GENE THERAPY IN GLAUCOMA - GLAUCOMA MANAGEMENT AHEAD

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Genetic manipulations of the mammalian CNS have progressed rapidly over the past 2 decades. Gene Transfer and the use of transgenic animals have been well characterized approaches in cellular biology.

To allow more controlled genetic manipulations, neurobiologists have used pathogenic viruses to develop and deliver transgenes to specific neuronal cell population.

For Glaucoma, targets of gene therapy include

Aqueous humor outflow modification in Trabecular meshwork

Neuroprotection of Retinal Ganglion Cells and Optic Nerve

Common Virus vectors employed :

Adeno Virus

A Ds-DNA virus with an icosahedral capsid (Fig 1) .The earlier Adenovirus vectors were engineered to render viral replication defective , but still some low level viral gene expression could cause inflammation as viral transcription still occurred.

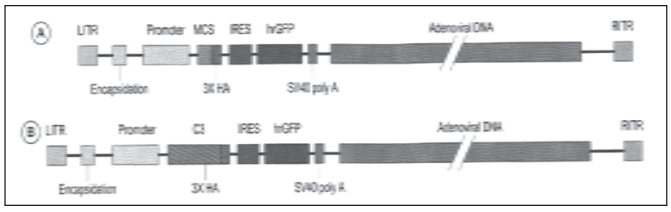

Figure1: A) Ad-GFP incorporating internal ribosome entry site expressing human recombinant GFP.
B) Ad-C3-GFP expresses C3 transferase and human recombinant GFP.
LITR/RITR:Left/Right inverted terminal repeat, MCS:Multiple cloning site

Adeno-Associated Virus (AAV)

AAV is a small helper dependant parvovirus with a SS-DNA genome surrounded by naked icosahedral capsid. It is attractive for use in gene therapy as a vector as it is efficient, long lived and nontoxic.

Absence of Rep and Cap sequences (Fig 2) implies the absence of viral protein synthesis occurring following transduction, minimizing the amount of foreign particle available to trigger immune responses. It has the ability to infect dividing and non dividing cells. Thus, recombinant AAV vectors have one of the highest safety ratings among all viral vectors.

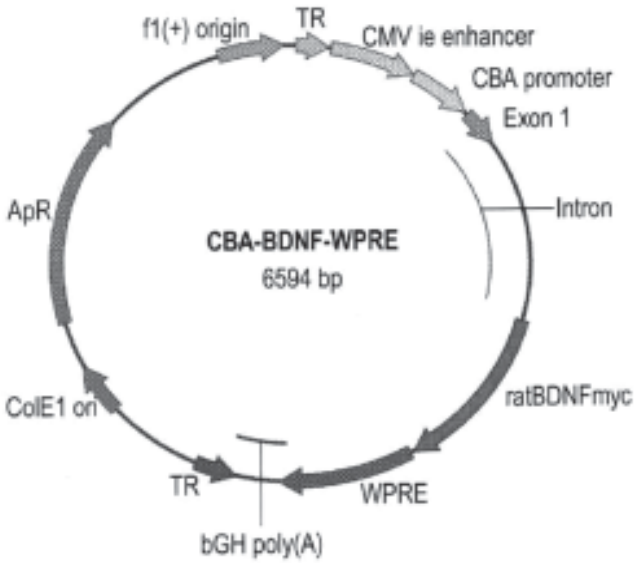

Figure 2: Map of the AAV-CBA-BDNF-WPRE virus
F1(+) origin: f1 bacteriophage origin of replication ; TR: terminal repeats ; CMV ie enhancer: CMV immediate early enhancer; CBA promoter: chicken ? actin promoter; rat BDNF myc: myc tagged rat brain derived neurotrophic factor sequence; bGH poly(A): bovine growth hormone polyA sequence; ColE1 ori: E.Coli origin of replication; ApR: Ampicillin resistance

LentiVirus

Retroviruses are RNA containing viruses. Viral coat proteins coded by viral env gene enable the retrovirus to interact with a receptor on the host cell membrane, enter the cell cytoplasm and uncoating of the virion occurs. Reverse Transcriptase encoded by the pol gene transcribes a Ds-DNA complementary to viral RNA and moves into the nucleus to get incorporated in host cell's chromosome .This DNA is used as a template for transcription of viral RNA and translation into viral proteins. These proteins interact with packaging sequences rev and psi resulting in encapsulation and release by budding from the surface of mature infectious virions (Fig 3).

Lentiviruses are non oncogenic retroviruses that

produce multiorgan diseases characterized by long incubation periods and persistent infection. To eliminate the possibility of lentivirus becoming a replication competent retrovirus, they are produced by cotransfection of septate plasmids that express the lentivirus transfer genome containing the transgene of interest, structural components and heterologous env protein that confers stability and broad cell tropism.

Deletion of the LTR region has increased biosafety by decreasing insertional mutagenesis.

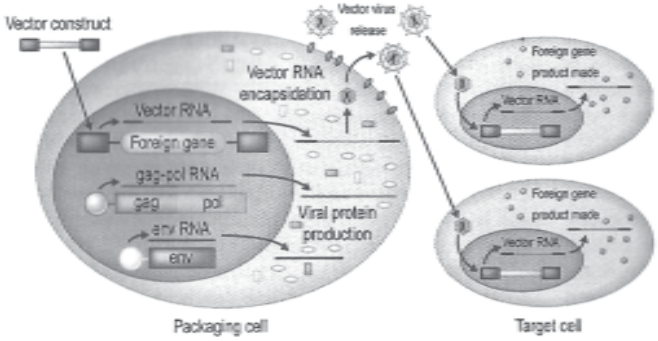

Figure 3: Retroviral Vector production.

Modulation of Viral Vector Expression

To initiate transcription, a DNA promoter sequence binds RNA polymerase. CMV promoter is efficient in driving expression in TM cells and chicken ? actin (CBA) promoter enables long term rAAV mediated gene expression in RGC's.

Downregulation of gene expression can be done using antisense oligonucleotides and (silencing/ short interfering) siRNA which degrades mRNA preventing protein translation.

Modulation of Aqueous outflow

Injection of viral vectors into anterior chamber is an efficient technique to express transgenes in the TM as aqueous outflow causes deposition of vector primarily in this location and modulates outflow by targeting extracellular matrix or cytoskeletal proteins. AC of perfused porcine and human anterior segment cultures were injected with replication deficient adenovirus constructs and transduction of all layers of TM and inner wall of Schlemm's canal was noted. Injection of high titres of vector caused an increase in outflow due to constipation of outflow channels with viral particles[1].

TM cultures transduced by adenovirus coding for Aquaporin 1 caused increase in mean resting cell volume and paracellular permeability in monolayers of TM cells suggesting aquaporin 1 as a modulator of outflow facility in vivo[2].

TM cultures transduced by adenovirus coding for stromelysin and RhoA showed histological evidence of cytoskeletal destruction and 32.5% mean increase in outflow facility after 72 hours[3].

Lentivirus vectors derived from feline immunodeficiency virus have been shown to efficiently transducer TM cells for upto 10 months after AC injection with minimum inflammation and cell loss[4,5].

Neuroprotection Of Retinal Ganglion Cells

Neurotrophins and their receptors were among the first transgenes explored in meuroprotective gene therapy experiments. TrkB is a receptor for neurotrophin BDNF (Brain derived Neurotrophic factor) which is important for RGC homeostasis. In an experimental monkey glaucoma model, interruption of BDNF retrograde transport and accumulation of TrkB at ONH was noted; suggestive of neurotrophin deprivation in the pathogenesis of RGC death in glaucoma[6].

2 weeks after Optic nerve axotomy RGC survival was compared between AAV-GFP eyes and AAV-TrkB eyes augmented with BDNF administration. Survival in the latter was 76% compared to <10% in the former7.

In a similar experiment, AAV incorporating the CBA promoter and WPRE (Woodchuck hepatitis post-transcriptional regulatory element) was used to direct expression of brain-derived neurotrophic factor (BDNF) or GFP in RGCs after intravitreal injection in rats (Fig 4).

This proves potential for neuroprotection of injured RGCs using neurotrophin replacement by AAV gene therapy.

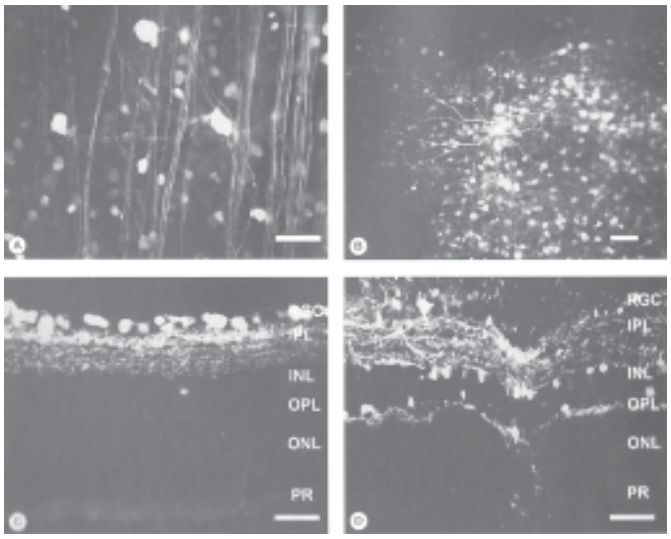

Figure 4: A) Individual RGCs and their axons express GFP
B) Dendritic trees of RGCs
C) GFP-labeled cells are localized almost exclusively to the RGC layerin retinal cross-sections.
D) In the immediate vicinity of injection site, inner retinal cells are also transfected.

Modulation of apoptosis can be an effective strategy for neuroprotection. Inhibitors of apoptosis proteins (IAP) inhibit caspases, which modulate apoptosis; thus increasing RGC survival.

Idiosyncratic immune responses leading to human deaths have been reported using viral vectors. Decreased amount of viral vectors have reduced the risk considerably but caution is still warranted. Non viral techniques like cationic lipid delivery systems (lipofection) or the use of electric current (electroporation) can be used to deliver DNA sequences into target cells, thus minimizing the immune responses which limit the viral vector gene trials.

Although these efforts are still in their relative infancy, successful gene therapy experiments have been directed towards modification of TM extracellular matrix and cytoskeletal elements.

References:

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