Potential of Müller Glial Cells in Regeneration of Retina; Clinical and Molecular Approach

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ABSTRACT

Retinal degenerative diseases are a group of heterogeneous eye diseases that affect a significant percentage of the world's population, i.e., age-related macular degeneration (AMD), diabetic retinopathy, retinitis pigmentosa (RP), and glaucoma. Regenerative medicines look for novel therapies for severe injuries or chronic diseases, e.g., retina degeneration. Müller glia is the only retinal glia type with a common embryonic origin, with retinal neurons derived from the neural crest. Also, the lack of neurons in the retina does not automatically regenerate. Therefore, Müller glial cells, which make up about 5% of retinal cells, are a potent source for retinal regeneration. Following the retinal damage, Müller glial cells dedifferentiate and re-enter the cell cycle, producing multipotent progenitor cells. This feature leads to applying Müller glial cells in the regeneration of the retina. This study reviews this feature's molecular and clinical approaches, focusing on the critical signaling pathways, generation and transplantation methods, and clinical and sub-clinical challenges.

Keywords: Retinal degenerative diseases; Müller glial cells; Regenerative medicine; Retina

INTRODUCTION

The retinal tissue of vertebrates is part of the central neural system (CNS) responsible for absorbing, processing, and sending visual information through the optic nerve to the brain. This tissue has different neuronal and glial cells [1, 2]. Rod and cone photoreceptors convert light into electrical signals and transmit it through synapses with bipolar cells to the retina's ganglion cells. These signals are then transmitted to the nuclei of the brain through the axons of the optic nerve via neurotransmitters. In addition to the different types of neurons, there are three types of non-neuronal cells, including Müller glia, retinal pigment epithelial (RPE) cells, and astrocytes. Müller glia spread to the surface of RPE cells and is similar to astrocytes located in other areas of CNS [3].

Retinal degenerative diseases are a group of heterogeneous eye diseases that affect a significant percentage of the world's population, including age-related macular degeneration (AMD), diabetic retinopathy, retinitis pigmentosa (RP), and glaucoma [4-6]. Although these diseases can be caused by different etiologies (hereditary or acquired), they will eventually have a common feature, "the lack of vision." AMD and RP are commonly associated with the destruction of photoreceptors and RPE, and glaucoma is associated with retinal ganglion cell (RGC) destruction [7]. AMD is an inflammatory disease of the retina and the third leading cause of blindness in the world after cataracts and glaucoma [8].

Regenerative medicines are looking for novel therapies for severe injuries or chronic diseases that the body cannot repair. The main focus of regenerative medicine is on developing and regenerating tissue alternatives to prevent

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pathological damage and restore the physiological function of the tissue. Various methods have been proposed to improve regeneration to date, i.e., endogenous regeneration using growth factors, exogenous transplantation of living cells of allogenous and autologous origin, tissue engineering, and artificial development [9-11]. Due to the potential of Müller glial cells in the regeneration of ocular tissues, these cells are involved in clinical trials, especially applying for the regeneration of the retina. This review article is aimed to introduce cellular, molecular, and clinical approaches to Müller glial cells in retinal regeneration.

MAIN TEXT

Retinal Regeneration using Müller Glial Cells

In The result of a novel medical approach to the regeneration of retinal degenerative diseases is based on two methods: (i) cell therapy with transplantation of exogenous photoreceptor cells generated from embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), or progenitor cells, and (ii) the self-regeneration of the damaged retinal tissue, which is performed by retinal stem (-like) cells. However, the process of self-regeneration is common in several species of non-mammalian vertebrates [12]. There are various cellular mechanisms and resources for retinal self-regeneration in different species using RPEs and Müller glia [13, 14].

Müller glial cells make up about 5% of retinal cells. They spread along the surface of the retina and coexisted with neurons. Numerous roles of these cells include retinal homeostasis and visual function through trophic support of retinal cells [15]. They are also considered the second source of regeneration of visual pigments, especially cone photoreceptors. Müller glia is the only retinal glia with a common embryonic origin, with retinal neurons derived from the neural crest [12]. The transcriptomic analysis bears many similarities between the molecular characteristics of Müller glia and late retinal progenitors because they are formally the last cells to form in all vertebrate retina [16].

As with other mammalian CNS, the lack of neurons in the retina does not automatically regenerate. However, the regeneration process is more efficient in many non-mammalian vertebrates, such as fish and amphibians. Following retinal damage, Müller glial cells dedifferentiate and re-enter the cell cycle, producing multipotent progenitor cells. These progenitor cells originate from Müller glia and RPE cells in fish and amphibians, respectively [17]. Adult birds, like mammals, are unable to regenerate after injury. Fischer et al. showed that after inducing a neurotoxicant, such as N-methyl-D-aspartic acid (NMDA), into postnatal chicks, Müller glial cells begin to proliferate and produce limited neurons [18]. In mammals, Müller glial cells can respond to damage and transcript the genes associated with retinal stemness, but in-vivo, they do not act as retinal progenitor cells. However, these characteristics suggest that under appropriate conditions, Müller glial cells may operate using the properties of a retinal precursor that can be used for regeneration [15, 19]. In this regard, several in-vitro and in-vivo studies have been performed. For the first time, two studies in mice [20] and rats [21] showed that after induction of damage by NMDA and stimulation of proliferation by fibroblast growth factor 1 (FGF1) and epidermal growth factor (EGF), Müller glial cells had the potential to proliferate and produce neurons. Therefore, Müller glial cells are potent in the regeneration of the retina in mammals. However, the lifespan of these new cells is not long, and they may not be able to integrate into the network of retinal neurons. As a result, researchers have decided to upgrade this regeneration with new methods.

Das et al. showed that Müller glial cells isolated from rat retina produce clonal neurospheres that are self-renewing and multipotent, thus showing the cardinal characteristics of stem cells. These neurospheres are also able to differentiate functional neurons in in-vitro and in-vivo. They stated that the neurogenic potential of these cells is inherent but dormant and is induced after damage [22]. Another

study used immortalized Müller glial cell line (MIO-M1) produced by the postmortem human neural network. In this study, these cells were transplanted into the retina of an animal model of glaucoma. As retinal detachment showed resistance to fusion, erythropoietin and ABC chondroitin increased the efficacy of fusion by altering the retinal environment and eventually observed differentiation. This cell line may not have the ethical concerns of ESCs [5, 23]. In another study, Giannelli et al. used adult human Müller glial cells. In in-vitro conditions, they differentiated these cells into photoreceptor cells and transplanted them to the rat model to follow the differentiation and the ability to integrate into the target tissue. This group showed that these cells integrate into the proper retinal layer and express the marker of rod cells [24].

Another study also showed that by isolating Müller glial cells from the adult human retina, these cells are characteristic of human Müller stem cells. They immortalized these cells and observed retinal neuronal function under treatment with various growth and differentiation factors. Singhal et al. distinguished Müller glial cells from retinal ganglion cell (RGC) precursors in in-vitro conditions by isolating them from human embryos and treating them with FGF2 and Notch inhibitors. Then, the precursors of RGC were transplanted into a retinal animal model induced by NMDA, which lacked RGCs. These cells migrated to the RGC layer in anti-inflammatory and destructive matrix agents and slightly improved RGC function [25-27]. Jayaram et al. differentiated Müller glial cells into photoreceptors with the exact origin with treatment by FGF2, retinoic acid, and insulin-like growth factor type 1 (IGF-1). Then, precursors of photoreceptors were transplanted to the animal model P23H rat, whose photoreceptors had been destroyed. Following transplantation, these cells can migrate and integrate into the outer nuclear layer (ONL) of the damaged retina, leading to a significant improvement in the recipient's optical function. This recovery increase is shown by the amplitude and slope of the wave by scotopic flash electroretinography [28]. In another study, feline embryonic Müller glial cells were used to transplant allografts. After differentiating these cells in in-vitro conditions and creating RGC precursors, these cells were transplanted to the damaged cat's retina. They observed an improvement in RGC performance, although the integration did not go well. However, researchers could not perform these tests in the long term because these animals are prone to side effects of immunosuppressants [29]. Shams Najafabadi et al. used Opto-mGluR6-engineered mouse retinal pigment epithelium and bone marrow mesenchymal stem cells to differentiate into retinal-specific neurons [30].

Sanges et al. treated hematopoietic stem and progenitor cells (HSPCs) with GSK-3 inhibitor 6-bromoindirubin-3-oxime to pre-activating Wnt signaling. Then, the cells were transplanted into the rd10 mice model, a model for hereditary retinitis pigmentosa. These cells were able to hybridize into the Müller glial cells of the animal. They showed that these Müller-HPS hybrid cells could re-enter the cell cycle, replicate, return to the neural pluripotency or precursor state, and finally differentiate into photoreceptors. Subsequently, electrophysiological rescue and retinal degeneration were observed. These findings suggest that reprogramming of Müller glial cells and subsequent differentiation into the photoreceptors via cellular fusion is a possible strategy for treating RP [31]. Eastlake et al. used Müller glial cells isolated from retinal organoids derived from human iPSC for transplantation in the NMDA-induced rat animal model. After transplantation, these cells were partially able to repair the function of the retinal ganglion neurons. It has been suggested that retinal organoids derived from human iPSC are a suitable origin for Müller glial cells for cell therapy [32]. Chang et al. applied human umbilical cord mesenchymal stem cells to differentiate into RPE-like cells in a transwell system using brain-derived neurotrophic factor and glial-derived neurotrophic factor [33]. Ameri et al. applied CRISPR-Cas9 ribonucleoprotein-mediated gene disruption to reduce the expression level of VEGF-A in RPE cells aimed at treating retinal vascular diseases [34] (Table 1).

Table 1: Efforts on retinal regeneration via Müller glial cells.

Scientists	Year	Origin	Experiment
Das *et al.*	2006	Müller glial cells separated from rat retina	Injection and transplantation to the damaged retina of rates and their ability to differentiate in in-vivo conditions [22].
Bull *et al.*	2008	Müller glial cell line (MIO-M1)	The transplantation of these cells into the ratus glaucoma model and the ability to differentiate in in-vivo conditions and improve integration via erythropoietin [23].
Giannelli *et al.*	2011	Müller glial cells separated from the human retina	Differentiation of these cells to photoreceptor precursors in the culture medium and merging to ratus retinal network [24].
Singhal *et al.*	2012	Human Müller glial stem cells	Differentiation of these cells to RGC precursors in the culture medium and transplanting them to the NMDA rat retina leads to improvement of RGC function [25].
Jayaram *et al.*	2014	Human Müller glial stem cells	Differentiation of these cells to photoreceptor precursors in the culture medium and transplantation to P23H rat model leading to merging to ratus retinal network [28].
Becker *et al.*	2015	feline Müller glial stem cells	Differentiating these cells to RGC precursors in the culture medium and transplanting them to cat retina [29].
Sanges *et al.*	2016	Hematopoietic stem/ progenitor cells - Müller glial stem cells (HSPC-MSC) hybrid	Hybridization of Hematopoietic stem/ progenitor cells - Müller glial stem cells (HSPC-MSC) and in-vitro differentiation to photoreceptors (31).
Eastlake *et al.*	2018	Müller glial cells isolated from hiPSC-derived organoid	Transplantation of Müller glial cells isolated from hiPSC-derived organoid to the NMDA rat retina leading to improvement of RGC function [32].
Ameri *et al.*	2020	RPE cells	CRISPR-Cas9 ribonucleoprotein-mediated gene disruption system were applied to reduce the expression level of VEGF-A in RPE cells aimed at treating retinal vascular diseases [34].
Shams Najafabadi *et al.*	2021	Mouse retinal pigment epithelium and bone marrow mesenchymal stem cells	Opto-mGluR6-engineered mouse retinal pigment epithelium and bone marrow mesenchymal stem cells were used to differentiate into retinal-specific neurons [30].
Chang *et al.*	2022	human umbilical cord mesenchymal stem cells	Human umbilical cord mesenchymal stem cells were significantly differentiated into RPE-like cells in a transwell system using brain-derived neurotrophic factor and glial-derived neurotrophic factor [33].

Critical Signaling Pathways Enrolled in Müller Glial Regeneration

Despite ESCs and iPSCs, Müller glial cells are a suitable internal source for retinal regeneration. Since the process of retinal regeneration is limited in the mammalians, characteristics of internal and external signaling pathways of the biological development of Müller glial cells are critical for the achievement of appropriate treatment methods in the regeneration of the retina [35].

Fausett et al. showed that the Achaete-scute homolog 1a (Ascl1a) protein is upregulated in the early stages of retinal damage. The inhibition of this transcription factor leads to the regeneration of Müller glial cells [36]. Numerous studies have shown that several growth factors are upregulated in retinal detachment in fish, including midkine-a and -b and ciliary neurotrophic factor (CNTF). CNTF is sufficient to stimulate the proliferation of Müller glial cells in the undamaged retina. Various neural precursor genes, i.e., Oligo2, Asc11a, Ngn1, Notch1, and Pax6, have a significant effect in the shift of Müller glial cells to retinal precursors. Müller glial cells have also been shown to normally express genes needed for pluripotency, including Sox2, Klf4, and c-Myc [17]. Therefore, more specific studies sorted by signaling pathways have been conducted on the regeneration of Müller glial cells in the retina.

JAK/STAT and MAPK Signaling Pathway

The first glial reaction occurring in mammals following retinal damage is glycolysis. This phenomenon is characterized by glial fibrillary acidic protein (GFAP) upregulation, limits proliferation, cell hypertrophy, glial scar formation, and decreases neurogenic responses [37]. Following damage to the retina and glycolysis in mammals, the release of several cytokines and mitogens, including EGF, FGF1, FGF2, BDNF, GFAP, IGF-1, and CNTF, has been reported. CNTF can phosphorylate JAK1/2 and STAT3 by binding to their receptors. The phosphorylated STAT3 goes to the nucleus by phosphorylation and dimerization of STAT1 and causes the transcription of glycolysis-related genes such as GFAP [38].

The MAPK pathway is activated by binding EGF and FGF1/2 to tyrosine kinase receptors or CNTF binding to CNTF receptor, followed by phosphorylation of a cascade of executive proteins including Ras, Raf, MEK, and ERK1/2. The phosphorylated ERK1/2 goes to the nucleus and transcribes genes for neuronal proliferation and fate, such as Ccnd1 [39]. Although Müller glia-derived progenitor cells (MGPCs) can produce all types of neurons after injury in fish, this neurogenic ability is limited in avians. However, the high proliferation of these cells has been reported after injury. Todd et al. observed that inhibiting the JAK/STAT signaling pathway by suppressing gp130, JAK2, and STAT3, and injection of CNTF and FGF2 in the NMDA retinal damage model lead to the glial differentiation of Müller glial cells and inhibition of neurogenic potency [40].

Jorstad et al. studied an RNAseq array of Müller glial cells and found that the cells, which could not convert to neurons, expressed high levels of STAT3. Also, they observed that STAT potentially leads Ascl1, which is a basic-helix-loop-helix (bHLH) neural transcription factor, to developmentally inappropriate targets, e.g., Id1, which is a negative regulator of bHLH, and their expression in this model keeps the Müller glial cells in a progenitor-like state and prevents them from differentiating to neurons. Along with the results of non-mammalian vertebrates, it appears that initial activation of the STAT pathway may be necessary to initiate the regeneration process, but stable activation of this pathway may be limited [41].

Wnt Signaling Pathway

As mentioned above, the expression of Ascl1 and Pax6 genes (essential for the proliferation of precursors derived from Müller glial cells) increases following retinal damage. However, the signaling pathways that activate them are still unknown. Ramachandran et al. stated that the Wnt signaling pathway in zebrafish is essential for the proliferation and dedifferentiation of Müller glial cells. On the second day of retinal injury, they observed β-catenin in proliferating Müller glial cells. Also, Dkk inhibits the proliferation of damaged Müller glial cells by inhibiting Wnt signaling pathways [42, 43]. Liu et al. showed that after applying damage to the transgenic mouse model, removing Axin2 in the Wnt signaling pathway reduces programmed death and increases the proliferation and expression of retinal progenitor cell (RPC) markers. Also, they showed that the proliferated cells that express Pax6 migrate from the inner nuclear layer (INL) to the site of damage [44]. Yao et al. showed that ShH10-GFAP-mediated β-catenin gene transfer could simultaneously induce the proliferation of Müller glial cells proliferation with the damage model. Following this transfer, they found that different β-catenin binding sites led to the transcription of the lin28a and lin28b genes, sufficient for the proliferation of Müller glial cells. Lin28, as an RNA-binding protein, inhibits miRlet7 biogenesis and ultimately causes the proliferation of Müller glial cells in adult mammals [45].

Notch Signaling Pathway

The studies in the fish retina have shown that there is upregulation of genes involved in the Notch signaling pathway that leads to proliferation and dedifferentiation of Müller glial cells. Also, it has been shown that in the early stages of the development of the retina, the Notch signaling pathway keeps the progenitor cells in an undifferentiated state. In contrast, in the final stages, they differentiate to glia.

The regulation of the Notch signaling pathway using a small molecule DAPT inhibitor reduces the proliferation of Müller glia-derived progenitor cells. DAPT operates shortly after acute damage when the Müller glial cells begin to dedifferentiate. After the generation of progenitors, the control of the Notch signaling pathway significantly increases the number of new neurons. These findings suggest that the Notch signaling plays two different roles in retinal regeneration: first, the Notch activity enhances the proliferation of Müller glia-derived progenitor cells, while after the proliferation, the Notch activity inhibits the neural differentiation of Müller glial cells [13].

Debbio et al. demonstrated that the Notch signaling pathway activates the Müller glial cells and re-enters them in the cell cycle by controlling the expression p27kip1 [46]. Also, Fgf8, a member of Fgf subclasses that include Fgf17 and Fgf18, is involved in the formation of the retina and the beginning of the differentiation of the progenitor cells. Wan et al. showed that Fgf8 expression in fresh tissues inhibits the Notch signaling pathway and induces the proliferation of Müller glial cells. In contrast, in the older tissue, the increased activity of Notch signaling inhibits the proliferation of Müller glial cells [47]. Also, Sharma et al. showed that TGF-β acts as an anti-proliferating molecule in zebrafish retinal regeneration. As in the early stages of regeneration, TGF-β signaling is a proliferating molecule, but it acts as an anti-proliferator in the final stages of regeneration [48].

Clinical and Sub-clinical Approaches to Generation and Transplantation

One of the issues with transplantation is the injection site in the retina, which is done in two ways: injection in subretinal space or intravitreal injection. Subretinal space is placed between RPE cells and photoreceptors, which injectable materials can communicate directly with the cell membranes. This method is more suitable for treating damaged RPE cells or photoreceptors. Intravitreal injections are prescribed for many high-dose medications and antiviral drugs to prevent infection but may be associated with complications such as retinal detachment or bleeding. In addition, more dose of medications is required due to the presence of membrane barriers between the retina and RPE cells. In contrast, subretinal injection is a direct method for the accurate location with minimal invasion [7, 49]. Preparing Müller glial cells for transplantation is an essential step that can be done in various ways. Many studies have been done on the initial differentiation of these cells in in-vitro conditions. In this method, human Müller glial stem cells (hMSCs) undergo further growth and differentiation factors after isolation. For example, in a study, hMSCs were treated with FGF2 and a Notch signaling inhibitor, DAPT, which eventually led to the generation of RGC progenitors. This phenomenon was confirmed by a decrease in CRALBP (Müller glial cells marker) expression and an increase in BRN3B (RGC marker) expression [25]. In another study, hMSCs underwent differentiation factors, FGF2 and IGF1. These factors differentiated hMSCs into photoreceptor progenitor cells [28]. Instead of using hMSCs, Giannelli et al. used feline embryonic Müller glial cells (fMSCs). After differentiating them to RGC, these cells were significantly transplanted to the feline retina, closer to humans in terms of anatomy and evolution [24]. Kubota et al. differentiated primary Müller glial cells to neurons using aggregated cultures and treatment with valproic acid (VPA), a neural differentiation factor, and PDGF. However, these cells did not express photoreceptor markers in in-vitro. However, they generated photoreceptors by transplanting them to the subretinal space in the retinal niches. This phenomenon indicates the critical role of the cellular niches of Müller glial cells in differentiating photoreceptors [50]. Ex-vivo methods can be a better option compared to in-vivo or in-vitro methods. It has been shown that the intravitreal cell transplant model can be mimicked by isolating the retina of mature rats and culturing it in a serum-free B27 neural culture medium using MIO-M1 Müller glial progenitor cells. This model can effectively determine the rate of cell migration, differentiation, and optimal dose [51]. In another study, hiPSCs were differentiated to adult retinal organoid tissue within 120 days. Then, Müller glial cells

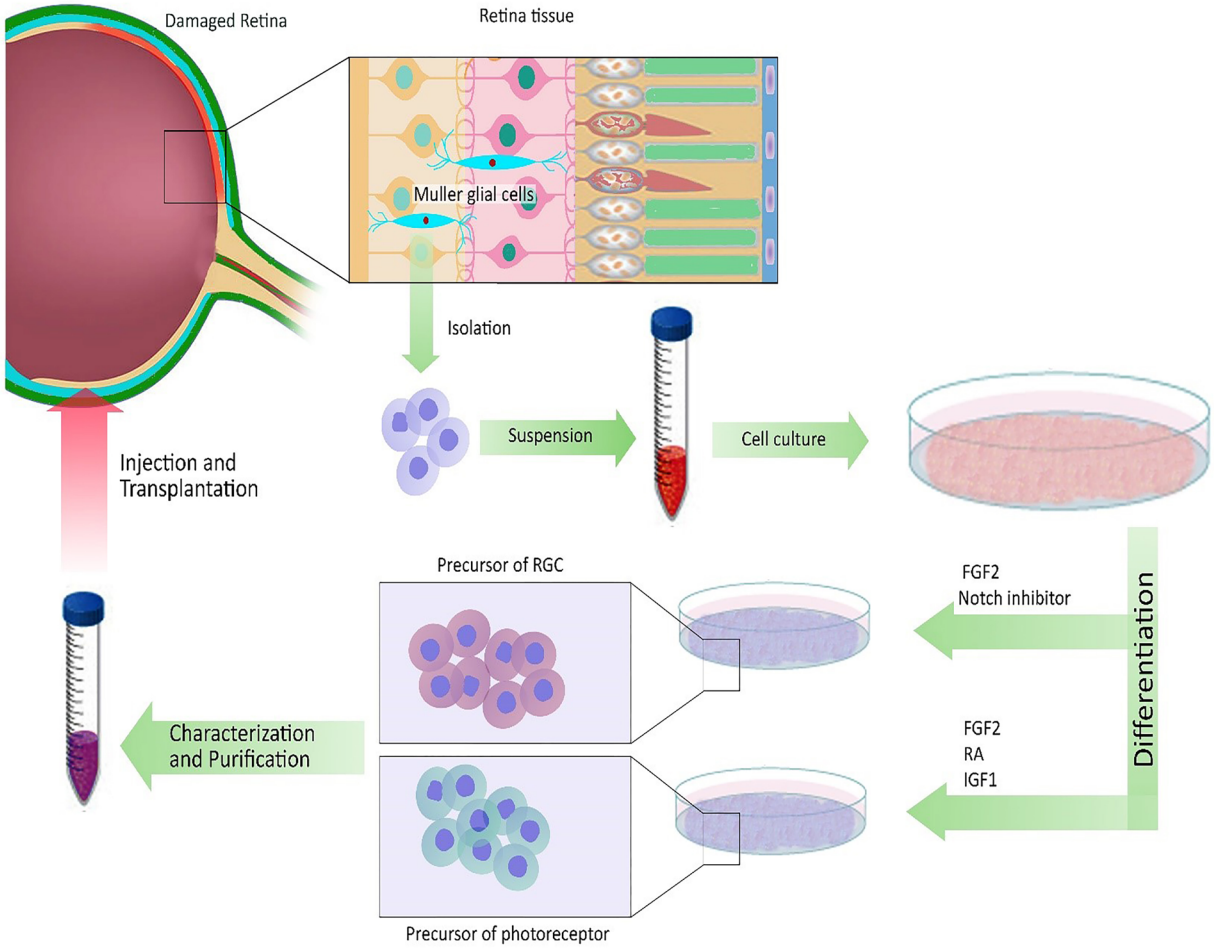

Figure 1: Overview of regeneration of retina by Müller glial cells. Müller glial cells could be isolated from healthy tissues. The suspended cells are cultured in differentiating medium and treated with different cytokines and growth factors to induce differentiation. Then, the differentiated precursor of RGC and photoreceptors are characterized and purified for transplantation.

were isolated, purified, and transplanted into the retina intravitreally, resulting in improvement [32]. As discussed, another way to in-vivo or in-situ activation of Müller glial cells is to use HSPCs, in which the Wnt pathway is activated. In this method, by fusing HSPCs to Müller glial cells and the formation of cellular hybrids, it is possible to differentiate them to photoreceptors within neural growth factors [31] (Fig 1).

Cell Therapy Challenges

Although cell therapy has become a hot topic in regenerative medicine today, there are still many challenges to achieve in the clinical application. As a result, researchers are trying to overcome existing problems by selecting appropriate animal models in the preclinical stages and achieving a reproducible experiment. These problems include safety issues and transplant rejection, tumorigenesis, proper integration with the target tissue, and the viability of transplanted cells.

The term "privilege" refers to the safety in the transplantation of an external antigen that does not trigger an individual's immune system. Among the various tissues, the brain and eyes and its subretinal region have privileged features [52], but this privilege is not an absolute feature. For example, the subretinal allogeneic transplant of RPE cells showed no transplant rejection in patients with RP. In contrast, another study in AMD patients showed transplantation rejection with the discontinuation of immunosuppressive drugs. Even an autologous iPSC transplant can trig-

ger an immune response [53]. It has been shown that the long-term survival of allogeneic transplantation in mice requires immune suppression. However, older patients will not tolerate consecutive doses of immunosuppressive drugs, such as prednisone or cyclosporine [54].

Another critical issue in Müller glial cell therapy is tumorigenesis of impure stem cells. The probability of tumor formation in stem cell transplantation cannot be ignored due to the similarity of stem cells to cancer cells in terms of longevity, resistance to apoptosis, and high replication. For example, human iPSCs are a suitable source for the differentiation of Müller glial cells, which prepare autologous transplantation for retinal regeneration. Despite all benefits, human iPSCs are potent to induction of teratoma due to the pluripotency state of these cells. Therefore, an impurity of human iPSCs in iPSC-derived Müller glial cell product leads to teratoma in the host. Different factors such as the location of injection and in-vitro culture can trigger the tumorigenicity of stem cells. These features occur due to chromosomal abnormality and loss of heterozygosity. Another critical issue is the desired number of cells and injection method to prevent the in-vivo aggregation of the cellular products [55, 56].

Another essential issue in transplantation is the migration of transplanted cells to the growing layer, in which the neurons can grow, expand and establish synaptic connections with neighboring cells. As a result, changes in the environment of the transplant site are essential. For this purpose, researchers are using ABC chondroitinase to break down extracellular matrix chondroitin proteoglycans [57]. Immunosuppressants such as cyclosporine A and prednisolone are also used in several stages to prevent transplant rejection problems [25].

CONCLUSION

Many in-vitro and in-vivo studies have shown the ability of spontaneous regeneration of Müller glial cells in posterior vertebrates and to a lesser extent in other vertebrates. These cells dedifferentiate to precursors during retinal damage and can differentiate into retinal neurons. Therefore, they are an option for regenerating retinal tissue. Also, because these cells ancestrally are the last differentiated cells during retinal neurogenesis and have a common evolutionary origin with retinal neurons, many researchers have conducted extensive research on these cells and their ability to regenerate retinal neurons. In in-vivo conditions, Müller glial cells could be generated from different sources, i.e., straight differentiation of iPSCs, and trans-differentiation of other stem cells. iPSCs are a suitable source of Müller glial cells with various challenges, i.e., teratoma formation. Finally, the generated/isolated Müller glial cells could differentiate into eye-specific retinal cells. After (pre-)differentiation, the cell compound should transplant or inject into the injured location. JAK/STAT, MAPK, Notch, and Wnt signaling pathways are critical in fetal development and in-vitro generation of Müller glial cells. Up-/down-regulation of secondary messenger molecules in these signaling pathways can control the ex-vivo differentiation of Müller glial cells. At the same time, various trans-mediators can induce the expression of signaling pathway molecules through epigenetic features. Numerous preclinical studies have shown that these cells can differentiate from other retinal cells and improve in the early stages of transplantation. To achieve clinical trials further researches on transplant safety, long-term viability, neural merging, and migration are needed.

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