Molecular Therapy
Editorial

Genome Editing for Sickle Cell Disease: A Little BCL11A Goes a Long Way

Gene therapy may finally lead to a cure for sickle cell disease, the most common form of inherited blood disorders. In particular, genome editing aimed at increasing fetal hemoglobin production or replacing the sickle cell mutation with a wild-type sequence has gained momentum. Recent publications demonstrate feasibility in human hematopoietic stem and progenitor cells (HSPCs) when subsequently evaluated in mouse transplantation studies. CRISPR/Cas9 or zinc finger nucleases (ZFNs) have proven to be useful tools to delete or replace sequences involved in the production of hemoglobin. The repair of DNA double-strand breaks by targeted nucleases occurs either through non-homologous end joining (NHEJ) or homology-directed repair (HDR; which is facilitated by providing the cells with therapeutic donor DNA).

BCL11A has emerged as a major repressor of γ-globin gene expression in the adult and may thus represent a target for therapeutic intervention. Expression of BCL11A in erythroid cells is governed by a cell-type-specific enhancer that contains a critical GATA1 binding site.[1,2] In a recent publication in *Molecular Therapy - Methods & Clinical Development*, Chang et al.[3] demonstrate that ZFN-mediated disruption of the erythroid-specific enhancer in the BCL11A gene locus by NHEJ led to increased expression of γ-globin without impairing the differentiation capacity of the edited HSPCs. In contrast, bi-allelic disruption of the BCL11A coding sequence negatively impacted the terminal differentiation of erythroid cells. These data demonstrate that reducing, but not eliminating, BCL11A expression represents a viable approach for the treatment of sickle cell disease and other β-hemoglobinopathies. Chang et al.[3] demonstrated further that edited HSPCs with a disrupted BCL11A enhancer were efficient in long-term engraftment in Nod-Scid-Gamma (NSG) immunocompromised mice. The study by Chang et al.[3] is elegant and impressive, because it shows that simply disrupting a GATA sequence in the BCL11A enhancer reduces expression of BCL11A sufficiently to achieve therapeutic levels of γ-globin expression without impairing the maturation of red blood cells.

To put this study in perspective, others reported correction of the SCD mutation in HSPCs using CRISPR/Cas9-directed HDR technology.[4,5] In these studies, the Cas9/guide RNA (gRNA) complex was electroporated into HSPCs as ribonucleoprotein (RNP) complexes and the homologous DNA was delivered via either adeno-associated virus (AAV) infection or co-electroporation of a single-stranded DNA oligonucleotide (ssODN) donor. Dever et al.[4] used AAV-mediated HDR to not only introduce a nucleotide change in the β-globin gene, but also to introduce a selectable marker gene (GFP or the truncated nerve growth factor receptor). This clever strategy allows significant enrichment of cells that have undergone HDR. In contrast, DeWitt et al.[5] took a selection-free approach and delivered the HDR donor template as an ssODN. Importantly, both studies showed efficient editing and long-term engraftment of gene-edited HSPCs in mice.

Using a different approach, two groups generated deletions in the β-globin gene locus by CRISPR/Cas9-mediated NHEJ that mimic the known hereditary persistence of fetal hemoglobin (HPFH) mutations.[6,7] Ye et al.[6] deleted 12.9 kb of DNA, including the adult δ- and β-globin genes in HSPCs to create the Sicilian HPFH mutation. This deletion eliminates a recruitment site of BCL11A and brings an enhancer located downstream of the β-globin gene in close proximity to the fetal globin genes. Traxler et al.[7] used lentivirus transduction to express gRNA and Cas9 targeting the −117 region of the two γ-globin genes, which is bound by a repressor in adult cells. Both studies showed efficient targeting frequencies and elevated γ-globin expression.

As encouraging as these recent studies are for the treatment of sickle cell disease, several limitations may prevent them from immediate broad application. First, there are concerns with respect to the off-target effects of CRISPR/Cas9 and ZFNs. This has been addressed to some degree by the current studies, but none of them involved genome-wide analysis of off-target cutting using, for example, GUIDE-sequencing.[8] DeWitt et al.[5] used both wild-type and high-fidelity (HF) Cas9 in their studies.[8,9] The data demonstrate that the HFCas9 exhibited reduced off-target cuts at specific sites, but also that these enzymes were reduced in their ability to cut the on-target site. A second limitation is the fact that genome editing is performed in autologous HSPCs, which will have to be transplanted back into patients. This is a complicated procedure that is not without risks, as discussed by DeWitt et al.[5] Future work improving the fidelity of the DNA-binding moieties and reducing the risks associated with autologous transplantations of edited HSPCs will likely weaken these concerns. In the meantime, it is also prudent to continue to screen for or develop new drugs (such as small molecule drugs) that can be systemically delivered and elevate γ-globin expression without impairing cellular functions.

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