Genotypic and phenotypic evolution in a patient with chronic myelomonocytic leukemia

Klaus Geisslera,b, Eva Jägerc, Michael Gurbisz

a Sigmund Freud University, Freudplatz 1, 1020 Vienna, Austria
b Department of Internal Medicine V with Hematology, Oncology and Palliative Medicine, Hospital Hietzing, Wolkersbergerstrasse 1, 1130 Vienna, Austria
c Department of Laboratory Medicine, Medical University of Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria

ARTICLE INFO

Keywords:
CMML
Genotype
Phenotype
Evolution

ABSTRACT

The correlation of molecular and phenotypic evolution in individual patients with chronic myelomonocytic leukemia (CMML) is poorly investigated. The longitudinal follow up of a CMML patient for more than 10 years illustrates that the emergence of clones harboring mutations in TET2, SRSF2, RUNX1, MPL, NRAS, and finally in multiple genes, respectively, was mirrored by thrombocytopenia, thrombocytosis, myeloproliferation and transformation into acute myeloid leukemia. Moreover, molecular aberrations of the RAS genes were associated with markedly increased spontaneous in vitro myeloid colony formation which has been shown to be a functional indicator of RAS pathway hyperactivation.

1. Introduction

Chronic myelomonocytic leukemia (CMML) is a hematopoietic malignancy of the elderly that is characterized by leukocytosis with monocyes and granulocytic cells in all stages of development, marked dysmeylopoiesis, a variable course, unresponsiveness to aggressive chemotherapy and an inherent risk of transformation to acute myeloid leukemia (AML) [1]. With regard to the presence of myeloproliferation CMML was originally subdivided into myeloproliferative disorder MP-CMML (WBC count >13 × 10⁹/L) versus myelodysplastic syndrome MD-CMML (WBC count ≤13 × 10⁹/L) by the FAB criteria. Since CMML is characterized by features of both a MDS and a myeloproliferative neoplasm (MPN) the World Health Organization (WHO) classification of 2000 assigned CMML to the mixed category MDS/MPN. In a large series of 1832 patients captured in the international CMML database that merged CMML registries from 8 tertiary cancer centers across 3 different countries between July 1981 and June 2014 the median age at diagnosis was 70 (16–93 years), with a male predominance (67%) [2]. The median overall survival of CMML patients is about 30 months, one third evolving to AML while the others die from the consequences of cytopenias or comorbidities.

CMML is a disease with significant molecular and phenotypic heterogeneity. Molecular abnormalities are seen in >90% of patients with CMML [1]. Many of these alterations, however, affect the same pathway and tend to not co-occur in the same patient. Thus, a large number of gene mutations in genes encoding epigenetic regulators (TET2, ASXL1, EZH2, UTX, IDH1, IDH2, DNMT3A), splicing factors (SF3B1, SRSF2, ZRSF2, U2AF1), and signaling molecules (NRAS, KRAS, CBL, JAK2, FLT3) have been found. Regarding clinical characteristics CMML patients with a MDS phenotype tend to present with peripheral blood cytopenias, effort intolerance, easy bruising, recurrent infections, and transfusion dependence. Patients with a MPN phenotype tend to present with leukocytosis, monocytes, hepatomegaly, splenomegaly, and features of myeloproliferation, such as fatigue, night sweats, symptoms of organomegaly, bone pains, weight loss and cachexia [1].

The clonal evolution of CMML has been extensively studied on a molecular level [3]. Moreover an association between certain clonal abnormalities and characteristic phenotypes has been described [3]. The association of molecular and phenotypic changes in individual patients during longitudinal follow up is poorly investigated.

2. Methods

Next Generation Sequencing (NGS) was performed using a capture-based target enrichment Kit (Myeloid Solution by SOPHIA GENETICS) containing 30 CMML-associated genes (Supplementary Table 1). Genomic DNA was isolated and purified from mononuclear cell fractions of peripheral blood (PB) or bone marrow (BM) samples using DSP DNA Midi Kit and QiAsymphony instrument. Variant annotation and interpretation was performed using the following databases and
During the next months the WBC count further increased, paralleled by an increased blast cell percentage and an increase in the VAF of mutated NRAS. From August 2016 on the patient was treated with azacitidine and hydroxyurea for cytoreduction. In 2017 when the patient transformed into secondary AML four additional molecular aberrations were found. In March 2017 the patient was treated with decitabine but died in May 2017 due to progressing disease.

4. Discussion

The phenotype of mild neutropenia and thrombocytopenia early in the course of disease may be considered as manifestation of oligomono-cytic CMML [5] which is supported by the mutations in the TET2 gene and the SRSF2 gene, respectively, which are common molecular findings in patients with CMML [1]. A mutation in the RUNX1 gene was also found which has to be considered as germline variant since the same variant was found in cells of the hair follicles raising the possibility that we retrospectively detected a myeloid neoplasm with germline predisposition and pre-existing platelet disorder. Familial platelet disorder with predisposition to AML is an autosomal dominant syndrome characterized by abnormalities in platelet number and function and enhanced risk of developing MDS/AML at a young age [6]. Moreover, it has been reported that CMML can develop on a background of germline mutations in RUNX1 [6]. Unfortunately, due to the lack of mutational data and clinical data of parents and siblings we cannot directly show the association between the RUNX1 mutation and a familial platelet disorder in this patient. However, the following findings provide some indirect evidence that our patient may belong to this category: the patient had some unexplained bleeding tendency in his history, he developed CMML at the age of 55 years which is earlier than in the majority of other CMML patients who have a median age around 70 years at diagnosis, and the same RUNX1 variant which was detected in our patient has been reported in a family with familial platelet disorder with associated myeloid malignancy (https://www.ncbi.nlm.nih.gov/clinvar/).

During the course of disease the thrombocytopenia improved without any therapeutic intervention, and, moreover, platelet counts even increased to supernormal values. This phenotypic change may be easily explained by a mutation in the MPL gene which can be found in 5% of patients with essential thrombocytopenia but, to the best of our knowledge, has not been described in patients with CMML so far.

The increase in the WBC counts which was observed in 2016 for the

| Table 1 | Correlation between genotypic and phenotypic evolution of chronic myelomonocytic leukemia in a 55 years old male patient. |
|---------|---------------------------------------------------------------------------------------------------------------------|
| Date    | Parameters | Genotypic evolution | Aug/2016 | Mar/2017 | Normal values |
|---------|-------------|---------------------|----------|----------|--------------|
| Feb/2006| TET2%VAF    | 44.58               | 38.50    | 46.72    | 45.54        | 46.15        | <5.0        |
|         | TET2%VAF    | 39.06               | 39.39    | 48.72    | 49.48        | 49.19        | <5.0        |
|         | SRSF2%VAF   | 37.44               | 39.33    | 44.28    | 48.74        | 49.38        | <5.0        |
|         | RUNX1%VAF   | 46.83               | 48.69    | 47.77    | 48.98        | 48.60        | <5.0        |
|         | MPL%VAF     | <5.0                | 40.93    | 48.58    | 48.25        | 50.50        | <5.0        |
|         | NRAS%VAF    | <5.0                | <5.0     | 19.09    | 40.58        | 11.87        | <5.0        |
|         | IDH2%VAF    | <5.0                | <5.0     | <5.0     | 35.42        | <5.0        |
|         | KRAS%VAF    | <5.0                | <5.0     | <5.0     | 34.24        | <5.0        |
|         | RUNX1%VAF   | <5.0                | <5.0     | <5.0     | 36.94        | <5.0        |
|         | TET2%VAF    | <5.0                | <5.0     | <5.0     | 35.31        | <5.0        |

| Phenotypic evolution | WBC G/L | Hb g/dL | PLT g/L | Granulocytes% | Monocytes% | Blasts% | Spon. CFU-GM/10^5 PBMCN |
|----------------------|---------|---------|---------|---------------|------------|--------|-----------------------|
|                      | 3.1     | 8.5     | 14.3    | 37            | 18         | 0      | ND                    |
|                      | 11.2    | 47      | 105     | 52            | 16         | 0      | 8                     |
|                      | 2.6     | 11      | 296     | 52            | 11         | 10     | 170                   |
|                      | 4.4–11.3| 14.0–17.5| 150–400 | 50–70         | 2–9        | 0–0    | 3.5–8.5               |

Abbreviations: VAF, variant allele frequency; WBC, white blood cell count; Hb, hemoglobin; PLT, platelet count; CFU-GM, colony-forming unit-granulocyte-macrophage; PBMCN, peripheral blood mononuclear cells.
first time was paralleled by a mutation in the NRAS gene. A correlation between myeloproliferation and mutations in signaling genes such as NRAS has been demonstrated previously [1]. The development of granulomonocytic hyperplasia as it has been seen in our patient concomitant with the emergence of a mutant NRAS clone has long been thought to result from hypersensitivity to GM-CSF [3]. We have originally reported that extensive in vitro formation of CFU-GM without exogenous growth factors can be found in a subset of patients with CMML. Later we have shown that spontaneous CFU-GM formation in CMML is a GM-CSF related in vitro phenomenon [4], and most importantly, GM-CSF hypersensitivity of CMML progenitors has been demonstrated by Padron et al. [8]. Recently, we were able to demonstrate a close correlation between high spontaneous colony formation in CMML patients and the presence of RAS pathway mutations [9]. Altogether these findings strongly suggest, that high spontaneous in vitro CFU-GM formation in CMML is a functional indicator of RAS pathway hyperactivation. In our patient we were able to perform sequential in vitro studies during follow up. We can demonstrate a gradual increase of spontaneously formed in vitro CFU-GM which was paralleled by an increase in VAF of the NRAS gene mutation.

At the final stage of the disease the increase in PB blasts indicated transformation into secondary AML. Aberrations in multiple genes were found at that time. An increase in the number of molecular alterations during the late stages of CMML has been previously reported [10]. Mechanistically, NRAS mutations have been shown to promote oxidative damage which may lead to genomic instability which may be causally linked to the emergence of multiple clones at the terminal stage of CMML [3].

5. Conclusion

In conclusion we can show in a CMML patient with long follow up that the emergence of clones harboring mutations in TET2, SRSF2, RUNX1, MPL, NRAS, and finally in multiple genes, respectively, were mirrored by thrombocytopenia, thrombocytosis, myeloproliferation and transformation into AML. Moreover, an increased activity of the RAS pathway could be shown not only at a molecular but also at a functional level. Current therapies in CMML aim to improve symptom burden using personalized treatment strategies. Our results show that phenotypic changes in patients with CMML may indicate changes at the molecular level which may be clinically relevant for these treatment concepts.

Declaration of Competing Interest

None.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.lrr.2019.100185.

References

[1] M.M. Patnaik, A. Tefferi, Chronic myelomonocytic leukemia: 2016 update on diagnosis, risk stratification, and management, Am. J. Hematol. 91 (6) (2016) 631–642, https://doi.org/10.1002/ajh.24396 Review. PMID:27185207.
[2] E. Padron, G. Garcia-Manero, M.M. Patnaik, R. Itzykson, T. Lasho, A. Nazha, R.K. Rampal, M.E. Sanchez, E. Jabbour, N.H. Al Ali, Z. Thompson, S. Colla, P. Fenaux, H.M. Kantarjian, S. Killick, M.A. Sekeres, A.F. List, F. Ondoa, R.S. Komrokji, A. Tefferi, E. Solary, An international data set for CMML validates prognostic scoring systems and demonstrates a need for novel prognostication strategies, Blood Cancer J. 5 (2015) e333, https://doi.org/10.1038/bcj.2015.53.
[3] R. Izyriksen, E. Solary, An evolutionary perspective on chronic myelomonocytic leukemia, Leukemia 27 (7) (2013) 1441–1450, https://doi.org/10.1038/leu.2013.100 Epub 2013 Apr 8. Review. PMID:23558522.
[4] K. Geissler, L. Ohler, M. Födinger, I. Virgolini, M. Leimer, E. Kabrna, M. Kollars, S. Skospy, B. Bohle, M. Rogy, K. Lechner, Interleukin 10 inhibits growth and granulocyte/macrophage colony-stimulating factor production in chronic myelomonocytic leukemia cells, J. Exp. Med. 184 (4) (1996) 1377–1384 PMID:8879210.
[5] P. Valent, A. Orazi, M.R. Savona, M.M. Patnaik, F. Ondoa, A.A. van de Loosdrecht, D. Haase, T. Haferlach, C. Lena, L. Pleyer, W. Kern, T. Pemovska, G.I. Vladimier, J. Schanz, A. Keller, M. Lubbert, T. Lion, K. Sodar, A. Reiter, T. De Witte, M. Pfeilstocker, K. Geissler, E. Padron, M. Deininger, A. Orfao, H.P. Hoeny, P.L. Greenberg, D.A. Arber, L. Malcovati, J.M. Bennett, Proposed diagnostic criteria for classical CMML, CMML variants and pre-CMML conditions, Haematologica (2019), https://doi.org/10.3324/haematol.2019.222059 pii: haematol.2019.222059 [Epub ahead of print] PMID:31048353.
[6] N. Shiba, D. Hasegawa, M.J. Park, C. Murata, A. Sato-Otsuho, C. Ogawa, M. Manabe, H. Arakawa, S. Ogawa, Y. Hayashi, CBL mutation in chronic myelomonocytic leukemia secondary to familial platelet disorder with propensity to develop acute myeloid leukemia (FPD/AML), Blood 119 (11) (2012) 2612–2614, https://doi.org/10.1182/blood-2011-02-333435 Epub 2011 Dec 2. PMID:22138511.
[7] K. Geissler, W. Hinterberger, P. Bettelheim, O. Haas, K. Lechner, Colony growth characteristics in chronic myelomonocytic leukemia, Leuk. Res. 12 (5) (1988) 373–377.
[8] E. Padron, J.S. Painter, S. Kunigal, A.W. Mailoux, K. McGraw, J.M. McDaniel, E. Kim, C. Bebbingman, M. Baer, G. Yarrast, J. Lanetz, R.S. Komrokji, O. Abdel-Wahab, A.F. List, P.K. Epling-Burnette, GM-CSF-dependent pSTAT5 sensitivity is a feature with therapeutic potential in chronic myelomonocytic leukemia, Blood 121 (25) (2013) 5068–5077, https://doi.org/10.1182/blood-2012-10-461070 Epub 2013 Apr 30. PMID:23632889.
[9] K. Geissler, E. Jäger, A. Barna, T. Alendar, E. Ljubuncic, T. Slivova, P. Valentin, Chronic myelomonocytic leukemia patients with RAS pathway mutations show high in vitro myeloid colony formation in the absence of exogenous growth factors, Leukemia 30 (11) (2016) 2280–2281, https://doi.org/10.1038/leu.2016.235 Epub 2016 Aug 18. No abstract available. PMID:27595952.
[10] C.C. Mason, J.S. Khorashad, S.K. Tantravahi, T.W. Kelley, M.S. Zabriskie, D. Yan, A.D. Pomicter, K.R. Reynolds, A.M. Eiring, Z. Kronenberg, R.L. Sherman, J.W. Tyner, B.K. Dalley, K.H. Dao, M. Yandell, J. Gotlib, T. O’Hare, J. Schanz, A. Keller, M. Lubbert, T. Lion, K. Sodar, A. Reiter, T. De Witte, M. Pfeilstocker, K. Geissler, E. Padron, M. Deininger, A. Orfao, H.P. Horns, Jr., K. Sotlar, A. Reiter, T. De Witte, M. Pfeilstocker, K. Geissler, E. Padron, M. Deininger, A. Orfao, H.P. Horny, P.L. Greenberg, D.A. Arber, L. Malcovati, J.M. Bennett, Proposed diagnostic criteria for classical CMML, CMML variants and pre-CMML conditions, Haematologica (2019), https://doi.org/10.3324/haematol.2019.222059 pii: haematol.2019.222059 [Epub ahead of print] PMID:31048353.