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Spliceosomal gene aberrations are rare, coexist with oncogenic mutations, and are unlikely to exert a driver effect in childhood MDS and JMML

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Somatic mutations of the spliceosomal machinery occur frequently in adult patients with myelodysplastic syndrome (MDS). We resequenced *SF3B1*, *U2AF35*, and *SRSF2* in 371 children with MDS or juvenile myelomonocytic leukemia. We found missense mutations in 2 juvenile myelomonocytic leukemia cases and in 1 child with systemic mastocytosis with MDS. In 1 juvenile myelomonocytic leukemia patient, the *SRSF2* mutation that initially coexisted with an oncogenic *NRAS* mutation was absent at relapse, whereas the *NRAS* mutation persisted and a second, concomitant *NRAS* mutation later emerged. The patient with systemic mastocytosis and MDS carried both mutated *U2AF35* and *KIT* in a single clone as confirmed by clonal sequencing. In the adult MDS patients sequenced for control purposes, we detected previously reported mutations in 7/30 and a novel *SRSF2* deletion (c.284_307del) in 3 of 30 patients. These findings implicate that spliceosome mutations are rare in pediatric MDS and juvenile myelomonocytic leukemia and are unlikely to operate as driver mutations. (*Blood*. 2012;119(11):e96-e99)

Introduction

Recent whole exome sequencing studies identified recurrent somatic mutations in spliceosome complex genes in a significant proportion of adult patients with myelodysplastic syndrome (MDS).[1-3] The biologic significance of such mutations remains to be fully elucidated; some mutations (eg, those involving *SF3B1*) may correlate with a better overall survival.[2] The spectrum of spliceosome genes commonly affected includes *SF3B1* (mutated in up to 83% of refractory anemia with ringed sideroblasts), *U2AF35* (affected in up to 18% of refractory cytopenia with multilineage dysplasia), and *SRSF2* (abnormal in up to 31% of chronic myelomonocytic leukemia [CMML] cases).[1] The lesions occur as somatic heterozygous missense mutations. They cluster to 2 hotspots in *U2AF35*, targeting protein residues S34 or Q157, or to 1 hotspot in *SRSF2* (targeting P95) or *SF3B1* (targeting K700) each. The genes code for components of the splicing machinery responsible for processing of pre-mRNA to mature RNA.[1,4] We hypothesized that the disruption of the spliceosome complex might play a central role in the pathogenesis of childhood MDS and juvenile myelomonocytic leukemia (JMML). We used targeted resequencing to investigate the 4 hotspots of 3 spliceosome genes in 371 pediatric and 30 adult cases.

Methods

Patients

Pediatric patients were enrolled in study 98 or 2006 (www.clinicaltrials.gov; NCT00047268 and NCT00662090) of the European Working Group of MDS in Childhood. Adult MDS samples were enrolled in multicenter treatment trials that investigated the use of all-trans retinoic acid,[5] antithymocyte globulin (NCT00004208),[6,7] deferasirox,[8] lenalidomide, or thalidomide for treatment of MDS. Informed consent to use biologic material had been obtained from patients or patients' guardians after approval by the institutional review board of each participating institution in accordance with the Declaration of Helsinki.

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Table 1. Study cohorts

Diagnosis, subtype at diagnosis	No.	Median age, y (range)	Female/ male	*SRSF2* P95H/L/R/del	*SF3B1* K700E	*U2AF35* (S34Y/Q157P)
Pediatric cohort, classified according to references 10-12						
Primary MDS						
RCC	123	10.5 (1.5-17.9)	54/69	0	0	1/0
RAEB/RAEB-T	56	10.4 (1.0-17.6)	24/32	0	0	0/0
MDR-AML	8	12.8 (5.8-22.4)	5/3	0	0	0/0
Secondary MDS	68	13.1 (0.6-25.4)	25/43	0	0	0/0
JMML	116	1.4 (0.1-7.0)	40/76	2	0	0/0
Adult cohort, classified according to reference 13						
CMML	5	76 (57-84)	3/2	1	0	0/0
RA	12	59 (42-73)	3/9	2	0	0/1
RCMD	5	68 (42-78)	2/3	1	1	0/0
RAEB-1/2	8	73 (68-83)	3/5	3	0	0/1

RCC indicates refractory cytopenia of childhood; RAEB, refractory anemia with excess blasts; RAEB-T, RAEB in transformation; MDR-AML, myelodysplasia-related acute myeloid leukemia; RA, refractory anemia; RCMD, refractory cytopenia with multilineage dysplasia; RAEB-1, refractory anemia with excess blasts-1; RAEB-2, refractory anemia with excess blasts-2; P95H/L/R/del, mutation of amino acid residue proline 95 to histidine/leucine/arginine/deletion; K700E, lysine 700 to glutamic acid; S34Y, serine 34 to tyrosine; and Q157P, glutamine 157 to proline.

Direct sequencing

Targeted resequencing was performed using genomic DNA as detailed in supplemental Table 1 (see the Supplemental Materials link at the top of the article). Mutations were confirmed in at least 2 independent runs.

Allele-specific clonal sequencing

TA subcloning and sequencing of bacterial colonies were performed as previously described.[9] On average, 26 colonies were sequenced per single ligated amplicon of *U2AF35* or *KIT*.

Denaturing high-performance liquid chromatography

Longitudinal denaturing high-performance liquid chromatography analysis of *NRAS* exon 1 and exon 2 was performed using primers listed in supplemental Table 1.

Results and discussion

We analyzed 4 spliceosome gene hotspots in 401 patients with MDS and JMML; the subtypes are listed in Table 1. All 371 children were enrolled in European Working Group of MDS in Childhood studies and 30 adult MDS patients participating in various clinical trials[5-8] were included. Because mutations in spliceosome genes occur somatically in leukemic cells and are not found in healthy controls (dbSNP and the 1000 Genomes databases), an additional control cohort was not included in this study. As expected, results in the adult MDS population were comparable with those reported previously[1]: 10 of 30 samples had a spliceosome mutation. The *SRSF2* gene was affected most frequently. Of 7 patients with *SRSF2* mutation, 4 carried the known mutations c.284C > T/A/G(p.P95L/H/R) and in 3 patients a novel deletion *SRSF2* c.284_307del(p.P95_R102del) was detected. The *U2AF35* and *SF3B1* mutations were discovered in 2 patients and 1 patient, respectively (Table 1).

By contrast, in the cohort of 371 children, spliceosome mutations were found in only 3 cases (Figure 1A; supplemental Figure 1). A heterozygous *SRSF2*(p.P95L) mutation, being the most prevalent mutation in adult CMML,[1] was identified in 2 JMML cases (patients SC092 and D361). Analysis of DNA from buccal epithelial cells confirmed the somatic nature of this mutation in SC092 (supplemental Figure 1). Hematopoietic cells of both children had a normal karyotype and concomitant mutations of the RAS pathway: *PTPN11*(p.E76Q) in SC092 and *NRAS*(p.G13D) in D361 (Figure 1A). Patient SC092 was successfully transplanted and is currently disease-free. Patient D361 carried a *SRSF2* and a singular *NRAS* mutation at diagnosis (Figure 1B; supplemental Figure 1D). He relapsed after hematopoietic stem cell transplantation (HSCT). At relapse, the *SRSF2*(p.P95L) mutation was no longer detectable, whereas the initial *NRAS*(p.G13D) mutation persisted. After a second HSCT, which failed to induce remission, splenectomy was performed because of acceleration of the disease. In spleen cells, the *SRSF2* mutation remained absent, but strikingly, a new *NRAS*(p.Q61K) mutation had appeared in addition to the preexisting *NRAS*(p.G13D) mutation. The hypothetical model of clonal evolution for this patient is shown in Figure 1B. At diagnosis, 2 distinct clones are postulated to exist: an *NRAS*(p.G13D)–bearing primary clone, which was outcompeted by a faster-growing but less refractory offspring clone carrying a *SRSF2*(p.P95L) mutation as an additional lesion. Although HSCT eradicated this secondary clone, the primary clone persisted and gave rise to disease recurrence. Under selective pressure exerted through second HSCT, a novel secondary clone with *NRAS* p.G13D/Q61K double mutation emerged.

The third patient with a spliceosome mutation (D717) was diagnosed at the age of 17 years with systemic mastocytosis with a *KIT*(p.D816V) mutation, normal cytogenetics, and associated clonal hematologic nonmast cell lineage disease, which resembled MDS, subtype refractory cytopenia (Figure 1C). Although the mastocytosis component of the disease responded to interferon-alpha2b, HSCT was performed for persistent thrombocytopenia and anemia. Two years after HSCT, the patient relapsed with 35% autologous cells. Allele-specific clonal sequencing of affected *U2AF35* and *KIT* exons revealed the presence of both mutations in the sample at diagnosis and at time of disease recurrence. Total allelic burdens of the 2 mutations were similar both at diagnosis and relapse (Figure 1C), indicating that the *KIT*(p.D816V) and the *U2AF35*(p.S34Y) mutations were likely present in the same clone. As previously postulated, it is probable that the systemic mastocytosis and associated clonal hematologic nonmast cell lineage disease compartments in this patient originated from a common neoplastic precursor.[14,15]

The drastically reduced frequency of spliceosome mutations in pediatric compared with adult MDS suggests a different pathogenetic mechanism in childhood disorders; the pathogenesis in children is probably more related to genetic determinants acquired prenatally and less to accumulation of somatic events during life. This view fits well with previous reports that somatic mutations of genes, such as *DNMT3A*, *TET2*, *IDH*, and *ASXL1*, are also much less prevalent in pediatric MDS.[16-19]

A

Patient	Diagnosis	Spliceosome mutation	Concomitant mutations	Age (Sex)	Karyo type	HSCT (Relapse)	Survival status
SC092	JMML	*SRSF2* c.284C>T(p.P95L)	*PTPN11* c.226G>C(p.E76Q)	4.5 years (female)	46,XX	MRD (No)	Alive 4.2 years
D361	JMML	*SRSF2* c.284C>T(p.P95L)	*NRAS* c.38G>A(p.G13D)	3.9 years (male)	46,XY	MRD (Yes)	Dead
D717	SM-AHNMD	*U2AF35* c.101C>A(p.S34Y)	*KIT* c.2447A>T(p.D816V)	17.5 years (male)	46,XY	MUD (MC)	Alive 3.5 years

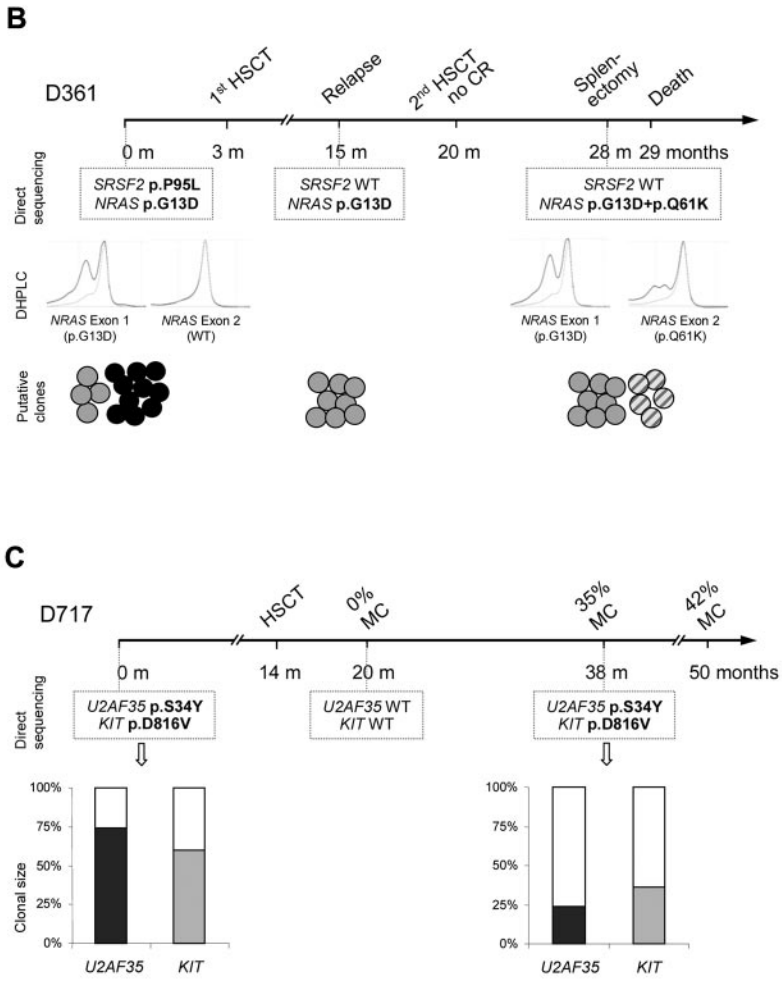

Figure 1. Spliceosome mutations and coexisting oncogenic mutations in children with MDS and JMML. (A) Mutations identified in 3 children. (B) Clinical timeline of patient D361. After second HSCT, the patient did not reach complete remission. Direct sequencing identified an *SRSF2* mutation that was present only at diagnosis but was undetectable at later time points. A mutation in *NRAS* exon 1 (p.G13D) was detectable at all time points. In contrast, the mutation in *NRAS* exon 2 (p.Q61K) was present only at time of disease progression after second HSCT. This was confirmed by denaturing high-performance liquid chromatography. Bold lines represent patients' samples; and dashed lines, a wild-type control DNA. The picture at the bottom illustrates the hypothetical clonal evolution; black circles represent a clone carrying both the *NRAS*(p.G13D) and the *SRSF2* mutation; gray circles, a primary clone harboring only the *NRAS*(p.G13D) mutation; and striped circles, a secondary clone with double *NRAS* mutation (p.G13D and p.Q61K). (C) Clinical timeline of patient D717. Mixed chimerism with 35% autologous cells occurred at 24 months after HSCT. Results from direct sequencing are presented within dashed boxes. The results from allele-specific clonal sequencing are shown at the bottom; the filled bars represent the proportion of mutant *U2AF35* and *KIT* alleles. Twenty-three *U2AF35* and 25 *KIT* subcloned alleles were sequenced in the diagnostic sample, and 25 *U2AF35* and 31 *KIT* subcloned alleles were sequenced in the post-HSCT sample. SM-AHNMD indicates systemic mastocytosis associated with a clonal hematologic nonmast cell lineage disease; RCC, refractory cytopenia of childhood; CR, complete remission; DHPLC, denaturing high-performance liquid chromatography; MRD, matched related donor; MUD, matched unrelated donor; MC, mixed chimerism; and WT, wild-type.

In conclusion, the data reported here suggest that alterations of the spliceosome complex do not constitute a fundamental etiologic factor in pediatric MDS and JMML. First, they are exceedingly rare in a group of diseases that is infrequent in itself. Second, in the positive cases identified here, spliceosome mutations did not occur independently of oncogenic mutations and in 1 case even disappeared during disease evolution. It is reasonable to assume that in children these mutations coexist as passengers in an oncogenic environment alongside primary mutations of signal transduction pathways, known to act as drivers in the malignant process.

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Authorship

Contribution: S.H. performed research and analyzed data; C.F. interpreted data and wrote the manuscript; J.M. performed research; M.H., H.H., B.G., T.K., and F.T. recruited patients; B. Schlegelberger reviewed cytogenetics; I.B. reviewed pathology; B. Strahm, J.S., F.L., M.Z., E.B., M.D., M.M.v.d.H.-E., and B.D.M. recruited patients; S.O. designed research; C.M.N. recruited patients and designed research; and M.W.W. designed and performed research and wrote the manuscript.

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