Brief Report

MYELOID NEOPLASIA

Tracing the development of acute myeloid leukemia in CBL syndrome

Heiko Becker,1 Kenichi Yoshida,2,3 Nadja Blagitko-Dorfs,1 Rainer Claus,1 Milena Pantic,1 Mahmoud Abdelkarim,1 Christoph Niemöller,1 Christine Greil,1 Björn Hackanson,1 Yuichi Shiraiishi,4 Kenichi Chiba,4 Hiroko Tanaka,5 Satoru Miyano,4,5 Konstanze Döhner,6 Susanne Schnittger,7 Philipp Henneke,8,9 Charlotte M. Niemeyer,9 Christian Flotho,9 Dietmar Pfeifer,1 Seishi Ogawa,2,3 and Michael Lübbert9

1Department of Medicine I, Medical Center–University of Freiburg, Freiburg, Germany; 2Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan; 3Cancer Genomics Project, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; 4Laboratory of DNA Information Analysis and 5Laboratory of Sequence Analysis, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan; 6Department of Internal Medicine III, University of Ulm, Ulm, Germany; 7MLL Munich Leukemia Laboratory, Munich, Germany; and 8Center of Chronic Immunodeficiency and 9Center for Pediatrics and Adolescent Medicine, Medical Center–University of Freiburg, Freiburg, Germany

Key Points

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

We describe the development of acute myeloid leukemia (AML) in an adult with CBL syndrome caused by 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 (eg, 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 generally, in genetically aberrant but clinically inconspicuous hematopoiesis. (Blood. 2014;123(12):1883-1886)

Introduction

Preceding hematologic disorders are documented in one-quarter of adults with acute myeloid leukemia (AML).1 However, an unknown proportion of AMLs that apparently arise de novo may have developed from undiscovered abnormal hematopoiesis.

Mutations in CBL, encoding an E3 ubiquitin ligase, are found in 10% to 20% of chronic myelomonocytic leukemia (CMML) or juvenile myelomonocytic leukemia (JMML) patients.2-7 Germline CBL mutations cause the CBL syndrome that recapitulates features of other RAS-MAPK pathway disorders and predisposes to JMML.8-10 In AML, CBL mutations are rare but associated with inv(16).11-14

Here we describe the development of AML in an adult with CBL syndrome and JMML-typical loss of wild-type (WT) CBL in bone marrow.

Methods

Written informed consent of the patient included in the present study was obtained for sample storage and analyses before 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.15-18 CBL mutated-to-WT allelic ratios were determined using the PyroMark Q96MD (Qiagen), and chromosomal copy numbers using CytoScanHD arrays (Affymetrix). Data were deposited at http://www.ebi.ac.uk/arrayexpress/ (E-MEXP-3997). Whole-exome sequencing was performed as reported19; variants were validated by Sanger sequencing. Methods are detailed in the supplemental data available on the Blood Web site.

Results and discussion

Characteristics of the AML

A 40-year-old man was diagnosed with AML in June 2011. His preexisting conditions were hereditary spherocytosis (diagnosed in 1996), coagulopathy (low FVII, X, XII, XIII), atrial fibrillation, and hypercholesterolemia; a splenomegaly was considered a consequence of the spherocytosis. At AML diagnosis, his white blood cell count was 19 390/µL, with approximately 30% blasts and 30% dysplastic monocytes (supplemental Figure 1). The marrow contained 50% CD117+ blasts and 30% CD14+ monocytes; the karyotype was 46, XY.add(4)(q?31),inv(16)(p13q22)[21]/46,XY.inv(16)(p13q22)[1].


The online version of this article contains a data supplement.

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CBFB-MYH11 (type D) was detected with a ratio of 46.23 in blood.

The patient received “3 + 7” induction followed by dasatinib (clinicaltrials.gov #NCT00850382). Six weeks after the start of therapy, complete remission (CR) was documented. The patient received 4 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 CBTL mutation

Between the treatment courses, the patient’s monocye counts rose to extraordinarily high levels. Although monocytes were within normal limits after treatment and in blood counts dating back to 1996 (supplemental Table 1), this observation prompted the question of whether the patient had an underlying monocytic disorder.

Because monocytosis is a hallmark of JMML and CMML, we examined the mutation status of CBTL exons 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 confirmed that the patient had previously undiagnosed CBTL syndrome, with the preexisting coagulopathy and atrial fibrillation being part of the phenotype (supplemental Table 2).8-10

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

Zygosity of the CBTL mutation

Copy number–neutral loss-of-heterozygosity (LOH) of the CBTL-containing chromosomal band 11q23.3 is common in children with CBTL 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 monocyes collected later during CR (Table 1 and supplemental Figure 2). This is reminiscent of the situation in children with CBTL 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 CBTL 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 and supplemental Figure 2).

Identification of cooperating mutations by whole-exome sequencing

Similar to our patient, children with CBTL syndrome and transient JMML feature normal blood counts and persistent homozygous CBTL 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 CBTL 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 (CAND1, NID2, PTPRT) were previously found mutated in AML.20-22 However, no gene has an established role in leukemogenesis (eg, as cooperating partner of mutant CBTL or CBFB-MYH11).

Biological impact of the CBTL 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 CBTL 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 CBTL syndrome in an adult, who, as observed in children with CBTL syndrome developing JMML,17 had lost the CBTL WT allele in the bone marrow. Whether this leads to overt JMML only under certain circumstances is not well understood.24

Table 1. Chromosome 11q aberrations assessed by single nucleotide polymorphism array and CBTL 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>CBTL 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%-95.6%)¶¶</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></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>

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

*LOH of chromosome 11 position 59764127-134942626.
†Gain of chromosome 11 position 88486678-134938470. CBTL D390V allele burden relative to combined D390V and WT alleles assessed by pyrosequencing.
‡Average of measurements from one time point.
§Average of range of measurements at 3 time points during CR 5 to 19 months after AML diagnosis.
¶The data suggest a small fraction of T lymphocytes with 11q-LOH (purity of T-lymphocytes in pyrosequencing 97%-98%).
#Concluded from Sanger sequencing.
Because 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. 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 eradicated by chemotherapy, leaving a hematopoiesis with homozygous CBL mutation.

Although 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 caused by 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.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 (eg, RUNX1 or CBPA) with germline mutations that were linked to a predisposition to AML.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. Because of potential health problems associated with a CBL syndrome, germline analyses may be generally warranted in younger adults with CBL-mutated neoplasias.

Acknowledgments

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Authorship


Conflict-of-interest disclosure: S.S. declares part ownership of the MLL Munich Leukemia Laboratory GmbH. The remaining authors declare no competing financial interests.

Correspondence: Michael Lübbert, Department of Medicine I, Medical Center–University of Freiburg, Hugstetter Straße 55, 79106 Freiburg, Germany; e-mail: michael.luebbert@uniklinik-freiburg.de.

References

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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>ARF3</td>
<td>12q13</td>
<td>NM_001659: c.303A&gt;G, p.N101S</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>16p23</td>
<td>NM_003069: c.968C&gt;T, p.T322M*</td>
</tr>
<tr>
<td>KIF14</td>
<td>1q32.1</td>
<td>NM_014875: c.1021G&gt;T, p.D341G</td>
</tr>
<tr>
<td>MIOX</td>
<td>22q13.3</td>
<td>NM_017584: c.6737G&gt;C, p.W225R*</td>
</tr>
<tr>
<td>MYOCD</td>
<td>17p11.2</td>
<td>NM_153604: c.847G&gt;T, 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>PTPRT</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.3370G&gt;A, p.D113N*</td>
</tr>
</tbody>
</table>

All mutations were identified by whole-exome sequencing of bone marrow mononuclear cells 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. *Missequences that are “probably damaging” according to PolyPhen-2 (v2.2.2r398, HumDiv-trained model).


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