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VIRAL INFECTION

A key host receptor for AAV

A transmembrane protein receptor that is critical for adeno-associated virus infection has been identified through an unbiased, genome-wide screen. Its role in viral entry could potentially be harnessed to develop enhanced gene therapy vectors and better animal models of human disease.

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Interest in adeno-associated virus (AAV) has surged in the past decade due to its strong clinical promise as a gene delivery vehicle, yet fundamental understanding of the virus's infectious pathway is incomplete. As viruses initiate infection by engaging specific surface receptors on the host cell, identifying these receptors is essential for understanding viral tropism, developing targeted anti-viral therapies, and harnessing viruses as therapeutics[1]. Prior studies in the field have indicated that many AAV serotypes initially bind primary receptors (including glycans and proteoglycans such as heparan sulfate) followed by membrane proteins as secondary receptors that facilitate internalization[2]. A range of secondary receptors has been reported, including the fibroblast growth factor receptor 1 (FGFR1) for AAV2 (ref. 3) and the hepatocyte growth factor receptor (c-MET) for AAV2 (ref. 4) and AAV3 (ref. 5). However, the at times discrepant reports in the literature about receptor usage and mechanisms of viral entry underscore the need to improve our understanding of AAV infection[2].

Carette and colleagues[6] have now discovered a protein receptor (hereafter AAVR) critical for the entry of numerous AAV serotypes and characterize its striking role in both *in vitro* and *in vivo* infection, thereby advancing our knowledge of how AAV penetrates cellular barriers to deliver its genetic cargo. To do so, they performed a loss-of-function, genome-wide screen to identify host factors that are important for infection by AAV2, the most studied serotype. Specifically, the authors used insertional mutagenesis to generate near-haploid human cells (HAP1) with null alleles in non-essential genes, an approach that overcomes the inability of RNA interference to completely silence gene expression. The resulting library of mutant HAP1 cells was iteratively selected for resistance to AAV2 infection, leading to enrichment for cells with mutations in AAVR, a transmembrane protein whose role in cellular function is not well understood.

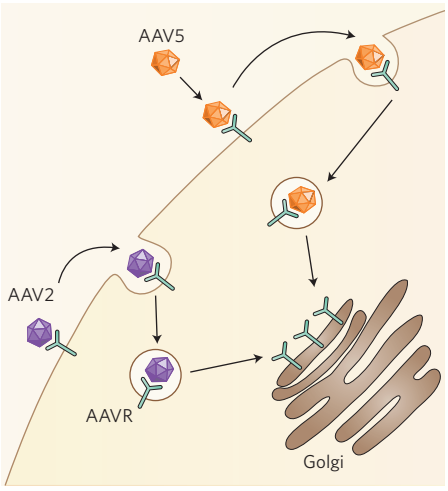

Figure 1 | The role of AAVR in AAV cellular entry. AAVR binds directly to AAV, undergoes rapid endocytosis, and relocalizes to the trans-Golgi network — an internalization and intracellular trafficking route also used by AAV. AAVR is critical for the entry of numerous serotypes, including AAV2 and AAV5, implicating its conserved role in AAV infectivity.

The authors then generated a panel of AAVR knockout (AAVR KO) human and mouse cell lines. All exhibited a dramatic decrease in susceptibility to AAV2, and ectopic AAVR expression rescued infectivity. AAVR was also essential for infectivity of a range of AAV serotypes (1, 2, 3B, 5, 6, 8 and 9) that utilize different glycan primary receptors, indicating that its role is both broad and conserved. In contrast, knocking out FGFR1 and c-MET in multiple cell lines did not significantly reduce AAV2 infectivity, although this serotype may use alternative receptors depending on the target cell type[2]. In addition, AAVR was found to be a co-receptor involved in viral internalization and intracellular trafficking (Fig. 1), as the protein naturally undergoes rapid endocytosis and localizes at the trans-Golgi network (TGN). Finally, AAVR was important for *in vivo* infection, as AAV9 administered to the abdominal cavity poorly infected AAVR KO mice, compared with wild type. Future work with systemic or other routes of administration will further elucidate the importance of AAVR in various tissues.

The discovery of a receptor that is vital for AAV infection has strong implications for both fundamental understanding of virus–host relationships and clinical applications of gene delivery. From a basic standpoint, mapping of the receptor-binding region on the capsid is of particular interest, considering the sequence and structural diversity among the serotypes that apparently utilize AAVR (for example, 60% sequence homology between VP1 of AAV2 and AAV5). Moreover, it will be intriguing to determine whether AAVR-dependence is evolutionarily conserved for all AAV clades, as well as for potential ancestral AAV variants[7,8]. Furthermore, the fact that infectivity is only modestly reduced when AAVR is modified to traffic to endosomes, rather than the TGN, may offer insights into mechanisms that trigger AAV's phospholipase activity and facilitate its escape into the cytosol. Finally, both the natural biological function of AAVR (for example, AAVR KO mice had no reported differentiating phenotype), and the extent to which its expression and localization in the brain[9,10] and elsewhere impact AAV infectivity, warrant further investigation.

From a translational viewpoint, AAV has enjoyed increasing success in several breakthrough clinical studies; however, better vectors are needed to build upon this success[11]. Two complementary approaches for vector engineering are rational design and directed evolution[11]. Deeper mechanistic insights into viral infection may guide rational design efforts to develop vectors with modified receptor targeting of clinically relevant tissues. In parallel, it will be interesting to see whether vectors engineered through directed evolution retain AAVR dependence or acquire the capacity to bind alternative receptors. Furthermore, the analysis of AAVR function

and tissue distribution across species may offer insights into the applicability of different preclinical models for human gene therapy. Finally, the authors suggest that AAV delivery to AAVR-expressing cells may allow the development of improved mouse models that mimic postnatal onset of certain human diseases. For example, analogous to the retroviral tumour virus A (TVA) system[12], murine strains with targeted AAVR expression in specific tissues on an AAVR KO background may be engineered.

In summary, the identification of AAVR as a key host receptor used by multiple AAV serotypes broadens and deepens our understanding of AAV–host cell interactions and potentially enables the development of more effective clinical therapies. ❐

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References

1. Grove, J. & Marsh, M. *J. Cell Biol.* **195,** 1071–1082 (2011).
2. Nonnenmacher, M. & Weber, T. *Gene Ther.* **19,** 649–658 (2012).
3. Qing, K. *et al. Nature Med.* **5,** 71–77 (1999).
4. Kashiwakura, Y. *et al. J. Virol.* **79,** 609–614 (2005).
5. Ling, C. *et al. Hum. Gene Ther.* **21,** 1741–1747 (2010).
6. Pillay, S. *et al. Nature* http://dx.doi.org/10.1038/nature16465 (2016).
7. Santiago-Ortiz, J. & Ojala, D. S. *et al. Gene Ther.* **22,** 934–946 (2015).
8. Zinn, E. *et al. Cell Rep.* **12,** 1056–1068 (2015).
9. Poon, M. W., Tsang, W. H., Waye, M. M. & Chan, S. O. *Histol. Histopathol.* **26,** 953–963 (2011).
10. Hawrylycz, M. J. *et al. Nature* **489,** 391–399 (2012).
11. Kotterman, M. A. & Schaffer, D. V. *Nature Rev. Genet.* **15,** 445–451 (2014).
12. Ahronian, L. G. & Lewis, B. C. *Cold Spring Harb. Protoc.* **2014,** 1128–1135 (2014).