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CASE REPORT

A diagnosis of discernment: Identifying a novel *ATRX* mutation in myelodysplastic syndrome with acquired α-thalassemia

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Abstract

Myelodysplastic syndromes (MDS) are a heterogeneous category of myeloid neoplasms that represent the most common class of acquired bone marrow failure syndromes in adults. MDS is typically associated with a hypoproliferative macrocytic anemia, but atypical findings on initial diagnostic evaluations can raise concern for a distinct pathophysiological process and lead to the investigation of alternative etiologies. Here, we report a case of MDS with a concomitant hypoproliferative microcytic and hypochromic anemia that led to the identification of acquired hemoglobin H due to a novel somatic *ATRX* mutation.

Keywords Myelodysplastic syndromes, ATRX, Acquired hemoglobin H.

Introduction

Myelodysplastic syndromes (MDS) are comprised of a heterogeneous group of clonal hematopoietic stem cell disorders characterized by ineffective hematopoiesis and dysplasia resulting in bone marrow (BM) failure, peripheral blood cytopenias, and increased risk of progression to acute myelogenous leukemia (AML) [1]. Though the genetic pathophysiology underlying MDS is not fully understood, multiple genes have been implicated in the development and progression of MDS, with massive parallel sequencing studies identifying at least one potentially oncogenic mutation in ~90% of cases [2]. The clinical significance of many of these mutations remains poorly understood.

Somatic mutations in the *ATRX* gene, which encodes the chromatin remodeling factor ATRX, occur in ~0.8% of MDS cases [3,4]. *ATRX* mutations in the setting of MDS have been associated with an acquired α-thalassemia syndrome (i.e., AT-MDS) [5]. In contrast to the typical macrocytic anemia in MDS [6], the anemia of AT-MDS is often characterized as microcytic and hypochromic [7,8]. About 25% of patients with AT-MDS will progress to AML; this risk of progression appears to be similar to that of the general MDS patient population. Though most commonly seen with MDS, acquired

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α-thalassemia has also been reported in other hematologic disorders such as ALL, de novo AML, myelofibrosis and essential thrombocythemia [7].

Here, we report the case of a patient with AT-MDS and progressive isolated microcytic and hypochromic anemia due to a novel acquired *ATRX* mutation.

Case presentation

A previously healthy 57-year-old male of European ancestry was found on routine testing in February 2011 to have isolated, mild, normocytic anemia (hemoglobin (Hgb) 12.3 g/dL, mean corpuscular volume (MCV) 86.1fL, mean corpuscular hemoglobin (MCH) 29.5pg) with normal white blood cell (WBC) count (5.8×10^3/uL) and WBC differential (absolute neutrophil count (ANC) 3.02×10^3/μL, absolute lymphocyte count (ALC) 1.51×10^3/μL, absolute monocyte count (AMC) 0.52×10^3/μL, absolute eosinophil count (AEC) 0.23×10^3/μL, absolute basophil count (ABC) 0.17×10^3/μL), and normal platelet count (PLT) 246×10^3/μL. In July 2012, he developed progressive isolated microcytic and hypochromic anemia (Hgb 9.3 g/dL, MCV 68.3fL, MCH 19.3pg). His WBC (8.4×10^3/μL) and WBC differential remained normal (ANC 5.32×10^3/μL, ALC 1.99×10^3/μL, AMC 0.33×10^3/μL, AEC 0.33×10^3/μL, ABC 0.33×10^3/μL), while his platelet count was minimally low (PLT 143×10^3/μL). Peripheral blood smear demonstrated hypochromic and microcytic erythrocytes with teardrop cells and significant anisopoikilocytosis. He had no family history of thalassemia and no evidence of iron deficiency (serum iron, 91 mcg/dL; total iron binding capacity, 310 mcg/dL; transferrin saturation, 29%; and ferritin, 325 ng/mL). On physical examination, there was no palpable lymphadenopathy or splenomegaly. A BM aspiration and biopsy revealed mild dyserythropoiesis and moderate dysmegakaryopoiesis with 6% myeloblasts, compatible with MDS-EB1 (myelodysplastic syndrome with excess blasts-1 per World Health Organization (WHO) criteria) [9]. Cytogenetic analysis demonstrated normal male karyotype. His erythropoietin level was 52 mU/mL. A hemoglobin electrophoresis performed to further evaluate his microcytic and hypochromic anemia revealed an acquired HgH (5.3%) in the setting of MDS (normally, HgH is not detected on hemoglobin electrophoresis).

Close observation and monitoring was recommended for our patient, as he had intermediate-I risk MDS per the International Prognostic Scoring System [10] and remained transfusion independent. He became red blood cell (RBC) transfusion dependent in December 2013, and a repeat BM aspirate and biopsy showed stable disease. He was initiated on erythropoietin stimulating agent (ESA) (darbepoetin injections) titrated up to 500 mcg weekly over the next three months in addition to continued weekly RBC transfusions.

Despite ESA therapy, his transfusion needs did not change, and in April 2014, a repeat BM aspiration and biopsy confirmed progression to AML. No cytogenetic alterations were identified and leukemic blasts maintained a normal male karyotype. Following leukemic transformation, his microcytosis resolved and a repeat hemoglobin electrophoresis showed normal results with no detectable hemoglobin H. He had persistent disease following CPX-351 (liposomal cytarabine:daunorubicin) double induction and partial response (i.e., $<5\%$ myeloblasts in the BM with occasional circulating myeloblasts) to FLAG (fludarabine, high-dose cytarabine, granulocyte colony stimulating factor) reinduction. He subsequently underwent matched related allogeneic peripheral blood hematopoietic cell transplantation (HCT), with the last BM aspirate and biopsy on post-HCT day $+154$ showing no morphologic evidence of leukemia. Post-HCT, his hemoglobin levels have been normal, the microcytosis has not recurred (the MCV has been maintained in the normal range), and the peripheral smear demonstrated normal RBC size and morphology; therefore, a hemoglobin electrophoresis was not repeated. He continues to be alive and well, demonstrating Karnofsky Performance Status of 70 at the last follow-up on post-HCT day $+1376$.

Materials and methods

Sample collection and DNA extraction

Informed consent was obtained, and all studies were performed according to a research protocol approved by the Institutional Review Board of the University of Michigan. Two milliliters of blood, buccal swabs (Buccal DNA Collection and Preservation Kit; Norgen Biotek, Ontario, Canada), and hair follicles were obtained from the patient. All samples were collected within a week of each other when the patient had MDS (prior to progression to AML and prior to administration of therapy). Genomic DNA was extracted from the blood sample (QIAamp DNA Blood Midi Kit (Qiagen, Venlo, Netherlands)), buccal swabs (DNA Isolation Kit (Norgen Biotek)), and hair follicles (Easy-DNA gDNA Purification Kit (Life Technologies, New York, USA)), per manufacturers' instructions.

Sanger sequencing

Polymerase chain reaction (PCR) was performed using Herculase II Fusion DNA Polymerase (Agilent Technologies, California, USA). Primer pairs used for PCR amplification are listed in Supplemental Table 1. PCR product was applied on 1% weight/volume agarose gel. Following gel electrophoresis, DNA was purified (QIAquick Gel Extraction Kit (Qiagen)), and subsequently subjected to Sanger sequencing. Sequences were analyzed using SeqMan Pro (DNASTAR, Wisconsin, USA). NM_000489.3 was used as reference sequence for *ATRX*.

Results

To determine the etiology of acquired HbH, the full protein coding sequence and all exon/intron junctions of *ATRX* (located on the X-chromosome) were sequenced. Sanger sequencing of DNA isolated from whole blood at diagnosis identified two *ATRX* alleles, a wild type allele and an allele with a single mutation, $5656C > G$ (p.P1886A) in exon 23 (Fig. 1(A)). No other previously reported AT-MDS causing *ATRX* mutations were identified by Sanger sequencing [11]. The $5656C > G$ mutation is non-synonymous and results in a change from proline to alanine at amino acid position 1886 (out of 2492 amino acids in the ATRX protein). This genetic variant is not

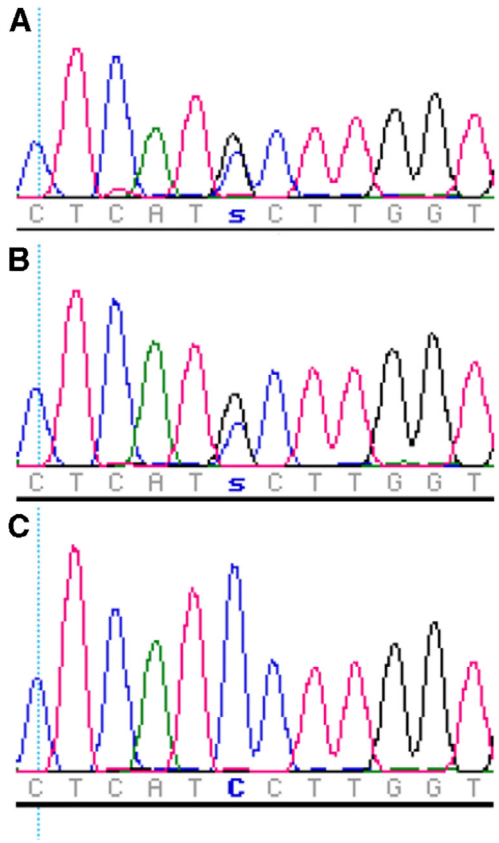

Fig. 1 Chromatograms illustrating the *ATRX* 5656C > G mutation (NM_00489.3) in DNA isolated from (A) blood and (B) Buccal swab, but not in (C) DNA isolated from hair follicle. Bases 5653–5664 are shown (Base 1 indicates the first coding base).

reported in the Single Nucleotide Polymorphism Database (dbSNP), in the exome variant server (which contains genetic information on > 10,000 human alleles sequenced) or in gnomAD (which contains sequencing data on > 120,000 exomes and > 15,000 whole genomes) [12]. The mutation is predicted to be damaging by both Sorting Intolerant from Tolerant (SIFT) [13] and Polymorphism Phenotyping-2 (PolyPhen-2) [14].

Sequencing for the identified 5656C > G mutation in DNA isolated from a buccal swab sample obtained at diagnosis [15] demonstrated both mutant and wild-type alleles as well (Fig. 1(B)). To rule out contamination of the buccal swab sample with blood cells as previously described in several reports [16,17], hair follicles were obtained from the patient. Sequencing for the 5656C > G mutation in DNA isolated from the hair follicles demonstrated presence of the wild type allele only (Fig. 1(C)).

Discussion

We report the identification of a novel *ATRX* mutation following investigation of atypical microcytic and hypochromic anemia in the setting of MDS. Anemia, with accompanying fatigue and lower activity levels, is one of the most common signs leading to a diagnostic workup for MDS [18,19]. However, the anemia associated with MDS is typically macrocytic, with greater than 80% of patients exhibiting elevated MCV on blood testing [18]. When new-onset microcytic and hypochromic anemia is detected in an iron-replete patient with the presence of other signs and diagnostic tests indicating MDS, it is important to consider a separate underlying etiology for the atypical RBC morphology.

In our case, a diagnosis of MDS (WHO subtype MDS-EB1) was confirmed on BM aspiration, but the etiology of the new-onset microcytic and hypochromic anemia remained unclear. The HgH detected on hemoglobin electrophoresis then suggested the presence of acquired α-thalassemia, as the patient had a normal MCV one year prior to his diagnosis. Further investigation of AT-MDS with genetic sequencing confirmed the presence of an *ATRX* mutation.

ATRX, a member of the SWI/SNF family of chromatin remodeling proteins, has been shown to have a role in repressing transcription [20] and to contribute to chromosome dynamics during mitosis [21]. ATRX exhibits ATPase activity that is encoded by highly conserved helicase domains at the C-terminal shown to be crucial to its function, which is severely attenuated by mutations in this region [22]. The identified 5656C > G exon 23 mutation lies within the ATPase region, suggesting that the ATPase activity of ATRX may have been altered [23,24]. The mutation identified in this report is novel and has not been described before in AT-MDS, and its position is consistent with the majority of known *ATRX* mutations that lie within the helicase or ADD domains [25].

Constitutional *ATRX* mutations are associated with the wide variety of clinical manifestations attributed to ATR-X syndrome, including developmental delay, skeletal deformities, and urogenital abnormalities [25–28]. Though patients with ATR-X syndrome also have α-thalassemia, their hematologic phenotype is much milder than that of patients with AT-MDS; the reason for this phenotypic difference is not known [7,8,28].

The severe hematologic phenotypes observed in AT-MDS are thought to reflect the critical role of *ATRX* in α-globin expression [5]. The known mutations of *ATRX* found in AT-MDS as well as the mutation identified in this report are illustrated in Fig. 2. Sequencing of *ATRX* in DNA isolated from peripheral blood identified both a wildtype and the 5656C > G mutant allele (Fig. 1), a finding consistent with Steensma et al.'s observation of two distinct RBC populations in AT-MDS, one characterized by low hemoglobin content and the other appearing normal [7]. The most likely explanation is that the peripheral blood cells are partly reconstituted from hematopoietic stem cells with wildtype *ATRX*, and partly from hematopoietic stem cell(s) with 5656C > G. Though the 5656C > G *ATRX* mutation was also identified in buccal swab DNA obtained prior to HCT, DNA isolated from hair follicles did not contain this mutation, essentially ruling out germline mosaicism for the *ATRX* mutation. Therefore, the presence of both mutant and wild-type alleles in the buccal swab sample prior to HCT reflects hematopoietic contamination of the buccal swab material [29].

No studies to date have compared the clinical outcomes of MDS patients with or without an acquired *ATRX* mutation. However, decreased *ATRX* expression has been suggested to contribute to poor survival outcomes in patients with AML [30,31], though there is no current evidence to support a change in management based on *ATRX* expression alone. A study of 132 consecutive adult patients with *de novo* AML demonstrated that patients with low *ATRX* expression were

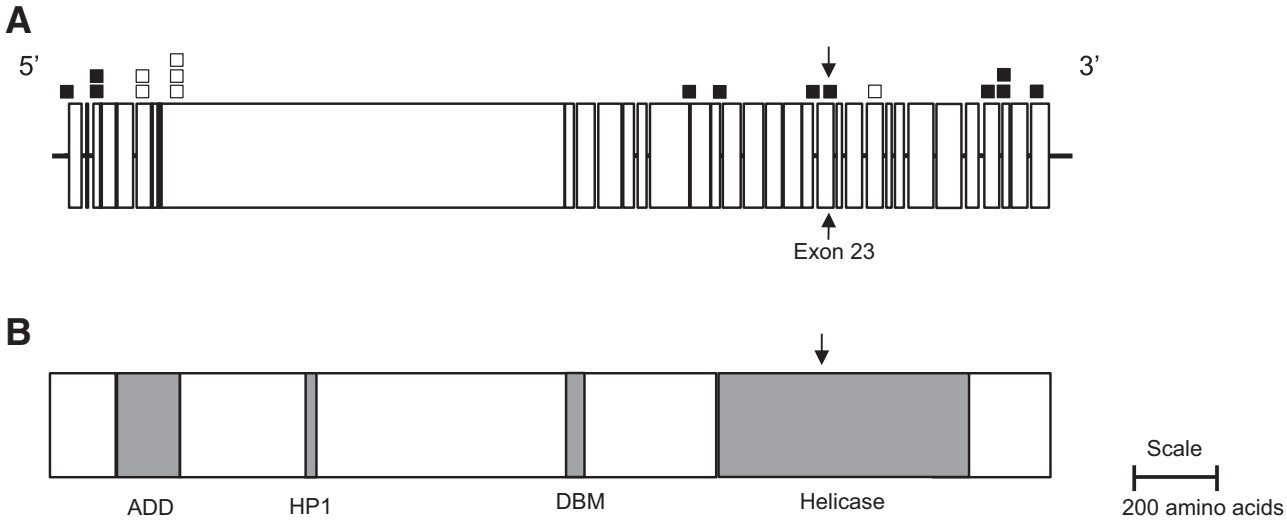

Fig. 2 *ATRX* mutations associated with AT-MDS reported to date. (A) *ATRX* exons (rectangles) and introns (horizontal lines between exons) are not drawn to scale. Untranslated regions are not shown. Mutations are represented by squares. Nonsense or frameshift mutations leading to protein truncation are darkened, while missense mutations or small deletions that maintain the reading frame are empty. Arrow indicates our reported 5656C > G exon 23 mutation. (B) ATRX protein, with ADD, HP1, DBM and helicase motifs. Arrow: Approximate location of structural modification resulting from 5656C > G exon 23 mutation.

more likely to have intermediate cytogenetic risk (vs. favorable cytogenetic risk for high *ATRX* expression), as well as the high-risk *FMS-like tyrosine kinase-3* internal tandem duplication (*FLT3*-ITD) mutation [30]. A separate study showed that *FLT3*-ITD AML patients with decreased ATRX and elevated *runt related transcription factor 3* (*RUNX3*) expression had higher rates of treatment failure and lower 3-year event-free and overall survival. Additional studies are needed to define the role of somatic *ATRX* mutations in the pathogenesis of MDS and to determine if somatic *ATRX* mutations confer an inferior clinical outcome in MDS.

Conflicts of interests

D.G. is member of the Board of Directors for Shire plc, and also benefits from license/patent royalty payments from Shire to Boston Children's Hospital (VWF) and the University of Michigan (ADAMTS13). He is also a member of the Scientific Advisory Boards for Portola Pharmaceuticals and Syros Pharmaceuticals.

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cancergen.2019.01.002.

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