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CASE REPORT

Natural history of chronic myelomonocytic leukemia: gene sequencing identifies multiple clonal molecular abnormalities associated with rapid progression to acute myeloid leukemia

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Key Clinical Message

Gene panel sequencing in a CMML patient without any detectable genetic abnormality by conventional genetic studies identified four concurrent somatic mutations in three genes. Gene panel mutation analysis is a rapidly emerging clinical tool to demonstrate the clonality in hematologic malignancies, and to identify the potential targets for therapy.

Keywords

Acute myeloid leukemia, chronic myelomonocytic leukemia, clonality, gene sequencing, genetic abnormality.

Introduction

The patient was a 58-year-old white male who was referred to our hematology clinic in January 2013 for a 3-months history of worsening anemia and monocytosis. Complete blood count (CBC) on the day of his first hematology visit showed WBC 15.1 × 10^9/L, 65% neutrophils, 21% monoctyes (absolute monocyte count 3171/μL), 14% lymphocyte and no eosinophils or basophils (Fig. 1). Hemoglobin was 7.0 g/dL, hematocrit 20.9%, mean corpuscular volume (MCV) was 121.4 and platelet count was 209 × 10^9/L. There were no signs of dysplasia and no circulating blasts on peripheral blood (PB) smear. Patient was asymptomatic. On general physical examination, he was pale but physical examination was otherwise unremarkable. Computed tomography (CT) scan of his chest, abdomen and pelvis did not show any lymphadenopathy, hepatosplenomegaly or any other abnormality.

A differential diagnosis of myelodysplastic/myeloproliferative neoplasm (specifically CMML), myeloproliferative neoplasm (specifically CML), and reactive monocytosis was considered. Bone marrow (BM) aspiration and biopsy done in March, 2013 (Fig. 2B and C) showed 75% cellularity with granulocytic and monocytic hyperplasia, decreased erythropoiesis, and decreased numbers of megakaryocytes. The myeloid to erythroid (M:E) ratio was 8:1. There were less than 3% blasts and smears were negative for eosinophilia or basophilia. Flow cytometry of the BM cells showed approximately 1% blasts expressing CD45, CD13, CD33, CD117, and CD34, and a population of approximately 12% monocytes expressing CD14. Cytogenetic analysis showed a normal male karyotype. Fluorescence in situ Hybridization (FISH) studies for *BCR-ABL*, *PDGFRA* (Platelet-Derived Growth Factor Receptor Alpha) and *PDGFRB* (PDGFR Beta), *FGFR1* (fibroblast growth factor receptor 1) and MDS (myelodysplastic

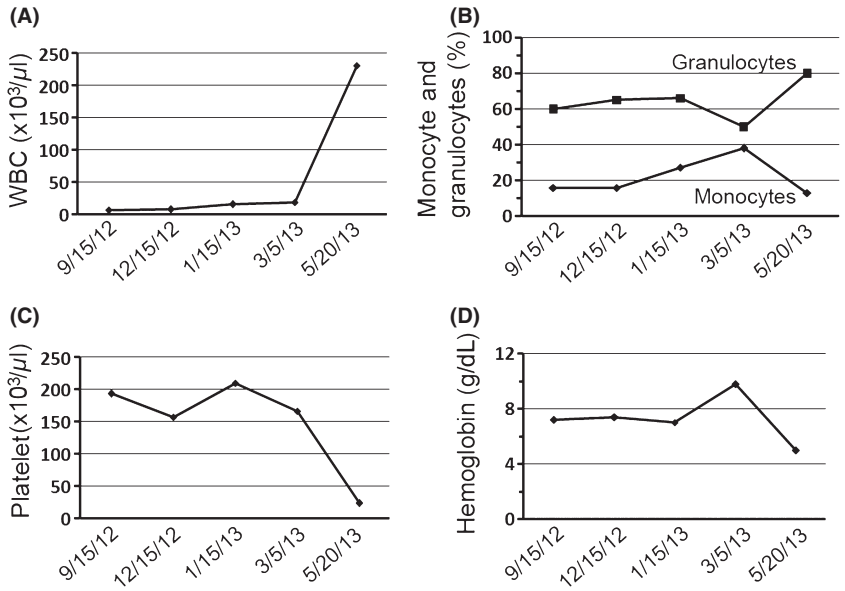

Figure 1. Patient's peripheral blood parameters over an 8-month time period prior to and after first hematology evaluation in January 2013. (A) White blood cell (WBC); (B) Monocytes and granulocytes percentages; (C) Platelet counts; (D) Hemoglobin.

syndrome) panel (5q−, 7q−, +8 and 20q) were all negative. The patient's CBC parameters on the day of BM biopsy were hemoglobin 9.8 g/dL, MCV 101.5, platelets 166 × 10^9/L, WBC 18.0 × 10^9/L with 43% neutrophils, 12% lymphocytes, 38% monocytes, and 7% myelocytes. The reticulocyte count was 0.29%.

CMML is a clonal hematopoietic malignancy characterized by dysplasia, peripheral monocytosis, ineffective hematopoiesis, and an increased risk of transformation to acute myeloid leukemia (AML). CMML represents ~10% of all MDS cases and median age at diagnosis varies between 65 and 75 years. In the 2008 World Health Organization (WHO) classification of hematologic neoplasms [1], CMML was assigned to a new category MDS/MPN disorders which includes CMML, atypical chronic myeloid leukemia (aCML) *BCR-ABL* negative, juvenile myelomonocytic leukemia (JMML), and refractory anemia with ring sideroblasts and thrombocytosis (RARS-T). Some cases of CMML with eosinophilia were relocated to the category "myeloid/lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA, PDGFRB or FGFR1*." Based on the percentage of blasts plus promonocytes in the PB and BM, CMML was subcategorized into 2 groups: CMML-1 (<5% blasts plus promonocytes in the PB and <10% blasts plus promonocytes in the BM) and CMML-2 (5% or more PB blasts plus promonocytes or 10% or more BM blasts plus promonocytes).

The diagnostic approach for CMML has been recently described [2]. CMML should be suspected if a patient has a persistent PB monocyte count greater than 1.0 × 10^9/L, dysplasia in at least one hematopoietic lineage, and less than 20% myeloblasts and promonocytes in PB and BM. If myelodysplasia is minimal or absent, CMML can still be diagnosed if an acquired, clonal cytogenetic or molecular cytogenetic abnormality is demonstrated in the hematopoietic stem cell, or if monocytosis has persisted for greater than 3 months, and all other causes have been excluded. The overall survival in CMML is poor with a 3-years survival rate of approximately 20% [3].

The diagnostic studies of our patient suggested CMML based on his persistent PB monocytosis, despite lack of dysplasia in all three lineages and lack of detectible clonal cytogenetic or molecular abnormality by conventional genetic studies. There were no secondary conditions by laboratory studies or CT scan to explain persistent monocytosis. The patient failed to return for follow up until 20 May 2013 when he presented to the hospital with confusion and seizure. His CBC showed WBC 230 × 10^9/L with marked neutrophilia (Fig. 1), and dysplastic granulocytes, myelocytes, and monocytes, WBC differential was: 42% granulocytes, 17% bands, 1% promyelocytes, 10% metamyelocytes, 11% myelocytes, 13% monocytes and less than 1% blasts. Hematocrit was 14% and platelet count was 29 × 10^9/L. Cytogenetic and molecular evaluation of PB showed normal male karyotype, no abnormalities on FISH studies for *PDGRFA, PDGFRB, FGFR1, BCR-ABL* and MDS panel, and no JAK2 V617F and exon 12 mutations analyzed by PCR. There were no genomic gains or losses by the whole genome SNP microarray.

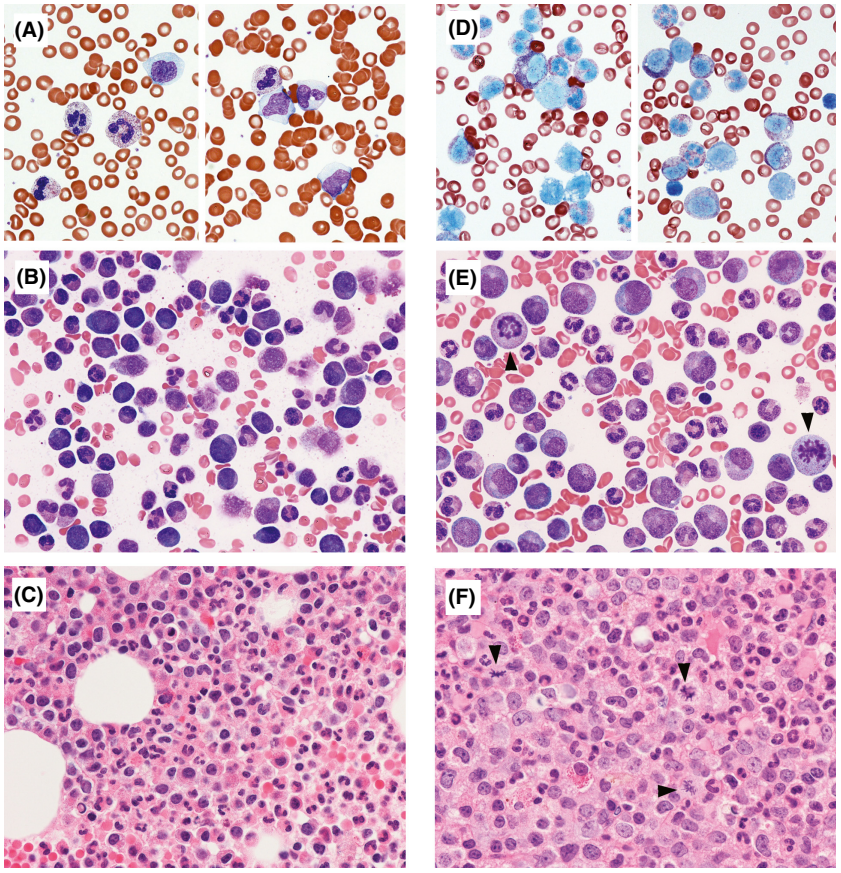

Figure 2. Peripheral blood smears and bone marrow (BM) biopsies. (A) PB smear on 5 March 2013 shows leukocytosis and monocytosis with mature monocytes. B and C BM aspirate (B) and biopsy (C) on 5 March 2013 shows hypercellular marrow with granulocytic and monocytic hyperplasia with no increase in blasts. (D) PB smear on 20 May 2013 shows marked leukocytosis with dysplastic granulocytes, monocytes and myelocytes. E and F. BM aspirate (E) and biopsy (F) on 20 May 2013 shows 100% cellularity with groups of blasts and multiple mitotic figures (arrow head).

At this point, the differential diagnosis of *BCR-ABL* negative aCML and chronic neutrophilic leukemia (CNL) were also entertained. Mutation analysis in *CSF3R* and *SETBP1*, recently reported recurrent mutations in CNL and aCML [4–6], were negative (kindly analyzed by JW Tyner's laboratory, Knight Cancer Institute, Oregon Health & Science University, Portland, OR).

BCR-ABL negative aCML is a rare leukemic disorder with myelodysplastic and myeloproliferative features characterized by leukocytosis with dysplastic neutrophils and their precursors. Clinical features of aCML include anemia, thrombocytopenia, and splenomegaly. Median WBC count ranges between 24 and 96 $\times$ 10^9/L, although some patients may have extremely high WBC count of >300 $\times$ 10^9/L. Blasts are usually <5%, but should be always <20% in PB and BM. Immature granulocytes (promyelocyte, myelocytes, and metamyelocytes) usually range between 10% and 20%. Monocytes can be increased, but are usually less than 10%. Basophilia may be present but is not prominent. Bone marrow examination should always show hypercellular marrow with myeloid hyperplasia and prominent dysgranulopoiesis; although multilineage dysplasia may be present, the pathological features such as hypogranular and hypolobated neutrophils, abnormal chromatin clumping, pseudo-Pelger–Hüet neutrophils may be observed. Megakaryocytes may be decreased, normal or increased in number with dysplastic features in most cases. Dyserythropoiesis is present in at at least 50% of cases. Karyotype abnormalities are common including trisomy 8, del(20q), and other abnormalities of chromosomes 12,13, 14, 17, and 19. Leukemic cells are negative for *BCR-ABL, PDGFRA*, and *PDGFRB*. Approximately 30% of cases are positive for *NRAS* or *KRAS* mutations. JAK2 V617F mutation has been reported in some cases. Most recent studies revealed recurrent *CSF3R* mutations in 40% of aCML cases [5, 6] and *SETBP1* mutations in 25% of cases [4, 6]. *CSF3R* and *SETBP1* are not mutually exclusive.

CNL is a rare myeloproliferative neoplasm characterized by sustained PB neutrophilia, hypercellular marrow due to neutrophilic granulocyte proliferation and hepatosplenomegaly. According to the 2008 WHO diagnostic criteria for CNL [1], leukocytosis is $\geq 25 \times 10^9$/L with >80% segmented neutrophils/band forms, <10% are immature granulocytes and <1% blasts. Granulocytic dysplasia is not present, and there is no monocytosis, eosinophilia or basophilia. Hypercellular marrow should always be observed with myeloid hyperplasia and full maturation and there should be <5% myeloblasts in BM with <1% blasts in the PB. Megakaryocytes are typically normal, but small hypolobated megakaryocytes can be present. Reticulin fibrosis is not significantly increased. Karyotype is usually normal. There are no *BCR-ABL, PDGFRA, PDGFRB*, and *FGFR1* abnormalities. Recent study has shown 89% of CNL patients harbor recurrent mutations in *CSF3R* gene [5, 6]. The differential diagnosis includes reactive neutrophilia, other MPNs, and MDS or overlapping MDS/MPN disorders.

The patient was started on hydroxyurea. Repeat BM aspiration and biopsy in May 2013 showed 100% cellularity (Fig. 2E and F). Although <1% blasts were identified by flow cytometry, multiple areas of the biopsy showed large groups and sheets of immature mononuclear cells with visible nucleoli consistent with blast morphology (Fig. 2E and F). Multiple mitotic figures were noted within these areas. Other regions of the biopsy showed granulocytic hyperplasia with abundant numbers of mature neutrophils and band forms. There was little erythropoiesis or megakaryocytopoiesis. These findings indicated transformation to AML. However, repeat cytogenetic and molecular studies were all negative. We sought to identify the molecular abnormalities by using genome sequencing. The patient's DNA was sent to Foundation Medicine (Foundation Medicine, Inc., One Kendall Square Ste B3501, Cambridge, MA) and a panel of 323 genes and 58 introns of 24 genes involved in rearrangement were sequenced (gene list is available upon request). This gene panel was designed to include all genes known to be somatically altered in human hematologic malignancies that are validated targets for therapy, either approved or in clinical trials, and/or that are unambiguous drivers of oncogenesis based on current knowledge. Four genomic alterations were identified including NRAS G12D and Q61K, ASXL1 G804 fs*14 and U2AF1 S34F. The patient declined therapy and died at home 4 weeks later.

Discussion

The clinical, hematological, and morphologic features of CMML are heterogeneous. Unlike CML, the diagnosis of CMML is based on clinical, morphologic, and histopathologic features after exclusion of other diseases that cause reactive monocytosis. Clonal cytogenetic abnormalities, including +8, −7/del(7q) and structural abnormalities of 12p have been reported in 20–40% of CMML cases. Using next-generation sequencing, somatic mutations can be detected in most CMML patients and numerous abnormalities have been recognized recently [7, 8]. Unfortunately, none of these abnormalities are pathognomonic for CMML.

Our patient had a rapid progression from CMML to AML. Despite a marked increase in granulocyte lineage with little change in monocyte counts, or circulating blasts, the BM, however, did show large groups of cells with blast morphology (Fig. 2E and F). Conventional cytogenetic, FISH and molecular studies, including whole genome SNP microarray, did not show any clonal genetic abnormalities; however, gene sequencing identified 4 mutations including 2 point mutations in *NRAS*, a frame shift mutation in *ASXL1*, and a point mutation in *U2AF1*.

RAS is the most common mutated oncogene in human cancer. In CMML, up to 40% of patients harbor *NRAS* mutations. Residues G12, G13 and Q61 of *RAS* genes are the most common targets for somatic mutations. Mutation of Q61 impairs GTP hydrolysis, whereas mutations in residues G12 and G13 prevent the formation of van der Waal bonds between RAS and GAP (GTPase-activating protein). These mutations perturb the proper orientation of the catalytic glutamine (Q61) in RAS, which results in the pronounced attenuation of GTP hydrolysis [9]. The consequence of these mutations is the persistence of the GTP-bound state of RAS and continuous activation of a multitude of RAS-dependent downstream effector pathways such as the RAF/MAPK/ERK, PI3K, and other pathways [9, 10]. Animal studies have shown that oncogenic *NRAS* can promote aberrant GM-CSF signaling in granulocytic/monocytic precursors [11], promote leukemogenesis by aberrantly regulating hematopoietic stem cell functions [12], and rapidly and efficiently induce CMML- and AML-like diseases in mice [13]. Clinical studies have shown that the *NRAS* mutation is frequently associated with the progression of MDS to AML [14, 15]. Concomitant mutations of G12, G13 and Q61 have been reported but are uncommon [14, 16]. Our patient had concomitant mutations in residues G13 and Q61. We speculate that these combined mutations may have a more potent oncogenic effect that could have led to the rapid disease progression that we observed.

Two additional mutations found in this patient are ASXL1 G804fs*14 and U2AF1 S34F. *ASXL1* is an epigenetic modifier and transcription regulator through interaction with Polycomb complex proteins and various

transcription activators and repressors [17–19]. The ASXL1 G804fs*14 mutation is predicted to truncate the ASXL1 protein prior to the PHD domain; similar truncations have been reported to be inactivating suggesting that this mutation is a loss-of-function mutation. Inactivating somatic mutations of *ASXL1* have been reported in a range of myeloproliferative disorders (MPNs/MDSs), and it is especially frequent in myelofibrosis (20%) and CMML (43%), particularly the myeloproliferative form CMML (63%) [20, 21]. Most importantly, mutations in *ASXL1* correlated with acute transformation of CMML to AML and with unfavorable outcome in myeloid diseases, including AML, MPN, and MDS [20]. In vitro studies have shown that *ASXL1* mutations promotes myeloid transformation through loss of Polycomb repressive complex 2 (PRC2)-mediated gene repression, and collaborates with $NRAS^{G12D}$ to promote myeloid leukemogenesis in vivo [19].

In MDS, U2AF1 mutations tend to associate with *ASXL1* mutations and increase the probability of progression to AML [22]. *U2AF1* encodes a pre-mRNA splicing factor required for accurate 3′ splice site selection [23, 24]. The S34F mutation is located in a zinc finger domain and has been shown to induce splicing alterations in cell-based in vitro assays [23, 25]. *U2AF1* mutations have been reported in 10% of CMML, 6–20% of MDS, 3% of AML, and 10% of AML associated with MDS.

Advances in genomic sequencing technology are revolutionizing our understanding of CMML and have identified an array of common mutations in genes encoding signaling, epigenetic, transcription, and splicing factors. Although none of these mutations is specific for CMML, the high frequency of these mutations suggests a distinct genetic identity for CMML [7, 8]. The most recent study by Itzykson et al. has found that *ASXL1* mutation status is an independent prognostic factor for CMML [8]. A new CMML-specific prognostic scoring system that includes *ASXL1* mutation status and clinical parameters was proposed which seems more discriminative than those systems based solely on clinical parameters [8]. However, the prognostic value of multiple co-existing mutations is unknown. For example, our patient had four mutations involving *NRAS*, *ASXL1*, and *U2AF* and each of these mutations has been reported to be associated with negative outcome [8, 14, 15, 22]. More comprehensive genomic analysis is needed to identify the true "driver" mutations for CMML, to identify mutations that carry diagnostic and prognostic information, and to identify potential therapeutic targets.

In this unfortunate patient, we were able to observe the natural history of CMML, and by using gene sequencing, to identify the molecular abnormalities which may be responsible for the rapid progression and transformation to AML. The routine clinical utilization of gene sequencing in CMML needs to be further studied and defined.

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