GENE THERAPY

A look at AAV toxicity in the mouse

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The eye is an easily accessible and immune-privileged organ, and therefore an ideal target for gene therapy. Adeno-associated virus (AAV) vectors are considered to be safe and are commonly used for ocular gene therapy, the main targets of which are photoreceptors (rods and cones) and retinal pigment epithelial (RPE) cells — the cell types affected by most genetic retinal diseases. Although clinical trials on ocular diseases such as Leber's congenital amaurosis 2 (LCA2) and choroideremia have proven that AAV gene therapy can be safe in humans, cases of AAV-induced ocular toxicity have been identified in both small and large animal models, depending on the viral construct or doses used in the study: subretinal injection of AAV2–*CNGA3* notably led to the loss of photoreceptors and RPE, and lymphocytic infiltration in sheep, whereas injection of AAV8–*CNGA3* activated both innate and adaptative immune responses in nonhuman primates. New findings in mice reveal a strong correlation between cis-regulatory sequences in AAV vectors and AAV-induced toxicity; these results could inform the design of safer AAV vectors for gene therapy.

The investigators designed several AAV vectors containing different cis-regulatory sequences that were injected subretinally into neonatal CD-1 albino mice. Histological analysis of harvested retinas and RPE 30 days after infection revealed that all AAVs tested incorporating broadly active promoters, including cytomegalovirus immediate-early promoter (CMV), human ubiquitin C promoter (UbiC), and chicken β-actin promoter (CAG), as well as an RPE-specific promoter (Best1), were toxic in a dose-dependent manner, as assessed by loss of photoreceptors and RPE cells. By contrast, vectors with photoreceptor-specific promoters, such as human redopsin (RedO), human rhodopsin (Rho) or mouse cone arrestin (CAR) were not toxic.

Similar results were obtained when an AAV with a CMV promoter (AAV8-CMV) was injected in pigmented C57BL/6J mice, which shows that AAV toxicity manifests across mouse strains. Imaging confirmed the alterations in retinal and RPE structure, and two techniques — electroretinogram and a behavioral optomotor assay — used to assess visual activity, showed a decrease in cone function in mice injected with a toxic vector.

"These data highlight the need to develop sensitive assays, specific to the organ and cell types that are being targeted, for each viral construct," say the investigators.

Further analysis of retinas infected with toxic vectors revealed that AAV-induced toxicity was associated with microglia activation and an increase in proinflammatory cytokines. Future genetic studies are needed to further delineate the specific pathways and cell types responsible for AAV-induced toxicity.

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