Chromothriptic cure of WHIM syndrome: Implications for bone marrow transplantation

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Abbreviations: WHIM, Warts, Hypogammaglobulinemia, Infections, and Myelokathexis; CXCR4, CXC Chemokine Receptor 4; CXCL12, CXC Chemokine Ligand 12; CCR5, CC Chemokine Receptor 5; HSC, haematopoietic stem cell; OMIM, Online Mendelian Inheritance in Man, www.omim.org; BMT, bone marrow transplantation; GVHD, Graft versus Host Disease.

We recently reported a 59 year old female, designated WHIM-09, who was born with the rare immunodeficiency disease WHIM syndrome but underwent spontaneous phenotypic reversion as an adult. The causative WHIM mutation CXCR4R334X was absent in her myeloid and erythroid lineage, but present in her lymphoid lineage and in epithelial cells, defining her as a somatic genetic mosaic. Genomic and hematologic analysis revealed chromothripsis (chromosome shattering) on one copy of chromosome 2, which deleted 164 genes including CXCR4R334X in a single haematopoietic stem cell (HSC) (Fig. 1). Experiments in mice indicated that deleting one copy of Cxcr4 is sufficient to confer a selective advantage for engraftment of transplanted HSCs, suggesting a mechanism for clinical cure in WHIM-09. Genome editing may allow autologous transplantation of HSCs lacking one copy of CXCR4 without bone marrow conditioning as a general cure strategy in WHIM syndrome, safely recapitulating the outcome in patient WHIM-09.

ADDENDUM

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abnormalities in hematopoiesis.\textsuperscript{16-20} CXCR4’s role in HSC biology has been known for over 15 years and in fact the reason that plerixafor has been licensed is that it promotes release of CD34\textsuperscript{+} HSC to the blood from the bone marrow facilitating harvesting of these cells by apheresis for bone marrow transplantation (BMT) in cancer.\textsuperscript{21,22} However, the role of CXCR4 gene copy number in HSC biology had not been previously fully explored or understood. Patient WHIM-09 provides an experiment of nature showing how CXCR4 might be manipulated in order to enhance BMT, much as transplantation of homozygous CCR5\textsuperscript{A32} bone marrow to an HIV+ leukemia patient (aka the Berlin patient) resulting in functional cure of HIV demonstrated the importance of CCR5 in maintenance of HIV disease.\textsuperscript{23}

Chromothripsis is thought to occur all at once in a single cell that either dies or acquires a selective advantage.\textsuperscript{24} Thus, we postulate that chromothripsis affecting one copy of chromosome 2 in patient WHIM-09 occurred in a single primitive HSC that subsequently possessed a strong selective advantage that eventually allowed that cell to engraft in the bone marrow replacing most if not all of her previous HSC.\textsuperscript{3} This is quite unprecedented. Even in BMT, where large numbers of HSCs are transplanted at the same time, engraftment requires pretreatment with a cytoreductive conditioning regimen.\textsuperscript{25} Conditioning often consists of non-specific chemotherapy and/or irradiation which directly or indirectly results in much of the toxicity of BMT either by initiating graft vs. host disease (GVHD) or damaging the host immune response creating lethal infections. Thus if BMT could be achieved by successfully engrafting autologous gene-corrected HSC that would replace the patient’s HSC without the need for conditioning, much of the morbidity, mortality and expense of BMT would be avoided.

However, our patient was actually haploinsufficient for at least 163 other genes that might also have contributed to efficient engraftment of her seminal chromothriptic HSC.\textsuperscript{3} Therefore, we turned to murine models of WHIM (Cxcr4\textsuperscript{+/S338X}) and Cxcr4 haploinsufficiency (Cxcr4\textsuperscript{+/-}), in which none of the other 163 genes was disrupted, and used competitive bone marrow repopulation experiments to define whether the competitive advantage apparent for chromothriptic Cxcr4\textsuperscript{+/-} HSC in WHIM-09 could be phenocopied by non-chromothriptic mouse Cxcr4\textsuperscript{+/-} HSC. We found that Cxcr4\textsuperscript{+/-} bone marrow cells are able to outcompete both wild-type (Cxcr4\textsuperscript{+/+}) and WHIM (Cxcr4\textsuperscript{+/S338X}) bone marrow cells after irradiation of the recipient mouse.\textsuperscript{3} Since CXCR4 mutations in WHIM patients increase signaling by the receptor, this suggested that increased CXCR4 signaling leads to decreased bone marrow engraftment (Fig. 2). We obtained the same result whether whole bone marrow cells or lineage-depleted bone marrow cells were used for transplantation. Together, the results are consistent with the notion that most if not all of ability of HSC from WHIM-09 to engraft can be attributed to the loss of one CXCR4 allele in that cell.

**Figure 1.** Chromothripsis (chromosomal shattering) resulted in clinical cure of a patient with a rare immunodeficiency (WHIM syndrome) by deleting the mutant copy of CXCR4.

**Figure 2.** Based on the patient described in this article, WHIM-09, and subsequent mechanistic murine bone marrow transplantation experiments, we have demonstrated an inverse correlation between CXCR4 signaling and bone marrow engraftment potential.
We are now following up these findings with additional tests of the hypothesis using more stringent models of HSC transplantation.

New techniques of genome editing have steadily been developed in the past 10 years to allow precise enzymatic cleavage of DNA (so called molecular scissors). Adaptation of these techniques to surgically target and inactivate the mutant CXCR4 allele in HSC from WHIM patients might recapitulate and phenotype outcome of chemokinopisis in WHIM-09 with little risk since fully edited CXCR4−/− cells would not be expected to engraft or survive and since conditioning might be obviated. This could allow WHIM syndrome to be cured as it was in WHIM-09, but by selectively targeting only CXCR4 using edited autologous HSC. Our murine experiments suggest this would be safe and efficacious. Of course, restricting editing to the mutant CXCR4 allele would be key to safety. Ultimately, as gene editing techniques develop further, it may be possible to delete one copy of CXCR4 while simultaneously correcting the underlying genetic defect with autologous HSC from patients with other haematopoietic diseases. This might work best with myeloid or erythroid genetic disorders such as chronic granulomatous disease (CGD) or sickle cell disease. Further murine experiments will clarify the safety and efficacy of this concept. It may also be possible to replicate the apparent engrafent enhancing effect of CXCR4 haploinsufficiency by using drugs, antibodies, or other blocking reagents to temporarily knockdown or inhibit CXCR4 function and then withdraw these engrafent stimulators. This could further enhance safety and broaden the application to nearly all diseases that BMT is currently used to treat. The safety of this approach is still a concern. However, WHIM-09 has been healthy with CXCR4 haploinsufficient HSC for at least 20 years.

Disclosure of Potential Conflicts of Interest

A provisional patent on CXCR4 knockdown as a method to enhance HSC engraftment has been filed by the US government with D.H.M, J.G. and P.M.M. as inventors.
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