Tracing the development of acute myeloid leukemia in CBL syndrome

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Running head: AML in CBL mutated hematopoiesis

Keywords: CBL syndrome, CBL mutation, acute myeloid leukemia, AML, juvenile myelomonocytic leukemia

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Key Points

• The *CBL* syndrome may predispose to myeloid neoplasias other than JMML
• Whole-exome sequencing identifies mutations that possibly cooperate with mutant *CBL* in AML development

Abstract

We describe the development of acute myeloid leukemia (AML) in an adult with *CBL* syndrome due to a heterozygous *de novo* germline mutation in *CBL* codon D390. In the AML bone marrow, the mutated *CBL* allele was homozygous after copy number-neutral loss-of-heterozygosity and amplified through a chromosomal gain; moreover, an inv(16)(p13q22) and, as assessed by whole-exome sequencing, 12 gene mutations (e.g., in *CAND1*, *NID2*, *PTPRT*, *DOCK6*) were additionally acquired. During complete remission of the AML, in the presence of normal blood counts, the hematopoiesis stably maintained the homozygous *CBL* mutation, which is reminiscent of the situation in children with *CBL* syndrome and transient juvenile myelomonocytic leukemia. No additional mutations were identified by whole-exome sequencing in granulocytes during complete remission. The study highlights the development of AML in an adult with *CBL* syndrome and, more general, in genetically aberrant but clinically inconspicuous hematopoiesis.
Introduction

Preceding hematologic disorders are documented in a quarter of adults with acute myeloid leukemia (AML). However, an unknown proportion of AMLs that apparently arise de novo may have developed from undiscoversed abnormal hematopoiesis.

Mutations in CBL, encoding an E3 ubiquitin ligase, are found in 10-20% of chronic (CMML) or juvenile myelomonocytic leukemia (JMML) patients. Germline CBL mutations cause the CBL syndrome that recapitulates features of other RAS-MAPK pathway disorders and predisposes to JMML. In AML, CBL mutations are rare, but associate with inv(16).

Here, we describe the development of AML in an adult with CBL syndrome and JMML-typical loss of wildtype CBL in bone marrow.
Methods

Written informed consent of the patient included in the present study was obtained for sample storage and analyses prior to sampling, as approved by the local ethics committee. This study was conducted in accordance with the Declaration of Helsinki. Karyotype, mutations in NPM1, FLT3 (tyrosine kinase domain, internal tandem duplication), CEBPA and CBL, and CBFB-MYH11 expression relative to ABL1 were assessed as described elsewhere.\textsuperscript{15-18} CBL mutated-to-wildtype allelic ratios were determined using the PyroMark Q96MD (Qiagen), and chromosomal copy numbers using CytoScanHD-arrays (Affymetrix). Data are deposited at \url{http://www.ebi.ac.uk/arrayexpress/} (E-MEXP-3997). Whole-exome sequencing was performed as reported;\textsuperscript{19} variants were validated by Sanger sequencing. Methods are detailed in the supplement.
Results and discussion

**Characteristics of the AML**

A 40-year-old male was diagnosed with AML in June 2011. Pre-existing conditions were hereditary spherocytosis (diagnosed in 1996), coagulopathy (low FVII, X, XII, XIII), atrial fibrillation and hypocholesterolemia; a splenomegaly was considered consequence of the spherocytosis. At AML diagnosis, the white blood cell count was 19,390/µl, with approximately 30% blasts and 30% dysplastic monocytes (Supplemental Figure S1). The marrow contained 50% CD117-positive blasts and 30% CD14-positive monocytes; the karyotype was 46,XY,add(4)(q?31),inv(16)(p13q22)[21]/46,XY,inv(16)(p13q22)[1]. *CBFB-MYH11* (type D) was detected with a ratio of 46.23 in blood. *NPM1*, *CEBPA* and *FLT3* mutations were absent.

The patient received “3+7” induction followed by dasatinib (ClinicalTrials.gov NCT00850382). Six weeks after therapy start, complete remission (CR) was documented. He received four consolidation courses with high-dose cytarabine. At last follow-up (September 2013), he was in continuous CR, with no *CBFB-MYH11* detectable.

**Identification of a germline CBL mutation**

Between the treatment courses, the patient’s monocyte counts rose to extraordinarily high levels. Although monocytes were within normal limits after treatment and in blood counts dating back to 1996 (Supplemental Table S1), this observation prompted the question whether the patient had an underlying monocytic disorder.
Since monocytosis is a hallmark of JMML and CMML, we examined the mutation status of CBL exon 8 and 9 in blood collected at the AML diagnosis. We indeed found a p.D390V-mutation, located in the frequently mutated RING finger domain. Assessing the germline origin of the mutation, we also identified it in buccal mucosa and hair follicles. We concluded that the patient had a previously undiagnosed CBL syndrome, with the preexisting coagulopathy and atrial fibrillation being part of the phenotype (Supplemental Table S2). 8-10

No CBL mutations were detected in blood of both parents, indicating de novo occurrence in the patient’s germline. The patient has no siblings.

**Zygosity of the CBL mutation**

Copy number-neutral loss-of-heterozygosity (LOH) of the CBL-containing chromosomal band 11q23.3 is common in children with CBL syndrome and JMML. 8,10 11q-LOH was also detectable in marrow mononuclear cells from our patient during AML. Moreover, the 11q-LOH persisted in B-lymphocytes, granulocytes and monocytes collected later during CR (Table 1, Supplemental Figure S2). This is reminiscent of the situation in children with CBL syndrome and JMML whose myeloproliferation spontaneously improves. 8 Notably, the AML in our patient exhibited an additional gain of 11q-material, indicating that the LOH had existed before the AML. In skin and T-lymphocytes, 11q retained heterozygosity.
To complement the LOH findings, we determined the allelic burden of the *CBL* mutation by pyrosequencing (Table 1). In agreement with the LOH data, the mutation was heterozygous in skin and T-lymphocytes but homozygous in AML cells and in granulocytes, monocytes and B-lymphocytes collected during CR, where it remained homozygous until last follow-up, underlining the stability of the genetically aberrant hematopoiesis. Notably, LOH and pyrosequencing data suggested the presence of a small fraction of T-lymphocytes also harboring the 11q-LOH (Table 1, Supplemental Figure S2).

**Identification of cooperating mutations by whole-exome sequencing**

Similar to our patient, children with *CBL* syndrome and transient JMML feature normal blood counts and persistent homozygous *CBL* mutation in their hematopoiesis.\(^8\) Little is known about mechanisms that could be responsible for normal hematopoiesis despite oncogenic features characteristic of JMML. We wondered whether this was associated with the acquisition of mutations that overcome the myeloproliferative impact of the homozygous *CBL* mutation. We therefore subjected granulocytes from CR and skin to whole-exome sequencing, but identified no additional mutations.

We also performed whole-exome sequencing of AML cells to identify mutations that were acquired during AML development, in addition to inv(16) and 11q-gain. We detected somatic mutations in 12 genes (Table 2), three of which (i.e., *CAND1*, *NID2*, *PTPRT*) were previously found mutated in AML.\(^{20-22}\) However, no gene has an established role in leukemogenesis, e.g., as cooperating partner of mutant *CBL* or *CBFB-MYH11*. 
**Biologic impact of the CBL mutation**

JMML features the formation of colonies at low concentrations of granulocyte-macrophage colony-stimulating factor (GM-CSF).23 We observed no spontaneous growth or hypersensitivity to GM-CSF of mononuclear cells collected from our patient during CR (data not shown), which underlines the lacking or only subtle impact of the homozygous CBL mutation on hematopoiesis. Moreover, granulocytes showed normal production of reactive oxygen species and interleukin-8 to stimuli, and adhesion and migration/chemotaxis were normal (data not shown).

In summary, we diagnosed a CBL syndrome in an adult, who, as observed in children with CBL syndrome developing JMML,8,10 had lost the CBL wildtype allele in the bone marrow. Whether this leads to overt JMML only under certain circumstances is not well understood.24 Since the LOH persisted in the various hematopoietic cell lineages in our patient, it likely conferred a clonal advantage at one point. Thus, the patient may have indeed gone through a JMML or related hematologic disorder during infancy, which spontaneously resolved and left behind normal blood counts. However, medical information to support this assumption is unavailable. Following the hypothesis that normal blood counts in our patient could be associated with the acquisition of mutations counterbalancing the mutant CBL, we performed whole-exome sequencing but identified no acquired mutations. On the background of the CBL mutation, the patient developed AML through the acquisition of inv(16), gain of 11q-material and at least 12 gene mutations. The AML was erased by chemotherapy, again leaving behind a hematopoiesis with homozygous CBL mutation.
While the CBL syndrome is known to predispose to JMML, this is the first description of a different myeloid neoplasia occurring at adult age. It cannot be determined whether the AML was mere coincidence, or due to a predisposition conferred by the CBL mutation. However, the latter is supported by the specific gain of CBL-encoding 11q-material and occurrence of inv(16), which associates with CBL mutations.\textsuperscript{11-14} If substantiated by future studies, the association between CBL syndrome and AML should be considered in clinical practice. CBL would then join other genes, e.g., RUNX1 or CEBPA, germline mutations in which were linked to a predisposition to AML.\textsuperscript{25}

Overall, the case highlights the possibility of genetically aberrant hematopoiesis despite normal blood counts and provides insight into myeloid neoplasias in the CBL syndrome. Due to potential health problems associated with a CBL syndrome, germline analyses may be generally warranted in younger adults with CBL-mutated neoplasias.
Acknowledgements

We wish to express our thanks to the patient for continued and interactive participation in the study. We also thank the German-Austrian AML Study Group (AMLSG), as the patient was enrolled on an AMLSG clinical trial.

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Authorship


Disclosure of conflicts of interest: S.S. declares part ownership of the MLL Munich Leukemia Laboratory GmbH. The remaining authors declare no competing financial interests.
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References


**Table 1.** Chromosome 11q-aberrations assessed by single nucleotide polymorphism (SNP)-array and *CBL* D390V allele burden determined by sequencing in different cell populations.

<table>
<thead>
<tr>
<th>Timepoint</th>
<th>Cell type</th>
<th>11q-LOH*</th>
<th>11q-gain†</th>
<th><em>CBL</em> D390V allele burden</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis of AML</td>
<td>BM MNCs</td>
<td>Yes</td>
<td>Yes</td>
<td>92.6%‡§</td>
</tr>
<tr>
<td>Complete remission of AML</td>
<td>PB Granulocytes</td>
<td>Yes</td>
<td>No</td>
<td>92.8% (90.1%-96.5%)‡</td>
</tr>
<tr>
<td></td>
<td>PB Monocytes</td>
<td>Yes</td>
<td>No</td>
<td>92.2% (88.9%-95.5%)‡</td>
</tr>
<tr>
<td></td>
<td>PB B-lymphocytes</td>
<td>Yes</td>
<td>No</td>
<td>83.3% (75.9%-94.1%)‡</td>
</tr>
<tr>
<td></td>
<td>PB T-lymphocytes</td>
<td>No†</td>
<td>No</td>
<td>55.4% (52.4%-59.3%)‡</td>
</tr>
<tr>
<td></td>
<td>Skin biopsy</td>
<td>No</td>
<td>No</td>
<td>48.6%§</td>
</tr>
<tr>
<td></td>
<td>Buccal mucosa</td>
<td>ND</td>
<td>ND</td>
<td>Heterozygous*</td>
</tr>
<tr>
<td></td>
<td>Hair follicle</td>
<td>ND</td>
<td>ND</td>
<td>Heterozygous*</td>
</tr>
</tbody>
</table>

LOH indicates loss-of-heterozygosity; BM; bone marrow; MNCs, mononuclear cells; PB, peripheral blood; and ND, not determined.

LOH data are also presented in Supplemental Figure S2. * LOH of chromosome 11 position 59764127-134942626; † Gain of chromosome 11 position 88486678-134938470; ‡ *CBL* D390V allele burden relative to combined D390V and wildtype alleles assessed by pyrosequencing; § average of measurements from one timepoint; || average and range of measurements at three time points during CR 5 to 19 months after AML diagnosis; ¶ data suggest small fraction of T-lymphocytes with 11q-LOH (purity of T-lymphocytes in pyrosequencing 97%-98%); # concluded from Sanger sequencing.
Table 2. Gene mutations in AML acquired in addition to the germline CBL mutation and the chromosomal aberrations.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene localization</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADAM12</td>
<td>10q26</td>
<td>NM_003474: c.665C&gt;T, p.A222V*</td>
</tr>
<tr>
<td>CAND1</td>
<td>12q14</td>
<td>NM_018448: c.1750G&gt;T, p.E584X</td>
</tr>
<tr>
<td>CMIP</td>
<td>16q23</td>
<td>NM_030629: c.968C&gt;T, p.T323M*</td>
</tr>
<tr>
<td>KIF14</td>
<td>1p32.1</td>
<td>NM_014875: c.1021G&gt;A, p.V341I</td>
</tr>
<tr>
<td>MIOX</td>
<td>22q13.3</td>
<td>NM_017584: c.673T&gt;C, p.W225R*</td>
</tr>
<tr>
<td>MYOCD</td>
<td>17p11.2</td>
<td>NM_153604: c.847G&gt;A, p.D283N*</td>
</tr>
<tr>
<td>NID2</td>
<td>14q22.1</td>
<td>NM_007361: c.955G&gt;A, p.D319N</td>
</tr>
<tr>
<td>PRSS16</td>
<td>6p21</td>
<td>NM_005865: c.1471C&gt;T, p.R491C*</td>
</tr>
<tr>
<td>PTPT</td>
<td>20q12-q13</td>
<td>NM_007050: c.2531C&gt;T, p.T844M*</td>
</tr>
<tr>
<td>TMEM125</td>
<td>1p34.2</td>
<td>NM_144626: c.337G&gt;A, p.D113N*</td>
</tr>
</tbody>
</table>

All mutations were identified by whole-exome sequencing of bone marrow MNCs from the AML. Their presence and somatic origin were validated by Sanger sequencing of AML and skin fibroblasts. The information on gene localization is based on Entrez Gene. * missense mutations that are "probably damaging" according to PolyPhen-2 (v2.2.2r398, HumDiv-trained model)
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