Targeting Gene Editing in Pluripotent Stem Cells: X-SCID Disease Now in a Dish

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Pluripotent stem cells (PSCs) are attractive cells to be used to develop new approaches based on regenerative medicine applications including cell and gene therapy, drug discovery and disease models. PSCs have self-renewal capacity and the ability to differentiate into all somatic cell types. Designer nucleases are attractive tools for therapeutic applications as they precisely edit the genome. Combining these two technologies is favorable to study diseases which are hard to study in patients due to shortage of available cell types. One example of such diseases is the X-linked severe combined immunodeficiency (X-SCID), which is a life-threatening immune disorder caused by mutations in the *IL2RG* gene, which encodes for the common gamma chain (GC), present in several interleukin receptors needed for lymphocytes' development and function. Impaired GC leads to early block in T cell development; as a consequence, patients do not have any mature T cells in their peripheral blood to fight infections and are likely to die in the first year of their life if left untreated. Here, we have developed a stage-specific T cell development protocol which could be used for various regenerative medicine applications. We verified the potential of this protocol by utilizing the X-SCID disease model *in vitro*. We have corrected a common hot spot mutation in the *IL2RG* gene in PSCs using designer nucleases. Next, we challenged both isogenic corrected and non-corrected clones to differentiate to mature T cells *in vitro*. While both non-corrected and corrected clones produced comparable levels of hematopoietic precursor cells, only corrected clones could overcome the block of differentiation and further differentiate to CD4+/CD8+ double positive (DP) T cells expressing the T cell receptor β-chain (TCRβ). Furthermore, mature CD8+ cells were produced from the DP T cells and IL2 dependent signaling was restored, confirming functional correction of X-SCID *in vitro*. In conclusion, our study emphasizes the significance of designer nucleases as a tool in generating isogenic disease models and demonstrates that pluripotent stem cells can be differentiated into mature T cells, with the prospect to produce genetically modified autologous transplants for various applications in the clinic.