319. Rationally Designed AAV Inverted Terminal Repeats Enhance Gene Targeting

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Basic and clinical applications of mammalian genetic engineering rely on selective pressure and/or, ironically, DNA damage at or near the modification site to obtain relevant efficiencies. In regards to the later situation, continuously evolving endonuclease technologies remain focused on the generation of a site-specific DNA double strand break to stimulate homology directed repair (HDR). The stimulation of HDR is thus dictated by the ability of the endonuclease platform to specifically recognize its cognate site, with safety concerns dictated by its promiscuity influenced by its persistence. To eliminate the serious safety concerns associated with designer endonucleases while maintaining efficient HDR, it was hypothesized that rational modification of a viral DNA repair substrate would enhance gene editing without the requirement for induced chromosomal damage. Specifically, modifications of the adeno-associated virus (AAV) inverted terminal repeat sequence (ITR), in a viral vector context, were evaluated for stimulation of HDR in human cells. The results demonstrate an approximate 30-fold enhancement of AAV gene targeting using a particular rationally designed ITR sequence. Characterization of this event suggests that differences in the inherent ITR-initiated transcriptional activity and altered interactions with host DNA repair proteins contribute to the enhancement in HDR. The collective results demonstrate for the first time that alterations in the ITR sequence can enhance AAV gene targeting. Importantly, the refinement of such vectors may offer a safer alternative to site-specific endonuclease technologies as on- and off-target DNA cleavage concerns are eliminated.

320. Transcriptional Silencing via Synthetic DNA Binding Protein Lacking Canonical Repressor Domains as a Potent Tool to Generate Therapeutics

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Transcription factors (TFs) function by the combined activity of their DNA-binding domains (DBDs) and effector domains (EDs). Here we show that in vivo delivery of an engineered DNA-binding protein uncoupled from the repressor domain entails complete and gene-specific transcriptional silencing. To silence RHODOPSIN (RHO) gain-of-function mutations, we engineered a synthetic DNA-binding protein lacking canonical repressor domains and targeted to the regulatory region of the RHO gene. AAV-mediate retinal delivery at a low dose (AAV2/8-CMV-ZF6-DBD, 1x10e10 vector genomes, vg) in the porcine retina resulted in selective transcriptional silencing of RHO expression. The rod photoreceptors (the RHO expressing cells) transduced cells when isolated by FACS-sorting showed the remarkable 90% RHO transcriptional repression. To evaluate genome-wide transcriptional specificity, we analyzed the porcine retina transcriptome by RNA sequencing (RNA-Seq). The differentially expressed genes (DEGs) analysis showed that only 19 genes were perturbed. In this study, we describe a system based on a synthetic DNA binding protein enabling targeted transcriptional silencing of the RHO gene by in vivo gene transfer. The high rate of transcriptional silencing occurring in transduced cells supports applications of this regulatory genomic interference with a synthetic trans-acting factor for diseases requiring gene silencing in a large number of affected cells, including for instance a number of neurodegeneration disorders. The result support a novel mode of gene targeted silencing with a DNA-binding protein lacking intrinsic activity.

321. Deletion of Mutated GAA Repeats from the Intron 1 of the Frataxin Gene Using the CRISPR System Restores the Protein Expression in a Friedreich Ataxia Model

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The CRISPR system is now widely used as a molecular tool to edit the genome. We used this technique in Friedreich Ataxia (FRDA), an inherited autosomal disease known to cause a decrease of the mitochondrial frataxin protein. Genetic analysis revealed a GAA repeat expansion within the intron 1 of the frataxin (FXN) gene. We used cells derived from the YG8sR mouse model where the mouse frataxin gene is knockout but contain a human FXN mutated transgene on one allele. We then deleted the GAA trinucleotide repeat using 2 specific guide RNAs (gRNAs) co-expressed with either *S. pyogenes* (Sp) or *S. aureus* (Sa) Cas9. We were able to monitored an increase up to 2-fold of frataxin mRNA and protein levels in clone cells. We also confirmed these results *in vivo* using DNA electroporation in the *Tibialis anterior* muscle of the YG8R mice. Ongoing *in vivo* investigation of a systemically injected AAV-DJ vector expressing the SaCas9 and 2 successful selected gRNAs in the mouse model YG8sR will hopefully provide more details answers on the efficacy of the approach and give us preliminary data to go forward for clinical trial. The deletion of the GAA repeats expansion then might be a highly valuable gene therapy approach for FRDA patients.

322. Genome Editing for Nucleotide Repeat Disorders: Towards a New Therapeutic Approach for Myotonic Dystrophy Type 1

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Myotonic dystrophy type 1 (DM1) belongs to the group of nucleotide repeat disorders. More specifically this autosomal form of muscular dystrophy is caused by the expansion of the CTG trinucleotide repeat located at the 3' untranslated region (3′-UTR) of the *DMPK* gene. Elongated CUG repeats of the mutated DMPK mRNAs become sequestration sites for splicing factors, and induce the formation of stable ribonucleoprotein complexes visualized as foci. As a consequence, the alternative splicing of numerous transcripts is dysregulated, which leads to the DM1 pathological alterations affecting various tissues. We have developed a strategy to delete the CTG repeat expansion in the human *DMPK* locus by using the CRISPR/Cas9 system. For that purpose, we constructed different expression platforms for small size Cas9 nucleases under either a ubiquitous or a muscle-specific promoter and guide RNAs (sgRNAs) targeting the 3'-UTR of the *DMPK* gene. Co-transfection of these