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Intravitreal Implantation of Genetically Modified Autologous Bone Marrow-Derived Stem Cells for Treating Retinal Disorders

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Abstract

A number of retinal degenerative diseases may be amenable to treatment with continuous intraocular delivery of therapeutic agents that cannot be delivered effectively to the retina via systemic or topical administration. Among these disorders are lysosomal storage diseases resulting from deficiencies in soluble lysosomal enzymes. Most cells, including those of the retina, are able to take up these enzymes and incorporate them in active form into their lysosomes. In theory, therefore, continuous intraocular administration of a normal form of a soluble lysosomal enzyme should be able to cure the molecular defect in the retinas of subjects lacking this enzyme. Experiments were conducted to determine whether genetically modified bone marrow-derived stem cells implanted into the vitreous could be used as vehicles for continuous delivery of such enzymes to the retina. Bone marrow-derived mesenchymal stem cells (MSCs) from normal mice were implanted into the vitreous of mice undergoing retinal degeneration as a result of a mutation in the *PPT1* gene. The implanted cells appeared to survive indefinitely in the vitreous without proliferating or invading the retina. This indicates that intravitreal implantation of MSCs is likely a safe means of long-term delivery of proteins synthesized by the implanted cells. Experiments have been initiated to test the efficacy of using genetically modified autologous MSCs to inhibit retinal degeneration in a canine model of neuronal ceroid lipofuscinosis.

1 Introduction

In recent years substantial research has been conducted to assess potential therapeutic applications of stem cells. The focus of much of this work has been on utilizing stem cells to regenerate tissues, including the retina, that have been damaged as a result of injury or disease (Ramsden et al. 2013). We implanted embryonic stem cell-derived neural precursors from normal mice into the vitreous of mice undergoing progressive retinal degeneration due to a mutation in *CLN8* (Meyer et al. 2006). The implanted cells migrated to and associated

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2.2 Intravitreal implantation of mouse MSCs

Mice used as recipients for intravitreal MSC implantation had a null mutation in *PPT1* that encodes the soluble lysosomal enzyme palmitoyl protein thioesterase-1 (Gupta et al. 2001). Via multiple backcrosses, the mutation was placed on a pure C57BL/6J strain background. The retina in these mice appears to develop normally and then undergoes a progressive degeneration (Lei et al. 2006).

For intravitreal implantation into the mutant mice, the eGFP-expressing normal C57BL/6J mouse MSCs were harvested after 4 to 12 passages and suspended in minimal essential medium at a concentration of 40,000 cells per μl . The recipient mice were anesthetized and approximately 2 μl of the cell suspension was injected into the vitreous. At various times up to 16 weeks after implantation, the recipient mice were euthanized. The eyes were enucleated immediately after death and prepared for and examined with either fluorescence or light microscopy (Meyer et al. 2006).

2.3 Characterization of canine MSCs

Canine MSC cultures were established and maintained as described for the mouse cells. Expression of GFP in the transduced cells was monitored with fluorescence microscopy. Expression of TPP1 by the cells transduced with AAV2-CAG-TPP1 was monitored by measuring TPP1 enzyme activity in the medium in which the cells were maintained using an established protocol (Tian et al. 2006).

All studies were performed in compliance with the ARVO Statement for the “Use of Animals in Ophthalmic and Vision Research” and were approved by the University of Missouri Animal Care and Use Committee.

3 Results

3.1 Mouse MSCs after intravitreal implantation

The mice tolerated the intravitreal injections with no apparent adverse effects, except in rare cases where the injection needle penetrated the lens capsule. In the latter cases, the mice developed cataracts within a few days of the injection. If the lens capsule was not ruptured the implanted cells formed net-like sheets within the vitreous (Fig. XX.1). The numbers of cells within these sheets remained stable over the 16 week evaluation period, with no evidence of donor cell proliferation or loss. Unlike neural precursor cells (Meyer et al. 2006), there was no evidence of donor cell migration toward or into the retina; the sheets of donor cells remained suspended in the vitreous. The presence of the donor cells in the vitreous did not appear to have a significant effect on the rate of retinal degeneration.

In cases where the lens capsule was damaged during the injection, many donor cells migrated to the lens. Some of these cells formed a layer that tightly adhered to the posterior side of the intact portions of the lens capsule. The majority of the donor cells migrated into the lens itself.

3.2 In vitro characterization of canine MSCs

Cells from the bone marrow aspirates were allowed to attach to culture plates for a period of 24 hours, after which the plates were washed repeatedly to remove non-adherent cells. Those cells remaining were identified as MSCs by their morphology and adherence to plastic (Williams and Hare 2011). The cells typically reached confluency by 96 hours after plating, at which time the cells were passaged. Subsequently the cells typically reached confluency by 48–72 hours after passaging. Cell morphology remained indicative of an MSC lineage through multiple passages (Fig. 1).

Canine MSCs were transduced with AAV2-CAG vectors at passage 4 by adding the vector to the culture media. The inoculated media was left on the plates for 96 hours, after which it was replaced with fresh media. GFP expression was detectable at 96 hours post-transduction and increased in intensity over time, reaching a stable high level of intensity approximately 5 days after transduction (Fig. 2). GFP expression remained stable for at least two passages post-transduction. P4 and P5 transduced cells kept at confluency without additional passaging maintained high levels of fluorescence for at least 70 days *in vitro*.

To gauge TPP1 expression *in vitro*, the culture medium was collected once every 24 hours starting 24 hours after transduction for up to 72 hours and each sample was analyzed for TPP1 enzyme activity. Based on the TPP1 activity in the conditioned media the estimated release of enzyme by the MSCs *in vitro* was approximately 3 to 5 pg per cell per 24 hours.

3 Discussion

The mouse studies suggest that intravitreal implantation of MSCs may be a safe means of long-term intraocular delivery of therapeutic agents. As long as there was no damage to the lens the donor cells appeared to survive indefinitely in the vitreous without proliferating or damaging the retina or lens. As the studies with the canine MSCs demonstrated, these cells can be genetically modified to produce and release therapeutic proteins, which are then likely to reach the target eye tissues adjacent to the vitreous.

In vitro the mouse MSCs proliferate indefinitely, yet after implantation into the vitreous, no proliferation was observed. This suggests that the vitreous contains factors that inhibit proliferation. These factors are present not only in eyes in which the retina is undergoing active degeneration, as the behavior of the MSCs was essentially the same when they were implanted into the eyes of normal C57BL/6J mice. Although the vitreous is not vascularized, the donor cells apparently received enough oxygen and nutrients from the adjacent retina to support their long-term survival. In the mouse eye, the vitreous is confined to a thin layer close to the retina due to the fact that the lens occupies most of the internal volume of the eye. The consequent close proximity of the donor cells to the retina may have aided in their long-term survival. However, preliminary studies of implantation of autologous bone marrow derived MSCs into the eyes of dogs indicate that such close proximity may not be necessary for donor cell survival. MSCs implanted into the vitreous of a dog far from the retina were still present several months after implantation. This will be important in developing implantation of these cells for human therapies as the anatomy of the dog eye is more similar to that of the human eye than is the mouse eye.

The migration of the donor cells toward and into the lens when the lens capsule was damaged suggests that the lens contains trophic factors to which the donor cells respond strongly. Potential donor cell responses to endogenous trophic factors is an important consideration when considering using intravitreal implantation of such cells for treating retinal degenerative diseases. Tissues undergoing degeneration release a variety of trophic factors to which the donor cells may respond, and different donor cell types may respond differently to such trophic factors. Indeed, we found that mouse neural precursors derived from embryonic stem cells migrate toward and into the degenerating mouse retina (Meyer et al. 2006), whereas no such migration is observed with the MSCs.

These studies indicate that intravitreal implantation of genetically modified MSCs is promising as a means of long-term delivery of therapeutic agents to the retina. While the mouse and preliminary dog studies support the safety of this approach, efficacy in treating retinal degenerative disease remains to be demonstrated. Such efficacy studies are currently under way using a canine model of neuronal ceroid lipofuscinosis which exhibits a slowly progressive retinal degeneration (Katz et al. 2008).

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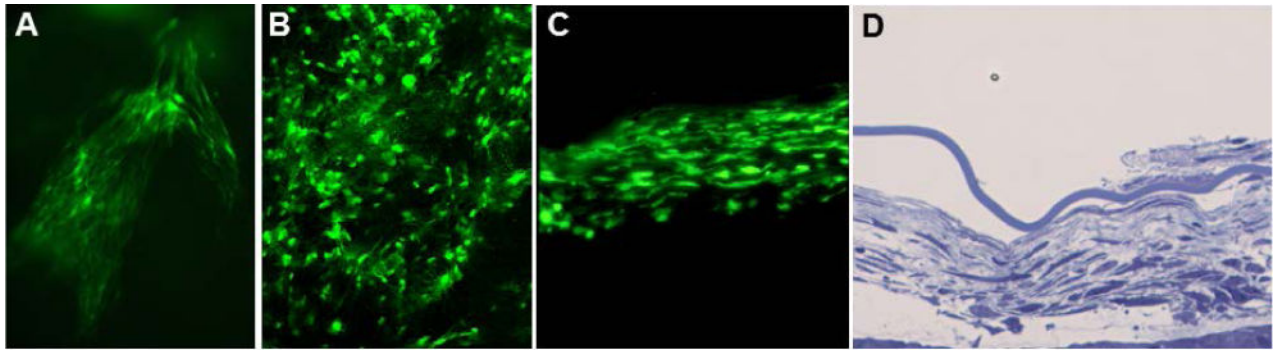


Fig. 1. Fluorescence micrographs of a sheet of GFP donor cells in the vitreous in the intact eye (A), in a retinal flat mount (B), and in a cryostat section of the eye (C). Light micrograph of a cross-section sheet of donor cells in the vitreous after fixation and embedding the eye in epoxy resin (D). All images were from eyes collected 16 weeks after MSC implantation.

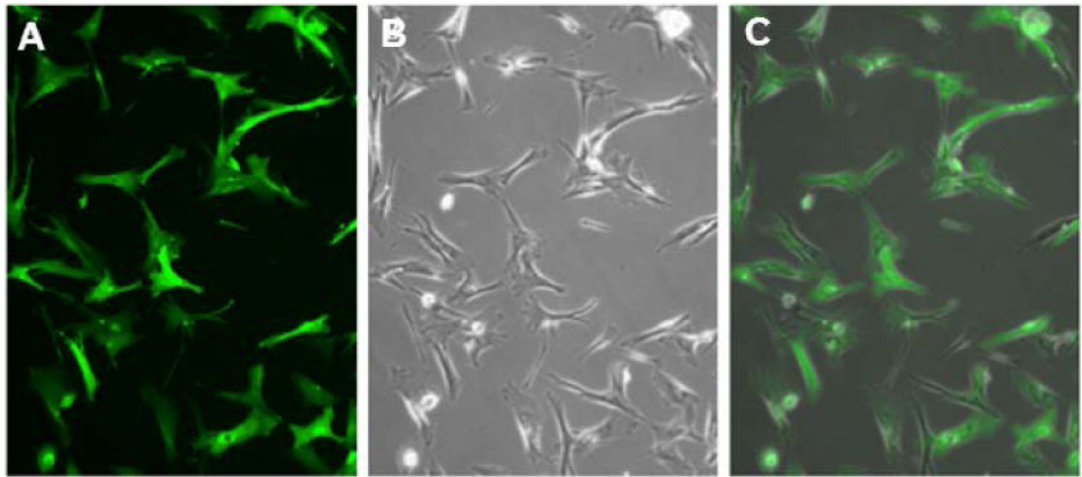


Fig. 2. Fluorescence (A), phase contrast (B) micrographs of rAAV2-CAG-GFP transduced passage 4 canine MSCs *in vitro*.