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Genotypic and phenotypic evolution in a patient with chronic myelomonocytic leukemia

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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 monocytes and granulocytic cells in all stages of development, marked dysmyelopoiesis, 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 $>13 \times 10^9$/L) versus myelodysplastic syndrome MD-CMML (WBC count $\leq 13 \times 10^9$/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, monocytosis, 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

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software tools: SOPHiA DDM, ExAC (Exome Aggregation Consortium), G1000 (1000 genomes), ESP (Exome Sequencing Project), COSMIC (*C*atalogue *o*f *S*omatic *M*utations *i*n *C*ancer), ClinVar, dbSNP, CG69, dbNSFP (database of human nonsynonymous SNPs and their functional predictions), GnomAD (Genome Aggregation Database). In case of conflicting results for the pathogenicity of a variant, the underlying data were manually rechecked. Variants were considered (likely) benign unless they satisfied all of the following conditions: the mutation occurred in a protein coding region, the mutation function was not synonymous, the annotation from ClinVar was not benign, and the change was not found at a frequency of 1% or higher in a population. Clearly pathogenic variants and variants of unknown significance were retained as potential mutations. A VAF of 5% or higher by a minimal depth of 1000 x was considered as positive for analysis. Colony-forming unit-granulocyte-macrophage (CFU-GM) growth was assessed in semi-solid cultures without growth factors as previously described [4].

3. Case report

Here we report a 55 years old male patient with CMML in whom the genotypic and phenotypic evolution of the disease could be reconstructed due to the fact, that molecular aberrations have been retrospectively analyzed by NGS from stored cell samples (Table 1, supplementary material). Because of thrombocytopenia and leukopenia the patient had BM puncture in 2006. At that time the patient had 18% monocytes in PB but otherwise no significant morphologic abnormality in BM. NGS showed somatic mutations in *TET2* and *SRSF2* and an additional abnormality in *RUNX1* which we could also detect in the hair follicles of the patient indicating a germline alteration. According to these results and the diagnostic criteria which have been proposed recently [5] the patient would be retrospectively classified as oligomonocytic CMML, which is defined by persistent (3 months) peripheral blood monocytosis of 0.5–0.9 $\times$ 10^9/L plus relative monocytosis of $>$10% of circulating PB leukocytes. Due to the low risk situation of CMML no specific treatment has been recommended in 2006. Surprisingly in 2011 the platelet count increased to supernormal values without clinical explanation. Interestingly, the thrombocytosis at that time was accompanied by the emergence of a *MPL* clone. In 2016 thrombocytosis disappeared and the WBC count continuously increased. At that time a mutation in *NRAS* was detected for the first time. 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 oligomonocytic 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 thrombocythemia 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	Feb/2006	Feb/2011	Feb/2016 Genotypic evolution	Aug/2016	Mar/2017	Normal values
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	<5.0	35.42	<5.0
KRAS%VAF	<5.0	<5.0	<5.0	<5.0	34.24	<5.0
RUNX1%VAF	<5.0	<5.0	<5.0	<5.0	36.94	<5.0
U2AF1%VAF	<5.0	<5.0	<5.0	<5.0	35.31	<5.0
		Phenotypic evolution				
WBC G/L	3.1	8.5	16.6	24.6	19.3	4.4–11.3
Hb g/dL	14.3	10.5	11.2	10.2	10.9	14.0–17.5
PLT G/L	130	574	296	351	273	150–400
Granulocytes%	37	43	52	52	21	50–70
Monocytes%	18	47	16	11	4	2–9
Blasts%	0	0	1	10	66	0–0
Spont. CFU-GM/10^5 PBMNC	ND	8	170	381	116	3.5–8.5

Abbrevations: VAF, variant allele frequency; WBC, white blood cell count; Hb, hemoglobin; PLT, platelet count; CFU-GM, colony-forming unit-granulocyte-macrophage; PBMNC, 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 [7]. 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.

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