Induced Pluripotent Stem Cells Restore Function in a Human Cell Loss Model of Open-Angle Glaucoma

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Abstract

The possibilities of novel stem cell therapies to curtail treat, or even cure diseases are enticing, yet safety and functional questions remain prior to clinical use. Currently the stem cell transplantation field for eye diseases is generally defined as being at the pre-clinical or early clinical trials stages. Pre-clinical studies can hopefully eliminate or determine possible transplantation complications prior to human trials. In our manuscript in Stem Cells, we investigated the possibilities of using a personalized cell therapy to treat glaucoma, and in this paper reported two crucial breakthroughs towards this end.

Keywords: Glaucoma; Stem cells; Eye diseases

Introduction

With this in mind, our pre-clinical study employed the well-established ex vivo human anterior segment perfusion culture system to study potential stem cell therapy for open angle glaucoma. The anterior segments were utilized to determine normal functioning of the trabecular meshwork or TM, a tiny tissue in the angle of the eye [1]. The TM has two important functions: 1) It regulates the intraocular pressure of the eye to keep it within a narrow, safe range, and 2) It filters debris by phagocytosis from the aqueous humor, as it moves from its production site, the ciliary body, through the TM, exiting via Schlemm's canal and into the venous system (Figure 1a).

Much of open angle glaucoma occurs as a result of elevated intraocular pressure or IOP. It is the normal TM’s job, possibly in conjunction with the inner wall cells of Schlemm’s canal, to regulate this pressure. As clinicians and glaucoma researchers, as well as many others are aware, elevated IOP is the primary risk factor for development of glaucomatous optic neuropathy. Sustained elevated IOP usually precedes the damages noted in the optic nerve and the retina in open angle glaucoma. This elevated IOP results when there is a loss of homeostatic regulation by the aqueous humor outflow pathway cells of the TM. Critical to this regulation is the concept of resistance to the aqueous humor outflow. The normal eye uses corrective adjustments of the aqueous humor outflow resistance in direct response to sustained pressure changes and these adjustments maintain IOP within acceptable physiological ranges [2]. By measuring the flow rate at constant pressure perfusion, we can assess the outflow resistance. By subjecting the system to a 2X pressure challenge, over several days the normal outflow system slowly reduces the outflow resistance, in an attempt to restore normal IOP levels. This ex vivo system (Figure 1b) can thus mimic the IOP homeostatic response of the normal eye. We applied this perfusion system to establish our human cell loss model for restoring function in open angle glaucoma.

Induced Pluripotent Stem Cells

Induced pluripotent stem cells (iPSCs) are adult stem cells that have the ability to develop into any other cell type in the body, like embryonic stem cells, but do not face the same legal and ethical complications. iPSCs were first made by Yamanaka [3], for which he won the Nobel Prize in Medicine in 2012. These cells are made by de-differentiating adult skin fibroblasts to a more primitive stem cell type, and then reprogramming them to a different terminal cell type. It is a major advantage of iPSCs that a patient’s own fibroblasts may be used, which negates immune rejection issues by allowing them to be patient-specific.

First Breakthrough

In our study, we first investigated the cell loss effect in TM function. Previously, Alvarado had noted significant TM cell loss in glaucoma, but this loss was associative [4]. The loss of cellularity in glaucoma is even greater than that which occurs with normal aging [5,6]. In our article in Stem Cells, we explored the hypothesis that the cell loss observed in glaucoma markedly disrupts IOP homeostatic regulation by the TM. We produced experimental eyes with cell loss comparable to glaucomatous eyes from eyes of human normal aged post-mortem donors. Under controlled conditions with a saponin detergent solution, we partially denuded the endogenous TM cells in normal anterior segments secured in the perfusion system to achieve an approximately 30% cell loss. To test for loss of IOP homeostatic function, we measured the resulting flow rate upon subjecting the anterior segments to a pressure increase or challenge, and compared normal and saponin-treated anterior segments. The normal TM compensated for the pressure challenge by decreasing the resistance to aqueous humor outflow, and increasing the flow rate gradually over several days. The saponin-treated anterior segments, however, showed no change, and were unable to mount a corrective IOP homeostatic response to the increased pressure, which corresponds to a glaucomatous situation. This breakthrough is the first experimental demonstration of a relationship between cellular loss and functional impairment in the TM in glaucoma.
Figure 1. Outflow pathway and perfused anterior segment organ culture model. a) Inset shows the human aqueous humor outflow pathway. Aqueous humor flows from the ciliary body through the pupil and out through the trabecular meshwork into Schlemm’s canal where it enters the venous drainage system. The TM outer beams are highly phagocytic. Aqueous humor percolates between the TM beams and then passes through the juxtacanalicular (JCT) region of the TM and across Schlemm’s canal inner wall endothelium [1-5]. Aqueous humor bathes the avascular cornea, lens and TM. Aqueous humor, formed by the ciliary body, flows at a relatively pressure-insensitive rate of around 2.75 μl/min. IOP is thus regulated by adjustments in the resistance to outflow that resides in the deepest portion of the JCT and Schlemm’s canal inner wall. (This diagram is modified significantly from an earlier review [1].) b) Anterior segment organ culture perfusion system [6,7]. Anterior segments, including the cornea, TM and approximately 5mm of sclera but without the iris, ciliary body or lens, are clamped into a polycarbonate flow cell [6]. Culture media is perfused through ports in the bottom of the flow cell, driven by a defined pressure head selected to mimic the physiologic IOP minus the episcleral venous pressure, which is missing in the model. Fluid flow rates are measured gravimetrically. The system is maintained in a standard CO₂ culture incubator at 37°C and 100% humidity [1].

Second Breakthrough

We then examined the effect of adding back normal cultured TM cells labeled with colored nanoparticles to saponin-treated anterior segments in the perfusion system. These TM cells were flowed into the perfusion system, and when the flow was stopped, the cells attached and integrated into the TM tissue. Upon re-starting the flow and testing these transplanted cells in saponin-treated anterior segments for an IOP homeostatic response in the perfusion system, we found that this homeostatic function was restored to normal. Next, we reprogrammed human iPSCs to a TM-like cell (TM-like iPSCs), with characteristics comparable to endogenous TM cells. These cells were labeled with the nanoparticles and injected into other saponin-treated anterior segments in the perfusion system, where they also homed to attach and integrate into all TM layers. Upon applying a pressure challenge to these transplanted anterior segments, the TM-like iPSCs also restored the IOP homeostatic response to normal. Several other cell types used as controls did not restore IOP homeostasis. This second breakthrough finding is the earliest report of successful and functional restoration using induced pluripotent human stem cells differentiated into TM-like cells. Additionally, phagocytosis of debris, a normal ability of TM cells, but not of iPSCs, likewise was found to occur in differentiated TM-like iPSCs.

Clinical Implications of The Study

This research study directly tested the feasibility of using patient-specific stem cells in a potentially promising and new personalized cell therapy strategy. Successful transplantation of TM-like iPSCs established the conceptual feasibility of using autologous stem cells for restoration of intraocular pressure regulatory function in open-angle glaucoma patients, thus providing a novel alternative treatment option.

References