ELSEVIER

Teaser This review reflects on the protective and deleterious response of Müller cells after injury and overviews their use as drug targets for novel retinal therapies based on their neuroprotective and regenerative functions.

Müller cells as a target for retinal therapy

Joke Devoldere[1], Karen Peynshaert[1], Stefaan C. De Smedt[1,2] and Katrien Remaut[1,2]

[1] Ghent Research Group on Nanomedicines, Laboratory of General Biochemistry and Physical Pharmacy, Ghent University, Ottergemsesteenweg 460, 9000 Ghent, Belgium
[2] Cancer Research Institute Ghent (CRIG), Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium

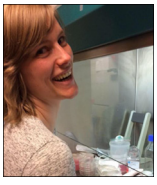
Müller cells are specialized glial cells that span the entire retina from the vitreous cavity to the subretinal space. Their functional diversity and unique radial morphology render them particularly interesting targets for new therapeutic approaches. In this review, we reflect on various possibilities for selective Müller cell targeting and describe how some of their cellular mechanisms can be used for retinal neuroprotection. Intriguingly, cross-species investigation of their properties has revealed that Müller cells also have an essential role in retinal regeneration. Although many questions regarding this subject remain, it is clear that Müller cells have unique characteristics that make them suitable targets for the prevention and treatment of numerous retinal diseases.

Introduction

In 1851, the German anatomist Heinrich Müller discovered a new cell type in the retina, which he described as thin fibers vertically extending throughout the vertebrate retina [1]. Named after their finder, it has now been demonstrated that Müller cells are vital for the proper functioning of vision through the support of retinal neurons. Indeed, Müller cells are one of the most important glial cell types of the retina besides astrocytes and microglia. They stand out because of their unique radial morphology, which spans the entire thickness of the retina, extending from the inner limiting membrane (ILM) to the outer nuclear layer, which allows interactions with all retinal neurons. Moreover, Müller glia are in proximity with the vitreous, blood vessels, and subretinal space and, thus, represent an anatomical and functional connection between these compartments and the retinal neurons. Each Müller cell is described to be coupled to one cone, approximately ten rods, and a varying amount of inner retinal neurons [2]. This columnar structure represents the smallest functional unit needed for the forward transduction of visual information [3,4]. As the core of this column, Müller cells interact with their associated neurons in a symbiotic way and are responsible for their functional, metabolic, and structural support [5].

Corresponding author: Remaut, K. (Katrien.Remaut@ugent.be)

Joke Devoldere obtained her MSc in pharmaceutical sciences at Ghent University (Belgium) in 2013. After graduating, she started her PhD in the Ghent Research Group on Nanomedicines under the supervision of Katrien Remaut and Stefaan De Smedt, and became a doctoral fellow of the Fund for Scientific Research-Flanders (FWO-Vlaanderen) in 2014. Her current research focuses on the nonviral delivery of chemically modified mRNA to the retina: overcoming immunogenicity and the need for multiple administrations.



Karen Peynshaert obtained her MSc in pharmaceutical sciences – drug development at Ghent University (Belgium) in 2012. In 2018, she received her PhD degree in pharmaceutical sciences from Ghent University under the supervision of Katrien Remaut and Stefaan De Smedt. Having obtained a postdoctoral grant from the Research Foundation Flanders, she is continuing her research on drug delivery barriers in the posterior segment of the eye, with a special focus on the inner limiting membrane. Karen has particular interest in the implementation of *ex vivo* models to optimize nonviral retinal drug delivery and has a passion for science communication.

Stefaan C. DeSmedt graduated from Ghent University in 1995 and joined Janssen Research Foundation. He has been a postdoctoral fellow at the universities of Ghent and Utrecht. In 1999, he became a professor in physical pharmacy and biopharmacy at Ghent University, where he founded the Ghent Research Group on Nanomedicines. He served as dean of his Faculty from 2010 till 2014. He is a member of the Board of Directors of Ghent University. Since 2004, Stefaan has served as the European Associate Editor of the *Journal of Controlled Release*; in 2015 he became Editor of *JCR*. His research is at the interface between drug delivery, biophysics, material sciences, and advanced optical imaging. Stefaan has received the Scott Blair Biorheology Award, the Controlled Release Society Young Investigator Award 2006, and the APV Research Award 2010 for Outstanding

https://doi.org/10.1016/j.drudis.2019.01.023

Therefore, Müller cells are ideally positioned to perform a variety of functions to maintain retinal homeostasis and initiate a protective response in case of retinal damage (Fig. 1) [2,3].

Given their unique anatomic and physiological features, researchers have shown increasing interest in the use of Müller cells as a target for novel therapeutic approaches. Indeed, a lot of their characteristics greatly favor their position as a therapeutic target. First, their close contact with the vitreous and subretinal space make them easily accessible to both intravitreally and subretinally injected drugs, which benefits various therapeutic applications. Second, Müller glia are resistant to pathological stimuli, allowing them to survive and remain a relevant target in advanced stages of retinal degenerative diseases, such as retinitis pigmentosa (RP) and age-related macular degeneration (AMD) [6,7]. Third, Müller glia are naturally involved in the synthesis and secretion of neuroprotective cytokines and growth factors, positioning them as an ideal target cell for the expression of these substances. Given that their many processes ensheath almost every retinal cell type, they are able to secrete neurotrophic factors towards the inner and outer retina and, therefore, are perfectly situated for the protection of degenerating neurons in, for example, glaucoma, diabetic retinopathy, or photoreceptor degenerations. Finally, the discovery that Müller cells have an evolutionarily conserved stem cell potential has opened the door for a range of new therapies that aim to induce self-renewal of the mammalian retina [8–10]. In this case, neurons that were lost through trauma or retinal diseases could be regenerated by the retina itself.

Here, we provide a short summary of the most established Müller cell functions and reflect on their unique response to retinal injury. We discuss how some of their natural characteristics can be used for the benefit of retinal therapy and how to avoid adverse effects by selectively targeting Müller cells with gene therapy. Finally, we summarize the current application of Müller cells for retinal neuroprotection and regeneration.

Müller cell functions

Structural, functional, and metabolic support to maintain retinal homeostasis

There is a range of important functions performed by the Müller cells, which nearly all assist in the functional, metabolic, or structural support of retinal neurons (Fig. 1). In the healthy retina, Müller cells participate in the establishment of the blood–retinal barrier (BRB), of which the integrity is essential for the health, functioning, and immune privilege of the retina [11]. The BRB comprises an inner and an outer barrier, the former represented by the tight junctions (TJs) of the inner retinal vasculature and the latter by TJs between the retinal pigment epithelium (RPE). These TJs restrict the movement of fluid and molecules between the blood and the retina and prevent entry of pathogens and other potentially harmful agents into the retinal tissue [12]. Müller cells were shown to enhance this barrier function because their selective ablation in transgenic mice resulted in severe BRB breakdown [13,14]. The precise mechanism by which they reinforce the BRB properties is not completely elucidated but includes the secretion of factors such as pigment epithelium-derived factor (PEDF) and thrombospondin-1, which increase the tightness of the endothelial barrier [15,16].

Besides their contribution to the BRB function, Müller cells are directly responsible for the light conduction in the retina. Given that photoreceptors are found at the outermost layer of the retina (Fig. 1, No 7 and 8), light has to pass all retinal layers before reaching its target. This retinal organization appears counter-intuitive because massive loss of light intensity could be expected because of light scattering by the multiple layers of retinal cells. However, Franze *et al*. discovered that the incident light is collected by Müller cells, which act as living optical fibers that guide the light through the retinal tissue toward the photoreceptors [17]. Thanks to their funnel-shaped endfeet and increasing refractory index along the different retinal layers, light reflection is reduced and a high-intensity signal is transported to the photoreceptors. It has been suggested that Müller cells are also crucially involved in the integrity maintenance of the photoreceptor outer segments, which are essential for proper light detection and visual function and are continuously being renewed [18]. Müller cells are reported to phagocytose cone outer segments [19,20] and contribute to the assembly of new outer segment discs [21,22]. Finally, they also participate in the recycling of the retinal chromophore, by converting cone-specific all-*trans*-retinal to 11-*cis*-retinol. This chromophore is then returned to the cones to restart the visual cycle (Fig. 1a) [23].

The strong connection with their surrounding neurons allows Müller cells to be involved in synaptic activity. During neurotransmission, Müller cells are responsible for the fast clearance of

Research Achievements in Pharmaceutical Sciences. He holds patents on carriers for drug delivery and diagnostics and is also a scientific founder of Memobead Technologies, a spin-off from Ghent University, whose technology is currently under further development by Biocartis (Lausanne and Mechelen) and Mycartis (Gent).

Katrien Remaut graduated as a pharmacist from Ghent University (Belgium) in 2001. She then started research in the Lab of General Biochemistry and Physical Pharmacy under the guidance of Stefaan De Smedt and Jo Demeester. In 2007, she received her PhD in pharmaceutical sciences and continued research as a postdoctoral fellow of the Research Foundation Flanders. In 2009, Katrien joined the Directors Research Lab under guidance of Ian Mattaj at the European Molecular Biology Laboratory for 6 months. She has received several scientific prizes, including the Prize of the Royal Academy of Medicine for Scientific Research in Pharmacy (2008–2011). In 2014, she was appointed tenure track professor at the Lab of General Biochemistry and Physical Pharmacy, Faculty of Pharmaceutical Sciences.

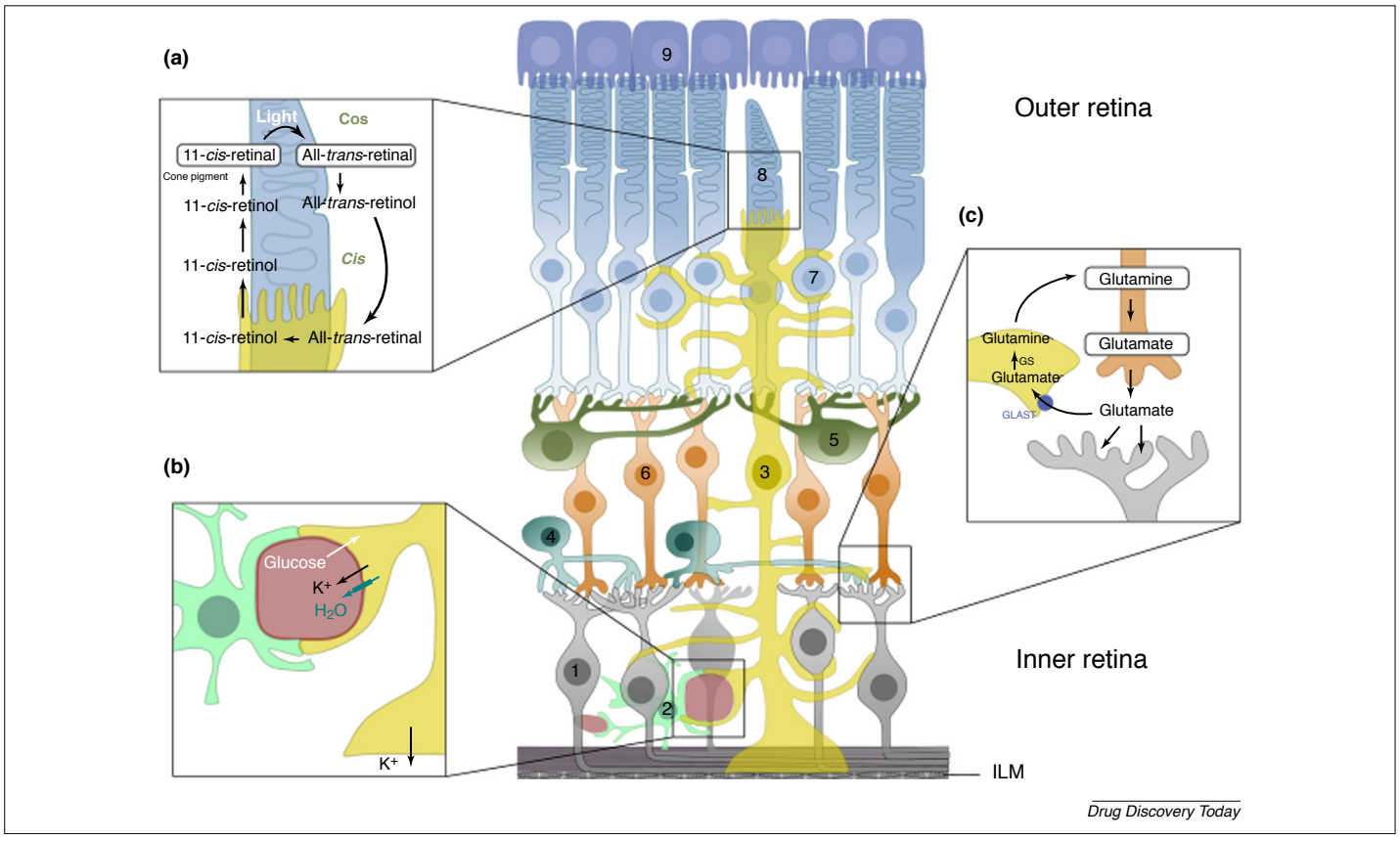


FIGURE 1

Schematic representation of the cellular components of the retina, highlighting some important Müller cell functions. **(a)** Müller cells have a key role in the cone-specific visual cycle: After photolysis, all-*trans* retinal is reduced to all-*trans* retinol in the cone outer segment (COS). All-*trans* retinol is transported to the Müller cells, where it is enzymatically converted to 11-*cis* retinol, which in turn is released for uptake by the cone inner segment (CIS). In the COS, 11-*cis* retinol is then oxidized back to 11-*cis* retinal for pigment regeneration. **(b)** Müller cells maintain retinal homeostasis by mediating K^+ and water transport: neuron-derived K^+ is taken up by Müller cells in the plexiform layers and redistributed into the blood, the vitreous and the subretinal space to avoid prolonged K^+ accumulation. Osmotically coupled to this K^+ transport, water from the inner retinal tissue is removed into the vasculature. **(c)** Müller cells are crucially involved in neurotransmitter recycling: during neurotransmission, Müller cells remove excess glutamate in the synaptic spaces via their glutamate aspartate transporter (GLAST) and enzymatically transform it into the non-neuroactive substance glutamine by means of glutamine synthetase (GS). Glutamine is then transported back to the neurons for the re-synthesis of glutamate. Abbreviation: ILM, inner limiting membrane. Key: 1, ganglion cell; 2, astrocyte; 3, Muller cell; 4, amacrine cell; 5, horizontal cell; 6, bipolar cell; 7, rod; 8, cone; 9, retinal pigment epithelium cell.

glutamate in the synaptic spaces, thereby protecting retinal neurons against excitotoxicity (Fig. 1c) [24,25]. Glutamate in Müller cells is also used for the production of glutathione, a crucial antioxidant that protects the retina against oxidative stress. When oxidative stress occurs, Müller cells rapidly release glutathione, a molecule that prevents neuronal damage by neutralizing reactive oxygen species (ROS) [25,26]. Moreover, they are the primary site of glycogen storage in the retina [5] and, in times of need, they access this storage to provide the neurons with glucose [6,27]. Similar to many other glial cell types, Müller cells mainly rely on anaerobic glycolysis, even when sufficient oxygen is present. This metabolic feature allows them to save oxygen for retinal neurons and renders them less susceptible to anoxia [6]. Furthermore, via anaerobic degradation of their own glucose, Müller cells produce large amounts of lactate, which is preferentially taken up by photoreceptors as an alternative energy source [28,29]. Finally, Müller cells are enriched with numerous ion channels to regulate the electrolytic balance. During neuronal activity, neurons release potassium (K^+) ions in the synaptic spaces, which can be taken up by Müller cells, which in turn redistribute the excess K^+ into the fluid-filled spaces outside the neuroretina (i.e., the blood, vitreous humor, and subretinal space), thereby buffering the K^+ imbalance [30–32]. Besides ion buffering, Müller cells also contribute to retinal homeostasis by regulating water clearance via the specialized aquaporin-4 (AQP4) water channels in their cell membrane (Fig. 1b) [33,34].

Importantly, Müller cells further support the survival of photoreceptors and other retinal neurons by the secretion of neurotrophic factors, growth factors, and cytokines [7]. One of the most studied growth factors released from Müller cells is vascular endothelial growth factor (VEGF). In hypoxic conditions, VEGF can induce the formation of new blood vessels to restore the retinal oxygen supply and, hence, prolong the survival time of retinal cells [7]. However, overexpression of VEGF can have detrimental effects on the retinal vasculature and can contribute to neurodegeneration [35]. In response to trauma, Müller cells have also been shown to produce other neuroprotective factors, such as basic fibroblast growth factor (bFGF) [36], glial cell line-derived neurotrophic factor (GDNF) [37], pigment epithelium-derived factor (PEDF) [16], neurotrophins [38,39], and insulin-like growth factor

1 (IGF-1) [40], as discussed further in this review. Binding of these factors to their cognate receptors, in an autocrine or paracrine manner, activates downstream signaling pathways, which promote cell proliferation, survival, or regeneration [41,42].

Remarkably, most of the current knowledge about Müller cell functions was only obtained over the past 20 years and it is likely that not all the roles of Müller cells have yet been identified. The many features described here are merely a selection, since an extensive overview can be found elsewhere [3].

Müller cell gliosis as a response to retinal imbalance

Besides their physiological roles, Müller cells have the unique capacity to respond to many types of retinal injury and disease in a process often referred to as gliosis [7,43]. Reactive gliosis is a complex response to any pathological alteration, including retinal detachment, photic damage, glaucoma, diabetic retinopathy, and retinal degeneration, considered to protect the retinal tissue from further damage and stimulate its repair. During this process, Müller cells undergo changes on a morphological, biochemical and physiological level, which are determined by the nature and intensity of the insult [43]. Müller cell gliosis is typically characterized by changes in gene and protein expression, and cellular hypertrophy. In particular, upregulation of the intermediate filaments (glial fibrillary acidic protein (GFAP), vimentin, and nestin) is a key feature in the gliotic response. This increase in intermediate filaments results in an increase in Müller cell stiffness, which is likely to prevent mechanical lesions in the retina [44]. Rapid upregulation of GFAP was found in response to retinal detachment [45], hypoxia [46], ischemia [47], and experimental glaucoma [48,49], and was shown to be highly variable and disease dependent [50]. Another prominent gliotic manifestation is the altered expression of glutamine synthetase (GS), a Müller cell-specific enzyme involved in neurotransmitter recycling (Fig. 1c). Although the expression of GS is reduced following the loss of photoreceptors, which normally produce lots of glutamate, GS levels are increased during hepatic retinopathy which requires GS for ammonia detoxification [2]. By contrast, in diabetic retinopathy and optic nerve crush, no difference is observed in the amount of GS [51]. In addition, individual Müller cells can respond to a harmful stimulus in different ways. Fischer *et al.* demonstrated such a heterogeneity between adjacent Müller cells in the chick retina, where ~65% of Müller cells started to proliferate following NMDA-induced damage, whereas ~35% did not [52]. Differences in Müller cell response are also observed between species. Whereas mammalian Müller cells mostly respond to retinal injury by increased GFAP expression, hypertrophy, and proliferation [2], Müller cells of teleost fish (such as zebrafish) undergo a reprogramming event that allows them to regenerate all major retinal cell types and repair retinal damage [53] (see later). Taken together, it is clear that the type of pathological injury and species strongly influence the glial response.

As a rapid response to injury, gliosis is a beneficial process activating different protective mechanisms aiming to buffer extra K^+ levels, take up excess glutamate, and release a variety of factors that protect neurons from degeneration [7]. Furthermore, in response to harmful stimuli, Müller cells are capable of dedifferentiating to cells reminiscent of stem cells and, in some species, redifferentiate to new retinal neurons (see later) [54]. This Müller cell dedifferentiation can be seen as an endeavor to regenerate the injured retina.

Unfortunately, in some severe cases and/or longer periods after injury, this Müller cell response can be excessive and long-lasting, thereby disturbing regular Müller cell functions, including their homeostatic mechanisms and their ability to support retinal neurons. Hence, a status of persistent Müller cell gliosis can be detrimental for neuronal function and survival, and often results in neuronal cell death [7]. For example, long-lasting hypoxia results in the persistent induction of GFAP and reduced levels of GS, thereby impairing glutamate detoxification and contributing to neovascularization (NV) and neuronal degeneration [55]. Chronic retinal ischemia, inflammation, and diabetic retinopathy lead to the downregulation of specialized K^+ channels in Müller cells, which normally release K^+ in the blood, without changes in the K^+ uptake. This increases the osmotic pressure within the Müller cell, causing Müller cell swelling and contributing to retinal edema [56,57].

Finally, persistent gliosis can lead to massive Müller cell proliferation throughout the entire retinal tissue, forming glial scars that fill the spaces left by degenerated neurons, RPE, and blood vessels [58]. This scar-like tissue prevents the renewing of photoreceptor outer segments, inhibits tissue repair, increases retinal stiffness, and exerts tractional forces, all of which can impede normal retinal function [2,59]. The formation of glial scars is thought to be one of the reasons for the limited degree of regeneration in the mammalian retina and can impede the integration of donor cells in the host retina following transplantation [2,60]. Thus, it is clear that gliosis is a complex process that can influence the therapeutic outcome of many retinal treatments, including the strategies discussed in this review. For a more detailed overview of the gliotic response in the diseased retina, readers are referred to Ref. [2].

Taken together, it is evident that Müller cells are an elemental part of the healthy retina and that any imbalance sensed by these cells can influence proper retinal functioning. The existing body of research on the involvement of Müller cells in the healthy and diseased retina illustrates that Müller cells are vital for retinal homeostasis and integrity on the one hand, yet can also accelerate the progress of neuronal degeneration on the other. This plethora of functions makes them an ideal target for therapeutic approaches to slow down, prevent, or even cure various retinal diseases. More specifically, if one could stimulate the neuron-supportive Müller cell functions and avert the destructive mechanisms of gliosis, it might be possible to use Müller cells in the development of new therapeutic strategies, such as neuroprotection or retinal regeneration.

In this review, we mainly address diseases accompanied by photoreceptor degeneration and, therefore, impaired light detection, such as RP [61] and AMD [62]; diseases with retinal ganglion cell (RGC) loss, in which photoreceptors might be able to detect light, but visual information is not transmitted to the brain, such as glaucoma [63] and optic nerve damage [64]; and diabetic retinopathy, in which abnormalities occur in both photoreceptor and RGCs as well as in amacrine cells and bipolar cells [59]. Although neuroprotective and regenerative strategies can be applied for a range of retinal diseases, it is the nature of the disease that will define which cell type requires protection or regeneration and which is the preferred therapeutic strategy.

Selective Müller cell targeting

Although Müller cell type-specific targeting is not a necessity for strategies such as retinal neuroprotection or regeneration, it can increase the success of a therapy and minimize unnecessary uptake of drugs and/or their carriers by other retinal cells. In this way, lower doses could be applied and adverse effects could be prevented. To restrict transgene expression to a specific retinal cell type, gene therapy is an attractive and promising approach. Especially for neuroprotective interventions, Müller cell-specific targeting has an advantage because Müller cells naturally participate in this process by the synthesis and release of neurotrophic factors to their surrounding neurons. Moreover, as ultimate survivors, they can continue to supply neurotrophic factors until the latest phases of the disease, when retinal neurons are lost to degeneration. Restricting expression to Müller cells allows the use of fewer viral particles and reduces the possibility of ectopic transfection and immune responses. In case of regenerative strategies, factors affecting Müller cell reprogramming can be delivered as such to the entire retina. However, targeting Müller cells directly eliminates the risk of adverse effects potentially caused by reaching untargeted cells. An overview of possible Müller cell target strategies is given in Table 1.

Over the past 20 years, myriad therapeutic vectors have been developed with recombinant viruses as leading players for retinal gene delivery [65]. The specificity of viral gene delivery greatly depends on four factors: the injection route; the virus type; the promoter; and the viral envelope or capsid. As such, reports indicate that subretinal injection of viral vectors mainly transduces cells that border the subretinal space, whereas intravitreal injections are preferable for transduction of the inner retina [66]. Nevertheless, because Müller cells span the entire retina, they can be targeted via both injection routes. Another factor influencing the cellular tropism is the choice of the virus type. Whereas the most widely used adenoviral vector, Ad-5, has been shown to transduce Müller cells after both subretinal [67] and intravitreal [68–71] injection, lentiviral vectors show more tendency toward transfection of photoreceptors and RPE rather than of glia [72]. Similarly, most natural adeno-associated viruses (AAVs) have a strong tropism for neurons with minimal transduction of glial cells [73,74]. Regardless, efficient Müller cell transgene expression can be driven by the use of glia-specific promoters, such as the cluster of differentiation 44 (CD44), GFAP, and vimentin promoter. For example, Greenberg *et al.* demonstrated that lentiviral vectors containing one of these three promotors yielded strong eGFP expression in Müller cells after subretinal injection in adult rodents, whereas the use of strong ubiquitous promoters [e.g., the human cytomegalovirus (CMV), human ubiquitin-C and hybrid chicken β-actin promoter] drove transgene expression mainly in the RPE [75]. However, intravitreal injection of lentiviral vectors commonly fails in transducing retinal cells, because these vectors are largely neutralized in the vitreous humor, are relatively instable, and are particularly large [76]. This is one of the reasons why AAVs have emerged as a favored tool for gene delivery to the retina. AAVs have been shown to lack pathogenicity, elicit a mild immune response, and mediate long-term transgene expression in retinal cells [77]. In addition, their small size (25 nm) is expected to facilitate diffusion across retinal barriers and, therefore, enhance delivery of genes into the inner retina following intravitreal injection [78]. For these vectors as well, it is possible to restrict transgene expression to Müller cells using Müller glia-specific promoters [79,80]. For instance, it was shown by Dorrell *et al.* that a GFAP promotor-driven AAV strictly transduced activated Müller glia after intravitreal injection in mice, whereas a ubiquitously CMV enhancer/chicken β-actin (CAG) promoter-driven AAV demonstrated nonspecific expression of GFP, mainly localized to ganglion cells [79]. However, the use of a CAG promoter still evoked stronger GFP expression compared with the use of the GFAP Müller cell-specific promoter. In addition, the research group of Flannery also reported possible cytotoxicity associated with the use of the GFAP promoter, because they noticed strong autofluorescence in fundus images when comparing subretinal injection of an AAV9 carrying a GFAP with that carrying a chicken β-actin (CBA) promoter [81]. Therefore, substantial research has been conducted to develop strategies that modify the viral envelope or capsid for improved targeting. For AAVs, for instance, engineering the capsid based on rational insertion of defined amino acid sequences has shown some success in increasing cell-specific targeting [82]. Nevertheless, this method of rational design requires prior knowledge of which capsid modifications to use for improved targeting, which is often unavailable. Therefore, the development of a technique called 'directed evolution', using mutant AAV capsid libraries in combination with high-throughput screening methods, drastically improved vector design, without the need for mechanistic knowledge of capsid properties. In principle, this approach involves the synthesis of AAVs with random capsid mutations, which are tested *in vitro* or *in vivo* followed by positive selection of their ability to transduce the desired cell type [83]. Using this strategy, Klimczak *et al.* identified a new AAV variant (ShH10) with enhanced and specific intravitreal Müller cell transduction *in vivo* [84]. Specific Müller cell targeting with this vector diminished the loss of vector genomes to neighboring cells unable to express the transgene, while maintaining the use of a strong promoter and, therefore, high transgene expression [81,85,86]. However, the ShH10 vector is substantially more selective in transducing Müller glia in rat retinas compared with mice retinas [87]. A potential explanation for this observation might be the difference in barrier function of the ILM. In mice, a thinner ILM could facilitate penetration and nonspecific transduction of the retina, whereas the thicker ILM in rats favors transduction of Müller cells. Nevertheless, the specificity of the ShH10 vector using a strong ubiquitous promoter remains controversial because other groups found that a Müller glia-specific promoter (e.g., GFAP) is necessary to bring about ShH10-mediated Müller glia specificity [88].

Although viral vectors are currently dominating ocular gene therapy trials, concerns regarding their immune response and high production cost have encouraged the development of nonviral alternatives. Especially lipid- [89,90], polymer- [91,92] and/or protein- [93,94] based nanoparticles (NPs) have been widely investigated for their ability to deliver drugs and genes to the retina. However, their lower transduction efficacy and short-lived gene expression are considered major drawbacks of the nonviral approach [66]. Nevertheless, their use might be beneficial in situations where short-term transgene expression is favorable, for example to boost neuronal survival. As for their viral counterparts, cell type-specific delivery of nonviral vectors can also be influ-

TABLE 1

Targeting expression to Müller cells *in vivo*[a]

Vector type	Promoter	Injection route	Gene	Specificity	Suggested mechanism	Animal model	Suggested application	Refs
Adenovirus								
Ad-5	CMV	IVT	BDNF	Selective transgene expression in MG	Basal end-feet of MG provide large surface for adsorption of viral particles from the vitreous chamber	WT rats	NP of RGCs; NP of PRs (for macular degeneration and RP)	[68,70]
Lentivirus								
HIV	CD44, GFAP, VIM	SR	eGFP	MG transduced with high efficiency, leaky expression in adjacent RPE cells	Proteins localized in MG, of which promoter could therefore confer specific glial expression	Rat model of RP	Retinal NP (for AMD, glaucoma and RP)	[75]
	CMV, ubiquitin	SR	eGFP	Expression restricted to RPE	Promoters strongly active in a range of cells			
	CD44	IVT	eGFP	No eGFP expression	Proteins at vitreal surface that bind and inactivate LV vectors; vectors too large for ILM penetration; polarized receptor profile on MG			
AAV-2	CAG	IVT	eGFP	Nonspecific expression in inner retina	Promoter strongly active in range of cells	Mouse model of subretinal NV; WT rats	NP; LCA and severe forms of RP	[79,84]
	GFAP	IVT	eGFP, NT-4	Expression in MG surrounding retinal blood vessels	Glial-specific promoter upregulated in MG located adjacent to subretinal NV	Mouse model of subretinal NV; Crb1 cKO mice		[79,186]
	CD44	IVT	eGFP	Subset of eGFP-positive RGCs	Natural tropism of AAV2 for RGCs	Rat model of RP	NP	[75]
	HRE-GFAP hybrid	IVT	eGFP	eGFP expression predominantly localized in MG	Combination of MG-specific promoter and several hypoxia-responsive and aerobically silenced elements	Mouse model of OIR	Diabetic retinopathy and AMD (retinal hypoxia)	[80]
Adeno-associated virus								
ShH10	CAG	IVT	eGFP; GDNF	Highly specific MG transduction; subset of RGCs and amacrine cells as well	Directed evolution for selection of IVT transduction of MG; suggested to better bind and transverse ILM and improved binding to EGFR on MG	WT rats; rat model of RP; mouse model with permeable BRB; WT mice	NP; gene therapy; retinal regeneration	[81,84–86,88,187]
	GFAP	IVT	eGFP,	β-catenin, Lin28; Otx2, Crx, Nrl	Highly specific MG expression	Vector selected by directed evolution + MG specific promoter	WT mice, cKO mice; mouse model of congenital blindness	Retinal
				regeneration	[88,171]			
	CD44	IVT	eGFP	Weak eGFP expression primarily in MG, but not specific		Crb1 cKO mice	CRB1 inherited retinal dystrophies	[87]
		SR	eGFP	Weak eGFP expression in MG, RPE, and PRs				
	RLBP1	IVT	eGFP	eGFP expression restricted to MG	Vector selected by directed evolution + MG specific promoter			
		SR	eGFP	eGFP expression restricted to RPE and MG				
CMV	IVT	eGFP; CRB2		Expression only at subapical region of MG		Crb1 cKO mice; Crb2 cKO mice	RP	[87,183]

[a] Abbreviations: CD44, cluster of differentiation 44; cKO, conditional knockout; Crb, Crumbs homolog; Crx, conr-rod homeobox; EGFR, epidermal growth factor receptor; HRE, hypoxia-responsive element; IVT, intravitreal; LCA, Leber's congenital amaurosis; MG; Müller glia; NP, neuroprotection; Nrl, neural retina leucine zipper; NT-4, neutrophin-4; NV, neovascularization; OIR, oxygen-induced retinopathy; Otx2, orthodenticle homeobox2; PR, photoreceptor; RLBP, retinaldehyde-binding protein; SR; subretinal; VIM, vimentin; WT, wild-type.

enced by the delivery method and the use of cell type-specific promoters [95]. In addition, specificity could be increased by rationally modifying the surface of the particle to target receptors on the cell membrane [96]. Although it has been suggested that some NPs, such as human serum albumin particles, are spontaneously taken up by Müller cells after intravitreal injection in rats [97], active Müller cell targeting of nonviral vectors has yet to be investigated.

Müller cells for neuroprotection

Müller cells are endowed with the ability to synthesize and secrete a wealth of neurotrophic factors and, hence, represent a natural target for expression of these proteins. Neurotrophic factors are a family of growth factors that promote the growth, survival, and differentiation of neurons. In many retinal diseases, deprivation of these factors contributes to neuronal cell death, promoting extensive research of neurotrophic factor supplementation therapy. Indeed, administration of neurotrophic factors could halt or slow down neuronal degeneration and help to maintain visual function. Most neurotrophic factors belong to one of three classic families: (i) neurotrophins, including the nerve growth factor (NGF), the brain-derived growth factor (BDNF), neurotrophin 3 (NT-3) and neurotrophin 4 (NT-4) [98]; (2) GDNF family ligands, including GDNF, neurturin, artemin, and persephin; and (3) interleukin-6 (IL-6) family of cytokines, including ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), and IL-6 [99]. Other proteins that have been identified as neurotrophic factors include bFGF, IGF-1, VEGF, transforming growth factor-β (TGF-β), and PEDF [100].

Of all neurotrophic factors, CNTF is the most extensively studied for therapeutic neuroprotection of the retina. Extensive research has shown that CNTF supports the survival of rod photoreceptors in almost all animal models of retinal degeneration [101]. In addition, CNTF has also been shown to significantly protect RGCs and inner retinal neurons from death in several disease models [102–104] as well as to promote axonal regeneration [105]. Despite these promising results, concerns have been raised in using CNTF as a retinal neuroprotective agent. This ambiguity is caused by the reported dose-dependent suppression of retinal function and decreased electroretinogram (ERG) amplitudes in response to delivery of the CNTF protein or transgene [106–109]. In addition to CNTF, the neurotrophin BDNF stands out owing to its powerful neuroprotective effect, particularly on RGCs [110]. Exogenous BDNF protein delivery as well as vector-mediated upregulation of BDNF expression promotes RGC survival after optic nerve axotomy, as described earlier [68,111,112]. Nevertheless, in most studies, neuroprotection of RCGs was only short-term and did not permanently rescue RCGs from cell death [68,113]. This transient survival effect of BDNF was attributed to a downregulation of the BDNF receptor (TrkB) on the RGC cell surface, triggered by prolonged BDNF exposure or the axotomy itself. In response to this observation, Cheng *et al*. successfully tested a combination approach of BDNF protein delivery and virus-mediated TrkB gene therapy, which greatly increased the extent and level of RGC survival [114]. Furthermore, molecules that selectively activate TrkB agonists were reported to enhance RGC survival following acute and chronic models of glaucoma [115,116]. Besides RGCs, BDNF delivery also results in photoreceptor survival [70], which is surprising considering their poor expression of the TrkB receptor. Research suggests that BDNF induces photoreceptor survival indirectly by activating Müller cells in an autocrine or paracrine fashion to secrete other neurotrophic factors, such as CNTF and bFGF, which in turn stimulate photoreceptors [70,117].

Other factors of interest include PEDF and GDNF. Increased expression of the latter has been reported to protect the retina from oxidative stress without altering normal retinal function [118] and to protect photoreceptors and RGCs from death in animal models of retinal degeneration [119–121]. A study investigating the long-term safety of AAV-mediated GDNF expression demonstrated no abnormalities in morphology or function of the retina after 1 year [122]. The neurotrophic factor PEDF was initially discovered to be secreted by RPE cells and was shown to exhibit antiapoptotic, antioxidative, and anti-inflammatory effects [123]. Intraocular injection of PEDF delayed photoreceptor cell degeneration and apoptosis in genetic and light-induced damage retinal models [124–126] and protected RGCs from ischemia-induced cell death [127]. Moreover, human clinical trials have been initiated with PEDF for the treatment of neovascular AMD, because it has proven activity against VEGF [128].

Although it is possible to achieve neurotrophic factor supplementation by delivering recombinant proteins, their short *in vivo* half-lives necessitate frequent ocular injections, which would hamper patient compliance [129–132]. By contrast, local and sustained delivery of neurotrophic factors within the retina by transfection of retinal cells with neurotrophic factor-encoding genes could avoid some of these limitations. Given that, in contrast to neurons, Müller cells are able to survive until the latest stages of retinal degeneration, they serve as ideal candidates for this strategy. In addition, because they have contact with all classes of retinal neuron, Müller cells can be directed to express the desired neurotrophic factors throughout the entire retina. The use of Müller cells as a secretion platform for neurotrophic factors was first investigated two decades ago, when Di Polo *et al*. demonstrated that the delivery of viral vectors containing a BDNF transgene resulted in the secretion of the BDNF protein and subsequent survival of injured RGCs in axotomized rats. More importantly, via intravitreal injection of Ad-5, it was possible to preferentially transduce Müller cells [68]. Using the same strategy, the research group of Di Polo demonstrated that Müller cell-mediated BDNF secretion also markedly increased the survival of photoreceptors following 10 days of light-induced photoreceptor degeneration [70]. However, the use of an adenoviral vector in both studies necessitated co-treatment with an immunosuppressant to prevent Ad-5-mediated inflammation and allow for sustained neurotrophic factor expression [68,70]. In 2001, Liang *et al*. successfully tested intravitreal injection of AAVs encoding CNTF for the protection of photoreceptors in animal models of RP [106]. However, the use of a CMV promotor did not restrict CNTF expression to Müller cells, because RGCs were also transduced [106]. By contrast, intravitreal injection of an AAV vector containing transgenes driven by a GFAP promotor more specifically transduced Müller glia, as demonstrated by Dorrell *et al*. Their GFAP-driven gene delivery of NT-4 resulted in NT-4 production in activated Müller cells and protected photoreceptors from oxidative stress in a mouse model of neovascularization [79]. To further enhance

the therapeutic effect of this approach, Dalkara *et al*. used the ShH10 AAV vector to overexpress GDNF, which resulted in strong, selective transduction of Müller cells after intravitreal injection. Moreover, the authors showed that Müller cell-mediated GDNF expression significantly slowed the rate of retinal degeneration in a rat model of RP. By targeting Müller glia, retinal degeneration was postponed for a longer period compared with previous reports using GDNF delivery without Müller cell targeting [81].

Taken together, the use of growth factors is an emerging strategy for retinal neuroprotection. Given the enormous variation in underlying genetic causes of retinal diseases, a mutation-independent strategy such as neuroprotection, can serve as a universal approach to halt or slow down the loss of retinal cells. This approach is based on secreted proteins and, therefore, neurotrophic gene therapy does not require cell type-specific delivery. Yet, targeting the ultimately surviving Müller cells, rather than losing transgenes to dying retinal neurons, can markedly increase retinal survival. Interestingly, supplementing a combination of different neurotrophic factors could provide even more benefit, as demonstrated by Koeberle *et al*. for the survival of RGCs [133]. In addition, in diseases where the underlying genetic cause is identified, neurotrophic factor delivery could have an added value to gene replacement strategies. An example of this combination is given by Buch e*t al.*, who observed enhanced photoreceptor survival when AAV-mediated GDNF expression was coupled to Prph2 gene replacement in *Prph2*$^{Rd2/Rd2}$ animal models of inherited retinal degeneration [107]. This strategy, where two viral vectors are simultaneously applied, one for gene replacement in the photoreceptors and one for neurotrophic factor secretion in Müller cells, underscores the value of selective Müller cell targeting for neurotrophic factor secretion. Indeed, by transferring the GDNF-secretion task to the Müller cells, neither photoreceptor machinery nor energy would be wasted on neurotrophic factor secretion, thus facilitating photoreceptor regeneration [81].

However, there are challenges to be overcome before vector-mediated neuroprotection can be translated into the clinic. First, each distinct factor should be optimally dosed to achieve optimal protection from degeneration. In this regard, it remains unknown whether data related to the duration of rescue from rodent models can be extrapolated to human diseases. Another important issue relates to the timing of the therapy because it is unclear at what time point during the progression of retinal disease neuroprotection would be the most beneficial. Finally, possible (adverse) effects of sustained, high-level neurotrophic factor expression in the eye should be taken into account, because some factors have shown to cause long-term detrimental effects. The use of viral vectors containing inducible promoters or the use of nonviral poly-(p)DNA or mRNA strategies to induce protein expression during a limited time frame, might however resolve some of these issues.

Müller cells for regeneration

One of the most intriguing Müller cell functions, revealed over the past decade, is their essential role in retinal regeneration. The observation that Müller cells have stem cell properties has positioned them as a primary target for endogenous retinal repair [134].

Unlike most cell types of the human body, differentiated retinal neurons do not have the ability to re-enter the cell cycle and divide. When traumatic injuries and diseases result in retinal cell death, lost neurons in the damaged area are not spontaneously replaced and apoptosis ultimately leads to vision loss. However, this is not the case for all species. Teleost fish, such as zebrafish, have the remarkable innate ability to regenerate retinal tissue and restore impaired sight [135–137]. The source of this regeneration was discovered in early studies, using goldfish, as a group of proliferating cells migrating from the inner (INL) to the outer nuclear layer (ONL), where they continue to divide and generate new rod photoreceptors [138,139]. Initially these clusters were assumed to originate from rod precursors [140,141], but the observation that INL cell proliferation preceded regeneration and that Müller nuclei, which reside in the INL, also proliferated and migrated into the empty spaces of lost photoreceptors following injury, suggested that Müller glia were an alternative source of regenerative neuronal progenitors [142]. Finally, several studies using transgenic zebrafish in which the fate of GFP-tagged Müller cells was followed shortly after retinal injury, were able to confirm Müller glia as the source of these progenitors [143–145]. Using a conditional expression system that allowed permanent labeling of Müller glia-derived progenitors, Ramachandran *et al*. demonstrated that these progenitors are responsible for regenerating all retinal cell types and that these cells are stably integrated into the retinal structure [146].

Despite considerable similarity between the mammal and the zebrafish retina, mammalian Müller glia do not respond to injury by means of dedifferentiation. Instead, they experience reactive gliosis, which is often associated with cell proliferation and scar formation (see earlier), failing to initiate regeneration [7]. Nevertheless, this response to injury together with their capacity to upregulate genes associated with retinal stem cells indicate that mammalian Müller cells might be stimulated to adopt a stem cell-like state and generate retinal neurons under appropriate circumstances. Rodent as well as human Müller cells have both been shown to generate both glial cells and neurons *in vitro* [147–150]. How mammalian Müller cells lost the capability to use this neurogenic potential after injury *in vivo* remains a pressing question. However, if one could unlock this restricted regenerative potential of mammalian Müller glia and mimic the self-healing capacity of their zebrafish counterparts, it might be possible to restore human retina after injury and regain vision in retinal degeneration diseases. In addition, the use of endogenous Müller cells to promote neuronal regeneration circumvents many risks associated with exogenous cell transplantation and prosthetic devices, such as immune rejection, potential ethical objections, and tumor formation [151]. To this end, several research groups have used the zebrafish as a model to gain a better understanding of the factors that control retinal regeneration.

In zebrafish, Müller glia reprogramming has been shown to be responsible for the regeneration of the retina in various cases of retinal damage, such as light exposure [145], chemicals [152] and mechanical injury [143]. Following retinal injury, Müller glia dedifferentiate, migrate towards the ONL, and undergo a single, asymmetric self-renewing division that preserves the Müller glia cell on the one hand and produces a multipotent progenitor cell on the other hand [153]. This progenitor cell proliferates to form a cluster of neural progenitors, which migrate along the radial process of the daughter Müller cell to the damaged retinal layer,

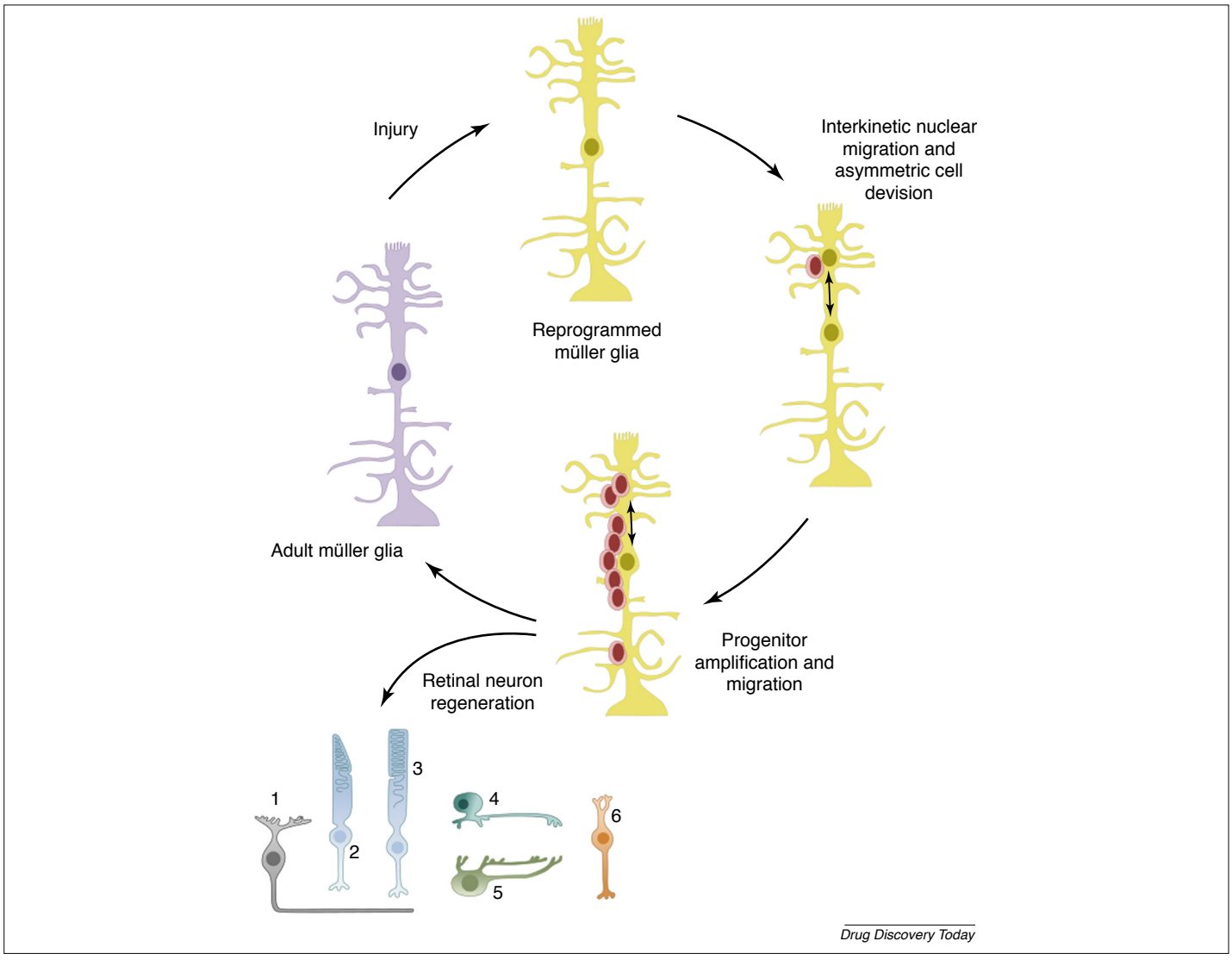


FIGURE 2

Retinal regeneration in zebrafish. Adult Müller glia (purple) respond to retinal injury with a gliotic response, accompanied by a reprogramming event in which they adopt retinal stem cell properties (yellow). The nuclei of the reprogrammed Müller glia migrate from the inner nuclear layer (INL) to the outer nuclear layer (ONL), where they divide asymmetrically and subsequently return to the INL, a process called interkinetic nuclear migration. This asymmetric division results in the formation of a multipotent progenitor, which amplifies to create a small population of progenitors capable of the regeneration of all major retinal neuron types. Key: 1, ganglion cell; 2, rod; 3, cone; 4, amacrine cell; 5, horizontal cell; 6, bipolar cell. Modified from [53,54].

where they withdraw from the cell cycle and can differentiate into all major retinal cell types [54,134] (Fig. 2).

Although further work is needed to shed more light on the molecular mechanisms driving Müller glia reprogramming in zebrafish, several proteins and signaling pathways involved in this process have been identified (for an excellent overview of the factors affecting Müller glial cell reprogramming and proliferation, readers are referred to Ref. [54]). Further studies now focus on how these proteins can be used to enable the mammalian retina to regenerate *in vivo* (Table 2). However, the first studies tried to stimulate the proliferative and neurogenic properties of mammalian Müller cells by simply inducing retinal damage. In 2004, Ooto and colleagues demonstrated that NMDA neurotoxicity of RGCs in the adult rat retina induced a small amount of Müller glia to proliferate and produce new cells with markers of early bipolar and rod photoreceptor cell differentiation [154]. Similarly, *N*-methyl-*N*-nitrosourea (MNU)-induced photoreceptor degeneration was reported to initiate Müller glia proliferation in rodents, associated with rare events of rhodopsin expression, indicating photoreceptor regeneration [155]. However, this concept was recently challenged by Kugler *et al.*, who demonstrated that NMDA-induced excitotoxic damage of the rodent retina did not induce Müller cell dedifferentiation [156]. Similar observations were reported by another research group using light exposure to induce neuronal damage without noticeable Müller cell proliferation [157]. These authors found that bromodeoxyuridine (BrdU) was colocalized with DNA Ligase IV, a marker for DNA repair. This suggests that BrdU, a nucleoside commonly used for the detection of proliferation, is incorporated in cells because of ongoing DNA repair, without the occurrence of cellular proliferation. Nevertheless, NMDA- or MNU-induced damage combined with the increased levels of key signaling factors, such as sonic hedgehog

TABLE 2

Müller cell regeneration in the mammalian retina *in vivo*[a]

Animal	Procedure	Effective factor (s)	Effect?	Newly generated cells	Refs
With injury					
Adult WT rats	IVT injection of NMDA followed by three injections of growth factors every other day, 2 days after injury	RA	NMDA caused cell death of RGCs and reduced thickness of IPL; MG proliferation 2 days post injury; dedifferentiation of a few MG; RA treatment promoted number of regenerated bipolar cells	Bipolar cells: marker: PKC, NSE. Rod PRs: marker: rhodopsin, recoverin	[154]
Wnt/β-catenin reporter mice	IVT injection of NMDA and Wnta3	Wnta3 (Wnt agonist)	Cell death observed in INL and GCL; Wnt3 treatment markedly increased MG proliferation; inhibition of Wnt signaling by Dkk1 prevented neuronal regeneration after injury	Treatment with RA promoted differentiation into rhodopsin-positive PRs (*ex vivo*)	[159]
Adult WT rat	I.p. injection of MNU followed by daily IVT injection of Shh (7 consecutive days)	Shh	MNU induced PR degeneration; treatment with Shh markedly increased proliferation of MG-derived progenitors; MG-derived progeny preferentially differentiated in rod-like cells	Rod PRs: Marker: rhodopsin	[158]
GAD67-GFP and Grm6-GFP transgenic mice	Intraocular NMDA injection followed by single injection of growth factors 2 days after injury	FGF1; EGF; FGF1 + insulin	NMDA caused cell death of RGCs and amacrine cells; MG re-entered cell cycle after injury and upregulated progenitor markers (Pax6, Notc, and DLL1); MG subset differentiated into amacrine cells	Amacrine cells: markers: Calretinin, NeuN, Prox1, and GAD67	[162]
Adult WT rat; newborn Z/EG mice	I.p. injection of MNU	N/A	MNU specifically damaged PRs; MG underwent gliosis and proliferation; MG subset differentiated in rod PRs and expressed synaptophysin, indicating possible synapse formation; transplantation into damaged retina led to MG migration and rhodopsin expression	Rod PRs: markers: rhodopsin, synaptophysin	[155]
Transgenic adult mice with increased sensitivity to Wnt through removal of negative regulator Axin2	Laser induced injury with two laser burns per eye	Wnt	After injury, MG proliferated more, but amount of proliferating MG was small; MG adopted expression patterns of retinal progenitor cells (PAX6); some MG expressed rod PR marker, rhodopsin	Rod PRs: marker: rhodopsin	[188]
Transgenic adult and young mice overexpressing Ascl1 in presence of tamoxifen	I.p. injections of tamoxifen (5 consecutive days) induced expression of Aslc1, followed by IVT injection of NMDA or continuous exposure to 10 000 lux light for 8	Ascl1	NMDA induced death of amacrine and RGCs; Ascl1 overexpression alone did not stimulate neurogenesis in MG; after injury, Ascl1 promoted dedifferentiation of MG (loss of Sox9) and initiated neurogenic response (proliferation, Otx2 expression), mature markers of neural/PRs were not expressed in adult mice; after injury, Ascl1 gave MG potential to regenerate neurons (amacrine, bipolar and PRs) in young mice	Amacrine cells: markers: HuC/D, Pax6 Bipolar cells: markers: Otx2, Cabp5 Rod PRs: markers: Otx2, recoverin	[160]
Transgenic adult mice overexpressing Ascl1 in presence of tamoxifen	I.p. injections of tamoxifen (5 consecutive days) induced expression of Ascl1, followed by IVT injection of NMDA (2 days later) and TSA (4 days later)	Ascl1 + TSA	NMDA led to loss of RGCs and reduction in IPL thickness; administration of tamoxifen + NMDA promoted dedifferentiation of MG (loss of Sox9) and initiated neurogenic response (Otx2 expression); MG-derived progeny preferentially differentiated in bipolar cells; MG-derived neurons synapsed with host retinal neurons and responded to light	Bipolar cells: marker: Cabp5 Amacrine cells: markers: HUC/D, Pax6	[161]
Without injury					
Adult WT mice	SR injection of glutamate or its analog α-AA	Glutamate; α-AA	Subtoxic levels of glutamate and α-AA directly stimulated MG to re-enter cell cycle and induced neurogenesis; single injection of α-AA upregulated progenitor markers (Chx10 and nestin); MG subset differentiated into rod PRs	Rod PRs: markers: rhodopsin and recoverin	[169]

TABLE 2 (*Continued*)

Animal	Procedure	Effective factor (s)	Effect?	Newly generated cells	Refs
Rat model of rod PR degeneration (S334ter rats)	Two-step method (IVT): Wnt2b and Jag1 for Müller cell activation; Shh and DAPT to promote differentiation	Wnt2b (Wnt agonist); Jag1 (Notch agonist); Shh; DAPT (Notch antagonist)	MG activated in response to Notch and Wnt signaling stimulation; rare population of activated MG differentiated along rod PR lineage; differentiation correlated with improvement in perception of light	Rod PRs: marker: Opsin	[170]
Adult WT; Lin28aflox/flox; Lin28bflox/flox mice	Shh10-GFAP-mediated gene transfer of WT β-catenin, Lin28a or Lin28b	□-catenin; Lin28	β-catenin gene transfer activated MG proliferation in injured retina; β-catenin gene transfer upregulated RNA levels of both Lin28a and Lin28b and downregulated let-7 miRNA levels; Lin28 regulated MG proliferation in both injured and uninjured retinas; a few MG differentiated to amacrine cells in all three treatment groups (β-catenin, Lin28a, or Lin28b gene transfer)	Amacrine cells: markers: PAX6, Syntaxin1, NeuN	[88]
$Gnat1^{rd17}Gnat2^{cpfl3}$ double-mutant mice, model of congenital blindness (lack PR-mediated light responses)	Two-step method (IVT); Shh10-GFAP-mediated gene transfer of β-catenin for Müller cell proliferation; Otx2, Crx and Nrl for PR differentiation	β-catenin; Otx2; Crx; Nrl	β-catenin gene transfer activated MG proliferation (mostly one cell division); after second injection, asymmetric cell division occurred with production of rod photoreceptor and daughter MG; newly formed rod PRs integrated into retinal circuits and rescued light response in mouse model of congenital blindness, from retina to visual cortex	Rod PRs: markers: rhodopsin, peripherin-2, GNAT1, recoverin, ribeye	[171]

[a] Abbreviations: α-AA, α-aminoadipate; ANT, Ascl1 + NMDA + Tamoxifen; Cabp5; Calcium-binding protein 5; Crx, cone-rod homeobox; Dkk1, Dickkopf WNT signaling pathway inhibitor 1; DLL1, delta-like canonical Notch ligand 1; GAD67, glutamate decarboxylase 1; GCL, ganglion cell layer; GNAT1, guanine nucleotide binding protein alpha transducing 1; HuC/D, ELAV-like RNA binding protein 3/4; i.p., intraperitoneal; IPL, inner plexiform layer; IVT, intravitreal; MG, Müller glia; NeuN, neuronal nuclei (hexaribonucleotide binding protein-3); NMU, *N*-methyl-*N*-nitrosourea; Nrl, neural retina leucine zipper; NSE, neuron-specific enolase; Otx2, orthodenticle homeobox 2; PAX6, paired box 6; PKC, protein kinase C; PR, photoreceptor cell; Prox1, prospero homeobox 1; RA, retinoic acid; RD, retinal degeneration; RGC, retinal ganglion cell; Shh, Sonic hedgehog; SR, subretinal; TSA, trichostatin-A; Wnt, Wingless/Integrated; WT, wild-type.

(Shh) [158], Wingless/Integrated (Wnt) [159], and achaete-scute homolog 1 (Ascl1) [160,161], or combined with growth factor treatment [162] was indicated to provide Müller glia with the ability to proliferate and regenerate neurons in the rodent retina. Indeed, the intraocular injection of NMDA followed by a single injection of epidermal growth factor (EGF) was shown to be a promising method for Müller glia regeneration [162]. The EGF receptor expression in Müller glia gradually declines as the retina matures, but appears to be upregulated after damage. Stimulation of this receptor by EGF treatment after injury promoted Müller cell proliferation and activated the expression of progenitor genes, similar to what has been reported for retinal regeneration in non-mammalian vertebrates. This was in contrast to injury or EGF injection alone, which failed to induce a proliferative response in the mouse retina [162]. A factor that was not upregulated after NMDA-induced damage in this study but has previously been shown to be required for reprogramming and proliferation in zebrafish, is the proneural transcription Ascl1 [163–165]. In zebrafish, Ascl1 is upregulated in proliferating Müller glia within 6 h of retinal injury [163,164] and its inhibition limits Müller glia dedifferentiation [164]. One mechanism by which Ascl1 affects retinal regeneration was reported by Ramachandran *et al.*, who demonstrated that Ascl1 is necessary for the expression of Lin28, a pluripotency mRNA-binding protein, highly expressed in embryonic stem cells. The authors demonstrated that Ascl1-dependent induction of Lin-28 supported Müller glia dedifferentiation partially by lowering let-7 miRNA levels, therefore removing repression of mRNAs crucial for Müller glia dedifferentiation [163]. The lack of Ascl1 upregulation in the mammalian retina following injury, led to the hypothesis that the Ascl1/Lin28/Let7 pathway might dictate the differences in the regeneration potential between mammalian Müller glia and Müller glia of other species. Indeed, Pollak *et al*. showed that virus-mediated overexpression of Ascl1 activated a neurogenic program in injured mouse Müller glia cultures and postnatal retinal explants, and stimulated generation of cells expressing retinal subtype-specific markers and displaying neuron-like physiological responses. Although Ascl1 drove expression of early markers of many retinal neurons, later markers were more restricted to bipolar neurons [166]. The same research group subsequently demonstrated that forced expression of Ascl1 in mouse Müller glia *in vivo* promoted proliferation and provided amacrine cells, bipolar cells, and photoreceptors after retinal injury [160]. However, this regeneration potential was shown to depend on the age of the animal [160,167]. The reprogramming of Müller glia by Ascl1 involves remodeling of the chromatin at the promoters of progenitor genes from a repressive to an active configuration [166]. Given that the accessibility of chromatin is limited in adults, mature mouse Müller glia lose their neurogenic capacity, despite Ascl1 overexpression [160]. Nevertheless, the research group of Reh recently reported that Ascl1 overexpression coupled with a histone deacetylase inhibitor treatment can circumvent this limitation and enables adult mice to generate inner retinal neurons from Müller glia after retinal injury [161]. In addition, these regenerated neurons express markers of inner retinal neurons, integrate into the neuronal circuit, and were shown to respond to light. This indicates that, although neuro-

genesis is still limited, the newly formed neurons are functional and could restore vision.

Besides activating lin28 expression, Ascl1 also contributes to Müller cell reprogramming by regulating Wnt signaling and its downstream target β-catenin, another major pathway in the regenerative response in zebrafish [168]. After injury of the zebrafish retina, this pathway was shown to be active in Müller cell-derived progenitors and to control their proliferation [165]. It was found that Asc1 suppresses the expression of Dkk, a Wnt signaling inhibitor, thereby increasing expression of Wnt genes, whereas Wnt in turn induces Asc1 expression in activated Müller cells [165]. Also in the murine retina, Osakada *et al*. previously provided evidence that Wnt/β-catenin signaling takes part in the small fraction of Müller cell proliferation that occurs following injury, which can further be enhanced by addition of Wnt receptor agonists [159].

While injuring the mammalian retina, whether or not in combination with growth or signaling factors, stimulates Müller glia proliferation and has led to limited neurogenesis, this injury inevitably causes cell death, which is unfavorable and counterproductive for regeneration. Therefore, a strategy free of injury, that would not require further damage to an affected retina, would be preferable. Remarkably, subretinal delivery of subtoxic doses of glutamate also stimulates adult murine Müller glia to re-enter the cell cycle and induce a rare population to regenerate without causing retinal damage [169]. Similarly, Del Debbio *et al*. demonstrated that stimulation of Wnt and Notch signaling in a rat model of rod photoreceptor degeneration led to the activation of Müller glia without neurotoxin-mediated retinal injury. Moreover, a small subset of activated Müller glia was observed to express rod photoreceptor-specific markers in degenerated outer nuclear layers, and a significant temporal improvement in light perception was demonstrated [170]. Although there was a strong correlation between the improvement in light perception and the number of activated Müller glia expressing opsin, the authors were unable to rule out that this functional improvement was (partly) because of the survival of host photoreceptors. More direct evidence is necessary to confirm the functionality of these cells. In this study, a two-step reprogramming method was used to first activate Müller glia by intravitreal injection of Wntb2 (activating Wnt) and Jag1 (activating Notch signaling) followed by injection of Shh and DAPT (inhibiting Notch) to promote differentiation along the rod photoreceptor line. recently, a similar approach was published by the group of Bo Chen, which provided evidence that, without injury, selective Müller glia gene transfer of β-catenin (using the previously discussed ShH10 AAV-variant) activates Wnt signaling and a single round of Müller cell division in a first step of their reprogramming method. Two weeks later, these activated Müller glia could subsequently be reprogrammed to generate rod photoreceptors by a second gene transfer of transcription factors essential for rod cell fate determination, namely *Crx*, *Otx2* and Nrl. To prove that the new rod photoreceptors were created from Müller glia, a tdTomato gene driven by a rhodopsin promoter was included in the first injection to label all transduced Müller glia. Finally, these Müller glia derived rods were shown to integrate into retinal circuits and restore visual responses in a mouse model of congenital blindness throughout the visual pathway (from the retina to the part of the brain that receives visual signals) [171].

Several important considerations must be taken into account when studying the neurogenic potential of Müller glia. So far, most research in this field has made use of BrdU injection and cell type-specific labeling for Müller cell lineage tracing. In these studies, the presence of BrdU+ in the neurons was generally seen as evidence that they derived from dedifferentiated Müller glia. Given that BrdU can be incorporated into newly synthesized DNA of replicating cells, BrdU+ Müller glia in these studies clearly demonstrate that retinal injury and/or signaling pathway alterations stimulate Müller glia to actively replicate their DNA and enter the cell cycle [172]. However, there is no direct evidence that BrdU+ neurons are generated from Müller glia, because BrdU can also be incorporated in cells repairing their DNA, as stated above [157,173]. In addition, also the use of promoter-inducible fluorescent labels (such as GFP, β-gal, and tdTomato) can lead to confounding results because of the process of 'material transfer', which was demonstrated in recent reports by four different research groups studying photoreceptor transplantation [174–176]. Using various techniques, the authors demonstrate that following transplantation most of labeled cells detected in the host retina do not represent integrated photoreceptors cells, but are in fact host cells that have exchanged RNA and/or proteins with the transplanted cells. Therefore, the results of studies transplanting Müller cell-derived neurons into partially degenerated retinas should also be interpreted with caution, because material transfer could lead to assumed retinal integration [148,150,177,178]. The fundamental mechanism of this process is currently unknown, but does not appear to be mediated by sustained donor-host cell contact or release of free protein or nucleic acid in the extracellular space [174]. By contrast, the material might be transferred by immune cells or via vesicle release, which should be investigated in future studies [179].

Although many lessons have been learned by studying the zebrafish model, the mechanisms underlying retinal regeneration are complex and many questions remain. Understanding why zebrafish Müller glia effectively initiate retinal regeneration and why their mammalian counterparts do not, will be of crucial importance. To identify factors that can unlock the mammalian regeneration-stimulating potential, gene expression in Müller cells of healthy and injured zebrafish could be compared with the transcriptome of mammalian Müller cells. Also, although Müller cells appear to obtain the first activating signals from injured cells, it cannot yet be excluded that other cells, such as microglia, might also influence the reprogramming process [8]. Therefore, examining the contribution of these cells to retinal regeneration and its capacity to activate Müller glia to a state of neuronal differentiation will be crucial for advancing mammalian regeneration.

Inducing the expression of transcription factors, which are important during cell fate determination, make it possible to direct the cell fate of Müller glia-derived progenitors and, therefore, replace the lost cells. At present, most research is focusing on two key factors with an integral role in the regenerative response in zebrafish, namely Ascl1 and Wnt. Induction or overexpression of these reprogramming factors and their downstream signaling pathways showed promising results for sight restoration in two recently published studies [161,171]. However, the study by Yao *et al*. stimulating Wnt signaling highlights important advantages over the studies addressing Ascl1 [171]. Indeed, gene transfer of

β-catenin alone is sufficient to activate the Wnt pathway and induce Müller glia proliferation in adult mice, whereas overexpression of Ascl1 requires the presence of injury to yield significant Müller cell proliferation. In addition, the authors demonstrated that stimulation of Wnt/β-catenin signaling combined with ectopic expression of photoreceptor transcription factors (Crx, Otx2, and Nrl) led to the generation of new rod photoreceptors. This in contrast to Ascl1-mediated induction of Müller glia dedifferentiation, which mainly results in the production of inner retinal neurons, such as bipolar and amacrine cells. However, it is currently unknown whether the same combination of photoreceptor fate-guiding transcription factors could also induce rod photoreceptors production, when coupled with Ascl1 stimulation. A more detailed understanding of the molecular mechanisms that drive retinal precursors to a particular cell type will be essential for the development of efficient therapeutic strategies. So far, for example, more is known about the commitment towards rod photoreceptors than about cone photoreceptor fate. Nevertheless, given their significance in human vision, identifying factors that will direct cone photoreceptor fate will also be of crucial importance.

Finally, because most studies of the regenerative potential of Müller cells have been performed in combination with acute injury, the question remains whether this potential will be maintained in chronic diseases, such as RP and AMD, in which Müller cell responses might differ. Given that the gliotic response of Müller cells strongly depends on the type and duration of the disease (see earlier), it is likely that chronic injury will also influence the regeneration process. Indeed, Osakada *et al*. reported that the progression of the disease might restrict the regenerative capacity of the retina, because BrdU incorporation in Müller cells appears to be lost as degeneration proceeds [159]. The study by Yao *et al*. offers hope for Müller cell-dependent regeneration therapies in mammals, but the use of this strategy in more advanced diseases in which larger numbers of cells are lost or which lack proper synaptic connection, still needs to be analyzed. The ultimate aim is to rescue vision in the latest stages of degeneration. Precise analysis of how new neurons rewire themselves into the retina after Müller cell regeneration, especially in the absence of host photoreceptor cell, will be necessary to generate a healthy new circuitry and visual function.

Concluding remarks

The Müller cell is one of the most multifunctional cells in the retina, and is attracting growing research interest because of its myriad functions related to the healthy and diseased retina. As described in this review, Müller cells strongly monitor retinal homeostasis and are vital for the proper functioning of the retina. However, in response to retinal imbalance, activated Müller cells can also contribute to retinal degeneration and impede regenerative processes by the formation of glial scars. Although the past couple of years have witnessed an enormous growth in our knowledge regarding the functional roles of Müller cells and their gliotic response, their use as targets for new therapeutic approaches is a nascent field. In particular, gene therapy could be an interesting method to selectively address Müller cells and modulate some of their cellular mechanisms to our advantage for medical treatment. In this review, we focused on two therapeutic fields in which Müller cells are becoming the central subject of many studies. First, their unique morphology, which allows them to interact with all neuronal cell types, renders them ideally located for interventions that aim to inhibit neuronal cell death, a strategy called neuroprotection. Second, their latent stem cell potential posits Müller cells as an excellent target for regenerative therapies that aim to stimulate endogenous replacement of injured retinal neurons.

However, the use of Müller cells for medical treatment is not restricted to these two applications. Their active participation in innumerous retinal processes makes them a possible intervention point for multiple ocular therapies. For instance, some strategies are investigating the suppression of VEGF secretion specifically secreted by Müller cells to control neovascularization in diseases such as retinopathy of prematurity, AMD and diabetic retinopathy [180–182]. In addition, Müller glial cells are also of interest for gene replacement therapy, because some recessive genetic mutations of genes expressed in Müller cells, such as *CRALBP* and *CRB1*, have been reported to cause retinal diseases [183–185]. Overall, it is clear that Müller cells are an intriguing retinal cell type, with high versatility for therapeutic interventions. Finally, as our knowledge of the functional roles of Müller cells continues to increase, together with improved targeting and the development of more potent and controllable viral- and nonviral delivery systems, Müller cells are expected to take a prominent place in the development of future therapeutic approaches to treat retinal diseases [7].

Acknowledgments

J.D. is a doctoral fellow and K.P. a postdoctoral fellow of the Research Foundation-Flanders, Belgium (FWO-Vlaanderen). The support of this institution is gratefully acknowledged. The authors further wish to thank An-Katrien Minnaert for her literature insights.

References

1 Müller, H. (1851) Zur Histologie der Netzhaut. *Zeitschr. Wiss. Zool.* 3, 234–237
2 Bringmann, A. *et al.* (2006) Muller cells in the healthy and diseased retina. *Prog. Retin. Eye Res.* 25, 397–424
3 Reichenbach, A. and Bringmann, A. (2013) New functions of Muller cells. *Glia* 61, 651–678
4 Reichenbach, A. *et al.* (1995) Muller glial cells of the tree shrew retina. *J. Comp. Neurol.* 360, 257–270
5 Vecino, E. *et al.* (2016) Glia-neuron interactions in the mammalian retina. *Prog. Retin. Eye Res.* 51, 1–40
6 Winkler, B.S. *et al.* (2000) Energy metabolism in human retinal Muller cells. *Invest. Ophthalmol. Vis. Sci.* 41, 3183–3190
7 Bringmann, A. *et al.* (2009) Cellular signaling and factors involved in Muller cell gliosis: Neuroprotective and detrimental effects. *Prog. Retin. Eye Res.* 28, 423–451
8 Xia, X. and Ahmad, I. (2016) Unlocking the neurogenic potential of mammalian Muller glia. *Int. J. Stem Cells* 9, 169–175
9 Chohan, A. *et al.* (2017) Muller stem cell dependent retinal regeneration. *Clin. Chim. Acta* 464, 160–164
10 Madelaine, R. and Mourrain, P. (2017) Endogenous retinal neural stem cell reprogramming for neuronal regeneration. *Neural Regen. Res.* 12, 1765–1767
11 Cunha-Vaz, J. *et al.* (2011) Blood-retinal barrier. *Eur. J. Ophthalmol.* 21 (Suppl. 6), S3–S9
12 Campbell, M. and Humphries, P. (2012) The blood-retina barrier: tight junctions and barrier modulation. *Adv. Exp. Med. Biol.* 763, 70–84

13 Tout, S. *et al.* (1993) The role of Muller cells in the formation of the blood-retinal barrier. *Neuroscience* 55, 291–301
14 Shen, W. *et al.* (2012) Conditional Muller cell ablation causes independent neuronal and vascular pathologies in a novel transgenic model. *J. Neurosci.* 32, 15715–15727
15 Eichler, W. *et al.* (2004) Angiogenesis-related factors derived from retinal glial (Muller) cells in hypoxia. *Neuroreport* 15, 1633–1637
16 Eichler, W. *et al.* (2004) PEDF derived from glial Muller cells: a possible regulator of retinal angiogenesis. *Exp. Cell Res.* 299, 68–78
17 Franze, K. *et al.* (2007) Muller cells are living optical fibers in the vertebrate retina. *Proc. Natl. Acad. Sci. U. S. A.* 104, 8287–8292
18 Young, R.W. (1967) The renewal of photoreceptor cell outer segments. *J. Cell Biol.* 33, 61–72
19 Long, K.O. *et al.* (1986) Disc shedding and autophagy in the cone-dominant ground squirrel retina. *Exp. Eye Res.* 43, 193–205
20 Bejarano-Escobar, R. *et al.* (2017) Muller glia and phagocytosis of cell debris in retinal tissue. *J. Anat.* 231, 471–483
21 Jablonski, M.M. and Iannaccone, A. (2000) Targeted disruption of Muller cell metabolism induces photoreceptor dysmorphogenesis. *Glia* 32, 192–204
22 Wang, X. *et al.* (2005) Contribution of Muller cells toward the regulation of photoreceptor outer segment assembly. *Neuron Glia Biol.* 1, 1–6
23 Wang, J.S. and Kefalov, V.J. (2011) The cone-specific visual cycle. *Prog. Retin. Eye Res.* 30, 115–128
24 Brew, H. and Attwell, D. (1987) Electrogenic glutamate uptake is a major current carrier in the membrane of axolotl retinal glial cells. *Nature* 327, 707–709
25 Bringmann, A. *et al.* (2013) GABA and glutamate uptake and metabolism in retinal glial (Muller) cells. *Front. Endocrinol. (Lausanne)* 4, 48
26 Huster, D. *et al.* (2000) The glutathione content of retinal Muller (glial) cells: effect of pathological conditions. *Neurochem. Int.* 36, 461–469
27 Hurley, J.B. *et al.* (2015) Glucose, lactate, and shuttling of metabolites in vertebrate retinas. *J. Neurosci. Res.* 93, 1079–1092
28 Poitry, S. *et al.* (2000) Mechanisms of glutamate metabolic signaling in retinal glial (Muller) cells. *J. Neurosci.* 20, 1809–1821
29 Poitryyamate, C.L. *et al.* (1995) Lactate released by Muller glial-cells is metabolized by photoreceptors from mammalian retina. *J. Neurosci.* 15, 5179–5191
30 Reichenbach, A. *et al.* (1992) K+ ion regulation in retina. *Can. J. Physiol. Pharmacol.* 70 (Suppl), S239–S247
31 Newman, E. and Reichenbach, A. (1996) The Muller cell: a functional element of the retina. *Trends Neurosci.* 19, 307–312
32 Newman, E.A. (1993) Inward-rectifying potassium channels in retinal glial (Muller) cells. *J. Neurosci.* 13, 3333–3345
33 Nagelhus, E.A. *et al.* (1998) Aquaporin-4 water channel protein in the rat retina and optic nerve: polarized expression in Muller cells and fibrous astrocytes. *J. Neurosci.* 18, 2506–2519
34 Bringmann, A. *et al.* (2005) Neuronal versus glial cell swelling in the ischaemic retina. *Acta Ophthalmol. Scand.* 83, 528–538
35 Tolentino, M.J. *et al.* (2002) Pathologic features: of vascular endothelial growth factor-induced retinopathy in the nonhuman primate. *Am. J. Ophthalmol.* 133, 373–385
36 Cao, W. *et al.* (1997) Induction of basic fibroblast growth factor mRNA by basic fibroblast growth factor in Muller cells. *Invest. Ophthalmol. Vis. Sci.* 38, 1358–1366
37 Zhu, X. *et al.* (2012) Expression of glial cell line-derived neurotrophic factor and its receptors in cultured retinal Muller cells under high glucose circumstance. *Anat. Rec. (Hoboken)* 295, 532–539
38 Taylor, S. *et al.* (2003) Glutamate stimulates neurotrophin expression in cultured Muller cells. *Brain Res. Mol. Brain Res.* 111, 189–197
39 Seki, M. *et al.* (2005) Muller Cells as a source of brain-derived neurotrophic factor in the retina: noradrenaline upregulates brain-derived neurotrophic factor levels in cultured rat Muller cells. *Neurochem. Res.* 30, 1163–1170
40 Morimoto, T. *et al.* (2005) Transcorneal electrical stimulation rescues axotomized retinal ganglion cells by activating endogenous retinal IGF-1 system. *Invest. Ophthalmol. Vis. Sci.* 46, 2147–2155
41 Paulus, Y.M. and Campbell, J.P. (2016) Neuroprotection and retinal diseases. *Dev. Ophthalmol.* 55, 322–329
42 Harada, T. *et al.* (2002) Microglia-Muller glia cell interactions control neurotrophic factor production during light-induced retinal degeneration. *J. Neurosci.* 22, 9228–9236
43 Bringmann, A. and Wiedemann, P. (2012) Muller glial cells in retinal disease. *Ophthalmologica* 227, 1–19
44 Lu, Y.B. *et al.* (2011) Reactive glial cells: increased stiffness correlates with increased intermediate filament expression. *FASEB J.* 25, 624–631
45 Lewis, G.P. and Fisher, S.K. (2003) Up-regulation of glial fibrillary acidic protein in response to retinal injury: its potential role in glial remodeling and a comparison to vimentin expression. *Int. Rev. Cytol.* 230, 263–290
46 Kaur, C. *et al.* (2007) Blood-retinal barrier disruption and ultrastructural changes in the hypoxic retina in adult rats: the beneficial effect of melatonin administration. *J. Pathol.* 212, 429–439
47 Kim, I.B. *et al.* (1998) Reaction of Muller cells after increased intraocular pressure in the rat retina. *Exp. Brain Res.* 121, 419–424
48 Layton, C.J. *et al.* (2006) The effect of insulin and glucose levels on retinal glial cell activation and pigment epithelium-derived fibroblast growth factor-2. *Mol. Vis.* 12, 43–54
49 Xue, L.P. *et al.* (2006) Muller glial cells express nestin coupled with glial fibrillary acidic protein in experimentally induced glaucoma in the rat retina. *Neuroscience* 139, 723–732
50 Hippert, C. *et al.* (2015) Muller glia activation in response to inherited retinal degeneration is highly varied and disease-specific. *PLoS One* 10, e0120415
51 Chen, H. and Weber, A.J. (2002) Expression of glial fibrillary acidic protein and glutamine synthetase by Muller cells after optic nerve damage and intravitreal application of brain-derived neurotrophic factor. *Glia* 38, 115–125
52 Fischer, A.J. and Reh, T.A. (2003) Potential of Muller glia to become neurogenic retinal progenitor cells. *Glia* 43, 70–76
53 Wan, J. and Goldman, D. (2016) Retina regeneration in zebrafish. *Curr. Opin. Genet. Dev.* 40, 41–47
54 Goldman, D. (2014) Muller glial cell reprogramming and retina regeneration. *Nat. Rev. Neurosci.* 15, 431–442
55 Ridano, M.E. *et al.* (2017) Galectin-1 expression imprints a neurovascular phenotype in proliferative retinopathies and delineates responses to anti-VEGF. *Oncotarget* 8, 32505–32522
56 Pannicke, T. *et al.* (2004) A potassium channel-linked mechanism of glial cell swelling in the postischemic retina. *Mol. Cell. Neurosci.* 26, 493–502
57 Bringmann, A. *et al.* (2004) Pathomechanisms of cystoid macular edema. *Ophthalmic Res.* 36, 241–249
58 Bringmann, A. and Wiedemann, P. (2009) Involvement of Muller glial cells in epiretinal membrane formation. *Graefes Arch. Clin. Exp. Ophthalmol.* 247, 865–883
59 Subirada, P.V. *et al.* (2018) A journey into the retina: Muller glia commanding survival and death. *Eur. J. Neurosci.* 47, 1429–1443
60 Hippert, C. *et al.* (2016) Gliosis can impede integration following photoreceptor transplantation into the diseased retina. *Adv. Exp. Med. Biol.* 854, 579–585
61 Parmeggiani, F. (2011) Clinics, epidemiology and genetics of retinitis pigmentosa. *Curr. Genomics* 12, 236–237
62 Bowes Rickman, C. *et al.* (2013) Dry age-related macular degeneration: mechanisms, therapeutic targets, and imaging. *Invest. Ophthalmol. Vis. Sci.* 54, ORSF68–ORSF80
63 Qu, J. *et al.* (2010) Mechanisms of retinal ganglion cell injury and defense in glaucoma. *Exp. Eye Res.* 91, 48–53
64 Bernstein, S.L. *et al.* (2011) Nonarteritic anterior ischemic optic neuropathy (NAION) and its experimental models. *Prog. Retin. Eye Res.* 30, 167–187
65 Bennett, J. (2017) Taking stock of retinal gene therapy: looking back and moving forward. *Mol. Ther.* 25, 1076–1094
66 Planul, A. and Dalkara, D. (2017) Vectors and gene delivery to the retina. *Annu. Rev. Vis. Sci.* 3, 121–140
67 Bennett, J. *et al.* (1994) Adenovirus vector-mediated *in vivo* gene transfer into adult murine retina. *Invest. Ophthalmol. Vis. Sci.* 35, 2535–2542
68 Di Polo, A. *et al.* (1998) Prolonged delivery of brain-derived neurotrophic factor by adenovirus-infected Muller cells temporarily rescues injured retinal ganglion cells. *Proc. Natl. Acad. Sci. U. S. A.* 95, 3978–3983
69 Isenmann, S. *et al.* (1998) Short communication: protection of axotomized retinal ganglion cells by adenovirally delivered BDNF *in vivo*. *Eur. J. Neurosci.* 10, 2751–2756
70 Gauthier, R. *et al.* (2005) Brain-derived neurotrophic factor gene delivery to muller glia preserves structure and function of light-damaged photoreceptors. *Invest. Ophthalmol. Vis. Sci.* 46, 3383–3392
71 Lamartina, S. *et al.* (2007) Helper-dependent adenovirus for the gene therapy of proliferative retinopathies: stable gene transfer, regulated gene expression and therapeutic efficacy. *J. Gene Med.* 9, 862–874
72 Balaggan, K.S. and Ali, R.R. (2012) Ocular gene delivery using lentiviral vectors. *Gene Ther.* 19, 145–153
73 Rolling, F. (2004) Recombinant AAV-mediated gene transfer to the retina: gene therapy perspectives. *Gene Ther.* 11, S26–S32
74 Lebherz, C. *et al.* (2008) Novel AAV serotypes for improved ocular gene transfer. *J. Gene Med.* 10, 375–382
75 Greenberg, K.P. *et al.* (2007) Targeted transgene expression in muller glia of normal and diseased retinas using lentiviral vectors. *Invest. Ophthalmol. Vis. Sci.* 48, 1844–1852
76 Cavalieri, V. *et al.* (2018) Non-primate lentiviral vectors and their applications in gene therapy for ocular disorders. *Viruses* 10, 316

77 Schon, C. *et al.* (2015) Retinal gene delivery by adeno-associated virus (AAV) vectors: strategies and applications. *Eur. J. Pharm. Biopharm.* 95, 343–352
78 Dalkara, D. *et al.* (2016) Let there be light: gene and cell therapy for blindness. *Hum. Gene Ther.* 27, 134–147
79 Dorrell, M.I. *et al.* (2009) Antioxidant or neurotrophic factor treatment preserves function in a mouse model of neovascularization-associated oxidative stress. *J. Clin. Invest.* 119, 611–623
80 Prentice, H.M. *et al.* (2011) Hypoxia-regulated retinal glial cell-specific promoter for potential gene therapy in disease. *Invest. Ophthalmol. Vis. Sci.* 52, 8562–8570
81 Dalkara, D. *et al.* (2011) AAV mediated GDNF secretion from retinal glia slows down retinal degeneration in a rat model of retinitis pigmentosa. *Mol. Ther.* 19, 1602–1608
82 Hickey, D.G. *et al.* (2017) Tropism of engineered and evolved recombinant AAV serotypes in the rd1 mouse and ex vivo primate retina. *Gene Ther.* 24, 787–800
83 Maheshri, N. *et al.* (2006) Directed evolution of adeno-associated virus yields enhanced gene delivery vectors. *Nat. Biotechnol.* 24, 198–204
84 Klimczak, R.R. *et al.* (2009) A novel adeno-associated viral variant for efficient and selective intravitreal transduction of rat Muller cells. *PLoS One* 4, e7467
85 Vacca, O. *et al.* (2014) AAV-mediated gene delivery in Dp71-null mouse model with compromised barriers. *Glia* 62, 468–476
86 Byrne, L.C. *et al.* (2014) Retinoschisin gene therapy in photoreceptors, Muller glia or all retinal cells in the Rs1h(-/-) mouse. *Gene Ther.* 21, 585–592
87 Pellissier, L.P. *et al.* (2014) Specific tools for targeting and expression in Muller glial cells. *Mol. Ther. Methods Clin. Dev.* 1, 14009
88 Yao, K. *et al.* (2016) Wnt regulates proliferation and neurogenic potential of Muller glial cells via a Lin28/let-7 miRNA-dependent pathway in adult mammalian retinas. *Cell Rep.* 17, 165–178
89 Apaolaza, P.S. *et al.* (2016) Structural recovery of the retina in a retinoschisin-deficient mouse after gene replacement therapy by solid lipid nanoparticles. *Biomaterials* 90, 40–49
90 del Pozo-Rodriguez, A. *et al.* (2008) Solid lipid nanoparticles for retinal gene therapy: transfection and intracellular trafficking in RPE cells. *Int. J. Pharm.* 360, 177–183
91 Andrieu-Soler, C. *et al.* (2005) Intravitreous injection of PLGA microspheres encapsulating GDNF promotes the survival of photoreceptors in the rd1/rd1 mouse. *Mol. Vis.* 11, 1002–1011
92 Peters, T. *et al.* (2017) Evaluation of polyesteramide (PEA) and polyester (PLGA) microspheres as intravitreal drug delivery systems in albino rats. *Biomaterials* 124, 157–168
93 Kim, H. *et al.* (2009) Investigating the movement of intravitreal human serum albumin nanoparticles in the vitreous and retina. *Pharm. Res.* 26, 329–337
94 Huang, D. *et al.* (2017) Hyaluronic acid coated albumin nanoparticles for targeted peptide delivery to the retina. *Mol. Pharm.* 14, 533–545
95 Miller, A.M. and Dean, D.A. (2009) Tissue-specific and transcription factor-mediated nuclear entry of DNA. *Adv. Drug Deliv. Rev.* 61, 603–613
96 Huang, D. *et al.* (2018) Hyaluronic acid coated albumin nanoparticles for targeted peptide delivery in the treatment of retinal ischaemia. *Biomaterials* 168, 10–23
97 Koo, H. *et al.* (2012) The movement of self-assembled amphiphilic polymeric nanoparticles in the vitreous and retina after intravitreal injection. *Biomaterials* 33, 3485–3493
98 Dawbarn, D. and Allen, S.J. (2003) Neurotrophins and neurodegeneration. *Neuropathol. Appl. Neurobiol.* 29, 211–230
99 Kolomeyer, A.M. and Zarbin, M.A. (2014) Trophic factors in the pathogenesis and therapy for retinal degenerative diseases. *Surv. Ophthalmol.* 59, 134–165
100 Pardue, M.T. and Allen, R.S. (2018) Neuroprotective strategies for retinal disease. *Prog. Retin. Eye Res.* 65, 50–76
101 Wen, R. *et al.* (2012) CNTF and retina. *Prog. Retin. Eye Res.* 31, 136–151
102 van Adel, B.A. *et al.* (2005) Ciliary neurotrophic factor protects retinal ganglion cells from axotomy-induced apoptosis via modulation of retinal glia *in vivo*. *J. Neurobiol.* 63, 215–234
103 Pease, M.E. *et al.* (2009) Effect of CNTF on retinal ganglion cell survival in experimental glaucoma. *Invest. Ophthalmol. Vis. Sci.* 50, 2194–2200
104 Takahata, K. *et al.* (2003) Retinal neuronal death induced by intraocular administration of a nitric oxide donor and its rescue by neurotrophic factors in rats. *Invest. Ophthalmol. Vis. Sci.* 44, 1760–1766
105 Hellstrom, M. *et al.* (2011) Post-injury delivery of rAAV2-CNTF combined with short-term pharmacotherapy is neuroprotective and promotes extensive axonal regeneration after optic nerve trauma. *J. Neurotrauma* 28, 2475–2483
106 Liang, F.Q. *et al.* (2001) Long-term protection of retinal structure but not function using RAAV.CNTF in animal models of retinitis pigmentosa. *Mol. Ther.* 4, 461–472
107 Buch, P.K. *et al.* (2006) In contrast to AAV-mediated Cntf expression, AAV-mediated Gdnf expression enhances gene replacement therapy in rodent models of retinal degeneration. *Mol. Ther.* 14, 700–709
108 McGill, T.J. *et al.* (2007) Intraocular CNTF reduces vision in normal rats in a dose-dependent manner. *Invest. Ophthalmol. Vis. Sci.* 48, 5756–5766
109 Wen, R. *et al.* (2006) Regulation of rod phototransduction machinery by ciliary neurotrophic factor. *J. Neurosci* 26, 13523–13530
110 Kimura, A. *et al.* (2016) Neuroprotection, growth factors and BDNF–TrkB signalling in retinal degeneration. *Int. J. Mol. Sci.* 17 (9), 1584
111 Chen, H. and Weber, A.J. (2001) BDNF enhances retinal ganglion cell survival in cats with optic nerve damage. *Invest. Ophthalmol. Vis. Sci.* 42, 966–974
112 Leaver, S.G. *et al.* (2006) AAV-mediated expression of CNTF promotes long-term survival and regeneration of adult rat retinal ganglion cells. *Gene Ther.* 13, 1328–1341
113 Mansour-Robaey, S. *et al.* (1994) Effects of ocular injury and administration of brain-derived neurotrophic factor on survival and regrowth of axotomized retinal ganglion cells. *Proc. Natl. Acad. Sci. U. S. A.* 91, 1632–1636
114 Cheng, L. *et al.* (2002) TrkB gene transfer protects retinal ganglion cells from axotomy-induced death *in vivo*. *J. Neurosci.* 22, 3977–3986
115 Hu, Y. *et al.* (2010) Neurotrophic effect of a novel TrkB agonist on retinal ganglion cells. *Invest. Ophthalmol. Vis. Sci.* 51, 1747–1754
116 Bai, Y. *et al.* (2010) An agonistic TrkB mAb causes sustained TrkB activation, delays RGC death, and protects the retinal structure in optic nerve axotomy and in glaucoma. *Invest. Ophthalmol. Vis. Sci.* 51, 4722–4731
117 Wahlin, K.J. *et al.* (2000) Neurotrophic factors cause activation of intracellular signaling pathways in Muller cells and other cells of the inner retina, but not photoreceptors. *Invest. Ophthalmol. Vis. Sci.* 41, 927–936
118 Dong, A. *et al.* (2007) Increased expression of glial cell line-derived neurotrophic factor protects against oxidative damage-induced retinal degeneration. *J. Neurochem.* 103, 1041–1052
119 Schlichtenbrede, F.C. *et al.* (2003) Intraocular gene delivery of ciliary neurotrophic factor results in significant loss of retinal function in normal mice and in the Prph2Rd2/Rd2 model of retinal degeneration. *Gene Ther.* 10, 523–527
120 Tschernutter, M. *et al.* (2005) Long-term preservation of retinal function in the RCS rat model of retinitis pigmentosa following lentivirus-mediated gene therapy. *Gene Ther.* 12, 694–701
121 Ishikawa, H. *et al.* (2005) Effect of GDNF gene transfer into axotomized retinal ganglion cells using *in vivo* electroporation with a contact lens-type electrode. *Gene Ther.* 12, 289–298
122 Wu, W.C. *et al.* (2005) Long-term safety of GDNF gene delivery in the retina. *Curr. Eye Res.* 30, 715–722
123 Polato, F. and Becerra, S.P. (2016) Pigment epithelium-derived factor, a protective factor for photoreceptors *in vivo*. *Adv. Exp. Med. Biol.* 854, 699–706
124 Cayouette, M. *et al.* (1999) Pigment epithelium-derived factor delays the death of photoreceptors in mouse models of inherited retinal degenerations. *Neurobiol. Dis.* 6, 523–532
125 Akiyama, G. *et al.* (2012) Photoreceptor rescue of pigment epithelium-derived factor-impregnated nanoparticles in Royal College of Surgeons rats. *Mol. Vis.* 18, 3079–3086
126 Imai, D. *et al.* (2005) Intraocular gene transfer of pigment epithelium-derived factor rescues photoreceptors from light-induced cell death. *J. Cell Physiol.* 202, 570–578
127 Takita, H. *et al.* (2003) Retinal neuroprotection against ischemic injury mediated by intraocular gene transfer of pigment epithelium-derived factor. *Invest. Ophthalmol. Vis. Sci.* 44, 4497–4504
128 Campochiaro, P.A. *et al.* (2006) Adenoviral vector-delivered pigment epithelium-derived factor for neovascular age-related macular degeneration: results of a phase I clinical trial. *Hum. Gene Ther.* 17, 167–176
129 Thorne, R.G. and Frey, W.H., 2nd (2001) Delivery of neurotrophic factors to the central nervous system: pharmacokinetic considerations. *Clin. Pharmacokinet.* 40, 907–946
130 Tria, M.A. *et al.* (1994) Pharmacokinetics of nerve growth factor (NGF) following different routes of administration to adult rats. *Exp. Neurol.* 127, 178–183
131 Dittrich, F. *et al.* (1994) Ciliary neurotrophic factor: pharmacokinetics and acute-phase response in rat. *Ann. Neurol.* 35, 151–163
132 Ejstrup, R. *et al.* (2010) Pharmacokinetics of intravitreal glial cell line-derived neurotrophic factor: experimental studies in pigs. *Exp. Eye Res.* 91, 890–895
133 Koeberle, P.D. and Ball, A.K. (2002) Neurturin enhances the survival of axotomized retinal ganglion cells *in vivo*: combined effects with glial cell line-derived neurotrophic factor and brain-derived neurotrophic factor. *Neuroscience* 110, 555–567
134 Ahmad, I. *et al.* (2011) Muller glia: a promising target for therapeutic regeneration. *Invest. Ophthalmol. Vis. Sci.* 52, 5758–5764
135 Mensinger, A.F. and Powers, M.K. (1999) Visual function in regenerating teleost retina following cytotoxic lesioning. *Vis. Neurosci.* 16, 241–251
136 Lindsey, A.E. and Powers, M.K. (2007) Visual behavior of adult goldfish with regenerating retina. *Vis. Neurosci.* 24, 247–255

137 Sherpa, T. *et al.* (2008) Ganglion cell regeneration following whole-retina destruction in zebrafish. *Dev. Neurobiol.* 68, 166–181
138 Johns, P.R. (1982) Formation of photoreceptors in larval and adult goldfish. *J. Neurosci* 2, 178–198
139 Raymond, P.A. and Rivlin, P.K. (1987) Germinal cells in the goldfish retina that produce rod photoreceptors. *Dev. Biol.* 122, 120–138
140 Hitchcock, P.F. *et al.* (1992) Local regeneration in the retina of the goldfish. *J. Neurobiol.* 23, 187–203
141 Raymond, P.A. *et al.* (1988) Regeneration of goldfish retina: rod precursors are a likely source of regenerated cells. *J. Neurobiol.* 19, 431–463
142 Braisted, J.E. *et al.* (1994) Selective regeneration of photoreceptors in goldfish retina. *Development* 120, 2409–2419
143 Fausett, B.V. and Goldman, D. (2006) A role for alpha1 tubulin-expressing Muller glia in regeneration of the injured zebrafish retina. *J. Neurosci.* 26, 6303–6313
144 Kassen, S.C. *et al.* (2007) Time course analysis of gene expression during light-induced photoreceptor cell death and regeneration in albino zebrafish. *Dev. Neurobiol.* 67, 1009–1031
145 Bernardos, R.L. *et al.* (2007) Late-stage neuronal progenitors in the retina are radial Muller glia that function as retinal stem cells. *J. Neurosci.* 27, 7028–7040
146 Ramachandran, R. *et al.* (2010) Conditional gene expression and lineage tracing of tuba1a expressing cells during zebrafish development and retina regeneration. *J. Comp. Neurol.* 518, 4196–4212
147 Das, A.V. *et al.* (2006) Neural stem cell properties of Muller glia in the mammalian retina: regulation by Notch and Wnt signaling. *Dev. Biol.* 299, 283–302
148 Giannelli, S.G. *et al.* (2011) Adult human Muller glia cells are a highly efficient source of rod photoreceptors. *Stem Cells* 29, 344–356
149 Lawrence, J.M. *et al.* (2007) MIO-M1 cells and similar muller glial cell lines derived from adult human retina exhibit neural stem cell characteristics. *Stem Cells* 25, 2033–2043
150 Zhao, J.J. *et al.* (2014) Induction of retinal progenitors and neurons from mammalian Muller glia under defined conditions. *J. Biol. Chem.* 289, 11945–11951
151 Wright, L.S. *et al.* (2014) Induced pluripotent stem cells as custom therapeutics for retinal repair: progress and rationale. *Exp. Eye Res.* 123, 161–172
152 Fimbel, S.M. *et al.* (2007) Regeneration of inner retinal neurons after intravitreal injection of ouabain in zebrafish. *J. Neurosci.* 27, 1712–1724
153 Nagashima, M. *et al.* (2013) A self-renewing division of zebrafish Muller glial cells generates neuronal progenitors that require N-cadherin to regenerate retinal neurons. *Development* 140, 4510–4521
154 Ooto, S. *et al.* (2004) Potential for neural regeneration after neurotoxic injury in the adult mammalian retina. *Proc. Natl. Acad. Sci. U. S. A.* 101, 13654–13659
155 Wan, J. *et al.* (2008) Preferential regeneration of photoreceptor from Muller glia after retinal degeneration in adult rat. *Vision Res.* 48, 223–234
156 Kugler, M. *et al.* (2015) Heterozygous modulation of TGF-beta signaling does not influence Muller glia cell reactivity or proliferation following NMDA-induced damage. *Histochem. Cell Biol.* 144, 443–455
157 Joly, S. *et al.* (2011) Pax6-positive Muller glia cells express cell cycle markers but do not proliferate after photoreceptor injury in the mouse retina. *Glia* 59, 1033–1046
158 Wan, J. *et al.* (2007) Sonic hedgehog promotes stem-cell potential of Muller glia in the mammalian retina. *Biochem. Biophys. Res. Commun.* 363, 347–354
159 Osakada, F. *et al.* (2007) Wnt signaling promotes regeneration in the retina of adult mammals. *J. Neurosci.* 27, 4210–4219
160 Ueki, Y. *et al.* (2015) Transgenic expression of the proneural transcription factor Ascl1 in Muller glia stimulates retinal regeneration in young mice. *Proc. Natl. Acad. Sci. U. S. A.* 112, 13717–13722
161 Jorstad, N.L. *et al.* (2017) Stimulation of functional neuronal regeneration from Muller glia in adult mice. *Nature* 548, 103–107
162 Karl, M.O. *et al.* (2008) Stimulation of neural regeneration in the mouse retina. *Proc. Natl. Acad. Sci. U. S. A.* 105, 19508–19513
163 Ramachandran, R. *et al.* (2010) Ascl1a regulates Muller glia dedifferentiation and retinal regeneration through a Lin-28-dependent, let-7 microRNA signalling pathway. *Nat. Cell Biol.* 12, 1101–1107
164 Fausett, B.V. *et al.* (2008) The proneural basic helix-loop-helix gene ascl1a is required for retina regeneration. *J. Neurosci.* 28, 1109–1117
165 Ramachandran, R. *et al.* (2011) Ascl1a/Dkk/beta-catenin signaling pathway is necessary and glycogen synthase kinase-3beta inhibition is sufficient for zebrafish retina regeneration. *Proc. Natl. Acad. Sci. U. S. A.* 108, 15858–15863
166 Pollak, J. *et al.* (2013) ASCL1 reprograms mouse Muller glia into neurogenic retinal progenitors. *Development* 140, 2619–2631
167 Loffler, K. *et al.* (2015) Age-dependent Muller glia neurogenic competence in the mouse retina. *Glia* 63, 1809–1824
168 Wan, J. *et al.* (2014) Retinal injury, growth factors, and cytokines converge on beta-catenin and pStat3 signaling to stimulate retina regeneration. *Cell Rep.* 9, 285–297
169 Takeda, M. *et al.* (2008) alpha-Aminoadipate induces progenitor cell properties of Muller glia in adult mice. *Invest. Ophthalmol. Vis. Sci.* 49, 1142–1150
170 Del Debbio, C.B. *et al.* (2010) Notch and Wnt signaling mediated rod photoreceptor regeneration by Muller cells in adult mammalian retina. *PLoS One* 5, e12425
171 Yao, K. *et al.* (2018) Restoration of vision after de novo genesis of rod photoreceptors in mammalian retinas. *Nature* 560, 484–488
172 Meyn, R.E. *et al.* (1973) Evaluation of S phase synchronization by analysis of DNA-replication in 5-bromodeoxyuridine. *Exp. Cell Res.* 82, 137–142
173 Kuan, C.Y. *et al.* (2004) Hypoxia-ischemia induces DNA synthesis without cell proliferation in dying neurons in adult rodent brain. *J. Neurosci.* 24, 10763–10772
174 Pearson, R.A. *et al.* (2016) Donor and host photoreceptors engage in material transfer following transplantation of post-mitotic photoreceptor precursors. *Nat. Commun.* 7, 13029
175 Ortin-Martinez, A. *et al.* (2017) A reinterpretation of cell transplantation: GFP transfer from donor to host photoreceptors. *Stem Cells* 35, 932–939
176 Singh, M.S. *et al.* (2016) Transplanted photoreceptor precursors transfer proteins to host photoreceptors by a mechanism of cytoplasmic fusion. *Nat. Commun.* 7, 13537
177 Singhal, S. *et al.* (2012) Human Muller glia with stem cell characteristics differentiate into retinal ganglion cell (RGC) precursors *in vitro* and partially restore RGC function *in vivo* following transplantation. *Stem Cells Transl. Med* 1, 188–199
178 Jayaram, H. *et al.* (2014) Transplantation of photoreceptors derived from human Muller glia restore rod function in the P23H rat. *Stem Cells Transl. Med.* 3, 323–333
179 Boudreau-Pinsonneault, C. and Cayouette, M. (2018) Cell lineage tracing in the retina: Could material transfer distort conclusions? *Dev. Dyn.* 247, 10–17
180 Craft, C.M. (2014) Targeting endostatin to potentially prevent retinal neovascularization using a hypoxia-responsive Muller glia cell-specific gene therapy. *Invest. Ophthalmol. Vis. Sci.* 55, 8054
181 Jiang, Y. *et al.* (2014) Targeting Muller cell-derived VEGF164 to reduce intravitreal neovascularization in the rat model of retinopathy of prematurity. *Invest. Ophthalmol. Vis. Sci.* 55, 824–831
182 Becker, S. *et al.* (2018) Targeted knockdown of overexpressed VEGFA or VEGF164 in Muller cells maintains retinal function by triggering different signaling mechanisms. *Sci. Rep.* 8, 2003
183 Pellissier, L.P. *et al.* (2015) Gene therapy into photoreceptors and Muller glial cells restores retinal structure and function in CRB1 retinitis pigmentosa mouse models. *Hum. Mol. Genet.* 24, 3104–3118
184 Zhao, M. *et al.* (2015) A new CRB1 rat mutation links Muller glial cells to retinal telangiectasia. *J. Neurosci.* 35, 6093–6106
185 Xue, Y. *et al.* (2015) CRALBP supports the mammalian retinal visual cycle and cone vision. *J. Clin. Invest.* 125, 727–738
186 Aartsen, W.M. *et al.* (2010) GFAP-driven GFP expression in activated mouse Muller glial cells aligning retinal blood vessels following intravitreal injection of AAV2/6 vectors. *PLoS One* 5 (8), e12387
187 Koerber, J.T. *et al.* (2009) Molecular evolution of adeno-associated virus for enhanced glial gene delivery. *Mol. Ther.* 17, 2088–2095
188 Liu, B. *et al.* (2013) Wnt signaling promotes Muller cell proliferation and survival after injury. *Invest. Ophthalmol. Vis. Sci.* 54, 444–453