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 Shiraishi,[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 Lübbert[1]

[1]Department of Medicine I, Medical Center–University of Freiburg, Freiburg, Germany; [2]Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [3]Cancer Genomics Project, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; [4]Laboratory of DNA Information Analysis and [5]Laboratory of Sequence Analysis, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan; [6]Department of Internal Medicine III, University of Ulm, Ulm, Germany; [7]MLL Munich Leukemia Laboratory, Munich, Germany; and [8]Center of Chronic Immunodeficiency and [9]Center 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 reported[19]; 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 hypocholesterolemia; 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].

Submitted October 20, 2013; accepted January 21, 2014. Prepublished online as *Blood* First Edition paper, February 3, 2014; DOI 10.1182/blood-2013-10-533844.

The online version of this article contains a data supplement.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

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

Timepoint	Cell type	11q-LOH*	11q-gain†	*CBL* D390V allele burden
Diagnosis of AML	BM MNCs	Yes	Yes	92.6%‡§
Complete remission of AML	PB granulocytes	Yes	No	92.8% (90.1%-96.5%)‡‖
	PB monocytes	Yes	No	92.2% (88.9%-95.5%)‡‖
	PB B lymphocytes	Yes	No	83.3% (75.9%-94.1%)‡‖
	PB T lymphocytes	No¶	No	55.4% (52.4%-59.3%)‡‖¶
	Skin biopsy	No	No	48.6%‡§
	Buccal mucosa	*ND*	*ND*	Heterozygous#
	Hair follicle	*ND*	*ND*	Heterozygous#

BM, bone marrow; LOH, loss-of-heterozygosity; MNCs, mononuclear cells; ND, not determined; PB, peripheral blood.
LOH data are also presented in supplemental Figure 2.
*LOH of chromosome 11 position 59764127-134942626.
†Gain of chromosome 11 position 88486678-134938470.
‡*CBL* D390V allele burden relative to combined D390V and WT alleles assessed by pyrosequencing.
§Average of measurements from one time point.
‖Average and 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.

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 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 *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 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 *CBL* 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 concluded that the patient had a previously undiagnosed *CBL* syndrome, with the preexisting coagulopathy and atrial fibrillation being part of the phenotype (supplemental Table 2).[8-10]

No *CBL* 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 *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 and supplemental Figure 2). 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 and supplemental Figure 2).

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 (*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 *CBL* or *CBFB-MYH11*).

Biological 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* WT allele in the bone marrow. Whether this leads to overt JMML only under certain circumstances is not well understood.[24]

Table 2. Gene mutations in AML acquired in addition to the germline *CBL* mutation and the chromosomal aberrations

Gene	Gene localization	Mutation
ADAM12	10q26	NM_003474: c.665C>T, p.A222V*
ARF3	12q13	NM_001659: c.302A>G, p.N101S
CAND1	12q14	NM_018448: c.1750G>T, p.E584X
CMIP	16q23	NM_030629: c.968C>T, p.T323M*
DOCK6	19p13.2	NM_020812: c.5616_5617insCCG, p.R1872_K1873insP
KIF14	1q32.1	NM_014875: c.1021G>A, p.V341I
MIOX	22q13.3	NM_017584: c.673T>C, p.W225R*
MYOCD	17p11.2	NM_153604: c.847G>A, p.D283N*
NID2	14q22.1	NM_007361: c.955G>A, p.D319N
PRSS16	6p21	NM_005865: c.1471C>T, p.R491C*
PTPRT	20q12-q13	NM_007050: c.2531C>T, p.T844M*
TMEM125	1p34.2	NM_144626: c.337G>A, p.D113N*

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.

*Missense mutations that are "probably damaging" according to PolyPhen-2 (v2.2.2r398, HumDiv-trained model).

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 *CEBPA*) 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

The authors thank the patient for continued and interactive participation in the study, and the German-Austrian AML Study Group (AMLSG), because the patient was enrolled in an AMLSG clinical trial.

The research was supported in part by the Research Committee of the University Freiburg, Germany (MER 785/10) (H.B.), the EHA and ASH Translational Research Training in Hematology (H.B.), the German Cancer Aid (DKH 110213) (B.H.), the Bundesministerium für Bildung und Forschung (P.H.), and the German Research Foundation (DFG Lu 429/7-1, CRC 992-C04 [M.L.] and DFG Fl 345/4-1, CRC 992-C05 [C.F.]).

Authorship

Contribution: H.B., K.Y., C.G., S.S., P.H., C.M.N., C.F., S.O., and M.L. contributed to the design and interpretation of the study; H.B., N.B.-D., M.P., M.A., C.N., B.H., K.D., S.S., and C.F. carried out laboratory-based analyses; K.Y., R.C., Y.S., K.C., H.T., S.M., and D.P. performed bioinformatic analyses; C.G., B.H., and M.L. were involved in patient care; H.B., S.O., and M.L. wrote the manuscript; and all authors contributed to and agreed on the final version.

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

1. Juliusson G, Antunovic P, Derolf A, et al. Age and acute myeloid leukemia: real world data on decision to treat and outcomes from the Swedish Acute Leukemia Registry. *Blood.* 2009;113(18):4179-4187.
2. Sanada M, Suzuki T, Shih LY, et al. Gain-of-function of mutated C-CBL tumour suppressor in myeloid neoplasms. *Nature.* 2009;460(7257):904-908.
3. Loh ML, Sakai DS, Flotho C, et al. Mutations in CBL occur frequently in juvenile myelomonocytic leukemia. *Blood.* 2009;114(9):1859-1863.
4. Makishima H, Cazzolli H, Szpurka H, et al. Mutations of e3 ubiquitin ligase cbl family members constitute a novel common pathogenic lesion in myeloid malignancies. *J Clin Oncol.* 2009;27(36):6109-6116.
5. Pérez B, Kosmider O, Cassinat B, et al. Genetic typing of CBL, ASXL1, RUNX1, TET2 and JAK2 in juvenile myelomonocytic leukaemia reveals a genetic profile distinct from chronic myelomonocytic leukaemia. *Br J Haematol.* 2010;151(5):460-468.
6. Kohlmann A, Grossmann V, Klein HU, et al. Next-generation sequencing technology reveals a characteristic pattern of molecular mutations in 72.8% of chronic myelomonocytic leukemia by detecting frequent alterations in TET2, CBL, RAS, and RUNX1. *J Clin Oncol.* 2010;28(24):3858-3865.
7. Itzykson R, Kosmider O, Renneville A, et al. Prognostic score including gene mutations in chronic myelomonocytic leukemia. *J Clin Oncol.* 2013;31(19):2428-2436.
8. Niemeyer CM, Kang MW, Shin DH, et al. Germline CBL mutations cause developmental abnormalities and predispose to juvenile myelomonocytic leukemia. *Nat Genet.* 2010;42(9):794-800.
9. Martinelli S, De Luca A, Stellacci E, et al. Heterozygous germline mutations in the CBL tumor-suppressor gene cause a Noonan syndrome-like phenotype. *Am J Hum Genet.* 2010;87(2):250-257.
10. Pérez B, Mechinaud F, Galambrun C, et al. Germline mutations of the CBL gene define a new genetic syndrome with predisposition to juvenile myelomonocytic leukaemia. *J Med Genet.* 2010;47(10):686-691.
11. Weissmann S, Alpermann T, Grossmann V, et al. Landscape of TET2 mutations in acute myeloid leukemia. *Leukemia.* 2012;26(5):934-942.
12. Abbas S, Rotmans G, Löwenberg B, Valk PJ. Exon 8 splice site mutations in the gene encoding the E3-ligase CBL are associated with core binding factor acute myeloid leukemias. *Haematologica.* 2008;93(10):1595-1597.
13. Reindl C, Quentmeier H, Petropoulos K, et al. CBL exon 8/9 mutants activate the FLT3 pathway and cluster in core binding factor/11q deletion acute myeloid leukemia/myelodysplastic

syndrome subtypes. *Clin Cancer Res.* 2009;15(7):2238-2247.

14. Haferlach C, Dicker F, Kohlmann A, et al. AML with CBFB-MYH11 rearrangement demonstrate RAS pathway alterations in 92% of all cases including a high frequency of NF1 deletions. *Leukemia.* 2010;24(5):1065-1069.
15. Fröhling S, Skelin S, Liebisch C, et al. Comparison of cytogenetic and molecular cytogenetic detection of chromosome abnormalities in 240 consecutive adult patients with acute myeloid leukemia. *J Clin Oncol.* 2002;20(10):2480-2485.
16. Schnittger S, Weisser M, Schoch C, Hiddemann W, Haferlach T, Kern W. New score predicting for prognosis in PML-RARA+, AML1-ETO+, or CBFBMYH11+ acute myeloid leukemia based on quantification of fusion transcripts. *Blood.* 2003;102(8):2746-2755.
17. Döhner K, Schlenk RF, Habdank M, et al. Mutant nucleophosmin (NPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: interaction with other gene mutations. *Blood.* 2005;106(12):3740-3746.
18. Schnittger S, Bacher U, Alpermann T, et al. Use of CBL exon 8 and 9 mutations in diagnosis of myeloproliferative neoplasms and myelodysplastic/myeloproliferative disorders: an analysis of 636 cases. *Haematologica.* 2012;97(12):1890-1894.
19. Yoshida K, Sanada M, Shiraishi Y, et al. Frequent pathway mutations of splicing machinery in myelodysplasia. *Nature.* 2011;478(7367):64-69.
20. Forbes SA, Bindal N, Bamford S, et al. COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. *Nucleic Acids Res.* 2011;39(Database issue):D945-D950. http://www.sanger.ac.uk/cosmic. Accessed September 24, 2013.
21. Ding L, Ley TJ, Larson DE, et al. Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing. *Nature.* 2012;481(7382):506-510.
22. Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med.* 2013;368(22):2059-2074.
23. Emanuel PD, Bates LJ, Castleberry RP, Gualtieri RJ, Zuckerman KS. Selective hypersensitivity to granulocyte-macrophage colony-stimulating factor by juvenile chronic myeloid leukemia hematopoietic progenitors. *Blood.* 1991;77(5):925-929.
24. Strullu M, Caye A, Cassinat B, et al. In hematopoietic cells with a germline mutation of CBL, loss of heterozygosity is not a signature of juvenile myelo-monocytic leukemia. *Leukemia.* 2013;27(12):2404-2407.
25. Owen C, Barnett M, Fitzgibbon J. Familial myelodysplasia and acute myeloid leukaemia—a review. *Br J Haematol.* 2008;140(2):123-132.