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Clonal evolution revealed by next-generation sequencing in a long-term follow-up patient with hypereosinophilia

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ABSTRACT

The natural history of primary hypereosinophilia remains poorly defined, given the underlying disease heterogeneity. Recently, targeted NGS helps to establish clonality in a subset of patients with hypereosinophilia. We first reported the clonal evolution in a long-term follow-up patient with hypereosinophilia. This case initially presented with chronic eosinophilic leukemia, not otherwise specified (CEL-NOS), successively transformed to myelodysplastic syndromes (MDS) and acute myeloid leukemia(s-AML). We identified three mutations at CEL-NOS phase, five and seven mutations at MDS and s-AML stages, respectively. Our data illustrate the clonal dynamic process associated with disease evolution from CEL-NOS to s-AML.

1. Introduction

Hypereosinophilia is observed in a range of secondary and clonal disorders. Clonal hierarchy in myeloproliferative neoplasms (MPNs), particularly in the context of disease progression, is not well defined. While next generation sequencing (NGS) studies can generate substantial dividends in dissecting the genetic basis of myeloid neoplasms with eosinophilia and suggest possible clonal populations [1,2]. Recurrent mutations in several genes (e.g. RUNX1, ASXL1, JAK2, U2AF1, IDH1,TET2) have also recently been identified in MPNs [2,3]. Many of these mutations have been identified in chronic as well as blast phase MPNs [3]. The specific role of these genes in disease initiation and/or progression remains incompletely understood. Here, we report the clonal evolution revealed by NGS in a long-term follow-up patient with hypereosinophilia.

2. Case report

A 59-year-old male was admitted to our clinic with a 7-month history of unexplained eosinophilia ($>1.5 \times 10^9$/L) and three-week history of muscle pain in September, 2009. The spleen was palpable 8 cm below the left rib cage. The complete blood count showed hemoglobin levels of 147 g/L, 362 $\times 10^9$/L platelets, and 22.7 $\times 10^9$/L leukocytes, with 36.7% eosinophils. A high lactate dehydrogenase was observed (493.5 U/L; normal, $<$240 U/L). An extensive workup for malignancy, allergies, parasitic, and autoimmune disease were all negative. Bone marrow (BM) specimen showed a hypercellular marrow with hypereosinophilia (26.0%) (Fig. 1A). No increase in blasts was detected. Cytogenetic analysis revealed the karyotype 46, XY in 20/20 of the metaphases examined. Fluorescence in situ hybridization analysis (FISH) showed no signals corresponding to BCR/ABL1gene fusion and myelodysplastic syndromes(MDS) markers [−5/del(5q), −7/del(7q), +8 and del(20q)]. FISH was also negative for rearrangements of PDGFRA, PDGFRB, FGFR1 and CBFB. Because of the lack of genetic mutations, the patient met the clinical criteria for idiopathic hypereosinophilic syndrome (HES) according to established diagnostic guidelines for eosinophilia in 2008 World Health Organization [4] and was treated with predinisone, which led to an improvement in eosinophils, and clinical symptoms.

Three years later, the man was examined for abdominal pain and confirmed to have pylethrombosis by computed tomograph. He was treated with thrombolytic therapy successfully. From then on, we managed him with low dose interferon alpha subcutaneously at 3MU per dose TIW (three times a week) and hydroxyurea. The leukocytes and eosinophils were ranged from 5×10^9/L to 10×10^9/L and 0.4×10^9/L to 1.0×10^9/L, respectively, during outpatient following-up. The man was again admitted to our clinic because of fatigue and weight loss in August, 2017. The leukocytes was 5.4×10^9/L (26.5% eosinophils), hemoglobin levels was 72 g/L, and the platelets was 187×10^9/L, BM smear showed slightly hypercellular marrow with

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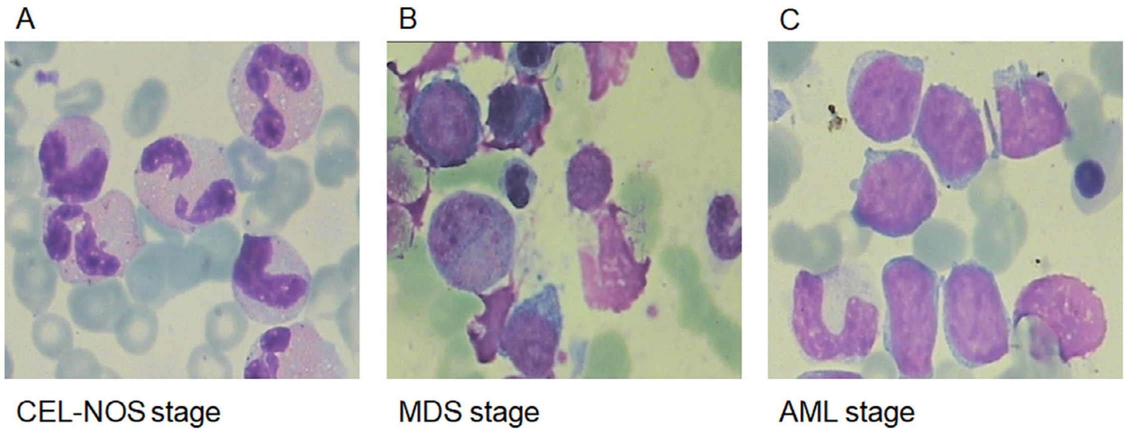

Fig. 1. Morphologic changes of bone marrow smear (WrighteGiemsa, ×1000). (A) Initial bone marrow showed slightly hypercellular marrow with marked eosinophil infiltrates; no definite blasts were found in this specimen. (B) Second bone marrow smear revealed multilineage dysplasia and markedly increased abnormal myeloblasts with eosinophils. (C) Third bone marrow smear showed hypercellular, with 80.5% blasts and 1% eosinophils.

eosinophilia(11.0%), multilineage dysplasia, and blasts comprised 7.5% of nucleated cells (Fig. 1B). Cytogenetics were normal, consistent with the findings obtained in September, 2009. MDS-EB-1(intermediate 1 according to the IPSS and intermediate according to the IPSS-R) was diagnosed based on the definition of 2016 World Health Organization (WHO) [5]. Therapy was changed to decitabine (20 mg/m^2 intravenously on days 1–5). He underwent severe pulmonary infection in the inhibition period of hematopoiesis, and didn't agree to receive cytotoxic chemotherapy any more, except of supportive cares.

In February, 2018, the patient complained of weakness and anemia (Hb 61 g/L). The leukocytes and platelets were 55.01 × 10^9 /L(1% eosinophils) and 10.0 × 10^9 /L, respectively. BM was hypercellular, with 80.5% blasts, 1% eosinophils (Fig. 1C). There is no new findings in cytogenetics. Flow cytometry analysis demonstrated that the BM blasts were positive for CD13, CD33, and CD117. The patient was diagnosed as acute myeloid leukemia with myelodysplasia related changes (s-AML) and prescribed with reduced intensity IA (idarubicin 6 mg/m^2 d1-3, cytarabine 100 mg/m^2 d1-7) regimen. Despite two courses of chemotherapy induction, the BM did not exhibit improvement. He expired in May, 2018.

Target-NGS of 51 genes, which were known or suspected to have a role in myeloid malignancies, was applied on BM genomic DNA to retrospectivly analyze the molecular evolution from HES to s-AML. We identified three mutations in HES phase, five and seven mutations in MDS and s-AML samples, respectively. The mutated genes included ETNK1, U2AF1, SETBP1, IDH2, and RUNX1. Based on the NGS results, the patient's diagnosis at presentation in 2009 was revised as chronic eosinophilic leukemia not otherwise specified (CEL-NOS), according to the new diagnostic criteria [6]. NGS on saliva DNA confirmed that all these alterations were somatic mutations. Additionally, variant allele fractions (VAFs) provided by NGS can be informative. VAFs represent the fraction of specific mutant sequences (the so-called “reads” provided by NGS) relative to total sequenced reads. Using a simplified model, the original “founding” clones can be identified based on their high VAF whereas clones acquired later in the development of disease would have a distinctly lower VAF. The mutated genes and its VAFs in different disease stages were listed in Table 1.

Table 1
The mutated genes and VAFs in different disease stages.

Stages	Mutated genes (VAFs,%)
HES	ETNK1p.Asn244Ser(44.5%), U2AF1p.Gln157Pro(42.0%), SETBP1p.Gly872Arg(22.0%)
MDS	ETNK1p.Asn244Ser(46.5%), U2AF1p.Gln157Pro(44.0%), SETBP1p.Gly872Arg(43.5%), IDH2p.R140Q(36.5%), RUNX1p.Tyr403fs (21.8%)
s-AML	ETNK1p.Asn244Ser(46.0%), U2AF1p.Gln157Pro(47.4%), SETBP1p.Gly872Arg(46.3%), IDH2p.R140Q(45%), RUNX1p.Tyr355fs (5.7%), RUNX1p.Ser389fs(16%), RUNX1p.Tyr403fs(38%)

VAFs, variant allele fractions.

3. Discussion

The target NGS revealed clonal evolution in our case who initially presented with CEL-NOS, successively transformed to MDS-EB-1 and post-MDS s-AML. The CEL-NOS stage was characterized by a founding clone harboring mutations in ETNK1 and U2AF1 and a subclone containing a mutation in SETBP1. One study showed the evidence of recurrent somatic ETNK1 mutations in the context of MDS/MPN disorders and may inhibit the catalytic activity of the enzyme [7]. Another study found ETNK1 mutations to be largely restricted to patients with systemic mastocytosis(SM) with associated eosinophilia and chronic myelomonocytic leukemia(CMML) [8]. These findings suggest that ETNK1 mutation may play a significant functional role in myeloid malignancies or in acidophilic differentiation. U2AF1 mutations documented in MDS, MDS/MPN and AML, analysis of serial samples from individual patients revealed that U2AF1 mutations occurred early in leukemogenesis and often persisted in clonal remissions [9]. Engle et al. also revealed that U2AF1 mutations preceded the other gene mutations in the evolution of the malignant clone [10]. Based on the stage-specific clonality, both ETNK1 and U2AF1 mutations were likely early events

SETBP1 mutations were a rare molecular event in AML and MDS, but relatively common in MDS/MPN overlap syndrome [11]. It was found to enhance ASXL1 mutation-induced differentiation block, and played a role as critical drivers in the leukemic transformation from MDS to AML [12]. In this case, SETBP1 mutation was present in a small subclone at the CEL-NOS stage, but its VAF increased with progression to MDS and s-AML stages. The exact role of SETBP1 in progression of MDS to AML is unknown.

Two additional mutations, IDH2p.R140Q and RUNX1p.Tyr403fs, were detected in a small subclone at the MDS stage, and the prior mutations in ETNK1, U2AF1, and SETBP1 were still observed. IDH1/2 mutations can be found in pre-leukemic clone in AML patients without concurrent presence of pathology-proven AML [13]. Makishima et al. demostrated that IDH2 mutation tended to be newly acquired, and were associated with faster s-AML progression [14]. Somatic mutations involving RUNX1 are frequently observed in MDS. Tsai et al. observed that MDS patients with RUNX1 mutations had a higher risk and shorter latency for progression to AML in comparison with MDS patients without RUNX1 mutations [15]. Collectively, these findings support the notion that newly acquired mutations on RUNX1 and IDH2 in this patient may be pathogenic, and contribute to disease evolution.

The s-AML stage was characterized by two more acquired RUNX1 mutations (RUNX1p.Tyr355fs, RUNX1p.Ser389fs). Both RUNX1 mutations were identified at a very low VAFs (5.7% and 16%, respectively) at the s-AML stage, but RUNX1p.Tyr403fs expanded (from 21.8% to 38%) and likely contributed to leukemic transformation. As RUNX1 is considered to be a high-risk mutation, its presence, even in a small subclone, at the s-AML stage is relevant to the biology of the patient's disease course. Notably, we also observed the eosinophil levels went down during disease evolution and remained in the normal range at s-AML stage, this observations suggest that the dominant clones at CEL-NOS and s-AML stages are different.

4. Conclusion

To our knowledge, this represents the first study to analyze the progression from a CEL-NOS to MDS and s-AML by NGS to model clonal evolution across the disease stages. ETNK1, U2AF1 and SETBP1mutations were likely early events, and acquired mutations on RUNX1 and IDH2 may be pathogenic, and contribute to disease evolution. Future studies are needed to determine whether consistent patterns of clonal evolution that drive CEL-NOS disease progression can be identified.

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Declarations of interest

None.

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