746 - AAVHSC Vectors Mediate Highly Precise and Efficient Homologous Recombination-Based Gene Editing

May 13, 2017, 10:30 - 10:45 AM Marriott Salon 2

Keywords
Gene Correction/Modification/Targeting,AAV Vectors,Hematopoietic Stem Cells

Authors
Laura Smith[1], Jason B. Wright[2], Gabriella Atallah[1], Thia St Martin[2], Taihra Ul-Hasan[1], Manasa Chandra[1], Abby Fong[1], Yuman Fong[3], Kamehameha K. Wong[1], Saswati Chatterjee[1]

[1]Surgery, Beckman Research Institute of City of Hope, Duarte, CA,[2]Homology Medicines Inc, Bedford, MA,[3]Surgery, City of Hope National Medical Center, Duarte, CA

Disclosures
L. Smith: 1; Commercial Interest ***i.e. Company X***; Homology Medicines Inc. 1; What was received? ***i.e. Honorarium***; Salary. 1; For what role? ***i.e. Speaker***; Current Employment.

Abstract
The permanent correction of pathogenic mutations by genome editing is a viable and attractive therapeutic strategy. To date, most editing platforms utilize the induction of nuclease-mediated DNA breaks of the genome as a first step in initiating the editing events. These breaks are then primarily repaired using the error-prone non-homologous end joining (NHEJ) pathway. The presence of a donor DNA correction template at the site of the DNA break, can result in a minor fraction of the breaks being repaired by the more precise homologous recombination (HR) pathway. However, NHEJ-mediated repair of the majority of nuclease-mediated breaks result in insertion/deletion errors at the site of breaks or inclusion of the entire vector genome including the inverted terminal repeats (ITRs), in both orientations via NHEJ. The off-target effects of nuclease-based DNA cleavage further pose unknown mutagenic risks. Thus, a precise, efficient and predictable genome editing technology based solely on HR pathways would represent a significant advance in the field. We recently described a group of novel natural AAVs isolated from CD34+ human hematopoietic stem cells known as AAVHSCs. Here we evaluated the capacity of AAVHSC editing vectors to mediate precise and efficient genome editing in vitro and in vivo. Editing vectors were designed to insert a promoterless GFP reporter cassette into the human PPP1R12C gene located in the safe harbor site, AAVS1, such that expression would be driven by the chromosomal PPP1R12C promoter. AAVHSC editing vector genomes included homology arms that flanked the promoterless reporter and specified the target genomic site to be edited. Editing was measured by GFP expression at the cellular level and confirmed by multiple molecular assays including Sanger and Next Generation sequencing. Phenotypic editing efficiencies up to >50%, were observed by flow cytometry for GFP expression in primary cells including human CD34+, hepatic sinusoidal endothelial cells and myotubes and myoblasts. Editing efficiencies of AAVHSC vectors were significantly higher than AAV2, AAV6 and AAV8, approximately 10-50-fold, ***in vitro***. Sequence analysis of edited genomes revealed no evidence of indels or inclusion of AAV ITRs, hallmarks of the utilization of the NHEJ pathway. BRCA2, an essential mediator of HR, was found to be critical for successful editing, indicating that HR was the underlying operative mechanism. In depth unbiased on and off target analyses revealed that editing was highly precise (Wright et al, ASGCT 2017). Similar editing outcomes were observed at other genomic loci. We additionally tested

the capacity of AAVHSC vectors to mediate in vivo targeted insertion of a promoterless luciferase gene into the murine safe harbor locus Rosa26, such that expression could only occur following accurate insertion downstream from the Rosa26 promoter. Intravenous injection of AAVHSC Rosa26 editing vectors led to rapid and sustained widespread expression of luciferase with no apparent toxicity. Mice injected with control AAVHSC vectors without homology arms did not express luciferase, confirming that expression resulted from accurate editing. Significantly, our results demonstrate that AAVHSC represent a uniquely HR-based, highly precise and efficient gene editing modality in a single component, nuclease-free platform with a built-in delivery component. This now enables the development of therapeutic in vivo genome editing for the treatment and potential cure of human diseases.