Deletion of the α Globin Gene Cluster as a Cause of Acquired α Thalassemia in Myelodysplastic Syndrome (ATMDS)

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ABSTRACT

Rarely, myelodysplastic syndrome (MDS) is complicated by an acquired form of α thalassemia (ATMDS) characterized by hypochromic, microcytic, anisopoikilocytic red cells with hemoglobin H (HbH) inclusions. Acquired mutations in *ATRX*, a chromatin remodelling gene, have recently been found in 12 patients with typical features of ATMDS, but have not been detected in MDS cases with similar red cell findings but little HbH. The α globin genes themselves have appeared normal in all ATMDS cases studied to date. Here we characterize the molecular defect in a unique MDS case with rare HbH inclusions where an abnormal clone lost a >1.9 Mb segment of the telomeric region of the short arm of one allele of chromosome 16, including both α globin genes. The red cell changes associated with this acquired somatic genotype (--/αα) are surprisingly severe, demonstrating that a minor globin chain imbalance may be unexpectedly deleterious during the abnormal erythropoiesis that occurs in the context of MDS.
INTRODUCTION

The myelodysplastic syndromes (MDS) are a heterogeneous group of bone marrow disorders characterized by ineffective clonal hematopoiesis, acquired genomic instability, and a variable risk of transformation to acute leukemia. Most patients with MDS have macrocytic or normocytic anemia, but occasional patients have microcytic red cell indices. Rarely, MDS patients with striking hypochromia, microcytosis and anisoipoikilocytosis are found to have acquired hemoglobin H (HbH) disease (α thalassemia); this constellation of findings has been called α thalassemia myelodysplastic syndrome (ATMDS). In most ATMDS cases a substantial proportion of red cells contain HbH inclusions after supravital staining, and severely decreased α globin chain synthesis is paralleled by diminished α globin cytoplasmic and nuclear mRNA levels.

The common inherited forms of α thalassemia are most frequently a consequence of deletions or point mutations affecting the duplicated α globin genes (αα/αα) on chromosome 16, or, less commonly, deletions of the remote regulatory elements which control α globin expression. Germline mutations of ATRX, an X-linked gene encoding a chromatin remodelling protein that regulates expression of diverse genes, cause mild α thalassemia associated with developmental abnormalities (ATR-X syndrome). ATRX mutations downregulate expression of all four α globin genes but the α-globin cluster itself is normal.

Recently we have shown that acquired, somatic mutations in the ATRX gene can also underlie ATMDS syndrome. To date, 12 unique pathologic ATRX mutations have been found in patients with typical features of ATMDS including striking “thalassemic” blood pictures, substantial amounts (>10%) of HbH, and severely reduced α/β globin chain biosynthesis ratios. However, we have also studied several untransfused patients with MDS who have hypochromic, microcytic red cell morphology but in whom HbH inclusions are only present in a small proportion of erythrocytes or cannot be detected at all. To date, none of these patients have been shown to have mutations in the ATRX gene; the underlying molecular defect remains unknown.
Here we report an MDS patient with acquired α thalassemia and rare HbH inclusions. In contrast to previously reported cases of ATMDS, an abnormal hematopoietic clone in this affected individual has an acquired deletion involving the telomeric region of one allele of chromosome 16, removing two of the four α genes (i.e., genotype --/αα). This case illustrates a second, less severe mechanism by which α thalassemia may occur as an acquired abnormality in the context of hematological malignancy.

PATIENT AND METHODS

A 72-year old Caucasian British man developed microcytic, hypochromic anemia (hemoglobin 10.4 g/dL, mean corpuscular volume 64 fL, mean corpuscular hemoglobin 21.7 pg) and neutropenia (leukocyte count 4.8 × 10^9/L with 14.7% neutrophils). Two years earlier, he had undergone partial pneumonectomy for localized lung carcinoma; at that time a complete blood count was normal. The patient had no Mediterranean or Asian ancestors, there was no family history of a hematological disorder, and iron studies were unremarkable.

Peripheral blood smears demonstrated a dimorphic red cell picture with striking anisopoikilocytosis, microcytosis, and severely hypochromatic “ghost” erythrocytes (Figure 1A). A bone marrow aspirate was hypercellular, with marked erythroid hyperplasia, trilineage dysplasia, rare ringed sideroblasts, and no excess of myeloblasts (MDS subtype: refractory cytopenia with multilineage dysplasia.) The patient consented to detailed hematologic and genetic analysis of his condition.

Fresh peripheral blood was incubated for 4 hours with 1% brilliant cresyl blue in 0.9% NaCl and examined for intraerythrocytic hemoglobin H inclusions. Hemoglobin subtype analysis was performed by an automated high-performance liquid chromatography unit using a manufacturer-recommended protocol.
Globin chain synthesis was measured by labelling an enriched reticulocyte preparation derived from fresh heparinized marrow with $^3$H leucine, precipitating globin chains, and measuring radioactivity on a liquid scintillation counter, as previously described.\textsuperscript{10}

Conventional G-banded cytogenetic analysis of unstimulated bone marrow was followed by FISH studies of metaphase spreads and interphase nuclei using standard hybridization protocols with cosmid probes specific for the telomeric region of the short arm of chromosome 16, including CRA36, GG1\textsuperscript{11}, 415C1, and 439A6 (the most proximal of the 4 cosmids, mapping 1.9 Mb from the telomere).

Peripheral blood was separated into granulocyte-enriched and mononuclear-cell-enriched fractions by double-density Ficoll-Hypaque\textsuperscript{TM} (Sigma, St. Louis, MO) centrifugation, and DNA was extracted from each fraction using the phenol-chloroform method. For analysis of the $\alpha$-globin 3’ hypervariable region (an array of 17-bp tandem repeats with high interallelic variability located 8 kb downstream of the $\alpha$ globin gene complex\textsuperscript{12}), DNA was digested with Hinfl restriction endonuclease, then the fragments were electrophoresed through a 1% agarose gel and identified by blot-hybridization using a $^{32}$P-labelled 4.0kb $Hinfl$ fragment as a probe and autoradiographed. The 5’ hypervariable region (100 kb upstream of the $\alpha$-globin genes\textsuperscript{13}) was analyzed in similar fashion after DNA digestion with Alul. In order to probe the $\alpha$ and $\zeta$ genes directly, DNA was digested with BamHI and BglII and hybridised on separate blots with radiolabelled 0.5 kb HindIII Psi  $\alpha$ globin probe and a $\zeta$ globin probe, then autoradiographed.

Haplotype analysis of the $\beta$ globin gene cluster was performed on unfractionated blood and granulocyte and mononuclear cell fractions as previously described\textsuperscript{14}, with the addition of the XmnI restriction site at position -158. The remaining $\alpha$ globin genes were sequenced to exclude point mutations\textsuperscript{15} using the BigDye\textsuperscript{TM} (ABI, Foster City, CA) technique.
RESULTS AND DISCUSSION

Severe microcytosis and hypochromia is unusual in MDS in the absence of iron deficiency but is typical of ATMDS. In the present case, a hypochromic microcytic blood film (Figure 1A) prompted further study to elucidate the underlying molecular defect. In contrast to most previously reported cases of ATMDS where untransfused patients have had >10% HbH containing red cells, here supravital staining demonstrated only 0.11% erythrocytes containing HbH inclusions (Figure 1B). Hemoglobin electrophoresis showed 2.0% hemoglobin A2 and 1.4% hemoglobin F; no clear peak corresponding to HbH was observed. The patient’s α/β globin chain synthesis ratio was reduced to 0.81 (normal 0.90-1.20), a value typical of heterozygous α thalassemia (genotypes -α/αα or --/αα).

The patient’s marrow karyotype was complex, including a rearrangement of the telomeric region of the short arm of chromosome 16: 45, X, -Y, add(3)(q?25), der(3)add(3)(?q25)del(3)(q?25), del(4)(p?15), der(5)add(5)(q?31)del(5)(p?14), del(6)(p?21), -7, der(11)add(p?11)add(q?23), der(13)t(13;21)(p11;q11), der(16)add(p?13)add(q?22), -18, -20, -21, +4mar [cp10]/46, XY[1]. FISH studies with the 4 cosmid probes demonstrated signals on the normal chromosome 16 only, indicating that the deletion extended at least 1.9 Mb from the 16p telomere (Figure 2A),

Southern blot analysis of DNA from the patient’s unFractionated blood with16p-specific polymorphic markers demonstrated moderately reduced intensity of one of the two bands corresponding to the closely linked α globin alleles when compared with normal controls (Figure 2B). Using DNA isolated from the patient’s purified granulocytes, the band corresponding to the abnormal allele was almost absent, consistent with deletion of one of two α globin clusters, but the band was preserved in the mononuclear cells (Figure 2B). In MDS, involvement of myeloid lineages - including granulocytes - in the neoplastic clone is more common than involvement of lymphocytes and it is likely that the granulocyte fraction in this case was enriched for the chromosomal abnormality compared with unFractionated blood and the mononuclear cell fraction. Southern blots
using probes for the $\alpha$ and $\zeta$ genes also demonstrated reduced band intensity compared with normal controls (data not shown), consistent with loss of one copy of the entire $\alpha$ globin cluster in myeloid cells. Therefore, the MDS clone exhibited a thalassemic ($-/\alpha\alpha$) genotype despite a normal ($\alpha\alpha/\alpha\alpha$) constitutional genotype. Haplotype analysis of the $\beta$ globin gene cluster was unremarkable, demonstrating no loss of heterozygosity, and the sequence of the remaining $\alpha 1$ and $\alpha 2$ genes in granulocyte DNA was also normal (data not shown).

Genomic instability in MDS is well recognized, although the underlying etiology remains unclear.\textsuperscript{17,18} It is probable that the clonal chromosomal loss in the present MDS case was a random genetic event, manifest as a dramatic red cell phenotype as a consequence of loss of two $\alpha$ globin genes; 16p13 is not a common breakpoint in MDS.\textsuperscript{19} Other examples of acquired red cell abnormalities associated with MDS include loss of glycosylphosphatidylinsitol (GPI)-anchored proteins including CD55 and CD59,\textsuperscript{20} enzymopathies (especially pyruvate kinase deficiency),\textsuperscript{21} membrane defects including elliptocytosis,\textsuperscript{22} and blood group isotype changes including exposure of cryptantigens.\textsuperscript{23,24}

To our knowledge this is the first report of a patient with ATMDS in whom an acquired $\alpha$ globin deletion is demonstrable. This case illustrates that loss of a single $\alpha$ globin cluster can cause a dramatic acquired thalassemic phenotype in MDS. It is not clear why the red cell changes in this patient are more severe than one would normally see associated with loss of only two of four $\alpha$ genes ($-/\alpha\alpha$); this may reflect an interaction between minor globin chain imbalance and the already markedly disordered hematopoiesis in MDS. Further molecular characterisation of ATMDS cases will help define the mechanisms responsible for this interesting association and may explain the unexpectedly severe red cell phenotype.
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FIGURE CAPTIONS

FIGURE 1A
Wright-Giemsa stained peripheral blood smear demonstrates severe anisopoikilocytosis with hypochromic “ghost cells” almost completely devoid of hemoglobin (400x).

FIGURE 1B
Brilliant cresyl blue stain of peripheral blood reveals a “golf ball” cell with classical hemoglobin H inclusions (1000x). Such cells represented only 0.11% of anucleate erythrocytes in the patient, but were observed on multiple occasions. Hemoglobin H-containing cells are not present in normal individuals.

FIGURE 2A
Metaphase spread and two interphase nuclei from the patient’s bone marrow hybridised with probe GG1 (green signal) reveal GG1 signal on the normal chromosome 16 only. Yellow arrowheads indicate the single GG1 signal in interphase, corresponding to the normal chromosome 16 short arm. Red arrow denotes the normal chromosome 16 homologue at metaphase, with paired GG1 signals on sister chromatids.

FIGURE 2B
Southern blot of the 3’ hypervariable region in the α globin cluster shows two bands for the two normal controls (one from each allele, corresponding to the normal diploid chromosome 16 short arm). In contrast, the second (upper) band from the patient’s sample shows reduced probe hybridization. “PB” represents DNA derived from unfractionated peripheral blood, “Mono” from the mononuclear cell fraction (predominantly lymphocytes), and “Gran” the granulocyte-enriched fraction. The superior band is virtually absent from the patient’s granulocyte-enriched DNA, corresponding to loss of one α globin cluster in these cells. However, both bands are present in the mononuclear fraction, which is likely to be enriched for normal cells. In the patient’s unfractionated peripheral blood, the superior band is less intense than the corresponding band in the mononuclear fragment, consistent with the expected admixture of clonal and nonclonal cells in unfractionated blood.
FIGURE 1A
FIGURE 1B
FIGURE 2A
FIGURE 2B
Deletion of the $\alpha$ globin gene cluster as a cause of acquired $\alpha$ thalassemia in myelodysplastic syndrome (ATMDS)

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