Cell-Based Therapies for Glaucoma

Subjects: Ophthalmology

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Glaucoma is clinically characterized by elevated intraocular pressure (IOP) that leads to retinal ganglion cell (RGC) and optic nerve damage, and eventually blindness if left untreated. Even in normal pressure glaucoma patients, a reduction of IOP is currently the only effective way to prevent blindness, by either increasing aqueous humor outflow or decreasing aqueous humor production. The trabecular meshwork (TM) and the adjacent Schlemm's canal inner wall play a key role in regulating IOP by providing resistance when aqueous humor drains through the tissue.

Keywords: glaucoma ; trabecular meshwork ; stem cells ; regeneration ; intraocular pressure

1. Introduction

Since glaucoma is a degenerative disease, where TM and RGC cellularity is decreased [1][2], using stem cells to regenerate tissue is a promising area of study to explore more efficient and long-term treatment for glaucoma. Stem cells are immature cells that have not yet committed to a particular lineage of differentiation [3]. They are an appealing tool because they can divide in an immature state, and they can be induced to differentiate into many types of mature and functional cells. Stem cells can be classified based on their ability to differentiate into different cell types. Pluripotent stem cells, like embryonic stem cells and induced pluripotent stem cells (iPSCs), can differentiate into all cell types theoretically [4][5]. Multipotent stem cells are found in different tissues of the adult body (i.e., bone marrow and fat tissue) and are farther along the path of differentiation. Unlike pluripotent cells, multipotent cells have already committed to some particular lineages, so they have a more limited set of possible cell fates. Transplantation of stem cells, both pluripotent and multipotent, or differentiated functional cells from stem cells, in degenerative diseases is an encouraging area of study because of their regenerative and malleable characteristics. In glaucoma, pluripotent and multipotent stem cells have been studied in the context of both RGC and TM regeneration [6][7].

2. Cell-Based Therapies for Trabecular Meshwork Regeneration by Using Trabecular Meshwork Stem Cells (TMSCs)

Various cell-based therapies for TM regeneration in glaucoma treatment have been proposed. The first and seemingly most effective one has been the TMSCs. These stem cells are tissue-specific stem cells in the TM [7][8][9][10][11][12], that can differentiate into phagocytic TM cells in vitro [12]. Upon being transplanted into the mouse anterior chamber, human TMSCs appear to be able to maintain mouse IOP in the normal range, improve aqueous outflow, home to the TM region, and suppress the inflammatory response [13]. Transplanted TMSCs can reconstruct the TM structure and rescue mouse eyes with normal range IOP in mice that had been treated with laser photocoagulation, which created similar conditions to POAG [14], but with an inflammatory response [15]. Our group has previously compared the TM localization and homing-in of TMSCs to human corneal stromal fibroblasts and a sham control in the healing of TM tissue wounds from laser photocoagulation [14]. TMSCs were seen to localize to the TM, specifically to the areas that received laser photocoagulation, while the fibroblasts seemed to localize non-specifically to the TM, the iris, and other tissues in the eye. The eyes injected with TMSCs also showed that there was very low-level apoptosis, as determined by terminal deoxynucleotidyl transferase-mediated dUTP-fluorescein nick end labeling (TUNEL) in the TM area, while the Sham and Fibroblast conditions showed a considerably greater level of apoptosis. TMSCs were also effective in reducing the inflammatory response induced by laser, as evaluated by the expression of the inflammatory markers CD45, CD11b, and F4/80, and also reducing fibrosis as assessed by the low expression of fibrotic markers SPARC and fibronectin (FN) in the eyes with laser photocoagulation [14]. On the contrary, the animals with sham and fibroblast injection were detected with higher inflammation and increased expression levels of SPARC and FN. Utilizing transmission electron microscopy (TEM), the structure of TM can be visualized. After 4 weeks of TMSC injection, in comparison to the sham and fibroblast conditions, there was a comparable number of organized cell-covered beams in the TM and giant vacuoles, like that of the control eyes, indicating structural and functional restoration of the TM [24

The major parameter that has shown TMSCs to be a possible viable treatment for POAG is the ability for TMSCs to improve the outflow facility, maintain normal IOP, and prevent RGC loss [16]. In the laser photocoagulation-damaged mouse model, the TMSC treatment group had significantly reduced IOP in comparison to the fibroblast and sham groups [14]. In addition to this, the aqueous outflow facility, as measured following procedures from Lei et al. [17], was also shown to be increased after TMSC treatment as compared to fibroblast and sham treatment groups. This decrease in IOP and increase in outflow facility shows that the TMSCs can induce significant regeneration in glaucomatous eyes. Another study on a mouse POAG model with transgenic myocilin Y437H mutation (Tg-MyocY437H) [18] showed similar results [8]. In this study, human TMSCs were injected into the anterior chamber of 4-month old Tg-MyocY437H mice when they had elevated IOP. The mouse IOP was significantly reduced within a month after TMSC transplantation, eventually reduced to a value similar to that of wild-type (WT) mice [8]. Accompanying the IOP reduction, the outflow facility of the eyes was significantly increased to the level of WT mice. To achieve the treatment purpose for preventing vision loss, the mouse RGC were protected from death and the RGC function was preserved by measuring and comparing the mouse pattern electroretinogram (PERG) [8]. These studies, although done in mouse models, indicate that TMSC transplantation can be a promising approach to reduce IOP, increase outflow facility, and prevent vision loss for glaucoma treatment.

3. Cell-Based Therapies for Trabecular Meshwork Regeneration by Other Stem Cell Types

As described above, the use of stem cells for TM regeneration is a promising therapy for glaucoma treatment. TMSCs were shown to be able to home to the TM region specifically, differentiate into functional TM cells to increase the TM cellularity and improve the ECM components, and reduce IOP in mouses models of glaucoma [8][14]. Although TMSCs can suppress inflammatory response without evoking immunorejection after xenotransplantation [8][14], some patients may prefer to use cells from themselves. However, the use of autologous TMSCs can have some limitations. Firstly, TMSCs are found in the insert region of the TM [7][9][11], and harvesting enough of these cells from a living patient is difficult. Secondly, it is possible that there are fewer TMSCs in glaucoma patients, or that TMSCs isolated from these patients may be impaired or with genetic mutations. Because of these limitations, there has also been extensive research performed on the use of other types of stem cells for TM regeneration.

One of these cell types is induced pluripotent stem cells (iPSCs). iPSCs are adult somatic cells that have been genetically reprogrammed to express four pluripotent transcription factors Oct4, Sox2, Klf4, and cMyc [4] or OCT4, SOX2, NANOG, LIN28 [5]. This creates a system in which almost any cell type of the body can be derived from patient-specific iPSCs, using a variety of co-culturing techniques, mixtures of supplements, bioactive small molecules, and growth factors to control cell fate. Successful differentiation of both mouse and human iPSCs into TM-like cells has been carried out in multiple ways [19][20][21][22]. One method for TM regeneration and reduction of IOP in mouse models can be achieved using iPSCs that can be differentiated into TM cells (iPSC-TM). iPSC-TM are morphologically like primary TM cells that express proteins similar to that of the regular TM cells found in human eyes [23]. To form glaucomatous iPSC-TM, iPSCs must first be derived from fibroblasts isolated from transgenic mice that exhibit the glaucoma phenotype expressing human myocilin Y437H (Tg-MYOCY437H). These iPSCs can be induced to differentiate into TM cells using conditioned media (CM) by primary TM cells. Another method described co-culturing of mouse dermal fibroblast-derived iPSCs with primary human TM cells for 21 days [20]. By this time, the iPSC-TM cells share many characteristics with primary TM cells, including the ability to phagocytose particles and upregulation of myocilin and MMP3 in response to dexamethasone treatment. They also show reduced expression of pluripotency markers Nanog, Oct4, and Sox2, which is important in assessing if these cells can give rise to a tumor in vivo. This co-culture method of iPSC-TM induction was also successful when human iPSCs derived from dermal fibroblasts and keratinocytes were cultured with human primary TM cells [19]. We described another method of creating iPSC-TM cells from human iPSCs following a two-step induction process, where iPSCs are first induced into neural crest cells (NCC) and then TM-like cells [21]. This more closely mimics the path of differentiation for TM cells in embryonic development [24]. First, iPSCs are grown on an extracellular matrix (ECM) derived from the cell line A549 in N2B27 and Y27632 containing medium [21]. This has been shown to induce iPSCs into NCCs, as they began expressing NCC markers NGFR and HNK1 and reduced expression of the pluripotent stem cell marker SSEA4. These iPSC-NC cells were then grown on an ECM derived from cultured primary TM cells and in primary TM conditioned media (CM) for 10 to 14 days. After this culture protocol, the iPSC-TM cells shared many characteristics with primary TM cells, including increased expression of the TM cell maker CHI3L1, and increased expression of myocilin, in response to five days of 100 nM dexamethasone treatment. After 14 days of dexamethasone treatment, these iPSC-TM cells formed cross-linked actin networks (CLANs), a structure predominately formed in TM cells after dexamethasone treatment. This method of iPSC-TM induction breaks up the protocol into two parts, allowing expansion and storage of iPSC-NC cells from which to derive iPSC-TM cells in the future. It also describes a method that relies on isolated ECM and CM from primary TM cells instead of direct co-culture.

Regardless of the method of iPSC-TM induction, these cells have successfully rescued glaucoma phenotypes in a transgenic mouse model of glaucoma [25][26]. As briefly described above, the transgenic-MYOCY437H mice express human myocilin with the disease-causing mutation Y437H. This mutation prevents myocilin from being transported out of the endoplasmic reticulum (ER), thus causing ER stress [18]. In one study, mouse-derived iPSCs were differentiated into iPSC-TM cells through co-culture with TM cells [25]. After a 14-day induction in the CM yielded cells that exhibited morphology and gene expression similar to that of primary TM cells. Specifically, a marked expression of laminin A4 and tissue inhibitor of matrix proteases 3 (TIMP3). iPSC-TM cells were then isolated and transplanted through intracameral injections into four-month-old Tg-MYOCY437H and WT mice. This was compared to mice injected with either PBS (vehicle control) or an equal number of fibroblasts. Six weeks after injection, the vehicle control Tg-MYOCY437H mice showed an increase in IOP, and a decrease in aqueous humor outflow compared to WT mice, confirming that the transgenic mice displayed a glaucoma phenotype. However, when Tg-MYOCY437H mice were transplanted with iPSC-TM cells, IOP decreased and aqueous humor outflow increased, matching WT levels. This observation was held for the remainder of the study (nine weeks). Twelve weeks after injection, mice were assessed for iPSC-TM integration and RGC preservation. Compared to the vehicle control, glaucoma mice injected with iPSC-TM cells showed both an increased RGC and TM cell density, similar to the levels seen in WT mice. Though injected iPSC-TM cells were able to integrate into the TM tissue, the increased TM cells in the host TM were not iPSC-TM cells, suggesting that injected cells may have induced proliferation of endogenous TM cells. To look at this observation in vitro, this group transfected the MYOCY437 mutant into primary mouse TM cells and co-cultured them directly and indirectly (using cell inserts) with iPSC-TM cells. They found that co-culture with iPSC-TM cells promoted TM cell proliferation, but only when these cells were in direct contact with each other. This study demonstrated that in the transgenic MYOCY437H mouse model, iPSC-TM injection rescued the glaucoma phenotype with decreasing IOP, increasing aqueous humor outflow, and maintaining RGC density by promoting the endogenous proliferation of TM cells. Similar effects were also observed in aged mice when transplantation of iPSC-TM cells was done at six months of age, instead of four months [26].

Another type of stem cell that has been studied within the context of TM regeneration are the adipose-derived stem cells (ADSCs). Like iPSCs, ADSCs can be obtained for autologous transplantation, but they do not require genetic perturbation. Additionally, large numbers of ADSCs can be isolated using minimally invasive procedures and can be differentiated into many cell types, making them another promising candidate for tissue regeneration. The method of TM regeneration using ADSCs begins like that of iPSCs, where ADSCs are differentiated into TM-like cells in vitro [27]. We have previously shown that ADSCs can be successfully differentiated in TM cells using TM-ECM and conditioned media. The ADSC-TM cells thus obtained showed increased expression of TM cell markers CHI3L1 and AQP1 [28]. In our recent study [27], ADSCs were isolated from three donors and subsequently cultured with either TM cells, ECM and CM obtained from TM cells, or only ECM from TM cells. After 10 days of culture, ADSCs in the first two groups began expressing TM markers CHI3L1 and AQP1, and reduced expression of stem cell marker OCT4 [27]. They also exhibited phenotypic traits of TM cells, including increased expression of myocilin and increased CLAN formation in response to dexamethasone treatment. ADSC-TM cells also showed more phagocytic activity compared to uninduced ADSCs. Interestingly, ADSCs incubated with TM-ECM only did not show obvious characteristics of the above-mentioned, implicating the importance of TM paracrine factors in this differentiation. Undifferentiated ADSCs and ADSC-TM from the ECM+CM group were then isolated and injected into wild-type (WT) mice, along with a fibroblast control. One month later, ADSCs and ADSC-TM cells integrated into the TM tissue, although some ADSCs were off target, while fibroblasts showed more off-target attachment to the iris and corneal endothelium, similar to previous reports [13][14]. Both ADSCs and ADSC-TM cells expressed the TM cell marker AQP1, which modulates aqueous outflow, while fibroblasts did not. Transplants of ADSCs and ADSC-TM maintained normal IOP and aqueous humor outflow levels in WT mice, while fibroblast injection increased IOP and reduced the aqueous humor outflow facility. This study describes another promising method for stem cell treatment of glaucoma, as ADSCs can be isolated in large quantitates with minimally invasive techniques and can be induced into ADSC-TM cells. After transplant into WT mouse anterior chambers, they preferentially integrate into the TM and maintain normal IOP and aqueous humor outflow. We have shown that ADSC conditioned media can induce regeneration in TM cells by increasing their wound healing potential and reducing fibrosis in vitro [28]. It would be interesting to see the effect of ADSC and ADSC-TM transplants specifically in a mouse model of glaucoma.

4. Mechanisms of Cell Mediated Glaucoma Treatment

The main mechanisms involved in the development of POAG is yet to be properly deduced. However, there are a few hypotheses about what may result in the development of POAG. One of these is the interaction between the chemokine SDF1 and its receptor CXCR4, which has been shown to have an important role in hematopoietic stem cell homing or localization [29]. The CXCR4 receptor has also been implicated in the signaling and homing of the cells involved in lymphopoiesis, myelopoiesis, embryogenesis, angiogenesis, cardiogenesis, neuron migration, and cerebral development

[30][31]. Due to its important function in all these processes, it could play a role in TMSC homing to TM tissue to repair any damage and/or induce proliferation. Gene expression studies of TMSCs and TM cells did indicate that there was a high expression of CXCR4, indicating a possibility that it was involved in TMSC signaling and localization. To confirm this interaction, Yun et al. cultured TMSCs on a TM feeder treated with recombinant SDF1α and 1β and anti-SDF 1 antibody to neutralize SDF1 in the TM cells [14]. The use of the anti-SDF 1 antibody neutralized the expression of SDF1, while the TMSCs cultured with the SDF1αβ displayed an increase in SDF1 expression. It was observed that the greatest number of TMSCs attached to the TM-SDF1αβ cells, while the least TMSCs attached to TM-SDF1Ab, indicating an increased affinity of TMSCs to the TM cells with upregulated SDF1. To inhibit the CXCR4 axis, TMSCs were treated with the inhibitor IT1t [32]. After the inhibitor treatment, TMSCs were not able to attach to TM and showed no significant difference in their affinity towards TM cells. It was also observed that chemotaxis between TMSCs and TM cells without direct contact utilized untreated TM cells, TM cells with SDF1αβ, and TM cells with SDF1Ab. A larger percentage of the TMSCs were found to migrate to the TM cells treated with SDF1αβ while showing a reduced migration towards TM cells treated with SDF1Ab. Further, the use of a CXCR4 antagonist, AMD3100, which reduced CXCR4 expression in TMSCs, and a short hairpin RNA which reduced SDF1 expression in TM, showed a decrease in the attraction between the TM and TMSCs, indicating that the CXCR4/SDF1 chemokine axis is part of the signaling involved in the TMSC homing-in and migration to the TM cells. This cell homing is essential to glaucoma treatment, as it ensures that the TMSCs can localize to the TM tissue to properly rebuild the TM network and reduce the IOP associated with POAG, to prevent any further RGC death and vision loss.

Another mechanism that is important in the TMSC-mediated protection of the TM tissue, as well as the maintenance of the TM Extracellular matrix (ECM) and protection of RGCs, is through the TMSCs upregulating certain genes related to these functions. Using transcriptomic analysis, Xiong et al. showed the involvement of three upregulated pathways in TMSCs that showed increased TM ECM interaction which included the focal adhesion pathway, the PI3K-Akt signaling pathway, and the ECM-receptor interaction pathway [8]. The focal adhesion pathway proteins are especially important as adhesion receptors for the ECM as well as involved in the signaling downstream for processes such as apoptosis, contraction, endocytosis, and phagocytosis [33][34]. The PI3K-Akt signaling pathway usually responds to oxidative stress in the regular TM cells as it is heavily involved with recovering from abnormal morphological changes and can cause cytoskeletal changes in the TM [25]. The ECM-receptor pathway has been shown to control the ECM of the TM cells which control the amount of outflow resistance that would in turn have an effect on controlling the IOP. These pathways were downregulated in the fibroblasts in comparison to TMSCs. This might be a reason that TMSCs were able to promote a regenerative effect on the damaged TM and reduced risk factors leading to POAG while the fibroblasts showed no such effect.

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