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Elevation of intraocular pressure in rodents using viral vectors targeting the trabecular meshwork

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ABSTRACT

Rodents are increasingly being used as glaucoma models to study ocular hypertension, optic neuropathy, and retinopathy. A number of different techniques are used to elevate intraocular pressure in rodent eyes by artificially obstructing the aqueous outflow pathway. Another successful technique to induce ocular hypertension is to transduce the trabecular meshwork of rodent eyes with viral vectors expressing glaucoma associated transgenes to provide more relevant models of glaucomatous damage to the trabecular meshwork. This technique has been used to validate newly discovered glaucoma pathogenesis pathways as well as to develop rodent models of primary open angle glaucoma. Ocular hypertension has successfully been induced by adenovirus 5 mediated delivery of mutant MYOC, bioactivated TGFβ2, SFRP1, DKK1, GREM1, and CD44. Advantages of this approach are: selective tropism for the trabecular meshwork, the ability to use numerous mouse strains, and the relatively rapid onset of IOP elevation. Disadvantages include mild-to-moderate ocular inflammation induced by the Ad5 vector and sometimes transient transgene expression. Current efforts are focused at discovering less immunogenic viral vectors that have tropism for the trabecular meshwork and drive sufficient transgene expression to induce ocular hypertension. This viral vector approach allows rapid proof of concept studies to study glaucomatous damage to the trabecular meshwork without the expensive and time-consuming generation of transgenic mouse lines.

1. Introduction

Modeling glaucoma in animals has been very challenging as not all animal models mimic all the clinical features associated with glaucoma in humans. One of the most important risk factors for the development and progression of glaucoma in man is elevated intraocular pressure (IOP) (AGIS, 2000; Gordon et al., 2002). Rodents have been increasingly used as glaucoma models. A number of approaches have been used to elevate IOP in rodent models, including: laser-induced outflow pathway photocoagulation (Levkovitch-Verbin et al., 2002; Aihara et al., 2003a), episcleral vein injection of hypertonic saline to sclerose the aqueous outflow pathway (Morrison et al., 1997; Walsh et al., 2009), microbead injection into the anterior segment (Sappington et al., 2010), injection of viscous materials into the anterior segment (Benozzi et al., 2002), extraocular vein cautery (Shareef et al., 1995), glucocorticoid-induced ocular hypertension (Shinzato et al., 2007; Whitlock et al., 2010; Overby et al., 2014; Zode et al., 2014), among others. IOP elevation in the DBA/2J mouse is due to iris stroma atrophy and pigment dispersion into the anterior segment angle (Anderson et al., 2002), which mimics some features of pigmentary glaucoma. This mouse strain has been extensively used to study the molecular mechanisms of glaucomatous optic neuropathy and

Abbreviations: AAV, adeno-associated virus; Ad, adenovirus; APC, adenomatosis polyposis coli; BCS, Baltimore Classification Scheme; BMP, bone morphogenetic protein; CK, casein kinase; CTGF, connective tissue growth factor; DKK1, Dickkopf-related protein-1; ECM, extracellular matrix; EIAV, equine infectious anemia virus; GFP, green fluorescent protein; GSK, glycogen synthase kinase; HSV, herpes simplex virus; IOP, intraocular pressure; JNK, c-Jun N-terminal kinases; LAP, latency-associated peptide; LRP, lipoprotein receptor-related protein; POAG, primary open angle glaucoma; PTS1, peroxisomal targeting signal-1; RGC, retinal ganglion cell; ROCK, rho-associated kinase; scAAV, self-complementary adeno-associated virus; SPARC, Secreted Protein Acidic and Rich in Cysteine; TGF, transforming growth factor; TM, trabecular meshwork; TU, transducing unit; VSV, vesicular stomatitis virus; VSV-G, vesicular stomatitis virus glycoprotein.

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retinopathy (Howell et al., 2011) as well as to identify novel therapeutic targets (Anderson et al., 2005; Howell et al., 2011, 2014). In addition, transgenic mouse models have been created that develop elevated IOP including the *Col1a1*$^{r/r}$ mouse (Aihara et al., 2003b), *Tg.hMYOC.Y437H* mouse (Zode et al., 2011), and *Tg.CTGF* (Junglas et al., 2012). However, developing relevant transgenic models of glaucoma takes considerable time and resources.

Another approach to elevate IOP in mice is to use viral transduction of the trabecular meshwork (TM) to overexpress glaucoma associated genes. Several different viral vectors have been used to transduce the TM including: Adenovirus serotypes 5 (Ad5) (Hoffman et al., 1997; Millar et al., 2008), 28 or 35 (Ueyama et al., 2014), self-complementary Adeno-Associated Virus (scAAV) (Buie et al., 2010), Herpes Simplex Virus (HSV) (Spencer et al., 2000), HIV pseudotyped with VEEV-G or VSV-G (Lipinski et al., 2014), and Equine infectious anemia virus (EIAV) pseudotyped with VSV-G (Balaggan et al., 2006).

Ad5 vectors have been used in mice to validate glaucoma associated pathogenic pathways, including: human myocilin mutations (Shepard et al., 2007), secreted frizzled-related protein-1 (sFRP-1) (Wang et al., 2008), transforming growth factor (TGF) β2 (Shepard et al., 2010; McDowell et al., 2013; Swaminathan et al., 2014), CD44 (Giovingo et al., 2013), and Gremlin (McDowell, submitted for publication). Prolonged transgene expression can lead to optic neuropathy in some mouse strains (McDowell et al., 2012). Aqueous humor dynamics can be measured *in vivo* in the mouse eye (Millar et al., 2011), allowing direct evaluation of transgene expression on the aqueous humor outflow pathway. For example, elevated IOP caused by TGFβ2-induced transgene expression is associated with decreased aqueous humor outflow facility (Shepard et al., 2010). Viral transduction of the TM could also be used to conditionally knockdown the expression of specific genes. This can be done using virus vectors expressing shRNA to knockdown expression of the targeted gene.

However, there are challenges associated with the use of viral transduction of the TM to elevate IOP. Oftentimes, the transgene expression is transient. Ocular inflammation often accompanies injection of viral vectors into the eye (Shepard et al., 2010). Partial suppression of the immune response using antibodies to CD40L can prolong transgene expression and suppress ocular inflammation after Ad5 vector injection (Millar et al., 2008). In addition, mouse strain and age appears to affect long term transgene expression and prolonged IOP elevation (McDowell et al., 2012).

2. Methods

2.1. Viral vectors

Viruses possess specialized molecular machinery for efficient transduction of their genomes to cells they infect. By replacing viral genes needed for viral replication with foreign (cargo) genes of interest, the resultant recombinant viral vectors can be used to transduce cargo genes, which can then be expressed by the target cell. Goff and Berg (Goff and Berg, 1976) were the first to describe such a process utilizing modified SV40 virus. Since then viruses of several phylogenetic types have been used for the generation of viral vectors. Each type, with their advantages and disadvantages are discussed below. A summary is presented in Table 1.

2.1.1. Herpes simplex virus (HSV)

HSV is a large enveloped dsDNA virus, 100–150 nm in diameter, Baltimore Classification Scheme (BCS) Group 1. With a capacity of ~150 kb, it is able to accommodate large genes (up to 74–84 kb). HSV is human neurotropic, and HSV vectors have been used to transfect mainly neurons including retinal ganglion cells (RGCs), but also other tissue types including TM and ciliary epithelia (Liu et al., 2005; Wang et al., 2005) in monkey and rat eyes. HSV vectors are helper-independent and can be prepared in titers of 10^9 to 10^{10} transducing units (TU)/mL.

Another HSV vector variant is the amplicon vector, a replication-defective and helper-dependent HSV-1 based vector. Amplicon vectors are plasmids that only require an HSV origin of replication and packaging sequence. They also incorporate a large cargo transgene capacity (>100 kb), as well as a broad range of cell tropism, and can infect both dividing and non-dividing cells (Epstein, 2005). HSV-1 amplicon vectors have facilitated efficient but transient reporter gene transfer in retinal pigment epithelial (RPE) cells in rats following sub-retinal injection (Fraefel et al., 2005).

However, the use of HSV and amplicon vectors for gene transduction has certain disadvantages, including: cytotoxicity, limited selection of suitable promoters, limited duration of gene expression, and a significant inflammatory response.

2.1.2. Adenovirus (Ad)

Ad is a non-enveloped icosahedral virus, 80–100 nm in diameter, incorporating a linear dsDNA genome, classified as BCS Group 1. It has a cargo capacity of 26–45 kb. There are 57 known serotypes of Ad. First generation recombinant Ad vectors with E1 and/or E1, E3 gene deletions (genes that assist in evasion of the immune response) have been investigated extensively, especially because they were one of the earliest recombinant DNA viral vector systems developed. They can be prepared to very high titers, can infect a variety of dividing and post-mitotic or terminally differentiated cells, and lead to expression of high levels of transgene product. A variety of TM cells have been efficiently transduced using recombinant Ad in human perfused anterior segments, as well as living monkey, dog, rat, and mouse eyes. Ad vectors also efficiently transduce iris epithelium and corneal endothelium (Budenz et al., 1995), as well as Müller cells and the RPE. But mature photoreceptors and RGCs have not been successfully transduced using these vectors.

E1, E3-deleted Ad vectors have been used extensively in animal models of experimental glaucoma, but complications have been reported, including immunogenicity which can lead to uveitis and/or corneal complications, and cataract (Shepard et al., 2010). High neutralizing antibody titers limit repeat applications (Yang et al., 1994; Ginsberg, 1996). Gene expression is often transient.

Ad vector developments known variously as: gutless or gutted Ad vectors, high capacity Ad vectors, helper dependent Ad vectors, mini Ad vectors, or encapsidated adenovirus minichromosomes promote less immunogenicity. They have a cargo-carrying capacity of up to 36 kb (Alba et al., 2005). Gutless Ad vectors have been used to successfully transduce liver, muscle, and CNS neurons, as well as retinal photoreceptors and TM cells in mice. Because gutless Ad vectors are devoid of all viral coding genes, they require viral proteins to be supplied *in trans* by a helper Ad. Subsequent removal of the contaminating helper Ad from the final preparation is required. This is usually achieved by excision of the helper packaging signal, most often using the Cre-loxP system. However contamination levels with helper Ad remain at 0.1–1%, which, although acceptable for basic research paradigms, render them as yet unsuitable for clinical trials. But better purification techniques are beginning to appear (Alba et al., 2005).

2.1.3. Adeno-associated virus (AAV)

AAV is the physically smallest (20 nm diameter) of all known primate viruses. It is a non-enveloped BCS Class II virus, possesses a linear ssDNA genome, and is derived from a replication-deficient, non-pathogenic parvovirus. AAV vectors are capable of