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The Structure of the 501 Residue on AAVHSC16 is Imperative to the Functional Binding to Cell Surface Glycans, which is a Key Step in Successful Transduction

Smith L, Schulman L, Van Lieshout L, Smith S, Behmoiras L, Seymour A and Francone O.

Homology Medicines, Inc.

AAVHSCs, derived from human hematopoietic stem cells, are 15 naturally occurring adeno-associated virus (AAVs) with one to four unique variations in the capsid when compared to fellow Clade F member, AAV9. Cell surface glycans play a key role in the virus-cell interactions as glycan binding is the first step of successful transduction. We investigated how the unique capsid residues of a panel of AAVHSCs contribute to cellular glycan binding and post internalization processes. We have demonstrated that all AAVHSCs tested preferentially bind to terminal galactose with the exception of AAVHSC16. While AAVHSC16 shares the unique variation 505R with several other AAVHSCs, AAVHSC16 has two additional unique variations, F501I and Y706C. Modeling revealed that the 501 and 706 residues are surface exposed on the AAVHSC16 capsid and that the 501 residue is in close proximity to key residues that comprise the galactose binding pocket on AAV9. Phenylalanine is conserved at the 501 position among the major AAV serotypes. To determine the contribution of the two AAVHSC16 residues to altered glycan binding, we mutagenized the 501 and 706 residues on AAVHSC15. Surface exposed glycan binding and expression of the AAVHSC15 F501I and Y706C modified capsids was tested on a panel of glycan mutant CHO cell lines. Transductions were performed on ice to prevent ATP mediated internalization of bound vector genomes (vgs) and cell surface bound vgs determined after unbound vgs were removed through a series of cold PBS washes. Bound vectors were quantified by quantitative PCR post-washing and successful internalization and transgene expression assessed by flow cytometry 24 hours post- washing. Binding of AAVHSC15 F501I to terminal galactose was significantly reduced to AAVHSC16 levels while the AAVHSC15 Y706C variation resulted in a similar glycan binding profile as wild-type AAVHSC15. While AAVHSC16 and AAVHSC15 F501I had similar levels of vgs bound to the cell surface, AAVHSC15 F501I had higher GFP expression than AAVHSC16. AAVHSC15 also has a unique T346A variation and it is possible this variation influences post internalization transgene expression. To further investigate the impact of the residue at the 501 position in the AAVHSC glycan binding, we substituted the isoleucine on AAVHSC16 with various amino acids including, leucine, threonine, valine, tryptophan and two different tyrosine codons. Each of these substitutions led to an increase in binding to terminal galactose, although full restoration only occurred with the addition of aromatic ring structured residues, suggesting aromatic ringed structures are required for strong carbohydrate recognition. The tyrosine modification at the 501 residue gave the best restoration in galactose binding most likely as it is structurally most similar to phenylalanine. Two tyrosine variants that differ at the codon level displayed differences in their binding to surface exposed glycans. Interestingly, the addition of alternative residues at the 501 position of the capsid also affected binding to non-galactose glycans including mannose and N-acetylglucosamine. While the aromatic ringed structures increased the number of vgs bound to the cell, GFP expression levels were not as high as AAVHSC15, again indicating the possible involvement of T346A on transgene expression. These data imply that the structure of the 501 residue is imperative to the functional binding to cell surface glycans. These studies also highlight the importance of the precise alterations made at key residues when investigating the structure and function relationship of the AAV capsid. Understanding of how the unique natural residues of the

AAVHSCs influence their function allows for rational-based selection of capsids for developing therapeutics.