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## LETTER TO THE EDITOR

# Putative RNA-splicing gene *LUC7L2* on 7q34 represents a candidate gene in pathogenesis of myeloid malignancies

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Acute myeloid leukemia (AML) is a myeloid malignancy that arises spontaneously or that may evolve from myelodysplastic syndrome (MDS). AML is characterized by somatic cytogenetic and molecular mutations associated with distinct clinical outcomes. In patients with normal cytogenetics, genetic techniques have been used to discover novel mutations.[1–5] In order to identify new candidate mutations involved in AML disease progression and pathogenesis, we conducted whole exome sequencing (WES) on DNA from a patient with cytogenetically normal-acute myeloid leukemia (CN-AML) evolving from MDS.

A patient in the seventh decade of life with CN-AML evolving from MDS was identified for exome sequencing. The patient initially presented with anemia and thrombocytopenia, and a bone marrow biopsy (BMBx) showed refractory anemia with excess blasts-1 (RAEB-1). Cytopenias worsened and a second BMBx was done 4 months after diagnosis, which also revealed RAEB-1. Six months later, cytopenias worsened and myeloblasts were detected in the peripheral blood. A third BMBx showed AML with 26% blasts. The cytogenetics were normal, and the *FLT3* internal tandem duplication (ITD), tyrosine kinase domain (TKD) and characteristic *NPM1* mutations were not detected. WES was performed on DNA obtained from bone marrow mononuclear cells (BMMNCs), representative of tumor sample. In addition, buccal mucosa and serially passaged bone marrow stromal cells (BMSCs), serving as germline controls, were also sequenced. Details of WES, data analysis and primers used are presented in the Supplementary Methods section.

We identified 16 single-nucleotide variations (SNVs) in the tumor sample, which were absent in the buccal and stroma DNA, and which were also absent in the dbSNP database. Fourteen SNVs were validated by Sanger sequencing (Table 1) while two were false positives. No insertions or deletions (indels) were identified in this sample. SNVs in two genes, *IDH2* and *RUNX1*, had been previously identified in patients with AML. The stroma did not contain unique SNVs or indels when compared with the buccal tissue. We examined two prior bone marrow samples from the same patient (collected at initial diagnosis and 4 months after initial diagnosis, both showing RAEB-1) using Sanger sequencing for the presence of the 14 mutations found in the secondary AML. The initial diagnostic sample did not carry the *IDH2* and *LUC7L2* mutations (Table 1). The second sample, which was obtained 6 months prior to the diagnosis of AML and which contained 5% blasts (RAEB-1), exhibited all 14 SNVs.

Homogeneous mass extension (hME, Sequenom) genotyping was performed on a panel of patients to rapidly screen for the presence of the novel SNVs. (PCPGM, Partners Center for Personalized Genetic Medicine; details in Supplementary Methods). *IDH2* and *RUNX1* were excluded from this analysis. HMe confirmed the presence of the remaining 11 mutations in all three of the index patient's samples with the exception that the *LUC7L2* nonsense mutation was absent in the initial sample, as previously confirmed by Sanger sequencing, and indicating that this mutation was acquired coincident with disease progression. Allelic frequencies of the SNVs found in the index AML patient ranged from 45 to 51% (as assessed by Sequenom), and were

**Table 1.** Non synonymous mutations found on whole exome sequencing

| *Gene* | *Gene symbol* | *SNV (chr_location hg19_original base/variant)* | *Allelic frequency of variant SNP % (by Sequenom)* | *Diagnostic sample (03/09) (Y/N)* | *Interim sample (7/09) (Y/N)* | *AML sample (02/10) (Y/N)* | *Amino acid change* |
|---|---|---|---|---|---|---|---|
| *Zinc finger protein 672* | *ZNF672* | 1_249142539_G/A | NA | ND | Y | Y | V356M |
| *Nuclear factor I/A* | *NFIA* | 1_61553933_G/A | 49 | Y | Y | Y | R47H |
| *Potassium voltage-gated channel, shaker-related subfamily, member 4* | *KCNA4* | 11_30034137_C/T | 45 | Y | Y | Y | R30Q |
| *Isocitrate dehydrogenase 2* | *IDH2* | 15_90631934_C/T | 45[a] | N[b] | Y[b] | Y[b] | R140Q |
| *Growth hormone 1* | *GH1* | 17_61995143_C/T | 46 | Y[b] | Y | Y | E145K |
| *Glucocorticoid receptor DNA binding factor 1* | *GRLF1* | 19_47422198_G/A | 51 | Y | Y | Y | C89Y |
| *Myosin binding protein C, fast type* | *MYBPC2* | 19_50964943_C/T | 50 | Y[b] | Y | Y | R1026C |
| *Syntrophin, gamma 2* | *SNTG2* | 2_1241691_G/A | 48 | Y | Y | Y | G251R |
| *Runt-related transcription factor 1* | *RUNX1* | 21_36259214_C/T | 45[a] | Y[b] | Y[b] | Y[b] | D93N |
| *Lysozyme-like 4* | *LYZL4* | 3_42438786_C/T | 50 | Y | Y | Y | A138T |
| *Dishevelled associated activator of morphogenesis 2* | *DAAM2* | 6_39836611_G/A | 49 | Y | Y | Y | E259K |
| *Phosphoglycerate kinase 2* | *PGK2* | 6_49754074_T/C | 51 | Y[c] | Y | Y | N276S |
| *LUC7-like 2 (S. cerevisiae)* | *LUC7L2* | 7_139102312_C/T | 45 | N | Y | Y | R279X |
| *Tensin 3* | *TNS3* | 7_47440393_T/A | | Y | Y | Y | N281I |

Abbreviations: NA, not applicable; ND- not determined. Luc7L2 mutation not identified in initial sample by Sanger and Sequenom techniques. [a]These values were obtained from next-generation sequencing, data as these two SNVs were not analyzed by Sequenom. [b]Determined only by Sanger sequencing [c]Determined only by Sequenom.

**Table 2.** Recurrent mutations in genes identified in an index patient in a cohort of 99 patients with myeloid malignancies analyzed by whole exome sequencing

| *Gene* | *Diagnosis* | *Karyotype* | *SNV* | *Amino acid change* | *Additionally mutated genes*[a] |
|---|---|---|---|---|---|
| *LUC7L2* ($n=3$) | | | | | |
| | MDS | 45,XY,add(5)(q15)[8], add(10)(q26),-12[8],-17, + mar1[8][cp9]/46,XY[11] | 7_139094327_C/A | Q236K | None |
| | MDS | 46XX, del(20)(q11.2) | 7_139060825_C/T | R27X | None |
| | MDS | 46,XX,add(15)(p11.1), add(22)(p11.2)[3] 47,idem, +19 [19] | 7_139097301_C/T | R262X | None |
| *LYZL4* ($n=1$) | CMML | — | 3_42448641_G/T | G35C | *RUNX1* |
| *TNS3* ($n=1$) | MDS | — | 7_47343019_C/G | P996A | None |

[a]Additionally mutated genes refers only to the 11 other genes identified in the index patient.

similar in all three bone marrow samples irrespective of the blast percentage (Table 1). Assuming these mutations are heterozygous, this allelic frequency points to the malignant clone comprising ~90% of the sample cellularity and far exceeding the blast percentage, a finding that has been seen in other studies.[6,7]

As all the novel mutations identified in our index patient were present prior to development of AML at the preceding MDS stage, we started by looking for recurrent mutations in the above identified genes in MDS patient samples. We analyzed a set of 29 MDS patients from our tumor bank using hME (Supplementary Table S1). None of the 11 point mutations found in our index patient were found in this heterogeneous group of 29 MDS patients (IDH2 and RUNX1 not assessed and ZNF672 technically unsuccessful). In order to determine if this set of genes was mutated at other locations, we conducted targeted exon sequencing on 8 of the 11 original genes (*DAAM2*, *NF1A*, *TNS3*, *GRLF1*, *LUC7L2*, *MYBPC2*, *PGK2* and *GH1*) by Fluidigm PCR-amplified DNA and Illumina sequencing in a subset of 12 of the above 29 MDS samples, which had a normal karyotype. No novel non-synonymous SNVs were detected in these 12 patients. Using identical methodology, we then analyzed a set of 111 patient samples (106 AML, 4 CMML, 1 biphenotypic leukemia) from the MGH tumor bank initially with hME followed by targeted exon sequencing on a subset of 47 patients with CN-AML (Supplementary Table S1). No recurrent or novel mutations were identified in the genes analyzed.

Our findings provide several insights into next-generation sequencing studies of samples from patients with myeloid malignancies. The first is that relevant sequence data may be obtained from patient samples containing only low levels of myeloblasts. The second is that many somatic SNVs identified using next-generation sequencing are unique to the patient, and of questionable relevance to pathogenesis given they are not recurrently identified at a high frequency in other patient samples. That is, there are likely only several relevant driver mutations and many additional passenger mutations.

Our study also points to the potential role of *LUC7L2* as a novel candidate gene in the pathogenesis of myeloid malignancies, as this mutation was acquired coincident with phenotypic progression of the index patient's disease.

*LUC7L2* resides on chromosome of 7q34. Indeed, chromosome 7 represents one of the most frequently altered chromosomes in patients with AML and MDS, with a variety of deletions and translocations described.[8] Indeed, expression and deletion analyses have identified decreased expression of a variety of genes on the long arm of chromosome 7 (7q) including *LUC7L2* in patients with MDS and CMML.[9] Furthermore, next-generation sequencing of DNA from a patient with secondary AML revealed a R27X mutation in *LUC7L2*.[10] An extension of this study by the same group disclosed two additional mutations in *LUC7L2* in a cohort of 99 patients with myeloid malignancies (70 MDS, 7 AML, 22 CMML) (Table 2) (Methodology included in Supplementary Methods). This work was presented at the 2012 American Society of Hematology Meeting.[11] These preliminary data also suggest a worse survival for patients with MDS/AML who carry loss of expression of *LUC7L2*. Therefore, while the frequency of *LUC7L2* mutations is unclear given the ongoing nature of this study, these findings raise the possibility that mutations in this gene may be found at a low frequency in patients with MDS and AML.

*LUC7L2* appears to participate in RNA-splicing though its function is still under evaluation.[12] However, its identification as possibly recurrently mutated is compelling given recent observations that other RNA-splicing molecules are mutated in MDS.[13–15] That the *LUC7L2* mutation was acquired coincident with disease progression and that *LUC7L2* mutations have been identified in other patients suggest that further study of this gene in myeloid malignancies is warranted.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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## AUTHOR CONTRIBUTIONS

Conception and design of study, data analysis, data interpretation, reviewed and revised manuscript: HS, AL, MC, DS, BP, JM and EA. Provided materials, reviewed and revised manuscript: KB and PA. Reviewed and revised manuscript: RS. All authors approved the final version of this manuscript.

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## REFERENCES

1 Ley TJ, Mardis ER, Ding L, Fulton B, McLellan MD, Chen K *et al.* DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome. *Nature* 2008; **456**: 66–72.
2 Mardis ER, Ding L, Dooling DJ, Larson DE, McLellan MD, Chen K *et al.* Recurring mutations found by sequencing an acute myeloid leukemia genome. *N Engl J Med* 2009; **361**: 1058–1066.
3 Ley TJ, Ding L, Walter MJ, McLellan MD, Lamprecht T, Larson DE *et al.* DNMT3A mutations in acute myeloid leukemia. *N Engl J Med* 2010; **363**: 2424–2433.
4 Greif PA, Eck SH, Konstandin NP, Benet-Pages A, Ksienzyk B, Dufour A *et al.* Identification of recurring tumor-specific somatic mutations in acute myeloid leukemia by transcriptome sequencing. *Leukemia* 2011; **25**: 821–827.
5 Bejar R, Stevenson K, Abdel-Wahab O, Galili N, Nilsson B, Garcia-Manero G *et al.* Clinical effect of point mutations in myelodysplastic syndromes. *N Engl J Med* 2011; **364**: 2496–2506.
6 Walter MJ, Shen D, Ding L, Shao J, Koboldt DC, Chen K *et al.* Clonal architecture of secondary acute myeloid leukemia. *N Engl J Med* 2012; **366**: 1090–1098.
7 Walter MJ, Ding L, Shen D, Shao J, Grillot M, McLellan M *et al.* Recurrent DNMT3A mutations in patients with myelodysplastic syndromes. *Leukemia.* 2011; **25**: 1153–1158.
8 Hussain FT, Nguyen EP, Raza S, Knudson R, Pardanani A, Hanson CA *et al.* Sole abnormalities of chromosome 7 in myeloid malignancies: spectrum, histopathologic correlates, and prognostic implications. *Am J Hematol.* 2012; **87**: 684–686.
9 Jerez A, Sugimoto Y, Makishima H, Verma A, Jankowska AM, Przychodzen B *et al.* Loss of heterozygosity in 7q myeloid disorders: clinical associations and genomic pathogenesis. *Blood.* 2012; **119**: 6109–6117.
10 Makishima H, Visconte V, Sakaguchi H, Jankowska AM, Abu Kar S, Jerez A *et al.* Mutations in the spliceosome machinery, a novel and ubiquitous pathway in leukemogenesis. *Blood* 2012; **119**: 3203–3210.
11 Hosono N, Makishima H, Jerez A, Gomez-Segui I, Przychodzen B, Sekeres MA *et al.* Mutation Screening Associated with Chromosome 7 Abnormalities Using Next Generation Whole Exome Sequencing. *ASH Annual Meeting Abstracts* **120**: 173.
12 Howell VM, Jones JM, Bergren SK, Li L, Billi AC, Avenarius MR *et al.* Evidence for a direct role of the disease modifier SCNM1 in splicing.. *Human molecular genetics* 2007; **16**: 2506–2516.
13 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.
14 Papaemmanuil E, Cazzola M, Boultwood J, Malcovati L, Vyas P, Bowen D *et al.* Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. *N Engl J Med* 2011; **365**: 1384–1395.
15 Narla A, Hurst SN, Ebert BL. Ribosome defects in disorders of erythropoiesis. *Int J Hematol* 2011; **93**: 144–149.



Supplementary Information accompanies this paper on Blood Cancer Journal website (http://www.nature.com/bcj)