301. Efficient Delivery of Nuclear and Cytoplasmic Proteins Fused to HIV-1 Gag Polypeptide By Means of Virus-Like Particles

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The expression of the retroviral polypeptide Gag can induce the formation of virus-like particles (VLP). Upon expression, the polypeptide is targeted to the cell membrane and incorporated in the VLP during membrane budding. Chimeric proteins consisting of Gag polypeptide fused to different proteins were engineered and the VLP produced were used as vehicles for protein delivery. One major advantage of this approach is the low mutation risk due to the absence of DNA transfer and genomic integration. In this study, the C-Terminus of Gag from HIV-1 was fused to the green fluorescent protein (GFP), a chimeric transactivator (cTA) and a reprogamming factor (KLF4). VLP were produced by transfection using a stable cell line (293SF-pacLV) that expresses VSVg and Gag-pol. Analysis of the supernatants from producing cells by western blot confirmed the presence of Gag-GFP, -cTA and -KLF4. Confocal microscopy showed that the vast majority of the cells (> 90%) treated with VLP-GFP/polybrene complexes was successfully transduced. The cells also displayed a GFP signal almost exclusively localized inside the cytoplasm. Additional VSVg expression during the production facilitated the endosomal escape of VLP-GFP in transduced cells. The insertion of a nuclear localisation signal (NLS) shifted the localization of the GFP to the cell nucleus demonstrating that a nuclear protein could be successfully delivered by VLP. The experiment was thus repeated using two transcription factors. Lentiviral vectors were used to make two stable pools of HEK293 cells each containing a specific GFP reporter cassette. The GFP gene was regulated either by the CR5 promoter (specifically activated by the cTA) or by a minimal promoter fused to KLF4 transcription response elements (TRE). Transduction of the CR5-GFP pool with VLP-cTA/polybrene complexes showed a powerful activation of the reporter (365-fold compared to the negative control) as measured by flow cell cytometry two days post-transduction. Surprisingly, no activation was observed in the TRE-GFP pool three days after transduction by VLP-KLF4/polybrene complexes. Evidence obtained by transfection suggested that the Gag fusion inhibits KLF4 activity. To augment activity, the activation domain of VP16 was fused to KLF4. Transfection of a plasmid encoding Gag-VP16KLF4 strongly activated the reporter by a factor of 126-fold (6-fold higher than wild type KLF4). The ability of VLP produced with Gag-VP16KLF4 to activate transcription is currently under investigation. In summary, VLP based on HIV-1 Gag can deliver nuclear and cytoplasmic proteins directly into cells with a low mutation risk. Therefore, our platform for VLP production could be useful for several applications including cell reprogramming and genome editing oriented toward cell therapies of diseases like Duchenne muscular dystrophy.

AAV Vectors II

302. Combinatorial Engineering of a Receptor Footprint on AAV Serotype 4 Yields Novel Vectors

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Most AAV strains in clinical development display a broad range of cellular tropisms within the central nervous system (CNS). As such, this current panel of vectors provides several options for efficient gene expression within neurons and/or glia in the brain. However, precise therapeutic interventions will require selective targeting of distinct cell populations within the brain and other organs, such as the eye. In this regard, a select few AAV serotypes have demonstrated unique tissue tropisms following systemic or peripheral routes of administration. One such example, originally isolated from African green monkeys, is AAV4. This serotype is one of the most divergent serotypes identified to date. From a structural perspective, AAV4 has several unique surface loop topologies that are consistent with the remarkably different usage of receptors, cellular tropism and unique antigenicity displayed by this serotype. Specifically, AAV4 is known to utilize O-linked sialoglycans, or mucins, for cell surface attachment. In the current study, through a combination of structural analysis and random mutagenesis, we identified several key amino acids for further genetic manipulation. Saturation mutagenesis of key residues within a broad receptor footprint on the AAV4 capsid surface yielded a highly diverse library, which was subjected to screening as well as directed evolution in a panel of cell lines in vitro. Additional screening and directed evolution studies in vivo in the mouse CNS yielded several novel AAV4 variants that display distinct transduction profiles in the brain. Extensive in silico analyses, structural modeling, and biological characterization of the novel panel of AAV strains is currently in progress.

303. AAV Capsid Evolution for Enhanced Antibody Delivery To Human Muscle for Use in Next-Generation HIV Vaccines

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Our goal is to develop a recombinant adeno-associated virus (AAV) vector that is both resilient to neutralization by pre-existing human antibodies, and can efficiently transduce human muscle after systemic or intramuscular administration at levels sufficient to express therapeutic quantities of human monoclonal antibodies with broad-spectrum protection against HIV for use as a prophylactic vaccine and as a treatment for HIV-infected individuals. We hypothesized that capsid library screens in human skeletal muscle cells both *in vitro* and *in vivo* would be an ideal route to accomplish these goals. We utilized wild-type replicating AAV libraries of 10e5 variants via DNA shuffling of eleven different parental AAV capsids. Our libraries selectively replicate in human cells when co-administered with wild-type adenovirus, making chimeric humanized muscle mice and human muscle cell cultures excellent tools to allow for selection of capsids with tropism for human muscle cells. Two *in vivo* screens are ongoing in chimeric humanized muscle mice. These screens involve transplanting immunodeficient mice with pooled primary human muscle stem cells FACS-purified from skeletal muscle biopsies from five human donors. Human muscle progenitors fuse with those from the mouse and create chimeric myofibers expressing human muscle proteins for AAV selection. AAV capsid libraries are used to serially