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COMMENT

AAV shuffles to the liver: commentary on Lisowski *et al*.

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The past several years have witnessed enormous strides in the development of clinical gene therapy platforms based on adeno-associated virus (AAV). Challenges remain, however, based on the very simple consideration that AAV did not evolve for our convenience to harness in biomedical applications. Indeed, there are often mismatches between the infectious properties of natural serotypes and the therapeutic delivery needs of human diseases. The properties of viral vectors thus require engineering, and in many situations, the delivery problem is so complex that mechanistic knowledge of virus structure–function relationships is insufficient to enable rational engineering of an intricate virus. Over the past decade, directed evolution has emerged as an alternative approach to enhance vector properties.[1,2] Analogous to its natural counterpart, directed evolution entails the iterative genetic diversification of a biomolecule to generate large variant libraries that are selected for enhanced delivery properties. This approach has led to AAV variants that can evade neutralizing antibodies,[2,3] efficiently infect different cell types *in vivo*,[4,5] and target delivery to specific cells *in vitro* and *in vivo*.[6] A recent advance by Lisowski *et al*. has now harnessed this approach to increase the infectivity of AAV for human hepatocytes,[7] raising the future possibility of enhanced gene expression levels or reduced dosages relative to vectors such as AAV8.

The development of successful gene delivery platforms—including vectors based on AAV and lentivirus—has enabled positive results in trials for numerous diseases, in the case of AAV including Leber's congenital amaurosis type 2,[8–10] hemophilia B,[11] lipoprotein lipase deficiency,[12–14] and very recently choroideremia.[15] These clear clinical advances are emerging in situations where the capabilities of a natural AAV serotype or variant are sufficient to meet the delivery requirements of a disease target. For example, the impressive Leber's congenital amaurosis type 2 and choroideremia studies entailed subretinal injections of comparatively low dosages of AAV2 to an immune-privileged site, and the recent hemophilia B trial harnessed the natural tropism of AAV8 for the liver. As a result, it is anticipated that clinical successes will increasingly emerge in situations where current delivery technologies are sufficiently effective to meet particular clinical needs.

That being said, numerous delivery hurdles pose challenges for extending current successes to future indications. For example, upon systemic administration, natural serotypes typically exhibit tropism for liver, or alternatively for multiple organs, which renders targeted delivery to other tissues difficult. Vector spread can also be limited following direct injection into a given organ, often because high concentrations of a primary AAV receptor (*e.g.*, heparan sulfate) retain the vector at the injection site. In addition, the majority of the human population is seroreactive to multiple AAV serotypes due to prior natural exposure to AAV, and vector injection into regions that are not immune privileged exposes the virus to anti-AAV antibodies that can readily neutralize it. Furthermore, the capacity for targeted delivery to specific cell types is currently very limited, posing concerns for off-target effects of transgene expression and for immune presentation of capsid or transgene epitopes. Finally, limited vector efficiency for a target cell can necessitate higher dosages, which can raise immune response risk and tax current vector manufacturing capabilities. The recent study by Lisowski *et al*. addresses this last problem.[7]

There are advantages for conducting selections of viral vectors in human cells, and doing so *in vivo* may further enhance physiological relevance. Given the importance of liver as a gene therapy target,[11] but the difficulty of maintaining mature hepatocyte phenotype *in vitro*, Lisowski *et al*. utilized an established model comprising human hepatocytes grafted into immunodeficient mice.[16] After four rounds of selection, in which a library of chimeric AAV capsids was administered intravenously followed by human adenovirus serotype 5 as a helper virus to induce AAV replication within the human hepatocytes, several novel AAV variants emerged. One variant in particular, rAAV-LK03, mediated 100-fold greater gene expression in human hepatocytes *in vitro* and transduced a 12-fold greater fraction of human hepatocytes in the xenograft model *in vivo*, when administered at the same dosage as AAV8.[7]

As noted, selections were conducted for the ability of AAV to replicate upon administration of a human adenovirus, which itself cannot replicate in mouse cells, and therefore, rAAV-LK03 selectively transduced the human cells within the mouse model. Subsequent analysis suggested that a single amino acid difference (S262C) relative to another isolated variant may have endowed rAAV-LK03 with the ability to transduce human hepatocytes via the hepatocyte growth factor receptor c-MET, a coreceptor for this variant's parent serotype AAV3B. Collectively, these results demonstrated that it is possible to increase AAV transduction of human hepatocytes in this model. If clinically translatable, this could enable reduction of vector dosage to achieve a given transgene expression level or take steps toward approaching the higher expression levels required for some transgenes (*e.g.*, α-1-antitrypsin).

As with any advance, this study raises intriguing questions. The xenograft system effectively maintains the human hepatocyte phenotype *in vivo*. However, given the importance of immunity in AAV transduction and transgene expression, it will be interesting to investigate the extension of these results to models with an intact immune system. As a related note, although the evolution increased hepatocyte infectivity, the resulting variant was just as susceptible as AAV8 to neutralization by human intravenous immunoglobulin (the pooled immunoglobulin G fraction from thousands of donors). Approximately 40–55% of the human population has antibodies to AAV8 (ref. 17 and Bryne, Conlon, and Agbandje-McKenna, personal communication). Further improvement to evade anti-AAV neutralizing antibodies

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would thus benefit AAV. Additionally, although the selectivity for human cells is intriguing, there are advantages to having a virus that can also infect mice in order to investigate therapeutic efficacy within the many murine models of human disease. Finally, clinical translation proceeds through large animal models, and investigation of vector biodistribution and tropism within a non-human primate model will be a key next step.

In summary, AAV is making major contributions toward the realization of the potential of human gene therapy. Advances in vector engineering, enabled by approaches including directed evolution, promise to enable and accelerate this translation.

CONFLICT OF INTEREST

D.V.S. is an inventor on patents related to directed evolution.

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