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CRISPR-directed gene editing creates genetic heterogeneity surrounding the sickle cell disease point mutation

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CRISPR-directed human gene editing in somatic cells and stem cells is rapidly transitioning from bench to bedside with clinical trials beginning to appear throughout the world. Our laboratory and others have demonstrated that the repair of a point mutation can be facilitated by the combined activity of a single-stranded oligonucleotide and the CRISPR/Cas9 system. Using K562 cells, we found that the presence of the appropriate CRISPR/Cas9 complex leads to an enhancement in the frequency of gene editing. While gene editing activity does in fact increase in a dose-dependent fashion, we find a heterogeneity of modified genetic sequences that are created and maintained even when repair at the sickle cell disease mutation in the human beta-globin gene is successful. Our data suggest that CRISPR complexes leave a genetic footprint at the target site by creating a DNA junction heterogeneity as a byproduct of the reaction. This heterogeneity could be called on-site mutagenesis and one of the most interesting is a curious pattern of DNA nucleotides adjacent to the double-stranded break site; footprints of the human delta-globin gene. A targeted population of cells contains one allele of apo appears to be a chimera of the HBB and the hemoglobin delta gene (HBD). Our results suggest the genes with similar sequences (related family members) such as HBB and HBD could be involved in template repair of double-strand DNA breaks independent of exogenously added donor DNA. We further evaluated the relationship among cellular delivery, nuclear uptake in CD34+ progenitor cells, often viewed as the benchmark metric of successful gene editing, and single base repair. We took a similar combinatorial approach using single-stranded oligonucleotide and a CRISPR/Cas9 ribonucleoprotein to convert wild-type HBB into the sickle cell genotype. Confocal microscopy data show that the CRISPR/Cas9 ribonucleoprotein tends to accumulate at the outer membrane of the CD34+ cell nucleus when NEON Transfection System is employed, while the ribonucleoproteins do pass into the cell nucleus when nucleofection is used. When the correct delivery system is maximized, we once again a similar genetic pattern of on-site heterogeneity created by the activity of the CRISPR Gene editing complex. Our results indicate that more stringent criteria must be established to facilitate the clinical translation and scientific robustness of gene editing for sickle cell disease, most critical in stem cell therapy.

Biography

Eric B. Kmiec is well-known for his pioneering work in the fields of molecular medicine and gene editing. Throughout his professional career, He has led research teams in developing gene editing technologies and genetic therapies for inherited disorders such as Sickle Cell Disease. He is the recipient of multiple research awards from the National Institutes of Health (RO1s, R21s), the American Cancer Society, and private foundations including the 2012 Proudford Foundation Unsung Hero Award in Sickle Cell Disease. He has been a member of numerous editorial boards, NIH study sections and review boards and is the (primary/senior) author of more than 145 scientific publications (mostly in genetic recombination and gene editing).

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