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Acquired RhD mosaicism identifies fibrotic transformation of thrombopoietin receptor-mutated essential thrombocythemia

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Abstract

Background—Acquired copy-neutral loss of heterozygosity (CN-LOH) has been described in myeloid malignant progression with an otherwise normal karyotype.

Case Report—A 65 year old woman with *MPL*-mutated essential thrombocythemia (ET) and progression to myelofibrosis was noted upon routine pre-transplant testing to have mixed field reactivity with anti-D and a historical discrepancy in RhD type. The patient had never received transfusions or transplantation.

Results—Gel immunoagglutination revealed group A red blood cells (RBCs) and a mixed-field reaction for the RhD phenotype, with a predominant RhD-negative population and a small subset of circulating RBCs carrying the RhD antigen. Subsequent genomic microarray SNP profiling revealed CN-LOH of chromosome 1 p36.33-p34.2, a known molecular mechanism underlying fibrotic progression of *MPL*-mutated ET. The chromosomal region affected by this CN-LOH encompassed the *RHD*, *RHCE*, and *MPL* genes. We propose a model of chronological molecular events that is supported by *RHD* zygosity assays in peripheral lymphoid and myeloid-derived cells.

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Author Contributions

Study concept and design (CM, AJB, SI); in vitro experiment and data collection (RC, MA, RU, PJ, SI) analysis and interpretation of data (CM, RC, MA, RU, PJ, SI); drafting of the manuscript (CM, HGK, AJB, SI); clinical data collection (CM, MB, SK, HGK, EK, AJB, SI); and obtained funding and study supervision (HGK, AJB, SI).

Conclusion—CN-LOH events that lead to clonal selection and myeloid malignant progression may also affect the expression of adjacent unrelated genes, including those encoding for blood group antigens. Detection of mixed-field reactions and investigation of discrepant blood typing results is important for proper transfusion support of these patients and can provide a useful surrogate marker of myeloproliferative disease progression.

Keywords

copy-neutral loss of heterozygosity; acquired RhD mosaicism; SNP microarray; post-ET myelofibrosis

Case Presentation

A 65 year-old female with a 17-year history of essential thrombocythemia (ET) with progression to secondary myelofibrosis was admitted for HLA-matched allogeneic stem cell transplantation under an IRB-approved clinical protocol. The patient had never received transfusions prior to transplant.

Routine pre-transplant evaluation using gel column agglutination (MTS ABD Gel Card, ID Gel System, Ortho, Raritan, NJ) revealed the patient's blood type as group A, with mixed-field reactivity detected for the RhD antigen (Figure 1A). The mixed-field reactivity appeared to represent a predominant RhD-negative red blood cell (RBC) population, with a small subset of circulating RBCs that agglutinated with addition of anti-D (MS20, Millipore Ltd, Livingston, United Kingdom) (Figure 1A, black and red arrows, respectively).

Transfusion history gathered from an outside facility showed that the patient had been classified as group A RhD-negative when undergoing a splenectomy two months prior to this admission, using an automated platform with microplate hemagglutination technique. A retained donor identification card produced by the patient revealed that she was typed as blood group A RhD-positive when she donated blood in her youth.

Review of the patient's prior bone marrow molecular studies revealed normal female 46XX karyotype by G-banding, as well as detection of *MPL* W515K mutation early in the course of her disease. The *MPL* gene, located in chromosome 1p, encodes for the thrombopoietin receptor and is mutated in approximately 5% of patients with ET.^{1,2} The patient's bone marrow always tested negative for *JAK2* mutation, and negative for monosomy 7, trisomy 8, and deletions 5q31, 7q31, and 20q12 by fluorescence in-situ hybridization.

To further explore the possibility of genomic dosage aberrations, high-density genomic microarray single nucleotide polymorphism (SNP) profiling of the patient's bone marrow was performed on admission (research sample collection protocol NCT00071045). This assay, based on genomic hybridization to 1.9 million probes to investigate ~750,000 SNPs, revealed copy-neutral loss of heterozygosity (CN-LOH) of chromosome 1p36.33-p34.2 (Figure 2A), a known molecular mechanism underlying fibrotic progression of *MPL*-mutated ET.³ The chromosomal region affected by CN-LOH contained the *RHD* and *RHCE* genes, explaining the mechanism for this acquired RhD mosaicism (also known as "RhD splitting"). The following chronologic molecular events are postulated and illustrated in Figure 2B: 1) congenital heterozygous *RHD*⁺/*RHD*[−] (*D/d*) genotype; 2) acquired W515K

MPL mutation (with associated ET) in *cis* with the *RHD*- (*d*) allele; 3) CN-LOH of chromosome 1p, leading to clonal acquired homozygosity for both the *RHD* deletion and the W515 *MPL* mutation, with associated myelofibrotic progression and a largely RhD-negative circulating RBC phenotype. Serologic testing confirmed concomitant mosaicism for the adjacent C antigen as demonstrated by mixed field reactivity with anti-C (MS24, Millipore Ltd, Livingston, United Kingdom). The patient typed as Fy(a+b+) with no mixed field reaction detected by routine serology (Gamma-clone anti-Fya and Immucor Gamma anti-Fby, Immucor, Inc, Norcross, GA); these antigens are encoded on the opposite arm of chromosome 1 by *ACKR1* and thus not affected by the LOH event (Figure 2A).

To test the proposed model, CD3+ T lymphocytes and CD11b+/CD14+ monocytes were isolated from the patient's peripheral blood by flow sorting. Genomic DNA was extracted from both populations and *RHD* zygosity was investigated as a quantitative ratio of *RHD* exons 5 and 7 to an internal control gene.⁴ T lymphocytes, which are not expected to arise from the myeloproliferative clone, revealed an *RHD*/control ratio of 0.5, consistent with congenital hemizygosity for the *RHD* gene. Circulating peripheral monocytes, on the other hand, had a lower *RHD*/control ratio of 0.3, consistent with complete absence of the *RHD* gene in a subset of these cells as predicted by our model.

The patient underwent myeloablative allogeneic stem cell transplantation with a conditioning regimen consisting of fludarabine, cyclophosphamide, and total body irradiation. She received an *ex vivo* T cell depleted, CD34⁺ selected peripheral blood stem cell graft (9.4 x10⁶/kg CD34⁺ cells and 5x10⁴/kg CD3 T cells) from a group A, RhD-positive donor. The patient achieved successful engraftment with 100% myeloid chimerism by day 14. Her bone marrow biopsy showed normal trilineage hematopoiesis with significantly reduced fibrosis, and genomic microarray SNP profiling was normalized 2 months after transplantation. RhD mosaicism was no longer identified 1 year after transplantation (Figure 1B).

Discussion

Several reports have previously documented RhD phenotype splitting in patients with hematologic disorders. Körmöcz et al⁵ reported RhD phenotype mosaicism in three cases of hematologic disorders secondary to LOH of chromosome 1. Orlando et al⁶ described a case of RhD phenotype splitting in a patient with myelofibrosis and a neoplastic clone lacking the *RHD* gene. This report is the first case to demonstrate acquired RhD mosaicism with concurrent *MPL* mutation and a clinical association to myelofibrotic progression of ET. Theoretically, a recipient with acquired RhD mosaicism can be managed with transfusion of RhD positive RBCs, however this patient received RhD negative blood products during the peri-transplant period until the mechanism of RhD splitting was elucidated.

Conclusion

Acquired changes in the RhD phenotype of untransfused patients may signal a progressing underlying myeloproliferative disease. In particular, identification of RhD mosaicism can serve as a simple biomarker of progression in high risk ET patients carrying a high burden of

MPL mutation. Detection of mixed-field reactivity in these patients requires a sensitive methodology and must be followed up by correlation with transfusion and transplantation history. This case illustrates the importance of thorough investigation of discrepant blood typing results, in order to avoid erroneous typing of these patients and unnecessary depletion of the RhD-negative blood supply.

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Figure 1. RhD and ABO blood typing by gel immunoagglutination

A. Blood typing results obtained pre-transplant. Arrows indicate a mixed-field reaction demonstrating 2 distinct circulating RBC populations: RhD-positive (red) and RhD-negative (black). **B.** Blood typing results obtained 1 year post-transplant. All circulating RBCs type as RhD-positive (black arrow).

B. Putative temporal progression of bone marrow myeloproliferative (MPN) clone

Figure 2. SNP microarray results and proposed molecular model

A. Graphical display of SNP microarray results from the patient's bone marrow pre-transplant. The location of the *RHD*, *RHCE*, *MPL* and *ACKR1* genes is indicated in relation to the G-banding pattern of chromosome 1. **B.** Proposed model for temporal progression of the myeloproliferative clone.