mutation at P95 frequently detected in the adult cases.10 Blue arrow head indicates a 6-bp in-frame deletion leading to deletion of S170 and K171.

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No mutations were identified in the 28 cases with pediatric MDS, which included 13 cases with refractory anemia with excess blasts, 5 with refractory cytopenia of childhood, 2 with Down syndrome-related MDS, 2 with Fanconi anemia-related MDS, 2 with secondary MDS and 4 with unclassified MDS. Similarly, no mutations were detected in 93 cases with de novo AML or in 17 with CML, as well as 19 leukemia-derived cell lines. Our previous study in adult patients showed the frequency of mutations in U2AF35, SF3B1, ZRSR2 or SRSF2 to be 60/155 cases with MDS without increased ring sideroblasts and 8/151 de novo AML patients, emphasizing the rarity of these mutations in pediatric MDS (P < 0.05) and AML (P < 0.02) compared with adult cases. We found mutations in two JMML cases, JMML 4 and JMML 17. JMML 4 carried a heterozygous U2AF35 mutation (R156M), whereas JMML 17 had a 6-bp in-frame deletion (c.518-523delAAGTCC) in SRSF2 that resulted in deletion of amino acids S170 and K171 (Figure 1). Both nucleotide changes found in U2AF35 and SRSF2 were neither identified in the 60 healthy volunteers nor registered in the dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/) or in the 1000 other SR proteins, again suggesting that the P95 mutation may have some role in the pathogenesis of JMML, although further evaluations are required.

CONFLICT OF INTEREST
The authors declare no conflict interest.

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Sequencing histone-modifying enzymes identifies UTX mutations in acute lymphoblastic leukemia

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Mutations affecting epigenetic regulators have long been known to have a crucial role in cancer and, in particular, hematological malignancies. One of the earliest epigenetic factors described altered in leukemia was the mixed lineage leukemia (MLL) protein, which has been found to be mutated in 10% of adult acute myeloid leukemia (AML), 30% of secondary AML and >75% of infants with both AML and acute lymphocytic leukemia (ALL). MLL is a SET domain-containing protein, which is recruited to many promoters and mediates histone 3 lysine 4 (H3K4) methyltransferase activity, thought to promote gene expression.

In addition to MLL fusions, recently, somatic mutations of UTX (also known as KDM6A), encoding an H3K27 demethylase, have been described in multiple hematological malignancies, including multiple myeloma and many types of leukemia cell lines. H3K27 methylation is generally thought to cause gene repression. Complimentary to UTX, mutations of EZH2, a H3K27 methyltransferase, have been reported in both lymphoid and myeloid tumors (Figure 1). These mutations lead to altered EZH2 activity and influence H3K27 in tumor cells. Mutations in EZH2, EED and SUZ12, which all cooperate in Polycomb repressive complex 2, have been recently detected in early T-cell precursor AML. Similarly, point mutations affecting the functional jumonji C (jmc) domain of UTX inactivates its H3K27 demethylase activity. In addition, UTX associates with MLL2 in a multiprotein complex, which promotes H3K4 methylation, and recently MLL2 has also been found mutated in cancer, further pointing to a common and complex epigenetic deregulation in cancer. In line with the growing evidence for epigenetic regulators as important in tumorigenesis, additional mutations affecting epigenetic regulators such as SETD2, a H3K36 methyltransferase, KDM3B, a H3K9 demethylase, and KDM5C, a H3K4 demethylase, have been reported and are associated with distinct gene expression patterns.

Though the clinical significance of these findings remains to be explored, it is evident that epigenetic deregulation is having an important role in both lymphoid and myeloid leukemogenesis. Furthermore, with novel drugs at hand, such as histone deacetylase inhibitors or demethylating agents that can target and reverse epigenetic alterations, understanding the underlying molecular aberrations is of growing interest. We therefore undertook an effort to examine the prevalence of somatic mutations in genes encoding histone-modifying proteins, in particular, KDM3B, KDM5C, UTX, MLL2, EZH2 and SETD2, which previously were reported mutated in cancer.

For an initial screen, we analyzed banked diagnostic primary leukemia samples from 44 childhood B-cell ALL and 50 adult AML patients, and, where available, used bone marrow samples obtained in complete remission to validate the somatic nature of the mutations. Samples had been collected with patient/parental informed consent from patients enrolled on Dana–Farber Cancer Institute protocols for childhood ALL (DFCI 00-001 (NCT00165178), DFCI 05-001 (NCT00400946)) or AML treatment protocols of the German-Austrian AML Study Group (AML5G) for younger adults (AML5G-HD98A (NCT00146120), AMLSG 07-04 (NCT00151242)), and the study was approved by the IRB of the participating centers.

Using conventional Sanger sequencing of primary leukemia sample-derived genomic DNA, we first screened all coding exons in which mutations have been reported previously. Initially, we analyzed a total of 36 of 174 exons (KDM3B (2/24), KDM5C (9/26), UTX (7/29), MLL2 (8/54), EZH2 (1/20) and SETD2 (9/21)) and found 7 non-synonymous tumor-specific aberrations. In AML, we found one EZH2 mutation (p.G648E) in a t(8;21)-positive, and two MLL2 missense mutations (p.R5153Q and p.Y5216S; Table 1) and one