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Gutted adenovirus

Gutted adenovirus: a rising star on the horizon?

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Gene therapy approaches for genetic diseases that are based on recombinant adenoviral vectors are often hampered by the fleeting nature of transgene expression after delivery of the therapeutic payload to the target cell. However, in the last March issue of Proceedings of the National Academy of Sciences, Toietta *et al*[1] described an exciting example for lifetime phenotypic correction of an autosomal recessively inherited genetic disease. A single dose of a recombinant adenoviral vector was sufficient to achieve stable correction of Crigler–Najjar (CN) disease type I in rats for more than 2 years.

The intriguing results presented by Toietta *et al* are very encouraging because they demonstrated safe and lifelong correction of a liver-based genetic disease. The authors utilized the well-established Gunn rat model for CN disease,[2] which provides an attractive animal model to test gene therapy protocols for genetic diseases. CN disease type I is a rare inherited metabolic disorder caused by lack of the hepatic bilirubin uridine diphosho-glucuronosyltransferase (UGT1A1) activity, an enzyme which is responsible for the metabolic breakdown of bilirubin. Affected individuals will develop jaundice (yellow skin and eyes) and kernicterus during adulthood. Although the life expectancy of patients can be extended by phototherapy on an ongoing basis and/or liver transplantation, alternative clinical protocols are needed.

Toietta *et al* demonstrated that a single i.v. injection of a medium dose (3×10^{12} viral particles per kg body weight) of a helper-dependent adenoviral vector (HD-Ad) with a transgene expression cassette encoding UGT1A1 results in normal bilirubin levels for more than 2 years. More importantly, there was no significant difference between the total bilirubin levels 1 week postinjection and 2 years post-treatment, strongly suggesting that phenotypic correction was stable throughout the length of study. Glucuronidation of bilirubin by UGT1A1, which is required for elimination of bilirubin from plasma, was still present at normal levels 2 years after injection.

This observation is very exciting because recombinant adenoviral vector genomes mainly persist as episomal DNA molecules and one would assume that the majority of episomal adenoviral DNA molecules and transgene expression will be lost during naturally occurring cell turnover of hepatocytes. Other studies in mice that received a single dose of a HD-Ad have shown that there is a very slow decline of up to 90% in gene expression over the period of 1 year,[3] but it needs to be emphasized that transgene expression levels remained in a therapeutic range. Moreover, this phenomenon appears not to be restricted to rodents: another study in non-human primates describes a 92% fall-off in transgene expression levels over a course of 24 months.[4] However, it is important to point out that the persistence of adenoviral vector genomes and transgene expression levels still remain a major obstacle and further studies need to shed light on the mechanism responsible for persistence of adenoviral DNA molecules in quiescent cells. It is yet unclear how long therapeutic levels of the transgene product would persist in humans. It is likely that for lifelong treatment, repeated therapy with alternative serotype vectors (to avoid humoral immunity against the vector capsid) will be required.

The article by Toietta *et al* has important implications for establishment of long-term and clinically relevant treatment protocols of genetic diseases and it may pave the way towards safer gene therapy approaches. Recent adverse events in using gene therapy vectors with the potential to integrate into the host genome caution that insertional mutagenesis is going to raise some serious safety concerns in clinical trials. The major advantage of recombinant adenoviral vectors compared to other commonly used viral vectors is their extremely low integration efficiency into the host genome. Thus, the risk of insertional mutagenesis in a gene therapy protocol based on adenoviral vectors is significantly lower than protocols that utilize retroviral vectors, which were shown to predominantly integrate into transcriptionally active genes.

Helper-dependent or 'gutted' adenoviral vectors are completely devoid of all viral coding sequences and were demonstrated to be significantly safer compared to early-generation adenoviral vectors, which still contain part of the adenoviral coding sequences. Remaining viral DNA sequences in first- and second-generation adenoviral vectors cause cytotoxic effects due to *de novo* production of immunogenic adenoviral proteins. Toxicity in transduced cells is mainly caused by a cell-specific immune response as a consequence of association of the synthesized adenoviral antigens with major histocompatibility complexes (MHC) class I on the cell surface. However, one major hurdle for using recombinant adenoviral vectors, including gutted adenoviral vectors, in the clinic is the acute immune response against the incoming viral particle itself shortly after systemic administration. Circumventing these limitations of HD-Ads will be the major challenge in the near future. A potential solution to reducing the immune response may be the use of alternative adenoviral vectors either based on different human serotypes or derived from another species. In addition, genetically modified adenoviral capsid structures and polyethylene glycolation of adenoviruses to protect the vehicle are currently being explored. Toietta *et al* performed limited toxicity studies by measuring serum alanine aminotransferase levels. All individuals showed negligible toxicity as demonstrated by normal liver enzyme levels, but for the medium-dose and high-dose (1×10^{13} viral particles per kg body weight) groups a transient platelet drop was detected. The lowest dose administered to rats did not result in a platelet drop but only partial correction of

hyperbilirubinemia was achieved. Although the reasons for this already previously observed phenomenon remain to be determined, it seems to be dose dependent and a common feature among recombinant adenoviral vectors after systemic delivery.

Finally, we would like to point out that very recently there were major improvements in the HD-Ad production protocol that significantly facilitated the generation of gene-deleted adenoviral vectors.[5] Less labor intensive methods are now available, which, in combination with the intriguing data from Toietta *et al*, will make the use of gene-deleted adenoviral vectors in the clinic more likely.

In summary, Toietta *et al* demonstrated that gutted adenoviral vectors hold great promise for a successful gene therapy approach for the treatment of genetic diseases. Over the past decade, various gene and cell therapies for CN disease were explored but only transient and/or partial correction of CN disease was observed, which makes the study by Toietta *et al* very important. However, various obstacles predominantly associated with the immune response against the incoming adenoviral capsid proteins remain to be resolved. We believe that the therapeutic window for adenoviral vectors needs to be carefully chosen because a viral vector dose that is too high may result in toxicity, but a lower dose might not be sufficient in achieving complete and long-term phenotypic correction. ■

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