553. Comparision of AAV Serotypes for Gene Delivery to iPSC Derived RPE

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Inherited retinal degenerations, such as retinitis pigmentosa (RP) and Leber congenital amaurosis (LCA), are characterized by progressive impairment of visual function associated with degeneration of retinal pigment epithelium (RPE) and photoreceptors. These monogenic diseases are genetically heterogeneous due to mutations in genes expressed affecting those cells. Gene therapy holds great potential for the treatment of inherited retinal diseases for which there currently is none. Efficient retinal gene transfer has been achieved with several recombinant viral vectors, including those derived from adenovirus, retrovirus, herpesvirus, and adeno-associated virus (AAV). Among these, AAV vectors appear particularly amenable to retinal gene transfer. However the success of any preclinical study depends on the availability of relevant animal models. In situations where suitable animal models are unavailable recent studies have generated proof-of concept data using personalized cell models: induced pluripotent stem cells (iPSC) derived from affected individuals and un-affected controls. With the increasing number of retinal gene therapy paradigms and recombinant vectors, *in vitro* bioassays characterizing vector transduction efficiency and quality are becoming increasingly important. To date, most *in vitro* assays using recombinant vector transduction have targeted iPSCs. To elicit additional features relevant to the disease, we differentiated iPSCs to RPE and evaluated the transduction efficiencies of a panel of AAV serotypes. Characterization of iPSC-derived RPE by qRT-PCR, immunohistochemistry, western blot analysis and flow cytometry showed the expression of typical RPE markers, phagocytic ability and gene-expression patterns similar to those of native RPE. Comparison of transduction efficiencies of different AAVs in iPSC-derived RPE was carried out using an enhanced green-fluorescent protein (eGFP) reporter gene driven by the cytomegalovirus immediate-early (CMV) promoter. At 24 to 48 hours post transduction, cells expressing GFP were identified by Typhoon scanner, and flow cytometry. Relative GFP expression was evaluated by using the software, Image-J. Of the tested AAV serotypes, AAV2 transduced iPSC-derived RPE cells most efficiently, followed by AAV7m8, AAV1 and AAV6. Differentiation into retinal neuronal cells types may require use of alternative AAV serotypes in order to obtain transduction efficiencies relevant to determination of therapeutic efficacy.

554. Serotype Comparison of AAV Transduction in Adipose-Derived Stem Cells

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Adipose-derived stem cells (ASC) demonstrate promising results in the treatment of many diseases. However, it is unlikely that a simple infusion of cells will provide the full range of desired treatment results. Transient genetic modification of ASC to turn them into drug-eluting depots is likely to enhance their reparative characteristics and accelerate healing. We hypothesized that adeno-associated virus (AAV), an approved gene therapy vector that has never been associated with disease, has several ideal characteristics needed for creating drug-eluting ASC. The most common recombinant AAV vectors were tested for transduction and duration of gene expression. rAAV5 demonstrated both the highest and longest term expression. The glycosylation profile of ASC was determined and we show that rAAV5 transduction requires plasma membrane associated sialic acid. Future studies will focus on rAAV5 as the vector of choice to deliver disease specific genes to drive biological drug delivery, engraftment, and disease correction.

555. Development of a Post-Exposure Treatment for Ebola Virus Infections Based on AAV Vectors and Zmapp Antibody Cocktail

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The recent Ebola outbreak in West Africa has been the deadliest in the history. To prevent future recurrence of such outbreak, better treatments and effective vaccines against Ebola virus are desirable. Among such promising treatments, the Zmapp cocktail containing neutralizing antibodies (13C6, 2G4 and 4G7) has successfully treated some patients. However, the feasibility of using it on large populations especially in developing countries is questionable. To address this potential issue, we propose to employ recombinant vectors derived from adeno-associated virus (rAAV). There are several advantages of using rAAV: because of 1) their safety profile; 2) only one injection (or a few) would be required; 3) the high stability of lyophilized rAAVs at ambient temperature and; 4) the panel of available serotypes. Because of these interesting features, we are currently developing a treatment based on three rAAVs to deliver the genes for the Zmapp cocktail of antibodies. We have already produced at small scale a rAAV expressing the 2G4 antibody. The DNA sequences for the heavy chain and light chains were codon-optimized for better expression in humans and were designed to be expressed from the same gene. A strong promoter (CAG) resistant to silencing in vivo was chosen to drive gene expression of the antibody. The rAAV were produced by transfection using our patented cGMP compatible HEK293 cell line. The production was performed in suspension culture in the absence of serum. Secretion of 2G4 antibody by rAAV transduced cells (HEK293 and CHO cells) was confirmed. The results demonstrated that rAAV-CAG-2G4 was functional and allowed for the correct assembly of the heavy and light chains of 2G4. Purification of 200 mL of rAAV-CAG-2G4 production was performed by ultracentrifugation on an iodixanol density-step gradient. Two other rAAVs coding 13C6 and 4G7 antibodies are in the processed of being constructed and produced in a similar manner. We are also in the process of comparing the efficacy of two serotypes of AAV (9 and DJ) in mice by intranasal delivery. Using the best serotype, the rAAVs will be produced and purified from a starting suspension culture of 20 L. Their efficacy for treating Ebola infections will then be evaluated in a mouse model infected by the virus.

556. Comparative *In Vitro* Transduction Efficiency of AAV Vector Serotypes 1-9 in Different Cellular Models

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Recombinant viral vectors can be useful tools for expressing transgenes in neuronal cells to study disease mechanisms and to test for therapeutic effect. Recombinant adeno-associated virus (AAV), generated from a nonpathogenic parvovirus, has become the most