Regeneration of trabecular meshwork in primary open angle glaucoma by stem cell therapy: a new treatment approach

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Abstract: Primary open angle glaucoma (POAG) is a worldwide disease with IOP being an important risk factor for the disease. Pharmacological and surgical treatments have been mainly targeted on lowering IOP by decreasing aqueous humor production or increasing aqueous humor outflow. Stem cell therapies may open new frontiers in regenerative ophthalmology branch. In POAG there is a strong association with pathologic degeneration of the trabecular meshwork (TM) and regenerative cell-therapy approaches have been focused mainly on modulation of the degeneration. Many different adult stem cell types have been discovered in different parts of the eye such as the corneal endothelium (CE) and anterior non-filtering portion of the TM called Schwalbe’s ring region. These stem cells may supply new cells for the TM and may regenerate the TM structure thus reducing IOP and restore the homeostatic function of the eye. In this paper, we report the studies’ latest findings and present our perspective on approaches that seem promising in the management of POAG.

Keywords: glaucoma, trabecular meshwork, stem cell therapy, POAG, iPSC

Introduction
Glaucoma is one of the most common causes of irreversible vision loss and blindness worldwide; 60 million suffer from this disease and of these, 7 million are blind. By definition, all glaucoma involves some degree of vision loss, which is because of the death of retinal ganglion cells (RGCs), as well as degeneration of the optic nerve head, the optic nerve, and the lateral geniculate nucleus. Primary open angle glaucoma (POAG) represents about 90% of all forms of glaucoma. POAG is characterized by a painless condition of IOP elevation with a normal-appearing anterior chamber angle. The IOP elevation is an important risk factor for the disease. Many RGCs, which are the projection neurons that carry signals from retina to brain, are irreversibly lost and by the time vision loss is detected. The IOP is the result of the balance between aqueous humor production by the ciliary body and the ability of the ocular outflow pathways to remove this fluid. IOP is determined by the continuous generation of aqueous humor by the ciliary processes (inflow) and its elimination by the trabecular meshwork (TM) which together with Schlemm’s canal, collector channels, and aqueous veins, constitute the major outflow pathways in the eye. TM and the endothelium of Schlemm’s canal are crucial to provide resistance to the aqueous humor outflow and thus control IOP levels.

The current treatment of glaucoma is based on IOP lowering by pharmacological reduction of aqueous humor production or by laser-based and other surgical procedures.
which provide an aqueous humor outflow bypass. An alternative or complementary strategy is to target TM to increase outflow by pharmacological agents. To this aim, many drugs are being developed and have shown encouraging results in clinical trials. Among these there are rho kinase (ROCK) inhibitors whose activity increases actomyosin contraction in smooth muscle cells, including the smooth muscle-like cells of the TM. Also, adenosine agonists can trigger a potentially important modulation of outflow resistance in vivo, particularly the interaction with A1 and A2 adenosine receptors. In the end, statins, more specifically lovastatin, can act as an important pharmacological suppressor of SPARC (secreted protein acidic and rich in cysteine) expressed in TM cells, that can provide further insight into the molecular mechanisms mediating statin enhancement of aqueous outflow facility.

Understanding the fundamental mechanisms leading to dysfunction and loss of TM cells in the glaucomatous eye may be useful for developing new strategies to target this primary pathological site.

The regenerative approach can give hope for numerous ocular pathologies, such as diabetic retinopathy and glaucoma.

**Stem cell overview**

Stem cells can be categorized into embryonic stem (ES) cells, induced pluripotent stem cells (iPSCs), and adult stem cells. iPSCs have rapidly obtained significance since their discovery by Takahashi and Yamanaka. One advantage of iPSCs is that these cells can be derived from readily available cell types of the intended recipient, thereby enabling autologous transplantation and avoiding immune rejection in the recipient. iPSCs present an advantage inasmuch as an autologous approach may be possible, circumventing the ethical and immunological disadvantages associated with ES cells therapy. However, there are safety concerns that involvement of retroviral vector integration for reprogramming iPSCs may cause oncogenesis.

It was demonstrated that transplantation of these cells into eyes of mice promotes aqueous humor outflow, lowers IOP, and prevents RGC loss. The improvement of aqueous humor outflow dynamics in iPSC-TM recipients is accompanied by a significant increase in TM cell density in vivo.

Considerable progress has also been made in developing human Tenon’s fibroblasts as feeder cells for culturing human stem cells.

**Structure, function, and embryology of TM**

The anterior chamber of the eye is bordered by the corneal endothelium (CE) in the front and the iris in the back. TM, scleral spur, ciliary body, and iris root, which form the anterior chamber angle are positioned at the periphery of the chamber.

TM stem cells located in the anterior chamber angle between the cornea and the iris, in the transition zone between the periphery of the CE and the anterior extension of TM, which is known as the Schwalbe’s ring region.

The TM can be anatomically divided into 3 regions (from inner to outermost). The uveal meshwork is the closest to the anterior chamber and consists of a network of collagen and elastin lamellae covered by TM cells with large spaces between lamellae. The corneoscleral meshwork, which is the middle layer, is composed of a series of perforated collagen and elastin plates covered by TM cells. The most external layer, the juxtaocular connective tissue is a loose connective tissue containing TM cells surrounded by extracellular matrix (ECM). These layers are considered part of the “filtering” TM. A fourth region of the TM is considered “non-filtering” since it resides just below Schwalbe’s line. Data suggest that this region contains a population of TM stem cells.

The development of the cornea begins at approximately 33 days of post fertilization. The primitive TM is formed at around the fourth month. It is formed from the first wave of neural crest which originated from mesenchymal cells migration between the surface ectoderm and the lens. It consists of a triangular mass of undifferentiated mesenchymal cells. During the seventh month, these cells flatten and become slightly separated from each other, and these cavities are filled with extracellular fibers. The fibers are then organized to form the trabecular lamellae. Some cells with a stellate phenotype form the juxtaocular lamellae. Some cells with a stellate phenotype form the juxtaocular layer of the TM. The complete morphogenesis of TM is completed around birth.

Specific gene regulatory networks are involved in tissue developments and these include many transcription factors and molecular signals such as PITX2, PITX3, PAX6, FOXC1, FOXE3, LMX1B, and MAF. PAX6 appears to be the most important eye development regulator in a number of organisms.

A number of studies have noted that TM was abnormally formed in Pitx2 c/c and Foxc1−− mice. The LMX1B gene was shown to have a direct link to the dysgenesis of the TM. Absent or hypoplastic TM and Schlemm’s canal, and profound ECM deficiencies in TM have been associated with heterozygous deficiency of BMP4.
the primary egress route for aqueous humor from the eye.
To assure the effective outflow resistance regulation, the
TM tissue has an important function of biological filter
self-cleaning by an activity of intercepting cellular debris
and reactive oxygen species (ROS). Thus, TM cells have a
macrophage-like activity to clear the cellular debris derived
from shade pigmented epithelia. The integrity of these cells
is important for the regulation of this passageway for the
maintenance of homeostatic IOP. The dysfunction of these
cells may generate an extra resistance that causes an IOP
elevation. TM cells create a link through cytoplasmic exten-
sions between the intertrabecular spaces while desmosomes
create a firm connection with the adjacent cells.32 Electron
microscopic observations have revealed that gap junctions
constitute the main intercellular bridge between TM cells.

TM cells express a variety of proteins, receptors and
abilities. Among the proteins and receptors expressed there
are vimentin, non-muscle actin, aquaporin-1, acetylated and
acetoacetylated low-density lipoproteins, and the alpha-2
adrenergic receptor. Moreover, the expression of myocilin
by TM cells increases after dexamethasone treatment. The
myocilin expression in the TM cells has been suggested to
play an important role in glucocorticoid-induced ocular
hypertension.33–35 Among the agents able to modify the activ-
ity of cells there are fibroblast growth factor and Hepatocyte
growth factor that cause TM cells mitosis in a dose-dependent
way.36–38 Platelet-derived growth factor can also increase cells
division in TM. Besides, it enhances the phagocytic activity
and promotes ECM secretion.39 On the contrary, vascular
endothelial cell growth factor can inhibit TM cells growth.40

TM cells have the ability to phagocytize and this ability
probably may help to eliminate debris from aqueous humor.
This characteristic demonstrates that TM is not a simple
passive filter.41 In addition TM cells are contact inhibited in
culture: when the cells were in contact they formed gap junc-
tions that caused a decrease of division rate. TM cells have
also contractile capability thanks to cytoplasm’s contractile
filaments.42

**TM cells loss and glaucoma**

It is important to highlight that the number of TM cells
decrease with age and a decrease is also associated with
plaques. There is also evidence of accumulation of ECM
associated with alteration of gap junction between cells of
the TM and Schlemm’s canal, leading to TM cell death and
IOP elevation. Moreover, aged and glaucomatous TM cells
present damages probably caused by ROS.43,44

Alvarado et al reported a cell loss rate of 0.58% per
year.45 This loss is similar to that observed in the CE. It was
calculated that there were 750,000 cells in the TM of 20 year
old people and around 400,000 in 80 year old people.46 There
are other age-related modifications such as TM thickening,
trabecular fusions, and alterations of the ECM in the juxta-
canalicular TM. All these changes would lead to an increase
of the aqueous outflow resistance and subsequently to IOP
increases. The main risk factor in POAG is the pathological
elevation of the IOP. The age-related changes are intimately
linked to the glaucomatous modifications observed in POAG
patients. It has been noticed that glaucomatous eyes have sig-
ificantly more cellular loss than age-matched normal eyes.47
This probably causes a diminished ability to drain humor
aqueous. Progressive cell loss leads to a TM thickening and
fusion probably due to adhesions of the naked portions of the
trabeculae. Some studies have documented accumulation of
ECM and meshwork cell hyperplasia in glaucomatous TM
that creates an obstruction of the outflow pathway. Conse-
Raviola in the region located just beneath the Schwalbe’s
line in rhesus monkeys. These cells have been called the
Schwalbe's line cells.48

Subsequently, it was noted that there is an increased TM
cell division localized in the anterior non-filtering portion
of the TM after argon laser trabeculoplasty (ALT).49 It is
not exactly known how this treatment is able to lower IOP;
probably, the repopulation of TM, stimulated by cell division,
is the main mechanism of action.50 Furthermore, there is evi-
dence that more than 60% of cell division is initially located
in the anterior non-filtering region of TM; those cells move
to the burnt lesion to repopulate. It seems that these cells
are probably stem cells that after ALT laser get stimulated
to repopulate TM, probably through the mediation of growth
factors and cytokines. Kelley et al have named them the “TM
insert cells” as they are located in the insertion region into
the cornea just beneath Schwalbe’s line.51

Gonzalez et al found cultured TM cells capable of form-
ing free-floating neurospheres, a function associated with
 neural stem cells. When the TM free-floating spheres were
incubated with serum, they evolved into monolayers of cells
morphologically indistinguishable from typically cultured

Evidence of therapeutic
implications for TM stem-cells

In 1982, a population of unusual cells was identified by
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incubated with serum, they evolved into monolayers of cells
morphologically indistinguishable from typically cultured
TM cells, indicating the possibility that they might differentiate spontaneously into TM cells.52

As previously stated, the number of TM cells decreases significantly in the patient with glaucoma, thus depressing the aqueous outflow. It is well documented that in the transition area between the periphery of the CE and the anterior non-filtering portion of TM there is a population of stem-like cells. Thus, using the stem cells to repopulate TM may be useful to enhance drainage in glaucoma.

Stem cell therapy potentially can restore TM function and protect the optic nerve from further damage.53 Replacing glaucoma-damaged TM cells with healthy stem cells may restore the micro environment of filtering structures, and this could create a reparative proliferation and restore the physiological aqueous outflow causing a reduction in IOP thanks to the TM cells property. TM stem cells were also observed in the region where the TM inserts and have been isolated by side-population cell sorting, clonal culture, and sphere culture (Table 1).51,52 Du et al have described the isolation and characterization of a population of stem cells from human TM that in culture present a homogeneous population displaying antigenic markers previously characterized for mesenchymal stem cells and expressing gene products associated with pluripotent stem cells. These cells are capable of differentiating into TM cells with phagocytic function and expressing TM markers. All these support the hypotheses that these cells represent a resident population of adult stem cells in the human TM. Moreover, those cells are multipotent and differentiate into TM cells in vitro and in vivo, they settle in the TM region and can regenerate the TM structure thus reducing IOP in mouse models, thanks to specific TM cells properties as phagocytosis activity.54,55 As we have shown previously Zhu et al demonstrate that iPSC can be induced to differentiate into a cell type (iPSC-TM) that strongly resembles TM morphologically, compositionally express a large number of proteins characteristic of TM, and functionally respond to various stimuli in a manner typical of TM cells. For example, iPSC-TM responds to exposure to glucocorticoids with enhanced synthesis and secretion of myocilin. For this purpose mouse’s iPSC-TM cells were induced from iPSC derived from fibroblasts isolated from transgenic animals; subsequently iPSCs were seeded and induced to differentiate by maintaining them in biopsy media previously conditioned by primary human TM cells. Finally, iPSC-TM cells were purified by removing cells still expressing markers of pluripotency from the iPSC-TM population to avoid tumor formation after transplantation. Therefore, 50,000 purified iPSC-TM cells were injected into the anterior chamber of mice that constitutively express human myocilin harboring a pathogenic mutation. The data demonstrate that intraocular injection of iPSC-TM prevents IOP elevation and aqueous humor outflow reduction and results in preservation of RGC density in treated mice. iPSC-TM cells have been observed to be able to restore homeostatic function in glaucoma mouse in vivo models.16,56 Moreover, Abu-Hassan et al observed that iPSC-TM cells can restore IOP homeostatic function in a human anterior segment ex vivo model. This remains the only study performed on human tissue, as shown in Table 2. In this case, human anterior segments were perfused for 48 hours to establish baseline flow and then treated with saponin for 7 minutes to remove approximately 1/3 of the TM cells. The anterior segments exposed to saponin lost the ability to adjust the outflow resistance when subjected to a pressure challenge; this capability was regained when

**Table 1** Trabecular meshwork stem cells (TMSCs)

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Cells</th>
<th>Donor</th>
<th>Vitro/vivo/ex-vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acott, Samples, Bradley, Bacon, Bylsma, Van Buskirk</td>
<td>1989</td>
<td>TMSCs</td>
<td>Human</td>
<td>Vitro</td>
</tr>
<tr>
<td>Gonzalez, Epstein, Luna, Liton</td>
<td>2005</td>
<td>TMSCs</td>
<td>Human</td>
<td>Vitro</td>
</tr>
<tr>
<td>Du, Roh, Mann, Funderburgh, Funderburgh, Schuman</td>
<td>2012</td>
<td>TMSCs</td>
<td>Human</td>
<td>Vitro</td>
</tr>
</tbody>
</table>

**Table 2** Trabecular meshwork stem cells (TMSCs); induced pluripotent stem cell-trabecular meshwork (iPSC-TM)

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Cell technology</th>
<th>Cell source</th>
<th>Host</th>
<th>Vivo, ex vivo</th>
<th>Transplantation site</th>
<th>IOP normalization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Du, Yun, Yang, Schuman</td>
<td>2013</td>
<td>TMSCs</td>
<td>Human</td>
<td>Mice</td>
<td>Vivo</td>
<td>Anterior chamber</td>
<td>+</td>
</tr>
<tr>
<td>Abu-Hassan, Li, Ryan, Acott, Kelley</td>
<td>2015</td>
<td>iPSC-TM</td>
<td>Porcine-Human</td>
<td>Human</td>
<td>Ex vivo</td>
<td>Anterior chamber</td>
<td>+</td>
</tr>
<tr>
<td>Zhu, Jain, Gramlich, Tucker, Sheffield, Kuehn</td>
<td>2017</td>
<td>iPSC-TM</td>
<td>Mice</td>
<td>Mice</td>
<td>Vivo</td>
<td>Anterior chamber</td>
<td>+</td>
</tr>
</tbody>
</table>
cultured human iPSC-TM were added back and allowed to attach and integrate into the anterior segments.57

Conclusion
In the eye, evidence reveals that there is a population of stem-like cells located in the transition area referred as Schwalbe’s ring. In 1982, Raviola identified a population of unusual cells located just beneath the Schwalbe’s line in the rhesus monkey. Not much attention was paid to Schwalbe’s line cells until there was more evidence supporting the presence of stem/progenitor cells in this transition zone. The observation of an increase in TM cell division localized to the anterior non-filtering portion of the TM after ALT suggested the possible mechanisms of action is the repopulation of the TM by stimulating cell division. Recent progress in stem cell research provides an optimistic prospect on their use in regenerative medicine and tissue engineering. Specifically, advances in iPSCs and adult stem cells research raise hope for personalized cell replacement therapies.

Prospectively, it will be necessary to establish the safety profile and potential activity of TM stem cells transplantation through preclinical studies before moving to clinical trial in patients with glaucoma. Moreover, TM stem cells models will offer a new possibility for a better understanding of trabecular outflow physiology, glaucoma pathophysiology, and drug testing.

Disclosure
The authors report no conflicts of interest in this work.

References