Brief report

Somatic mutations in the transcriptional corepressor gene *BCORL1* in adult acute myelogenous leukemia

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To further our understanding of the genetic basis of acute myelogenous leukemia (AML), we determined the coding exon sequences of ~18 000 protein-encoding genes in 8 patients with secondary AML. Here we report the discovery of novel somatic mutations in the transcriptional corepressor gene *BCORL1* that is located on the X-chromosome. Analysis of *BCORL1* in an unselected cohort of 173 AML patients identified a total of 10 mutated cases (6%) with *BCORL1* mutations, whereas analysis of 19 AML cell lines uncovered 4 (21%) *BCORL1* mutated cell lines. The majority (87%) of the mutations in *BCORL1* were predicted to inactivate the gene product as a result of nonsense mutations, splice site mutation, or out-of-frame insertions or deletions. These results indicate that *BCORL1* by genetic criteria is a novel candidate tumor suppressor gene, joining the growing list of genes recurrently mutated in AML. (*Blood*. 2011;118(22):5914-5917)

Introduction

Acute myeloid leukemia (AML) will occur in approximately 13 000 new cases per year in the United States and result in 9000 untimely deaths. Recurrent chromosomal alterations provided some of the first clues to the pathogenesis of AML and contributed to improved classification of its subtypes. The molecular characterization of these translocations, along with identification of genes harboring intragenic mutations, has further elucidated disease mechanisms and provided new diagnostic and therapeutic approaches.[1,2] These genes include *FLT3*, *NPM1*, *CEBPA*, *TP53*, *IDH1* and *IDH2*, *TET2*, *WT1*, *RUNX1*, *ASXL1*, *EZH2*, *NF1*, and *DNMT3A*.[2-18] Historically, most mutated genes driving cancers have been thought to regulate specific, well-defined pathways, such as those associated with *RAS/RAF*, *PTEN/PIK3CA*, and *APC/*β-catenin.[19] More recently, unbiased genome-wide studies have identified mutated genes that appear to deregulate global patterns of gene expression through effects on chromatin structure or DNA methylation (eg, *IDH1/2, TET2, EZH2*, and *DNMT3A*).[20-24]

To extend our knowledge of subtle genetic alterations involved in AML, we have studied 8 secondary AML cases by exomic sequencing and have extended findings into a well-characterized unselected cohort of 173 AML cases as well as 19 AML cell lines. Through these efforts, we have identified novel somatically acquired inactivating mutations in the transcriptional corepressor gene *BCORL1* in AML.

Methods

Patients and samples

The AML patients studied in this report were consecutively enrolled between March 2005 and October 2010 at the University of Michigan Comprehensive Cancer Center. The study was approved by the University of Michigan Institutional Review Board (IRBMED #2004-1022), and written informed consent was obtained from all patients before enrollment in accordance with the Declaration of Helsinki. Clinical and other characteristics of the 8 secondary AML cases used in the discovery screen are detailed in supplemental Table 1 (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). For the prevalence screen, 165 additional unselected AML cases (exclusive of AML-FAB-M3) were analyzed; the clinical characteristics of this AML cohort are detailed in supplemental Table 2. (For all other methods, see supplemental Methods.)

Results

Patient characteristics

Characteristics of the 173 AML patients (all non-M3 French-American-British subtype) analyzed in this study are summarized in supplemental Tables 1 to 3. Of these patients, 58% had de novo AML, 33% had AML with myelodysplasia-related changes (World Health Organization 2008 MRC1-3), and 9% had treatment-related AML (the latter category does not include patients that received MDS-directed therapies only). Eighty percent of patients had not been treated for AML at trial enrollment, whereas 20% had been treated for AML and relapsed at the time their samples were obtained.

Discovery screen

We determined the sequences of approximately 18 000 protein-encoding genes in 8 patients with secondary AML (supplemental Table 1). Massively parallel sequencing of captured tumor and matched normal DNA resulted in an average depth of coverage of

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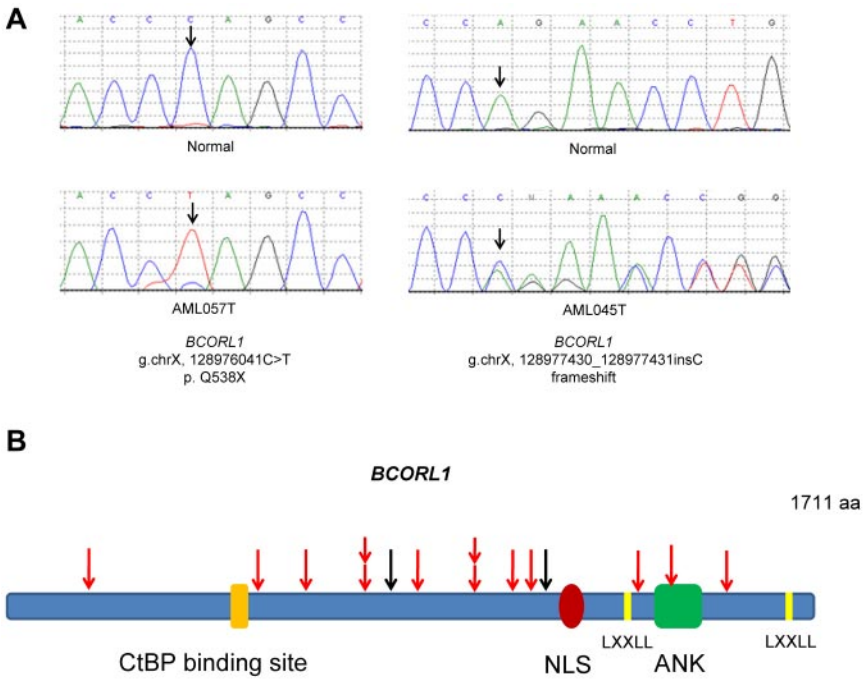

Figure 1. Schematic representations of the location of somatic mutations identified in the *BCORL1* gene. (A) Examples of sequence chromatograms showing inactivating somatic mutations of *BCORL1* in blast cells (bottom panels) but not in their matched normal buccal mucosa (top panels). (B) Schematic of somatic mutations identified in the *BCORL1* gene in primary AML cells and AML cell lines. Red arrows indicate truncating mutations; and black arrows, missense mutations. NLS indicates nuclear localization signal; LXXLL, Leu-Xaa-Xaa-Leu-Leu motif; ANK, tandem ankyrin repeats; aa, amino acids; and CtBP, C-terminal binding protein.

each base in the targeted regions of 92-fold; 94.1% of the bases were represented by at least 10 reads (supplemental Table 4).

Using stringent criteria, we found 5 genes to be somatically mutated in 2 tumors in the discovery set, including *BCORL1, NRAS, IDH1, DNMT3A*, and *RUNX1*. We also identified one *IDH2* mutation and one *TP53* mutation in 1 of 8 discovery tumors.

Prevalence screen

Based on the results of the discovery screen, we initially evaluated the *BCORL1, NRAS, IDH1, IDH2, DNMT3A, RUNX1, TP53, FLT3*, and *NPM1* genes for mutations in an additional 87 AML cases that were intentionally enriched for secondary AML (AML with myelodysplasia-related changes; supplemental Table 5). In each case, we amplified each exon of each gene from purified blast cell DNA and used conventional Sanger sequencing to search for mutations. Any mutations identified in the tumors were tested to determine whether they were somatic by similar sequencing of paired normal DNA from the same patients. Through these efforts, we identified 6 additional cases of AML with *BCORL1* mutations. Patient AML 35 was preceded by CMML1. We tested 2 consecutively procured bone marrow samples from patient 35 in the CMML1 phase of the illness and detected the *BCORL1* mutation at both times.

In addition to *BCORL1*, several of the other tested genes had mutations in these additional 87 cases (supplemental Tables 6 and 7). Whereas mutations in *FLT3* and *NPM1* were more prevalent in primary compared with secondary AML, mutations in *BCORL1, DNMT3A, RUNX1, IDH1*, and *IDH2* were equally distributed. As expected, *TP53* mutations were more common in secondary and t-AML than in de novo AML.

Next, we evaluated the *BCORL1* gene in an additional 78 AML cases, thus complementing the analysis for this gene for all AML in our consecutively enrolled AML cohort (N = 173). Two additional *BCORL1* mutated cases were identified, bringing the total to 10 (of 173 cases analyzed) and providing a *BCORL1* mutation prevalence estimate of 5.8%. Characteristics of the 10 AML cases with *BCORL1* mutations are summarized in Table 1. None of the *BCORL1* mutated cases was *TP53*, *CEBPA*, or *NPM1* mutated.

The coding exons of the *BCORL1* gene were also sequenced in 24 cell lines, of which 19 were AML-derived. In total, 5 *BCORL1* sequence variants were identified, including 3 nonsense, 1 frameshift, and 1 splice junction mutation (located in the conserved nucleotide immediately preceding exon 10). Data are summarized in supplemental Table 8.

BCORL1 is a transcriptional corepressor encoding a protein that is found tethered to promoter regions by DNA-binding proteins. *BCORL1* contains a putative bipartite nuclear localization signal, tandem ankyrin repeats, and a classic C-terminal binding protein (CtBP)–binding motif, PXDLS. Functional studies[25] have shown that BCORL1 can interact with class II histone deacetylases, interact with the CtBP corepressor through the CtBP binding motif-PXDLS and affects the repression of E-cadherin.[25]

The *BCORL1* gene is located on the X-chromosome at cytoband Xq25-q26.1 at approximately physical position 129.14 to 129.19 Mb. Eight of 10 (80%) *BCORL1* mutations found in primary cells and all *BCORL1* mutations in cell lines truncated the encoded protein as a result of nonsense mutations, splice site mutation, or out-of-frame insertions or deletions. All the truncating mutations in *BCORL1* were predicted to result in severely shortened polypeptides lacking the LXXLL nuclear receptor recruitment motif and the C-terminus (Figure 1). These data not only provide strong evidence that *BCORL1* mutations are important to the pathogenesis of AML but demonstrate that *BCORL1* is, on the basis of genetic criteria, a tumor suppressor gene that is inactivated by mutations.

We also measured normalized mRNA levels for *BCORL1* in cDNA made from RNA isolated from FACS-sorted AML blasts in an expanded cohort of AML cases. In summary, we found relatively uniform expression of *BCORL1* and all studied cases expressed the gene, including those with *BCORL1* mutations (supplemental Table 9).

Analysis of the *BCORL1* gene status in the AML cohort using SNP 6.0 array profiling uncovered no microdeletions or gains

Table 1. Characteristics of AML cases with BCORL1 mutations

Sample	Age, y	Sex	Previous treatment for AML	FAB subclass	WHO type	Prior MDS or MDS/MPN	Therapy-related	SWOG risk group	Cytogenetic risk
AML14	70	Female	No	M1	AML-NOS	No	No	Intermediate	47,XX,+8[19]
AML151	51	Male	No	M4	AML-MRC-1,2	RAEB-1	No	Unfavorable	45,XY,-7,i(21)(q10)[12]/46,XY, +i(21)(q10)[6]
AML33	68	Male	No	M6	AML-MRC-1	RAEB-2	No	Intermediate	46,XY,del(3)(q13q27)[16]/46,XY[4]
AML35	67	Male	No	M2	AML-MRC-1	CMML-1	No	Intermediate	47,XY,+14[16]/46,XY[4]
AML45	63	Female	No	M4	AML-TR	No	Yes	Intermediate	46,XY[20]
AML216	50	Female	No	M5	AML-MRC-1	RCMD	No	Intermediate	47,XX,+8[5]/46,XX[24]
AML257	63	Female	No	M4	AML-NOS	No	No	Intermediate	46,XX[20]
AML57	69	Male	No	M4eo	AML-RGA (16p13q22)	No	No	Favorable	46,XY,inv(16)(p13q22)[14]
AML87	55	Female	No	M0	AML-NOS	No	No	Unfavorable	46,XX,t(3;9;22)(q21;q34;q11.2) [15]/46,XX[5]
AML182	59	Male	No	M0	AML-NOS	No	No	Intermediate	48,XY,+21,+21[15]

FAB indicates French-American-British; WHO, World Health Organization; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; SWOG, Southwest Oncology Group; NOS, not otherwise specified; MRC, Medical Research Council; RAEB, refractory anemia with excess blasts; CMML, chronic myelomonocytic leukemia; RCMD, refractory cytopenia with multilineage dysplasia; and WT, wild-type.

spanning the gene. Furthermore, copy-neutral loss of heterozygosity was not identified (supplemental Figure 1).

DNMT3A mutations have recently been reported in 22.1% (62 of 281) of de novo AML[5] and in 20.5% of predominantly new diagnosed AML.[6] Similarly, somatic mutations in *DNMT3A* were identified in 23.2% (22 of 95) of the cases we analyzed (supplemental Table 10). The novel *DNMT3A*-related finding in our study was a frameshift mutation in normal buccal mucosa and in remission marrow and neoplastic cells of AML patient 89 (supplemental Figure 2). No additional mutations of *DNMT3A* were identified in the patient's tumor; the patient had no other tumors and was 78 years old. These data raise the possibility that germline *DNMT3A* mutations can weakly predispose persons to the development of AML but not to other tumors. There was no history of AML in this patient's family, and the parents were unavailable to determine whether the mutation was de novo.

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Authorship

Contribution: M.L., B.V., and S.N.M. conceived the study and supervised the work; M.L., P.O., Y.J., S.N.M., N.P., K.W.K., and B.V. performed the laboratory research and genomic data analysis; R.C., H.E., D.B., and S.N.M. enrolled patients and contributed and analyzed clinical data; and M.L., B.V., and S.N.M. wrote the paper.

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Table 1. Continued.

BCORL1 mutations	*KRAS* mutations	*NRAS* mutations	*CEBPA* mutations	*IDH1* mutations	*IDH2* mutations	*DNMT3A* mutations	*TP53* mutations	*RUNX1* mutations	*FLT3* mutations	*NPM1* mutations
p.S1158A	WT	WT	WT	WT	p.R140Q	WT	WT	Frameshift	p.D835Y	WT
p.P810T	WT	p.Q61K	WT	WT	WT	WT	WT	p.W106L	WT	WT
Frameshift	WT	WT	WT	WT	WT	p.V716I	WT	WT	WT	WT
Frameshift	WT	WT	WT	WT	WT	p.Y448X	WT	WT	WT	WT
Frameshift	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
Frameshift	WT	WT	WT	p.R132C	WT	p.S714F, p.G685R	WT	WT	WT	WT
Frameshift	WT	WT	WT	WT	WT	WT	WT	Frameshift; p.F131V	ITD	WT
p.Q538X	p.G13GD	WT	WT	WT	WT	WT	WT	WT	WT	WT
p.E1112X	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
p.R784X	WT	WT	WT	p.R132G	WT	p.R693H	WT	Frameshift	ITD	WT

FAB indicates French-American-British; WHO, World Health Organization; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; SWOG, Southwest Oncology Group; NOS, not otherwise specified; MRC, Medical Research Council; RAEB, refractory anemia with excess blasts; CMML, chronic myelomonocytic leukemia; RCMD, refractory cytopenia with multilineage dysplasia; and WT, wild-type.

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