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Molecular Therapy

AAV Empty Capsids: For Better or for Worse?

Adeno-associated virus (AAV) vectors expressing therapeutic genes continue to demonstrate great promise for treatment of a wide variety of diseases. A peculiar feature of AAV vector generation in cell culture is the formation of an excess of "empty" capsids, which lack the vector genome and are therefore unable to provide a therapeutic benefit. The effect of the empty capsids on clinical outcome is unclear, but the potential for increasing innate or adaptive immune responses to the vector is a major concern. At the same time, very recent data show that empty AAV capsids can actually enhance gene transfer by mitigating the problem of preexisting humoral immunity to AAV. A conclusive answer to the question of whether empty capsids are beneficial or detrimental may emerge from forthcoming data from several clinical trials, using different AAV vector preparations, combined with those from additional preclinical studies on biological consequences of their presence as an impurity or deliberate inclusion of empty capsids.

AAV virions comprise a small DNA genome packaged into a protein capsid. Sixty subunits comprising three amino terminal variants of the AAV capsid protein (mostly VP3) form the icosahedral capsid structure. Several large-scale manufacturing methods are available to support clinical trials, which for many diseases require large vector doses per patient. For example, approximately 10^{14} vector genome–containing AAV (serotype 8) particles are being infused into the livers of patients with hemophilia B, who have a deficiency in coagulation factor IX. This ongoing clinical investigation has demonstrated promising long-term efficacy despite using a vector infusate that contains empty capsids at an estimated 10-fold particle excess over the "full" vectors.[1] However, T-cell responses to capsid antigen were observed in some subjects, causing low-grade hepatotoxicity and partial loss of expression. If empty particles have no therapeutic value and capsid antigen triggers T-cell responses, one could conclude that they should be removed from clinical-grade vector preparations. However, when empties were carefully removed by centrifugation in an earlier trial using AAV2-based vectors, a capsid-specific T-cell response was observed,[2] suggesting that such a conclusion would be based on an oversimplification of the problem.

Indeed, as noted above, empty AAV capsids can serve as highly effective decoys for AAV-specific antibodies that are prevalent in the human population owing to natural infection with the wild-type virus.[3,4] To enhance the safety of the empty capsids, Mingozzi and colleagues[3] engineered the capsid amino acid sequence so as to prevent their entry into the cells transduced by the full vector, thereby reducing the potential for targeted immunotoxicity. An interesting report to be published in *Molecular Therapy—Methods & Clinical Development* from Guangping Gao and colleagues[5] adds a further twist to this evolving story. The authors show that empty capsids can contribute to hepatic transaminase elevations in certain mouse strains. Interestingly, partially empty capsids cogenerated with vectors via transient transfection of cultured mammalian cells, then separated from bona fide vectors by density-gradient ultracentrifugation and subsequently admixed with "full" vector, resulted in higher elevations of liver enzyme levels than did vector alone or vector admixed with "completely" empty capsids. The latter were generated by transfection with plasmids encoding all functions necessary to assemble viral particles but lacking the plasmid encoding the vector genome—i.e., a plasmid containing the therapeutic expression cassette flanked by viral inverted terminal repeats (ITRs). Therefore, the authors conclude that empty capsids prepared for such beneficial uses should be generated in the absence of ITR templates (as opposed to cogenerated and then separated from the desired vector).

Several key questions emerge from this study. What is the precise composition of these partially empty capsids, and which component or structure is causing the toxicity? Moreover, do they induce T cell–mediated immunotoxicity or some other toxic response? Whether empty particles contribute to a CD8 T-cell response to AAV capsid remains unclear, and this latest study suggests that there may be different kinds of empty particles with different biological effects, depending on how they are produced.[5]

Another confounding factor is the presence of other types of vector-related impurities generated in the biosynthetic milieu of vector production, albeit at much lower levels. For example, heterogeneous nucleic acid fragments derived from the host cell and helper components encapsidated in AAV particles by nonspecific mechanisms are in part copurified with vectors. A more abundant and homogeneous species appears to result from reverse packaging from the ITRs that flank the intended vector-expression cassette in production plasmids or, in the case of insect cell/baculovirus-based production systems, bacmid constructs. Encapsidated baculovirus DNA was one of six major product quality concerns identified during licensure assessment of Glybera;[6] it was noted therein that the sequences adjacent to the expression cassette were preferentially packaged, consistent with reverse packaging from the ITRs. A key concern was whether open reading frames in these DNA impurities could be expressed and thereby contribute to immunotoxicity. Hence, an important challenge for those responsible for clinical-grade manufacturing of recombinant AAV is how to minimize vector-related impurities—both the abundant empty capsids and the partially empty capsid-containing fragments of other nucleic acids that may represent disproportionate risks for immuno- and genotoxicity. Given that recent studies in the field support a dependence of T-cell responses against AAV on innate immune sensing of the vector genome, it may be possible to develop empty capsids that do not contribute to immunotoxicity and provide the benefit of overcoming preexisting humoral immunity—an interesting challenge for AAV vectorology!

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