Functional correction of large factor VIII gene chromosomal inversions in hemophilia a patient-derived iPSCs using CRISPR-Cas9

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Hemophilia A is an X-linked genetic disorder caused by mutations in the F8 gene which encodes the blood coagulation factor VIII. Almost half of all severe hemophilia-A cases result from two gross (140-kbp or 600-kbp) chromosomal inversions that involve introns 1 and 22 of the F8 gene, respectively. We derived induced pluripotent stem cells (iPSCs) from patients with these inversion genotypes and used CRISPR-Cas9 nucleases to revert these chromosomal segments back to the WT situation. We isolated inversion-corrected iPSCs with frequencies of up to 6.7% without detectable off-target mutations based on whole-genome sequencing or targeted deep sequencing. Endothelial cells differentiated from corrected iPSCs expressed the F8 gene and functionally rescued factor VIII deficiency in an otherwise lethal mouse model of hemophilia. Our results therefore provide a proof of principle for functional correction of large chromosomal rearrangements in patient-derived iPSCs and suggest potential therapeutic applications.

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Novel approaches to treat hemophilia inhibitor formation: Engineered specific human regulatory and cytotoxic T-cells

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Anti-FVIII antibody (inhibitor) formation is a major complication in the treatment of Hemophilia A. Thus, it is necessary to develop effective tolerogenic therapies to prevent as well as reverse inhibitor formation. Our lab has focused on developing novel approaches to modulate inhibitor formation at multiple levels for example by modulating the function of FVIII-specific T effector cells as well as by directly targeting anti-FVIII B cells. We have generated engineered antigen-specific regulatory T-cells (Tregs), created by transduction of a recombinant T-cell receptor (TCR) isolated from a hemophilia A subject’s T-cell clone. The resulting engineered T-cells are specific for the C2 domain of FVIII. These Tregs bind MHC tetramers, proliferate in response to a specific FVIII epitope and suppress effector responses to FVIII. Moreover, these Tregs can inhibit the secondary antibody response to multiple epitopes in FVIII in-vitro. We have now generated additional human Tregs by transduction of a single chain Fv (scFv) directed against the A2 domain of FVIII. These scFv Tregs (ANS8) can effect bystander suppression of the response to C2 when FVIII is present. Finally, we have developed a novel chimeric antigen-like receptor called B-cell antibody recognizing receptor (BAR) to target and directly kill the FVIII-specific B-cells and antibody-producing cells. CD8 T-cells transduced to express C2-BAR caused suppression of antibody secretion of C2-specific hybridoma cells suggesting that BAR CD8 T-cells expressing FVIII domains can be used to target specific B-cells to suppress inhibitor formation and may also provide an additional platform to treat other undesirable antibody responses.

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