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 $FLT3$, $NPM1$, $CERPA$, $TP53$, $IDH1$ 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 $APC/\beta$-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...
7). Whereas mutations in mutations in these additional 87 cases (supplemental Tables 6 and our consecutively enrolled AML cohort (N
173 cases analyzed) and providing a
estimate of 5.8%. Characteristics of the 10 AML cases with mutations in both times.

Patient AML 35 was preceded by CMML1. We tested 2 consecu-
ditionally procured bone marrow samples from patient 35 in the
dual normal DNA from the same patients. Through these efforts,
mutations. Any mutations identified in the tumors were tested to
cell DNA and used conventional Sanger sequencing to search for
each case, we amplified each exon of each gene from purified blast

tions identified in the
tions identified in the

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 mutations.

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

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 frame-shift, 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 studies25 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.

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

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.

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
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 and in 20.5% of predominantly new diagnosed AML. 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.

**Acknowledgments**

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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.B., 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.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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**References**

7. Mardis ER, Ding L, Dooling DJ, et al. Recurring mutations have recently been reported in 22.1% of de novo AML and in 20.5% of predominantly new diagnosed AML. 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.

**Table 1. Characteristics of AML cases with BCORL1 mutations**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Age, y</th>
<th>Sex</th>
<th>Previous treatment for AML</th>
<th>FAB subclass</th>
<th>WHO type</th>
<th>Prior MDS or MDS/MPN</th>
<th>Therapy-related</th>
<th>SWOG risk group</th>
<th>Cytogenetic risk</th>
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<tbody>
<tr>
<td>AML14</td>
<td>70</td>
<td>Female</td>
<td>No</td>
<td>M1</td>
<td>AML-NOS</td>
<td>No</td>
<td>No</td>
<td>Intermediate</td>
<td>47.XX, +8[19]</td>
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<tr>
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<td>No</td>
<td>M4</td>
<td>AML-MRC-1.2</td>
<td>RAEB-1</td>
<td>No</td>
<td>Unfavorable</td>
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</tr>
<tr>
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<td>No</td>
<td>M5</td>
<td>MRC-1</td>
<td>RAEB-2</td>
<td>No</td>
<td>Intermediate</td>
<td>46.XY,del(3)[q13q27][15]/46,XY[4]</td>
</tr>
<tr>
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<td>67</td>
<td>Male</td>
<td>No</td>
<td>M2</td>
<td>MRC-1</td>
<td>CMMML</td>
<td>No</td>
<td>Intermediate</td>
<td>47.XY, +14[16]/46,XY[4]</td>
</tr>
<tr>
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<td>63</td>
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<td>No</td>
<td>M4</td>
<td>AML-TR</td>
<td>No</td>
<td>Yes</td>
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<td>46,Y[20]</td>
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<tr>
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<td>No</td>
<td>M4</td>
<td>AML-NOS</td>
<td>No</td>
<td>No</td>
<td>Intermediate</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td>AML57</td>
<td>69</td>
<td>Male</td>
<td>No</td>
<td>M4eo</td>
<td>AML-RGA</td>
<td>(16p13q22)</td>
<td>No</td>
<td>No</td>
<td>Favorable</td>
</tr>
<tr>
<td>AML87</td>
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<td>Male</td>
<td>No</td>
<td>M0</td>
<td>AML-NOS</td>
<td>No</td>
<td>No</td>
<td>Intermediate</td>
<td>46,XX[i(13;22)[q21;q34]/q11.2]</td>
</tr>
<tr>
<td>AML182</td>
<td>59</td>
<td>Male</td>
<td>No</td>
<td>M0</td>
<td>AML-NOS</td>
<td>No</td>
<td>No</td>
<td>Intermediate</td>
<td>48,XY, +21[21]</td>
</tr>
</tbody>
</table>

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; CMMML, chronic myelomonocytic leukemia; RCMD, refractory cytopenia with multilineage dysplasia; and WT, wild-type.
Table 1. Continued.

<table>
<thead>
<tr>
<th>BCR/ABL1 mutations</th>
<th>KRAS mutations</th>
<th>NRAS mutations</th>
<th>CEBPA mutations</th>
<th>IDH1 mutations</th>
<th>IDH2 mutations</th>
<th>DNMT3A mutations</th>
<th>TPS3 mutations</th>
<th>RUNX1 mutations</th>
<th>FLT3 mutations</th>
<th>NPM1 mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frameshift</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>p.Y448X</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>Frameshift</td>
</tr>
<tr>
<td>Frameshift</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>Frameshift</td>
<td>p.F131V</td>
<td>ITD</td>
<td>WT</td>
<td>Frameshift</td>
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<tr>
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<td>p.G538X</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>ITD</td>
<td>WT</td>
<td>Frameshift</td>
<td>WT</td>
</tr>
<tr>
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<td>p.G13GD</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>ITD</td>
<td>WT</td>
<td>Frameshift</td>
<td>WT</td>
</tr>
<tr>
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<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>Frameshift</td>
<td>Frameshift</td>
<td>ITD</td>
<td>Frameshift</td>
</tr>
<tr>
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<td>p.R784X</td>
<td>WT</td>
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<td>p.R132G</td>
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<td>Frameshift</td>
<td>ITD</td>
<td>Frameshift</td>
</tr>
<tr>
<td>Frameshift</td>
<td>p.R969H</td>
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<td>WT</td>
<td>p.R132G</td>
<td>WT</td>
<td>Frameshift</td>
<td>WT</td>
<td>Frameshift</td>
<td>WT</td>
<td>Frameshift</td>
</tr>
</tbody>
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