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A common alternative splicing signature is associated with SF3B1 mutations in malignancies from different cell lineages

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blocks hERG1B-sustained currents, with no adverse cardiac effect (Gasparoli L, unpublished results). This, or similar drugs, could hence be proposed for a patient's tailored therapeutic approach especially in nonresponsive pediatric T-ALL, such as ETP-ALL, with a high *hERG1B* expression.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

SP, BA and VS performed the research and biological assays; PR performed and MGV supervised the statistical analysis and reviewed the manuscript; AA and SP designed the research and wrote the manuscript; GB contributed primary samples and edited the manuscript.

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REFERENCES

1 Redaelli A, Laskin BL, Stephens JM, Botteman MF, Pashos CL. A systematic literature review of the clinical and epidemiological burden of acute lymphoblastic leukaemia (ALL). *Eur J Cancer Care* 2005; **14**: 53–62.
2 Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. *N Engl J Med* 2006; **354**: 166–178.
3 Goldberg JM, Silverman LB, Levy DE, Dalton VK, Gelber RD, Lehmann L *et al.* Childhood T-cell acute lymphoblastic leukemia: the Dana-Farber Cancer Institute Acute Lymphoblastic Leukemia Consortium experience. *J Clin Oncol* 2003; **21**: 3616–3622.
4 Sanguinetti MC, Tristani-Firouzi M. hERG potassium channels and cardiac arrhythmia. *Nature* 2006; **440**: 463–469.
5 Pillozzi S, Brizzi MF, Bernabei PA, Bartolozzi B, Caporale R, Basile V *et al.* VEGFR-1 (FLT-1), β_1 integrin and hERG K^+ channel form a macromolecular signaling complex in acute myeloid leukemia: role in cell migration and clinical outcome. *Blood* 2007; **110**: 1238–1250.
6 Arcangeli A, Pillozzi S, Becchetti A. Targeting ion channels in leukemias: a new challenge for treatment. *Curr Med Chem* 2012; **19**: 683–696.
7 Pillozzi S, Masselli M, DeLorenzo E, Accordi B, Cilia E, Crociani O *et al.* Chemotherapy resistance in acute lymphoblastic leukemia requires hERG1 channels and is overcome by hERG1 blockers. *Blood* 2011; **117**: 902–914.
8 London B, Trudeau MC, Newton KP, Beyer AK, Copeland NG, Gilbert DJ *et al.* Two isoforms of the mouse ether-a-go-go-related gene coassemble to form channels with properties similar to the rapidly activating component of the cardiac delayed rectifier K+ current. *Circ Res* 1997; **81**: 870–878.
9 Jones EM, Roti Roti EC, Wang J, Delfosse SA, Robertson GA. Cardiac IKr channels minimally comprise hERG 1a and 1b subunits. *J Biol Chem* 2004; **279**: 44690–44694.
10 Crociani O, Guasti L, Balzi M, Becchetti A, Wanke E, Olivotto M *et al.* Cell cycle-dependent expression of HERG1 and HERG1B isoforms in tumor cells. *J Biol Chem* 2003; **278**: 2947–2955.
11 Conter V, Bartram CR, Valsecchi MG, Schrauder A, Panzer-Grümayer R, Möricke A *et al.* Molecular response to treatment redefines all prognostic factors in children and adolescents with B-cell precursor acute lymphoblastic leukemia: results in 3184 patients of the AIEOP-BFM ALL 2000 study. *Blood* 2010; **115**: 3206–3214.
12 Coustan-Smith E, Mullighan CG, Onciu M, Behm FG, Raimondi SC, Pei D *et al.* Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. *Lancet Oncol* 2009; **10**: 147–156.
13 Lastraioli E, Bencini L, Bianchini E, Romoli MR, Crociani O, Giommoni E *et al.* hERG1 channels and Glut-1 as independent prognostic indicators of worse outcome in Stage I and II colorectal cancer: a pilot study. *Transl Oncol* 2012; **5**: 105–112.
14 Luo X, Xiao J, Lin H, Lu Y, Yang B, Wang Z. Genomic structure, transcriptional control, and tissue distribution of HERG1 and KCNQ1 genes. *Am J Physiol Heart Circ Physiol* 2008; **294**: 1371–1380.
15 Tosello V, Ferrando AA. The NOTCH signaling pathway: role in the pathogenesis of T-cell acute lymphoblastic leukemia and implication for therapy. *Ther Adv Hematol* 2013; **4**: 199–210.
16 Chiaretti S, Vitale A, Cazzaniga G, Orlando SM, Silvestri D, Fazi P *et al.* Clinico-biologic features of 5202 acute lymphoblastic leukemia patients enrolled in the Italian AIEOP and GIMEMA Protocols and stratified in age-cohorts. *Haematologica* 2013; **98**: 1702–1710.

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A common alternative splicing signature is associated with *SF3B1* mutations in malignancies from different cell lineages

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The RNA maturation is an important and complex biological process. It requires several small nuclear ribonucleoproteins (snRNPs) that comprise the two forms of spliceosomes. The major form of spliceosome (U2-type) is composed of U1, U2, U4/6 and U5 snRNPs, and catalyzes most splicing events in metazoans.[1]

Mutations of genes, such as *SF3B1*, *SRSF2*, *U2AF1*, *ZRSR2*, and to a lesser extent *SF1*, *SF3A1*, *U2AF2* or *PRPF40B*, encoding spliceosome compounds have been found to occur at high frequencies in myelodysplastic syndromes (MDS) and chronic lymphocytic leukemia (CLL).[2–4] Subsequently, *SF3B1* mutations were also found in solid tumors such as endometrial, lung, bladder, pancreatic and breast carcinomas and cutaneous melanomas.[5] We and others also reported that 15–20% of uveal melanoma (UM) carry

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SF3B1 mutations.[6–8] SF3B1 (splicing factor 3 B subunit 1, also named SF3B155) is a central compound the U2 snRNP complex in direct contact with the branch site of the pre-mRNA. SF3B1 contains 22 HEAT repeats (Huntingtin, Elongation factor 3, protein phosphatase 2A, Targets of rapamycin 1). Major hotspots of missense mutations of SF3B1 target one of the HEAT repeats at positions R625, K666 and K700, with variability according to diseases, R625 and K700 mutations being the most prevalent in UM and hematologic disorders, respectively. Prognosis value of these mutations vary according to diseases, *SF3B1* mutations are associated with poor outcomes in CLL and favorable outcomes in UM.[9] *SF3B1* mutations are especially associated with the refractory anemia with excess of ring sideroblasts (RARS), a mild form of MDS with little impact on patient's survival. Consequences of these mutations on splicing are less clear. Aberrant splicing associated with *SF3B1* was reported by two landmark publications.[2,3] However, little overlap was found between MDS and CLL. Furthermore, Harbour *et al.*[6] found no splicing effect of *SF3B1* mutations in a series of UM patients analyzed by RNA-seq. By combining exon array and RNA-seq analyzes, we recently reported alternative transcripts of eight genes statistically associated with *SF3B1 R625* mutations and linked to various RNA processing mechanisms: alternative terminal exons (*UQCC*, *ADAM12*, *GAS8*), alternative 3′ acceptor splice sites (*CRNDE*, *ANKHD1*), alternative cassette exons (*GUSBP1*), alternative first exon (*F8*) and intron retention (*ABCC5*).[7]

Five of these differential splicing events (*CRNDE*, *GUSBP11*, *UQCC*, *ABCC5* and *ANKHD1*) were most consistently associated with the *SF3B1*-mutated status. Here we explored whether this splicing signature was also associated with *SF3B1* mutations in malignancies other than UM, namely in MDS and CLL.

Series of CLL (26 cases), MDS of various subtypes (48 cases) and UM (87 cases) were assembled. These samples were genotyped for the recurrent mutated genes of the spliceosome, and biological and clinical data were obtained (see Supplementary Table for sample characteristics). After RNA extraction and reverse transcription, samples were analyzed for the two splice forms of *UQCC*, *CRNDE*, *ANKHD1*, *GUSBP1* and *ABCC5* by reverse transcription quantitative PCR (RT-QPCR) as described previously.[7] *CRNDE* was poorly expressed in CLL, below the detection sensitivity of the RT-QPCR in most cases. Unsupervised hierarchical clustering was applied on the splicing ratio of the five expressed genes obtained for the series of MDS and CLL data, as well as with that of the series of UM previously reported.[7] Whereas no disease aggregation was observed in the clustering, all *SF3B1*-mutated samples clustered in a single branch (Figure 1 and Supplementary Table for complete sample status). More specifically, this group of samples included 8 CLL *SF3B1*-mutated samples (K700E (6), K666T (1) and G742D (1)), 24 MDS *SF3B1*-mutated sample cases (K700E (14), H622Q (4), D781G (2), R625L/H/D/C (4)) and 19 *SF3B1*-mutated UM samples (R625H/C/L/P (16), K666M/T (2), K700E (1)). As expected, most *SF3B1*-mutated MDS were classified as RARS or refractory cytopenia with multilineage dysplasia and ring sideroblasts (RCMD-RS) (16 and 4, respectively out of 23 cases). No *SF3B1*-mutated samples were misclassified, except one MDS sample, MDS44 and one UM case, UM48, with low but detectable frequencies of mutated SF3B1 allele, which segregated with wild-type cases. Five cases without detected splicesome mutations (one MDS, two UM and two CLL) clustered in this branch corresponding to the mutated samples. The three *SRSF2*-mutated MDS cases, bearing the P95H hotspot mutation, segregated in the second branch with the wild-type samples, suggesting that this mutant-splicing factor differently affects the splicing process than the *SF3B1* mutants. Two MDS samples bearing ZRSR2 missense mutations of unknown functional consequences (K413E and V304M) segregated each in a different branch.

Several conclusions could be drawn from these results. First, it is the first time that consistent splicing abnormalities are associated with *SF3B1* mutations. The fact that the same splicing pattern is observed in tumor cells from different lineages and embryonic origins, strongly argues for a direct consequence of *SF3B1* mutation. Second, the same pattern is observed irrespective of the mutated HEAT repeats of SF3B1. The reason for different hotspot mutations in different malignancies is largely unexplained but no different consequence was detected in the limited splicing events explored here. Third, the other mutated spliceosome compounds present in our series, including SRSF2 and ZRSR2, did not share the splicing abnormalities of the *SF3B1* mutants. Finally, while no false negative was present in the clustering, the splicing pattern of 5 out of 161 samples from the three series was undistinguishable from that of SF3B1 samples, suggesting either experimental limitation of our splicing analysis or undetected abnormalities of the splicing machinery.

Our data clearly associate specific splicing abnormalities to *SF3B1* mutations but these abnormalities are most probably not limited to those analyzed here. To statistically demonstrate splicing variants among more than 200 000 different splicing

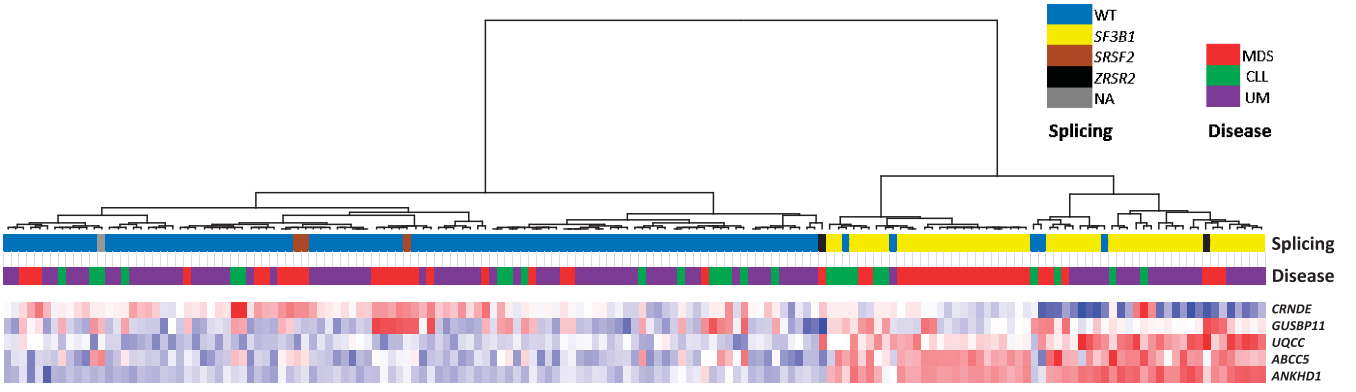

Figure 1. Hierarchical clustering of tumor samples from different pathologies according to the splicing signature. An unsupervised hierarchical clustering and a heatmap are performed using the difference in Ct (threshold cycle) between the two splicing forms expression for the five genes, measured by RT-QPCR for a series of 161 tumor samples representing 3 pathologies: uveal melanoma (UM), myelodysplastic syndrome (MDS) and chronic lymphocytic leukemia (CLL). Data were normalized on mean and standard deviation within each pathology and research center. Clustering was performed based on Euclidian distance by Ward's method. Splicing status is indicated by color code, representing: WT, wild-type status of the tested spliceosome compounds; SF3B1, SRSF2 and ZRSR2, mutated status of these genes, respectively. NA, not available.

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events remains challenging. It requires reliable molecular tools such as the latest generation of exon array or RNA-seq, efficient bioinformatics and large series of cases. It is thus likely that only the most consistent events were picked by the analysis of a limited number of mutated cases. Splicing forms yet to be discovered may better explain the oncogenic pathways activated by mutant SF3B1. Finally, our results do not rule out functions of SF3B1 besides splicing, which deregulations could intervene in oncogenesis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1 Wahl MC, Will CL, Lührmann R. The spliceosome: design principles of a dynamic RNP machine. *Cell* 2009; **136**: 701–718.
2 Wang L, Lawrence MS, Wan Y, Stojanov P, Sougnez C, Stevenson K *et al.* SF3B1 and other novel cancer genes in chronic lymphocytic leukemia. *N Engl J Med* 2011; **365**: 2497–2506.
3 Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, Yamamoto R *et al.* Frequent pathway mutations of splicing machinery in myelodysplasia. *Nature* 2011; **478**: 64–69.
4 Quesada V, Conde L, Villamor N, Ordóñez GR, Jares P, Bassaganyas L *et al.* Exome sequencing identifies recurrent mutations of the splicing factor SF3B1 gene in chronic lymphocytic leukemia. *Nat Genet* 2011; **44**: 47–52.
5 Scott LM, Rebel VI. Acquired mutations that affect pre-mRNA splicing in hematologic malignancies and solid tumors. *J Natl Cancer Inst* 2013; **105**: 1540–1549.
6 Harbour JW, Roberson EDO, Anbunathan H, Onken MD, Worley LA, Bowcock AM. Recurrent mutations at codon 625 of the splicing factor SF3B1 in uveal melanoma. *Nat Genet* 2013; **45**: 133–135.
7 Furney SJ, Pedersen M, Gentien D, Dumont AG, Rapinat A, Desjardins L *et al.* SF3B1 mutations are associated with alternative splicing in uveal melanoma. *Cancer Discov* 2013; **3**: 1122–1129.
8 Martin M, Maßhöfer L, Temming P, Rahmann S, Metz C, Bornfeld N *et al.* Exome sequencing identifies recurrent somatic mutations in EIF1AX and SF3B1 in uveal melanoma with disomy 3. *Nat Genet* 2013; **45**: 933–936.
9 Rossi D, Spina V, Bomben R, Rasi S, Dal-Bo M, Bruscaggin A *et al.* Association between molecular lesions and specific B-cell receptor subsets in chronic lymphocytic leukemia. *Blood* 2013; **121**: 4902–4905.

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Looking for *CALR* mutations in familial myeloproliferative neoplasms

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Diseases commonly referred to as chronic myeloproliferative neoplasms (MPNs) comprise essential thrombocythemia (ET), polycythemia vera (PV), primary myelofibrosis (PMF) and chronic myeloid leukemia (CML), and are acquired, clonal disorders arising at the stem-cell level characterized by overproduction of terminally differentiated myeloid cells and a variable propensity to transform into acute leukemia.[1] Although the underlying genetic defect in CML has been known for different decades, the genetic complexity of Philadelphia-negative MPNs has only more recently been unraveled. The somatic gain-of-function mutation *JAK2*V617F has been described in the vast majority of patients with PV and in 50–60% of those with ET and PMF.[2] Subsequently, several additional mutations have been reported in a fraction of MPN patients (that is, mutations in *JAK2* exon 12, *MPL*, *CBL*, *TET2*, *DNMT3A*, *IDH1/2*, *EZH2* and *ASXL1*, among others).[3–7] Very recently, two seminal publications reported on new mutations in the *CALR* gene in a substantial proportion of sporadic ET and PMF patients lacking the *JAK2*V617F mutation.[8,9]

Even though the majority of MPN cases described appears to be sporadic, the existence of familial MPNs (that is, pedigrees in which at least two members have an MPN) is well known.[10–12] The reported prevalence of familial cases within a population of MPN patients is ~7.6–11.0% and might even be underestimated.[11,13] A large Swedish population-based study has identified an approximately five to sevenfold increased relative risk of developing an MPN in first-degree relatives of affected patients.[10] Familial MPN patients may harbor somatically acquired *JAK2*, *MPL* and *TET2* mutations, and different MPN phenotypes and genotypes may coexist within a pedigree without a specific segregation pattern.[13,14] Patients seem to inherit a hitherto scarcely characterized 'predisposition' to develop MPN-related mutations. Patterns of inheritance are heterogeneous and disease anticipation between generations has been described.

The aim of the present study was to investigate *CALR* mutations in familial MPNs, as no information is available to date on the occurrence of this mutation in this context. The Institutional Review Board of Varese approved the study and procedures followed were in accordance with the Declaration of Helsinki. All patients provided written informed consent prior to acquisition

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