Experimental Eye Research 90 (2010) 546–554

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Self-complementary AAV5 vector facilitates quicker transgene expression in photoreceptor and retinal pigment epithelial cells of normal mouse

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ARTICLE INFO

Article history:
Received 25 November 2009
Accepted in revised form 25 January 2010
Available online 4 February 2010

Keywords:
transgene expression
self-complementary AAV
scAAV5
AAV5
subretinal injection
intravitreal injection
photoreceptor
RPE

ABSTRACT

To clarify whether transduction efficiency and cell type specificity of self-complementary (sc) AAV5 vectors are similar to those of standard, single-stranded AAV5 vectors in normal retina, one micro liter of scAAV5-smCBA-GFP vector (1×10^{12} genome-containing particles/ml) and AAV5-smCBA-GFP vector (1×10^{12} genome-containing particles/ml) were subretinally or intravitreally (in both cases through the cornea) injected into the right and left eyes of adult C57BL/6J mice, respectively. On post-injection day (PID) 1, 2, 5, 7, 10, 14, 21, 28 and 35, eyes were enucleated; retinal pigment epithelium (RPE) whole-mounts, neuroretinal wholemounts and eyecup sections were prepared to evaluate green fluorescent protein (GFP) expression by fluorescent microscopy. GFP expression following trans-cornea subretinal injection of scAAV5-smCBA-GFP vector was first detected in RPE wholemounts around PID 1 and in neuroretinal wholemounts between PID 2 and 5; GFP expression peaked and stabilized between PID 10–14 in RPE wholemounts and between P14 and P21 in neuroretinal wholemounts with strong, homogeneous green fluorescence covering the entire wholemounts. The frozen sections supported the following findings from the wholemounts: GFP expression appeared first in RPE around PID 1–2 and soon spread to photoreceptors (PR) cells; by PID 7, moderate GFP expression was found mainly in PR and RPE layers; between PID 14 and 21, strong and homogenous GFP expression was observed in RPE and PR cells. GFP expression following subretinal injection of AAV5-smCBA-GFP was first detected in RPE wholemounts around PID 5–7 and in neuroretinal wholemounts around PID 7–10; ssAAV5-mediated GFP expression peaked at PID 21 in RPE wholemounts and around PID 28 in neuroretinal wholemounts; sections from AAV5 treated eyes also supported findings obtained from wholemounts: GFP expression was first detected in RPE and then spread to the PR cells. Peak GFP expression in RPE mediated by scAAV5 was similar to that mediated by AAV5. However, peak GFP expression mediated by scAAV5 in PR cells was stronger than that mediated by AAV5. No GFP fluorescence was detected in any retinal cells (RPE wholemounts, neuroretinal wholemounts and retinal sections) after trans-cornea intravitreal delivery of either scAAV5-GFP or AAV5-GFP. Neither scAAV5 nor AAV5 can transduce retinal cells following trans-cornea intravitreal injection. The scAAV5 vector used in this study directs an earlier onset of transgene expression than the matched AAV5 vector, and has stronger transgene expression in PR cells following subretinal injection. Our data confirm the previous reports that scAAV vectors have an earlier onset than the standard, single strand AAV vectors (Natkunarajah et al., 2008; Yokoi et al., 2007). scAAV5 vectors may be more useful than standard, single-stranded AAV vector when addressing certain RPE and/or PR cell-related models of retinal dystrophy, particularly for mouse models of human retinitis pigmentosa that require rapid and robust transgene expression to prevent early degeneration in PR cells.

1. Introduction

With the progress of molecular biology, more and more homologous genetic defects in human and animals have been characterized. This has greatly added in the development of gene therapies for different kinds of inherited human diseases. Gene therapy for retinal diseases is particularly attractive due to intrinsic

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doi:10.1016/j.exer.2010.01.011

features of the retina that lend themselves to gene mediated therapies; specifically, isolated anatomical structure and immunological privilege (Bainbridge et al., 2006; Dinculescu et al., 2005). Though there are several ways to transfect the target ocular tissues and cells with genes, viral vectors, especially the utilizing recombinant adeno-associate virus (rAAV), have become widely used. rAAV has proven to be a good vector for retinal gene therapy due to the lack of pathogenicity, ability to transfect quiescent cells (i.e., neurons, including retinal photoreceptors) and ability to promote long term expression of delivered transgene (Dudus et al., 1999; Bennett et al., 1999). AAV also has the additional advantage of serotype selectivity. By changing the nature of the capsid protein AAV can be targeted to specific cells type and the relative intensity of transduction efficiency can be modulated (Pang et al., 2008a; Yang et al., 2002; Rabinowitz et al., 2002; Davidson et al., 2000; Bainbridge et al., 2003; Auricchio et al., 2001).

Among the 12 reasonably well-studied serotypes, AAV2 and AAV5 are commonly used in the development of gene therapies for retinal diseases in animal models and in ongoing human clinical trials (Bainbridge et al., 2008; Maguire et al., 2008; Cideciyan et al., 2008, 2009a; Pang et al., 2006a; Alexander et al., 2007). In these studies cell specificity was strongly dependent on the site of administration and the AAV serotype used (Pang et al., 2008a, 2006a; Alexander et al., 2007). Subretinal injection of AAV2 and AAV5 resulted in either retinal pigment epithelium (RPE) or photoreceptor (PR) cell transduction, or both, depending on the nature of the promoter and transgene delivered (Pang et al., 2008a, 2006a; Alexander et al., 2007).

In our previous comparative study of AAV2 and AAV5 vector transduction, we found that AAV2 transduced inner retinal cells, including retinal ganglion and Müller cells following trans-sclera intravitreal injection, but only weak gene expression was found following trans-sclera intravitreal injection of AAV5-CBA-GFP vector, and this expression was limited to the injection site (Pang et al., 2008a). This expression pattern may have been mediated by the physical injury associated with injection (Pang et al., 2008a).

Since the leaking of AAV2 vector into the vitreous cavity is a likely scenario during or after subretinal injection, it is important to determine whether there is AAV5-mediated gene expression in the inner retinal cells following trans-cornea intravitreal injection. By utilizing a trans-cornea approach to the vitreous we can avoid injection-related retinal injury, and thereby mitigate vector transduction mediated by retinal injury.

Among the animal models of inherited retinal diseases, mouse models are favored for their ease of handling, high reproductive rate and short generation time. Mouse models of retinal degeneration exhibit a high degree of variation i.e., different cellular targets of the gene mutation (RPE versus PR cells versus ganglion cells), as well a variety of mutations within a cell type (for example, PR cells) and different timeframe for the course of retinal degeneration. There are several well-studied mouse models with rapid photoreceptor degeneration. For example, PR degeneration starts around postnatal day (P) 7 in *rd1* (Caley et al., 1972) and P16 in *rd10* mice (Chang et al., 2007; Gargini et al., 2006), and within the following two weeks most of the PR cells are gone. Our previous studies show that cone degeneration also starts very early in *rd12* mice (Pang et al., 2005; Pang et al., IOVS 2007 (48): ARVO E-Abstract 1688) and in other cone photoreceptor function loss mouse models (Pang et al., 2008, IOVS 49: ARVO E-Abstract 5355). Because of the technical difficulty and low success rate of subretinal injections in neonatal mice, it is extremely important to achieve therapeutic gene expression as soon as possible in mouse models with aggressive retinal degenerations (Pang et al., 2008b). Rapid expression may also be critical for devastating episodic retinal diseases such as retinal detachment.

When addressing these rapidly degenerating models with tradition, single-stranded AAV vectors, arresting the progression of PR degeneration and restoring ERG responses has proven difficult. This is presumably due to the lag in transgene expression intrinsic to these vectors. It has been established that the lag in expression mediated by AAV is at least in part due to the time required to convert the single-stranded viral DNA genome into a double strand DNA molecule in-vivo, which can then serve as a platform for transcription (Ferrari et al., 1996; Fisher et al., 1996).

To overcome this rate-limiting step, self-complementary AAV vector (scAAV) have been developed that package an inverted repeat genome that subsequently folds into a double stranded DNA molecule that is covalently linked on one end (Yokoi et al., 2007; McCarty et al., 2003). Several studies have shown faster onset of expression and overall increased transduction efficiency with scAAV vectors relative to matched AAV vectors. Cell types analyzed have included brain (Fu et al., 2003), liver (Nathwani et al., 2006), muscle (Wang et al., 2003), cancer cells (Xu et al., 2005), and the trabecular meshwork of the eye (Borrás et al., 2006). It has also been shown that scAAV vectors generate quicker and more efficient transgene expression in retinal cells (Yokoi et al., 2007; Natkunarajah et al., 2008) compared with standard AAV. It is well established that AAV5 has better transduction efficiency in PR cells than AAV2 (Pang et al., 2008a; Yang et al., 2002; Rabinowitz et al., 2002; Davidson et al., 2000), therefore we chose to compare scAAV5 versus AAV5 matched vectors in the mouse retina. By utilizing a long acting, ubiquitous promoter, smCBA (Pang et al., 2008b), and evaluating both wholemounts (RPE and neuroretinal) and eyecup cross-sections we were able to compare efficiency in both RPE and PR cells.

2. Methods

2.1. Animals

C57BL/6J mice were obtained from the Jackson Laboratory (Bar Harbor, ME) and bred at Wenzhou Medical College (Wenzhou, China). All mice were maintained in the Animal Facilities of Wenzhou Medical College under a 12-h light/12-h dark cycle. One hundred and forty, 6–8 weeks old C57BL/6J mice were used in this study; at least three eyes were evaluated for each time point of the experiment. All experiments were approved by the Wenzhou Medical College's Institutional Animal Care and Use Committee and were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

2.2. Production of AAV5 and scAAV5 vectors

Standard and self-complementary versions of pseudotyped AAV5 capsid were manufactured and purified in accordance with previously published methods (Flannery et al., 1997; Zolotukhin et al., 1996, 2002). The matched vectors contained the small version of the hybrid CMV-chicken beta-actin promoter (smCBA) driving the humanized green fluorescent protein (hGFP) cDNA. smCBA (Pang et al., 2008b) has been shown to have identical transduction and tropism characteristics to the full chimeric CMV-chicken beta-actin (CBA) promoter when driving transgene expression in mouse retina (Pang et al., 2008b; Haire et al., 2006; Petrs-Silva et al., 2009). The vector plasmid containing modifications to allow for self-complementary AAV packaging has been previously described (McCarty et al., 2003).

Viral preparations had an average titer of 10^{13} genome-containing viral particles per milliliter (ml). Vector titer was determined by real-time polymerase chain reaction (PCR) (Petrs-Silva et al., 2009) and final aliquots were resuspended in balanced saline solution