AAV Biology

37. Capacity of Viral Genome Packaging and Internal Volumes of AAV Viral Particles

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Adeno-associated virus (AAV) vectors have widely been used in many preclinical and clinical studies, showing promise as the most effective in vivo gene delivery vector. Despite successful application of AAV vector-mediated gene therapy to the treatment of certain genetic diseases such as hemophilia, the inability to package genomes of longer than 5 kb has precluded broader applications of this approach. Whether or not longer genomes would become able to be packaged by viral capsid engineering has yet to be determined; however, what is lacking in this field is comprehensive understanding of the maximal capacity of genome packaging in various AAV serotypes through a systematic analysis. Here we developed a novel computational algorithm to calculate the capsid internal volume that minimizes approximation and determine the internal volumes of 22 eukaryotic viruses with T=1 icosahedral symmetry including 16 viruses that belong to the family of Parvoviridae. In addition, we systematically determine the maximal packaging capacity of both VP1/VP2/VP3 capsids and VP3 only capsids derived from various serotypes using a panel of recombinant AAV2 vector genomes of varying sizes from 4.80 kb up to 6.00 kb by an increment of 0.05 kb, termed ruler genomes. To determine capsid internal volumes, three dimensional structure coordinates of each viral capsid were retrieved from the Protein Data Bank, and then, using an algorithm we have developed, each atom was inflated in such a way that interstices between adjacent atoms in the protein shell are effectively filled with protein while keeping the shape of the inner surface of the capsid unchanged. The internal volume of the capsid was then calculated by a numerical integration of coordinates in the non-protein space that were closer to the center than the atom that is most distant from the center of the capsid. By this method, we find that the genome lengths and internal volumes of the 22 eukaryotic T=1 viruses that we analyzed vary from 0.83 kb to 6.04 kb and from 6.72×10^5 to 3.46×10^6 Å^3, respectively, showing a strong non-linear allometric relationship with an exponent of 1.30 (R^2=0.97). In the following AAV serotypes, AAV1, 2, 3B, 4, 5, 6, 8 and 9, AAV5 is found to have a distinctively smaller volume (2.48×10^6 Å^3) compared to the other 7 serotypes ($2.62 \pm 0.03 \times 10^6$ Å^3, mean ± SD). This analysis also reveals that AAV2 VP3 only capsid has a 7% larger volume than AAV2 VP1/VP2/VP3 capsid. In keeping with this observation, a preliminary study using the ruler genomes has indicated that the AAV2 VP3 capsid can package a genome that is longer than that can be packaged in the AAV2 VP1/VP2/VP3 capsid by several hundred nucleotides, which appears to correspond to the difference in the volume of the two different capsids. Investigation of other serotypes and capsid mutants is currently underway. Thus, the approach presented here will help understand the genome packaging and provide insights into how to design capsids to overcome packaging limitations.

38. Pharmacological Regulation of Vesicular Trafficking as a Strategy to Enhance Recombinant AAV Transduction

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Vector dose-related toxicity has been highlighted as a potential safety concern in recent preclinical studies and clinical trials with recombinant AAV vectors. Thus, investigation of strategies to enhance AAV transduction may allow for administration of lower vector doses, thereby mitigating toxicity. Several host cell factors have been shown to restrict viral infection during post-entry trafficking steps through a variety of mechanisms. In the case of AAV, the 26S proteasome is thought to reduce transduction efficiency by restricting nuclear entry via ubiquitin-dependent capsid degradation. The AAA+ ATPase p97/valosin-containing protein (VCP) is a segregase and unfoldase which is closely associated with the proteasomal degradation pathway and disassembles multimeric protein complexes for subsequent processing. VCP modulates numerous cellular processes ranging from endosomal sorting to autophagy and ER-associated degradation. In the current study, we sought to determine whether VCP plays a role as a restriction factor in the AAV infectious pathway. Chemical inhibition of VCP by Eeyarestatin I (EerI) increased AAV transduction by nearly an order of magnitude in a cell type and serotype independent manner. This effect was associated with increased recovery of viral genomes and capsid accumulation within the nucleus, but not cell surface binding or uptake. Interestingly, VCP appears to restrict AAV infection by regulating vesicular trafficking of viral particles. In particular, VCP inhibition redistributes AAV capsids to Rab7 and LAMP1 positive vesicles, while simultaneously disrupting capsid association with syntaxin-5 positive vesicles recently shown to be essential for Golgi transport. These results support the notion that AAV vesicular transport can be redirected to enhance transduction efficiency. In summary, our results identify VCP as a novel host restriction factor that regulates the post-entry trafficking of recombinant AAV vectors, and highlight VCP as a potential target for modulating AAV transduction in the clinic.

39. Modulation of Histone Dosage and Chromatin Dynamics Regulates AAV Transduction

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Recombinant adeno-associated vectors (AAVs) have gained momentum due to their combined characteristics of safety and efficiency. Nonetheless, the identity of the host functions intercepting the AAV transduction pathway still needs to be thoroughly understood.

To address this issue on a genome-wide scale, we previously performed an unbiased RNAi high throughput screening (HTS; 18,120 human target genes) and identified 710 negative and 414 positive regulators of AAV efficiency. Based on the results obtained and with the purpose to identify factors involved in single-stranded (ss) AAV genome processing, we compared, again in an HTS format, siRNAs affecting ssAAV, but dispensable for scAAV transduction. One of the genes identified was ERI-1. While the effects of this protein were negligible on scAAV transduction, its knock down or overexpression lead to a 5 fold decrease or 2-7 fold increase of ssAAV efficiency, respectively.

ERI-1 is a 3'-exoribonuclease known to degrade endogenous miRNAs and histone mRNAs. Indeed, we observed that AAV transduction negatively correlated with histone mRNA levels. Chromatin immunoprecipitation (ChIP) studies aimed at assessing the extent of chromatinization of the AAV genome revealed that

the overexpression of ERI-1 determined an over 10-fold, selective reduction on ssAAV genome association with H3 and H4, while changes were negligible for scAAV DNA and for control cellular genes. Consistent with chromatin exerting a repressive role on ssAAV transduction, we also noticed that the downregulation of the main replication-dependent histone chaperone CAF-1 induced an over 20-fold increase in transduction. Increase of ssAAV2 transduction by ERI-1 also decreased the association, with the viral DNA, of proteins of the cellular DNA damage response (DDR; e.g. Nbs1 and Mre11), which our previous work had indicated as inhibitory of AAV transduction.

Interestingly, DNA damage per se induced downregulation of histone gene expression. In particular, hydroxyurea, a drug markedly increasing AAV transduction, also determined histone mRNA degradation, an effect that required integrity of ERI-1.

These results underline the importance of chromatin and its dynamic regulation in determining the fate of productive AAV transduction. These findings can be exploited for the development of more effective AAV-mediated gene delivery strategies.

40. Transgene Product-Specific Treg Are Initially Induced in the Celiac Lymph Node During Tolerance Induction By Hepatic AAV Gene Transfer

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Previous work by us and several other laboratories has shown that liver-directed gene transfer can induce transgene product-specific immune tolerance. Induction of CD4+CD25+FoxP3+ regulatory T cells (Treg) is a critical component of the tolerance mechanism. Gaps in knowledge exist about the identity of the antigen-presenting cells (APCs) and the origin of peripherally induced Treg. To characterize the interaction of adeno-associated virus (AAV) antigen expression in the liver with immune cells, we used AAV8-EF1a vectors, which have a high tropism for murine liver, expressing either a secreted ovalbumin (AAV8-ova) or cytoplasmic ovalbumin (AAV8-Cyto-ova). In Treg-deficient mice transgenic for an ova-specific CD4+ T cell receptor (DO11.10-tg Rag-2-/- BALB/c), Treg were induced with either vector. For secreted ova, multiple organs (liver, spleen, lymph nodes [LN]) showed a similar level and time course of Treg induction, with greater than 1% of the CD4+ T cells being Treg starting at 4 weeks. Substantial T cell activation using the very early activation marker CD69 was also observed in these tissues starting at 3 weeks after AAV8-ova. However, Treg induction was observed in the celiac LN (one of the liver-draining LN) as early as 3 weeks after AAV8-Cyto-ova gene transfer, followed by induction of Treg in other tissues by 4 weeks. Interestingly, T cell activation was notably higher in liver and celiac LN after AAV8-Cyto-ova with over 70% activated CD4+ T cells after 4 weeks. In addition, T cell activation was observed earlier in all tissues after AAV8-Cyto-ova when compared to AAV8-ova. Literature data document a role for tolerogenic antigen presentation in the liver itself via sinusoidal endothelial cells and possibly hepatocytes. Our data suggest an important role of conventional mechanisms of adaptive immunity involving draining LNs that survey tissue antigens leading to early T cell activation and Treg induction of hepatic expressed non-systemic antigens. To start defining the critical APCs, BALBc mice were given AAV8-ova vector two weeks prior to adoptive transfer of CellTrace Violet-labeled CD4+ T cells from DO11.10 donor mice and their proliferation was measured using flow cytometry after 5 days. Half of these mice had their Kupffer cells and M1 macrophages inactivated with Gadolinium chloride for two days prior to DO11.10 adoptive transfer. DO11.10 cells proliferated in an antigen-specific manner, and proliferation was blocked by Gadolinium chloride, supporting a role for professional APCs in presentation of hepatic transgene product. Our data suggest and reinforce the importance of Kupffer cells as key APCs for hepatic antigens and further characterize the impact of the route of antigen expression on T cell activation and Treg induction.

41. U2 snRNP Spliceosome Proteins Block Recombinant AAV Vector Transduction

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The recent approval, continued success and safety of recombinant adeno-associated viral (AAV) vectors in gene therapy clinical trials have paved the way for therapeutic modalities for a range of genetic disorders. However, efficient therapeutic gene expression by AAV vectors can often require high vector doses, presenting potential challenges related to production and dose-related toxicity. In this regard, a particularly well known barrier to AAV transduction is the host proteasomal machinery. To identify specific host restriction factors, we screened an siRNA library that revealed several candidate genes including the PHD finger-like domain protein 5A (PHF5A), a U2 snRNP-associated protein. Disruption of PHF5A expression selectively increased transduction by multiple AAV serotypes. Notably, genetic disruption of U2 snRNP and associated proteins, such as SF3B1 and U2AF1, also increased AAV vector transduction, suggesting the critical role of U2 snRNP spliceosome complex in this host-mediated restriction. Moreover, pharmacological inhibition of U2 snRNP by meayamycin B, a potent SF3B1 inhibitor, substantially enhanced AAV vector transduction of clinically relevant cell types. Further study indicated that U2 snRNP proteins suppress AAV vector transgene expression through recognition of incoming AAV capsid and enhanced histone recruitment to the vector genome, independently of the cellular splicing machinery. In summary, we postulate a novel cellular mechanism involving spliceosome U2 snRNP proteins in regulating AAV vector epigenetic modification and its manipulation to enhance transgene expression for human gene therapy.

42. Differential Transduction Profiles of AAV Vectors in a Mouse Model of Human Glycosylation

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Recent studies have demonstrated that AAV gene transfer efficiency and tropism, particularly with regard to the liver, can vary markedly across animal species. A mechanistic understanding of such host-specific AAV transduction profiles is essential for translating preclinical studies to the clinic. In this regard, virus-glycan interactions are key determinants of tissue tropism displayed by various AAV vectors. It is now known that AAV serotypes 1, 4, 5 and 6 utilize glycans terminating in sialic acid (Sia) as a primary receptor. Amongst various forms of Sia, N-glycolylneuraminic acid (Neu5Gc) is the most prevalent in most mammals; however humans are unable to synthesize Neu5GC from its precursor, N-acetylneuraminic acid (Neu5AC), due to a loss-of-function mutation. Consequently, Neu5AC is the predominant Sia in humans. In the current study, we first observed that metabolic incorporation of the nonhuman Sia, Neu5GC selectively reduces transduction efficiency of AAV1 and 6 in vitro. Utilizing a mouse model genetically engineered to express only the human-specific Sia, Neu5AC, we assessed the effects of sialic