Prospects for the application of Müller glia and their derivatives in retinal regenerative therapies

Eastlake K, Lamb WDB, Luis J, Khaw PT, Jayaram H, Limb GA

NIHR Biomedical Research Centre at Moorfields Eye Hospital and UCL Institute of Ophthalmology, London, UK

Corresponding author:
G Astrid Limb, PhD, FRCPath
UCL Institute of Ophthalmology
11-43 Bath Street, London EC1V 9EL
Phone: 020 7608-6974
Email: g.limb@ucl.ac.uk

Contents

Abstract

Neural cell death is the main feature of all retinal degenerative disorders that lead to blindness. Despite therapeutic advances, progression of retinal disease cannot always be prevented, and once neuronal cell damage occurs, visual loss cannot be reversed. Recent research in the stem cell field, and the identification of Müller glia with stem cell characteristics in the human eye, have provided hope for the use of these cells in retinal therapies to restore vision. Müller glial cells, which are the major structural cells of the retina, play a very important role in retinal homeostasis during health and disease. They are responsible for the spontaneous retinal regeneration observed in zebrafish and lower vertebrates during early postnatal life, and despite the presence of Müller glia with stem cell characteristics in the adult mammalian retina, there is no evidence that they promote regeneration in humans. Like many other stem cells and neurons derived from pluripotent stem cells, Müller glia with stem cell potential do not differentiate into retinal neurons or integrate into the retina when transplanted into the vitreous of experimental animals with retinal degeneration. However, despite their lack of integration, grafted Müller glia have been shown to induce partial restoration of visual function in spontaneous or induced experimental models of photoreceptor or retinal ganglion cell damage. This improvement in visual function observed after Müller cell transplantation has been ascribed to the release of neuroprotective factors that promote the repair and survival of damaged neurons. Due to the development and availability of pluripotent stem cell lines for therapeutic uses, derivation of Müller cells from retinal organoids formed by iPSC and ESC has provided more realistic prospects for the application of these cells to retinal therapies. Several opportunities for research in the regenerative field have also been unlocked in recent years due to a better understanding of the genomic and proteomic profiles of the developing and regenerating retina in zebrafish, providing the basis for further studies of the human retina. In addition, the increased interest on the nature and function of cellular organelle release and the characterization of molecular components of exosomes released by Müller glia, may help us to design new approaches that could be applied to the development of more effective treatments for retinal degenerative diseases.

1. Introduction

The retina is a neurosensory organ formed by a complex neural network supported by radial Müller glia (Reichenbach et al., 1993). Müller glia not only exert key metabolic and supportive functions for retinal neurons (Reichenbach et al., 2007), but also modulate neuronal excitability by releasing and recycling neurotransmitters (Newman and Zahs, 1998, Reichenbach and Bringmann, 2013). In the zebrafish, Müller glia are responsible for the regeneration of the retina after injury, a phenomenon observed throughout the life of this species (Raymond et al., 2006, Bernardos et al., 2007), and although Müller glia in the rodent eye share many characteristics with those in the zebrafish, they only possess a limited regenerative potential in early postnatal life (Karl et al., 2008, Ooto et al., 2004, Osakada et al., 2007). Müller glia with neural stem cell characteristics have been also identified in the adult human retina (Bhatia et al., 2009, Lawrence et al., 2007), yet, there is no evidence that they are capable of regeneration. Nonetheless, research undertaken in recent years on the cellular and molecular events underlying the regenerative ability of Müller glia in the zebrafish and lower vertebrate species, may help us to unlock the regenerative potential of these cells in the human eye. Due to the neurogenic and active metabolic function of Müller glia, and recent progress in the stem cell field, the potential use of these cells in the development of retinal regenerative therapies has generated significant scientific interest.

Currently, pharmacological and surgical interventions used to treat retinal degeneration are aimed at minimizing disease progression, but in many cases disease progression cannot be prevented and patients often develop significant and permanent visual loss. Experimental transplantation approaches utilizing Müller glia (Eastlake et al., 2019), Müller glia-derived retinal neurons (Becker et al., 2016, Jayaram et al., 2014, Singhal et al., 2012), or pluripotent stem cell-derived retinal neurons (Cuevas et al., 2019, Gasparini et al., 2019), have shown improvement of retinal function without evidence of true regeneration. Transplantation of iPSC and non-retinal cells, such as mesenchymal stem cells, have also shown evidence of neuroprotection in the retina (Hu et al., 2017, Fang et al., 2014, Johnson et al., 2010), for which it has been suggested that improvement of visual function by cell transplantation may be ascribed to the release of neuroprotective factors by grafted cells. The source of cells can be an important factor to consider when designing retinal cell-based therapies, and

autologous cells would be preferred to allogeneic cells as they are likely to elicit minimal immunological responses. Due to the complexity of neural networks within the retina, developing methods to promote retinal regeneration through stem cell transplantation have proved difficult. True regeneration would entail functional cell replacement and synaptic integration of grafted cells within the neural retina network. However, to regenerate the retina, we will not only require a deep understanding of the molecular mechanisms that promote stem cell differentiation into functional neurons and glia, but also the appropriate protocols to promote successful survival, migration, and synaptic integration of the transplanted cells. Thus far, migration and integration of stem cells have been exceptionally challenging as a retina that needs regeneration may have lost the developmental cues that facilitate the permissive microenvironment necessary for neural integration. Diseased retina is likely to exhibit pro-inflammatory features characterized by microglia accumulation and cytokine release (Roque et al., 1996), as well as glial scarring (Asher et al., 2001, Lewis and Fisher, 2003), all of which have shown to form a barrier that prevents successful regeneration by transplanted cells (Singhal et al., 2008). On this basis, unless transplantation is solely aimed at promoting neuroprotection, much research is still needed to achieve functional integration of transplanted stem cells into pre-existing neural circuits.

With much knowledge gained in the regenerative field from studies in the zebrafish and lower vertebrates, an alternative option that may be explored for therapeutic approaches is the induction of endogenous regeneration by stimulating the neurogenic ability of Müller glia *in situ*. In addition, recent advances on the identification of the genomic and secretomic profiles of Müller glia in the zebrafish may be explored for designing non-invasive therapies without the need for cell transplantation. Here we discuss various aspects of Müller glia research aimed at exploring the potential of these cells for application in retinal regenerative therapies.

2. Neural degeneration as a feature of retinal disease

Retinal degenerative conditions are a leading cause of blindness in the world's population after cataract and untreated refracted error (Flaxman et al., 2017). In developed countries such as the United Kingdom, where blindness caused by cataract is rare, retinal degenerative diseases such as age-related macular degeneration (AMD), glaucoma, diabetic

retinopathy and hereditary retinal conditions, including Retinitis Pigmentosa (RP), Stargardt's Disease and Leber's Congenital Amaurosis, are more prominent (Rahman et al., 2020). These inherited retinal dystrophies manifest as a result of defects or absence of key proteins of the visual cycle, and their progression can vary from person to person depending on the severity of the genetic defect. For example, RP typically presents with the onset of night blindness and loss of peripheral vision within the first two decades of life, followed by variable progression to potentially involve central vision, leading to severe visual impairment (Ferrari et al., 2011). Despite the complex nature of these diseases, many shared histopathological endpoints are common as the retina degenerates. Findings such as atrophy of the photoreceptors and RPE cells as well as thinning of the remaining neuroretinal layers are not dissimilar in appearance to AMD, or indeed other forms of acquired retinal degeneration. The optic nerve, which is formed by the clustering of axons from RGC located in the ganglion cell layer of the retina, carries visual signals from the eye to the brain, and when optic nerve damage or deterioration disrupts the transfer of information, vision loss occurs (Vrabec and Levin, 2007). This is illustrated in glaucoma, an optic neuropathy involving structural damage of the optic nerve, death of RGC and defects of the visual field, which are often associated with raised intraocular pressure (Weinreb and Khaw, 2004, Park et al., 2012). In addition to these common degenerative diseases, retinal neurodegeneration can be secondary to, or exacerbated by problems with the ocular vascular system, such as in diabetic retinopathy. In this condition, abnormal angiogenesis or increased permeability of vessels lead to leakage or macular oedema (Ozawa et al., 2011, Gardner et al., 2011).

In the context of neural degeneration, it is important to consider that regardless of the underlying cause, the final common pathway of retinal degenerative disease is high metabolic stress that leads to insult to the respective target structures in the retina and optic nerve, resulting in neural cell death. Once cell death occurs in a specific neural cell population, reorganization of the neural circuit follows, leading to subsequent remodelling and cell death of all types of retinal neurons (Marc et al., 2003). These pathological features are clearly illustrated in AMD, in which photoreceptor cell loss occurs as a consequence of RPE cell death (Bhutto and Lutty, 2012, Kauppinen et al., 2016), and is followed by marked remodelling and rewiring of bipolar and amacrine cells, preventing

appropriate connections with RGC and leading to loss of retinal function (Cho et al., 2011). In other conditions, such as retinitis pigmentosa, secondary changes in the cones and RPE cells follow degeneration of rod photoreceptors, eventually leading to RGC loss due to axon compression and neural reorganization (Villegas-Pérez et al., 1998), as well as glial hypertrophy and the formation of a glial scar that isolates the neural retina from RPE and choroid (Jones et al., 2016). Interestingly, in a mouse model of retinitis pigmentosa, defects in rod and cone outer segments, bipolar cells, amacrine cells and photoreceptor synapses, as well as Müller glial changes, have been observed in early stages of postnatal development before photoreceptor death occurs (Roche et al., 2016). There is also evidence that damage to the optic nerve and RGC in advanced glaucoma leads to photoreceptor and horizontal cell degeneration (Janssen et al., 1996, Vincent et al., 2010), which leads to loss of retinal function and consequently blindness.

3. Concepts of stem cell-based retinal regenerative therapies

Early transplantation studies aimed to repair the degenerated retina involved subretinal grafting of foetal retina (Aramant and Seiler, 1991) or cells dissociated from new-born retina (del Cerro et al., 1989). Although it was reported that they integrated into the host retina, recovery of function was never demonstrated. Other studies involving embryonic retina transplantation into neonatal rat brain reported axonal outgrowth into the superior colliculus (Hankin and Lund, 1987, Hankin and Lund, 1990), leading to investigations into various cell sources to induce regeneration in animal models of retinal disease. Importantly, identification of retinal progenitors in the foetal human (Kelley et al., 1995) and embryonic rat (Ahmad et al., 1999) retina established the basis for subsequent use of these cells in retinal transplantation research (Klassen et al., 2004).

Synaptic remodeling of neural circuits observed during retinal degeneration (Jones et al., 2003a) is recognized as a major challenge for integration of transplanted cells into the retina. During early retinal disease, successful approaches for regeneration of a single neural cell type may be a more realistic goal than in late disease, when extended damage to the neural circuitry has occurred. It has been suggested that some residual plasticity is observed earlier in degenerative diseases and may offer a window for transplantation approaches, with the replacement of unidirectional sensory neurons, such as photoreceptors potentially

being more feasible, than the replacement of RGC in glaucoma, which involves complex afferent inputs and distant synapses (MacLaren and Pearson, 2007). With recent progress in the establishment and characterization of several pluripotent stem cell lines, it is thought that these cells could be valuable tools to regenerate the retina. The original concept behind the use of stem cell lines for retinal therapies was to deliver therapeutic cells as single cell suspensions or as cellular scaffolds on suitable biomaterials (Tomita et al., 2005, McHugh et al., 2013, Sodha et al., 2011). The success of retinal cell replacement and regeneration would therefore rely on the migration of transplanted cells, and their ability to undergo *in situ* differentiation and development of appropriate synaptic connections with host neurons. However, to date, functional integration of transplanted cells has not yet been unequivocally shown. Initial studies demonstrated that injection of green fluorescent protein (GFP)-tagged photoreceptor precursors integrated into the host retina and improved visual function (MacLaren et al., 2006, Homma et al., 2013, West et al., 2008). However, this was later disproved by findings that GFP signals observed in the transplanted retina were due to material exchange between donor cells and host photoreceptors (Ortin-Martinez et al., 2017, Pearson et al., 2016, Decembrini et al., 2017, Santos-Ferreira et al., 2016). Although these findings suggest that transplantation may not necessarily lead to cell integration or replacement, it supports the view that transplanted cells can provide trophic support to remaining and partially damaged cells in the host retina, sending survival signals such as antioxidants and neurotrophins that may help to treat retinal degenerative diseases.

Other factors are known to limit the development of transplantation approaches in retinal degeneration, including the glial scar, which is rich in extracellular matrix proteins such as the chondroitin sulphate proteoglycans (Jones et al., 2003b, Reinhard et al., 2017). These proteins have been shown to inhibit axon guidance (Jones et al., 2003c) and regeneration of the injured rat optic nerve (Sellés-Navarro et al., 2001), which is supported by findings that degradation of these proteins by chondroitinases promote neurite outgrowth and axon regeneration in the brain (Bradbury et al., 2002, Li et al., 2013) and spinal cord (Bradbury et al., 2002, Kim et al., 2006). Experimental data has shown that injection of chondroitinase and anti-inflammatory drugs at the time of cell transplantation into the retina, can facilitate the survival of transplanted Müller glia and promote restoration of visual function in animal models of retinal degeneration (Singhal et al., 2008). This suggests that similar treatments

may enable migration of transplanted stem cells into the degenerated human retina. Despite much research in the field, transplantation therapies to regenerate the retina still require further elucidation of molecular mechanisms that promote stem cell differentiation into functional neurons and glia within the diseased retina, as well as approaches that could effectively promote migration, integration and survival of transplanted neurons.

4. Stem cell sources for retinal transplantation

Various cell sources have been explored for their ability to improve visual function in various experimental models of retinal degeneration. These have included amongst others Müller glia with stem cell characteristics (Lawrence et al., 2007, Singhal et al., 2012, Jayaram et al., 2014), brain derived stem cells (Young et al., 2000), neural precursors derived from embryonic stem cells (Banin et al., 2006), ciliary epithelium from the postnatal eye (Chacko et al., 2003), umbilical cord tissue cells (Zhao et al., 2011), mesenchymal stem cells derived from the bone marrow (Inoue et al., 2007) and photoreceptors derived from pluripotent stem cells (Gonzalez-Cordero et al., 2017). Advances in the stem cell field during the past decade has led to the generation of several human embryonic stem cell (hESC) and induced pluripotent stem cell (hiPSC) lines, which have been used for derivation of cells bearing characteristic tissue phenotypes as preferred cells for use in transplantation therapies (de Miguel-Beriain, 2015, Lengner, 2010). Embryonic stem cells (ESC) are obtained from the inner cell mass of the blastocyst (Evans and Kaufman, 1981, Bongso and Tan, 2005). Due to their pluripotency and ability to self-renew while retaining the capacity to differentiate into all the body cell types, these cells have the potential to yield large numbers for use in regenerative therapies. One of the first tissues to be differentiated *in vitro* from ESC was the retinal pigment epithelium (RPE), which opened the possibility for translational development of retinal therapies (Oswald and Baranov, 2018).

Unlike RPE cells, derivation of neurons from ESC for retinal transplantation involves longer and more complex methods. It was originally suggested that hESC may differentiate into a neuronal phenotype by default (Hemmati-Brivanlou and Melton, 1997), and extensive research has allowed the development of protocols to generate enriched populations of specific retinal neurons (Kobayashi et al., 2018, Markus et al., 2019) as well as retinal organoids (Kim et al., 2019, Lakowski et al., 2018). Since all retinal neurons and Müller glia

derive from the same precursors, several protocols have been developed and refined to reproducibly generate retinal cells for transplantation. Towards this aim, several methods have been used to obtain enriched populations of photoreceptor precursors (Lu and Barnstable, 2018, Gonzalez-Cordero et al., 2013) and RGC (Jagatha et al., 2009, Chang et al., 2019). Protocols to derive Müller glia have not been widely explored, but since these cells are key elements of retinal organoids, ESC constitute a potential renewable source for generating neuronal cell types and Müller glia for regenerative therapies. Transplantation of photoreceptors derived from ESC have been widely investigated in rodent models of retina degeneration (Lamba et al., 2009, West et al., 2012, Zhu et al., 2017) and normal non-human primate retina (Chao et al., 2017), however, they have not yet been tested in the clinic.

hiPSC lines have been generated from adult cells such as dermal fibroblasts (Lowry et al., 2008), keratinocytes (Aasen et al., 2008) or hematopoietic cells (Eminli et al., 2009) by retroviral transfection and upregulation of Oct3/4, Klf4, Sox2 and c-Myc. Due to their derivation from adult tissue cells, iPSC share many features with ESC, including DNA methylation, gene expression and chromatin properties (Wernig et al., 2007). One of the main advantages of these cells is the potential for autologous use, removing the need for immunosuppression after transplantation. However, hiPSC lines can exhibit different genetic and epigenetic signatures (Chhabra, 2017), indicating the need to derive hiPSC lines with stable genomic profile for safe use in human therapies (Kamao et al., 2014, Kanemura et al., 2014, Kawamata et al., 2015). Due to the tumorigenic potential of c-Myc, frequently used to induce iPSC generation, an alternative protocol replacing c-Myc with L-Myc has been developed to induce pluripotency in adult cells to reduce this risk (Nakagawa et al., 2010). More recent methodologies avoid the use of viral vectors by co-transfecting cells with two expression plasmids, one containing complementary cDNAs of Oct3/4, Sox2, and Klf4 and the other containing c-Myc cDNA. This has led to the generation of iPSC without evidence of plasmid integration, thus reducing the risk of tumorigenicity and increasing the prospects for use of these cells in regenerative therapies (Okita et al., 2008)

Genomic instability has been a feature of several experimentally generated hESC and hiPSC lines. Enriched culture conditions and rapid expansion of hESC *in vitro* do not resemble the *in vivo* environment of the developing blastocyst, causing these cells to rapidly develop

karyotypic abnormalities (Draper et al., 2004). These abnormalities appear to be caused by increase in cell proliferation rates and resistance to apoptosis or senescence (Mantel et al., 2007), and are thought to pose risks for the therapeutic applications of stem cells due to their tumorigenic potential (Moon et al., 2011). However, new culture strategies to minimize the appearance of genetic variants in hESC cells have been developed, and several cell lines have been established, which exhibit chromosomal stability and comply with regulatory requirements for human cell therapies (De Sousa et al., 2016b)

5. Müller glia- the stem cell population of the developed retina

Initial investigations to characterize the stem cell niches in the zebrafish showed that in response to injury, Müller glia present in the peripheral retina acquire phenotypic and molecular features that characterize multipotent retinal progenitors (Raymond et al., 2006). These observations subsequently led to the demonstration that Müller glia in the zebrafish function as multipotent retinal stem cells that generate retinal neurons in response to injury (Bernardos et al., 2007). Müller glia can be induced to proliferate *in vivo*, express neurogenic factors, and generate a progeny that express neural or glial markers in all vertebrate species including early post- natal chick (Fischer and Reh, 2001) and mice (Karl et al., 2008), as well as adult rat retina (Ooto et al., 2004). Müller glia with stem cell characteristics have also been identified and isolated from the adult human retina (Lawrence et al., 2007, Bhatia et al., 2009) (Fig.1), and while they are able to proliferate and differentiate into cells expressing markers of neural precursors *in vitro*, their endogenous regenerative ability is not yet understood. Based on the observations that Müller glia can regenerate the zebrafish retina but not the adult mammalian retina, it has been suggested that these cells may have become quiescent upon maturation of the mammalian retina, and this could provide the basis to develop new strategies to induce these cells to regain their regenerative capability in humans.

During the early 1990's, much controversy arose from the nature and sources of stem cells in the adult human retina. Since the epithelium of the ciliary body derives from the neuro-epithelium of the optic vesicle during embryogenesis, it was thought that this region of the eye could contain cells that retained neurogenic potential. Early demonstrations indicated

that pigmented ciliary epithelium isolated from mouse (Tropepe et al., 2000) and rat (Ahmad et al., 2000) eyes could be induced to proliferate and express nestin and other neural phenotypes in the presence of FGF2. Later studies showed that the non-pigmented ciliary epithelium (CE) in the chicken eye was capable of generating neurons following intraocular injection of insulin, epidermal growth factor (EGF) and fibroblast growth factor-2 (FGF2) to stimulate their proliferation and neuronal differentiation (Fischer and Reh, 2003). These cells were shown to express the neural transcription factors Pax6 and Chx10 in the region where newly generated amacrine cells, Müller glia and retinal ganglion cells emerged in response to growth factor treatment (Fischer and Reh, 2003). These observations were followed by reports that in response to photoreceptor damage induced in early post-natal mouse retina, non-pigmented CE proliferated and expressed markers of immature retinal neurons (Nishiguchi et al., 2009). Since CE with neural progenitor characteristics had been also identified in the human eye (Coles et al., 2004), and in the hope that CE could be used as a source of neural progenitors for retinal regenerative therapies, extensive studies were conducted over many years. However, the existence of 'true retinal stem cells' in the CE was later questioned by many investigators (Frøen et al., 2013, Bhatia et al., 2009, Cicero et al., 2009, Gualdoni et al., 2010), and despite many publications in the field, the use of CE for regenerative therapies never proved successful, causing research in this field to gradually decline. Due to the close proximity of the ciliary body to the neural retina, and given the size of the ciliary body in small rodent eyes, it might have been possible that techniques employed by investigators for the isolation of CE with neural progenitor characteristics, yielded a mixture of CE and Müller glia with stem cell characteristics present in the marginal region of the retina. Taking into consideration that the ciliary marginal zone (CMZ) - a neuro-epithelial germinal zone first identified in fish and amphibians (Hollyfield, 1968, Harris and Perron, 1998)- is localized at the interface between the retina and the CE, investigations into the possibility of neurogenesis occurring in this region in various animal species showed that CMZ cells gradually diminish during vertebrate evolution (Kubota et al., 2002). Despite these findings, previous studies had indicated that young primate retina possess a CMZ (Fischer et al., 2001), prompting investigations in the human eye to further understand the nature of the progenitor cells identified in the CE.

Observations in the primate retina were later replicated in the adult human retina, where it was observed that the neural retinal margin harboured cells forming bundles of spindle cells

(resembling glial cells) 300-1000µm in size, and lacking of lamination (Bhatia et al., 2009) (Fig. 2). They co-stained for the neural progenitor markers SOX2, CHX10 and SHH as well as Nestin and cellular retinaldehyde-binding protein (CRALBP), clearly distinguishing this cell population from the CE, which expressed the same progenitor markers, but did not stain for Nestin or CRALBP, the key markers of Müller glia (Sarthy et al., 1998, Lewis et al., 1995, Limb et al., 2002). Interestingly, the intensity of expression of Nestin and CRALBP observed in the peripheral retina gradually decreased towards the posterior region, where it anatomically identified a population of Müller glia. In addition, cells of the retinal margin and the inner nuclear layer, where the soma of Müller glia is located, re-entered the cell cycle upon retinal explant culture with EGF, suggesting that these cells have the ability to proliferate *in situ* (Bhatia et al., 2009). The absence of lamination and abundance of cells expressing stem cell markers in the marginal region of the adult human retina resembling the CMZ of fish and amphibians, taken together with observations that cells expressing Müller cell markers in the CM-like zone and inner nuclear layer of the human retina proliferate in response to EGF, suggest that human Müller glia may have regenerative potential and merits extensive exploration. Because of their molecular characteristics and ability to proliferate and differentiate *in vitro*, it has been suggested that human Müller glia with progenitor characteristics may constitute an important source of cells for use in retinal regenerative therapies (Limb et al., 2002, Lawrence et al., 2007). Genetic profiling studies of Müller glia in various lower vertebrate species, including mice, indicate that these cells are very similar to retinal progenitor cells (Jadhav et al., 2009, Roesch et al., 2008).

5.1 Transplantation of Müller glia-derived neurons in animal models of retinal degeneration

Müller glial cells isolated from the adult human retina often show indefinite cell renewal *in vitro*, and express phenotypic and genotypic markers of neural stem cells, including βIII tubulin, Sox-2, Pax-6, Chx10 and Notch-1 (Lawrence et al., 2007, Becker et al., 2013). In addition, depending on the presence of growth and differentiation factors used to culture these cells, they can be induced to express characteristic protein markers of post-mitotic retinal neurons, including peripherin, recoverin, calretinin, S-opsin and Brn3 (Singhal et al., 2012, Jayaram et al., 2014). Because of their stem cell characteristics and the neuroprotective functions of Müller glia in the normal retina, we examined the potential ability of enriched

populations of retinal neurons derived from Müller cells to improve visual function in animal models of photoreceptor degeneration (Jayaram et al., 2014) and retinal ganglion cell depletion (Singhal et al., 2012, Becker et al., 2016). It was originally thought that undifferentiated stem cells were ideal candidates for transplantation strategies to replace neurons, however, this concept was challenged by a landmark study in which an optimal ontogenetic stage was shown to be important for the successful transplantation of rod photoreceptor precursors (MacLaren et al., 2006). In order to apply this principle to the setting of experimental transplantation to replace retinal ganglion cells, the cell type predominantly damaged in glaucoma, Müller glia were induced to differentiate towards committed RGC precursors *in vitro* upon culture in the presence of FGF and the Notch inhibitor (2*S*)-*N*-[(3,5-Difluorophenyl)acetyl]-L-alanyl-2-phenyl]glycine 1,1-dimethylethyl ester (DAPT) (Becker et al., 2013, Singhal et al., 2012). These differentiated RGC precursors expressed Brn3b and showed neuronal functionality as determined by a rise in cytosolic calcium in response to nicotine, a neurotransmitter known to stimulate RGC precursors (Singhal et al., 2012). Transplantation of Müller glia-derived RGC precursors into an experimental rat model of RGC depletion showed limited migration and integration of these cells within the host retina 4 weeks after grafting. Cells expressing Brn3b however, aligned within the ganglion cell layer and established limited synapses with local axons, as shown by the co-expression of both synaptic markers and other markers of ganglion cell fate including Islet-1 and neurofilament protein. Although the transplanted cells did not send axons to the optic nerve, partial recovery of retinal ganglion cell function was seen, as judged by an improvement in the scotopic negative threshold response (nSTR) of the electroretinogram (ERG) (Singhal et al., 2012).

When developing stem cell therapies, the translational potential of these approaches may depend upon successful engraftment of transplanted cells and improvement of retinal function in experimental models with anatomical and functional features resembling those of the human eye. In order to advance the understanding on the potential use of Müller cells for human therapies to treat glaucoma, a method to efficiently deliver these cells to a larger mammalian eye was later examined, in which a feline model of RGC depletion by N-methyl-d-aspartate (NMDA) was used (Becker et al., 2016). In this model, intravitreal injection of human Müller glial-derived RGC precursors into the feline vitreous elicited a severe inflammatory response without improving visual function, suggesting a xenogeneic response

to transplantation. In contrast, allogeneic transplantation of feline Müller glia-derived RGC into the vitrectomized eye of this species caused a recovery of RGC function as deduced by improvement in the nSTR of the ERG (Becker et al., 2016). In these experiments, despite causing functional improvement, grafted cells did not attach onto the retina but formed aggregates with vitreous remnants after vitrectomy, suggesting that the vitreous may not support graft viability and prevents cell attachment onto the retina. The limitation of cell attachment and the need for direct contact between cell graft and the retina for cell survival and function was confirmed by findings that enriched preparations of allogeneic feline Müller glia-derived RGC attached to scaffolds of compressed collagen facilitated strong cell attachment onto the retina. In addition, transplantation significantly improved the STR and photopic negative response of the ERG, indicating an improved RGC function (Becker et al., 2016). Improvement of retinal function only by attachment onto the inner retina without evidence of cell integration leads to the suggestion that Müller glia may be conferring neuroprotection to the damaged RGCs.

Various studies have indicated that Müller glia with the ability to differentiate into cells expressing rod specific markers can be isolated from adult human retinectomy specimens and mouse retina. These cells were reported to adopt a rod photoreceptor commitment with an efficiency as high as 54% when grown under differentiating conditions, which caused an increase in their expression of NRL and CRX early in culture, followed by recoverin and rhodopsin at a later stage (Giannelli et al., 2011). Subretinal transplantation of these rod progenitors into neonatal mouse retinae were shown to integrate within the appropriate retinal lamina and to express rod-specific markers albeit without the presence of a classic rod outer segment. Functional characterization of these photoreceptor precursors by patch-clamp recordings revealed that their electrical properties were comparable to those of adult rods, providing evidence that Müller glia retain their neurogenic potential in the adult human retina (Giannelli et al., 2011). Additional investigations have shown that culture of human Müller glial cells with FGF2, taurine, retinoic acid and insulin growth factor caused these cells to differentiate into cells expressing markers of photoreceptor precursors, including CRX, Nr2E3, recoverin and rhodopsin, as well as genes associated with the photo-transduction cascade (Jayaram et al., 2014). Transplantation of Müller glia-derived photoreceptor precursors into the subretinal space of the PH23 rat, an experimental model of photoreceptor

degeneration, resulted in limited migration of these cells within the host retina. Although grafted cells did not exhibit classical outer segment morphology, they expressed synaptophysin, supporting evidence of limited synaptic connectivity. Furthermore, as judged by scotopic electroretinography, animals who received human Müller glia-derived photoreceptor precursors showed significant improvement in the rod photoreceptor function, when compared to animals who received undifferentiated cells or un-operated controls (Jayaram et al., 2014). This suggests that as that observed with RGC precursors derived from Müller glia, this effect may be ascribed to factors released by these cells. It is therefore likely that as reported in photoreceptor precursor transplantation studies (reviewed in section 4 above), transfer of cytoplasmic molecules from Müller glia to damaged neurons may be responsible for the functional improvement observed after transplantation. These observations further support the notion that human Müller glia with stem cell characteristics may be regarded not only as a cell source for the development of transplantation strategies to treat photoreceptor degenerations in humans, but also, as a cell source for derivation of neuroprotective products, offering some potential for the development of new neuroprotective therapies.

5.2. Human pluripotent stem cells (hPSC) as a source of Müller glia for therapeutic applications.

Whilst exploring the therapeutic potential of Müller glia to treat retinal degenerations, it is clear that isolation of these cells from adult retina for allogenic transplantation presents major difficulties for clinical application due to the risks of disease transmission caused by prions and unidentified pathogens, as well as histocompatibility issues. hPSC, however, can help to overcome these issues as any progeny derived from PSC generated for therapeutic use under GMP conditions could be traced to the original cells, thus complying with regulatory requirements for clinical use. In addition, there have been suggestions amongst the scientific community of establishing a global network of haplobanks of hESC (Taylor et al., 2005) and iPSC (Taylor et al., 2012), which would facilitate HLA-type matching between the recipient and a cell line used for potential therapy. Various hESC and hiPSC lines that comply with regulatory requirements for human use have become recently available and this will facilitate the derivation of cells for retinal therapies (De Sousa et al., 2016a, McGrath et al., 2019). The generation of retinal organoids for use in the development of

cell transplantation therapies, as well as pharmacological or toxicological studies, would ideally require these organoids to be laminated, contain solely retinal cells and be physiologically and metabolically functional. Recent studies have supported this requirement and have indeed shown that in retinal organoids, RGC (Hallam et al., 2018) and cone photoreceptors (Kim et al., 2019) demonstrate electro-physiological responses to light. This supports the use of cells derived from retinal organoids as a valuable tool that could be explored in the development of retinal stem cell therapies, and could be facilitated by new approaches, such as bioreactors, that would allow upscaling of retinal organoids or cells derived from such sources. Using this technology, investigators have not only shown a significant increase in organoid yields but also increases in the number of photoreceptors neurons bearing cilia and nascent outer-segment-like structures (Ovando-Roche et al., 2018).

Upon culture with retinal differentiation factors, PSC can be induced to adopt a retinal fate, and different methods for the generation of retinal tissue from hPSCs have been developed by investigators in the field. Initial studies led to protocols that produced free-floating hESC aggregates to create 2D like structures which were then exposed to various growth and differentiation factors such as Wnt antagonists, retinoic acid and taurine, to induce differentiation of retinal progenitor cells, retinal pigment epithelium and at later stages photoreceptor differentiation (Osakada et al., 2008). Other reports showed that upon stimulation with a neural induction medium, hESC aggregates generated rosette like structures that could be further stimulated by addition of B27 supplement to encourage neural retinal differentiation (Meyer et al., 2009). Although this method induced the generation of various types of retinal cells, the resulting cellular assembly did not show evidence for the presence of Müller glia, amacrine, horizontal or ganglion cells (Meyer et al., 2009). Methods to develop a cellular structure that more closely resembles the cellular organization of the retina *in vivo* were later established by inducing mouse ESC to produce 3D like retinal structures, which are now known as 'retinal organoids'. A reliable method first developed by Nakano et al, induced a defined number of hESCs to form aggregates in a v-bottomed well plate, which were then cultured with extracellular matrix, Wnt signalling antagonists, and Hedgehog agonists to induce early neural differentiation. Aggregates were then cultured with N2 supplement and retinoic acid to encourage photoreceptor

development (Nakano et al., 2012). This led to the generation of retinal organoids that undergo self-organisation into a laminated retina and that contains rod and cone photoreceptors, RPE, ganglion cells, horizontal cells, amacrine cells and Müller glia (Nakano et al., 2012), and that closely recapitulates the development of neural retina *in vivo* (Völkner et al., 2016). Since this method was pioneered, selective modifications of the protocol have favoured differentiation of specific neuronal cell types, and although retinal organoid research for generating neural cells for transplantation appears to have been initially weighted towards the generation of high photoreceptor numbers (Eldred et al., 2018, Kim et al., 2019), recent studies have developed protocols for the derivation of RGC for potential treatments of glaucoma and optic neuropathies (Fligor et al., 2018, Chavali et al., 2020).

Müller glia have been reported to be one of the last-born cells during the 'postnatal development' of the vertebrate retina *in vivo* (Turner and Cepko, 1987, Bassett and Wallace, 2012, Clark et al., 2019) and to share the same lineage with neurons, with multipotent retinal progenitors giving rise to both cell types (Turner and Cepko, 1987). It is important to note that the study from Turner and Cepko (1987), which concluded that Müller cells were the last cells to emerge in the retina, only examined the proliferation of retinal neurons and Müller glia immediately after birth. Since the retina has already developed at the time of birth, tracing the proliferation of retinal cells in the early postnatal period does not prove that Müller glia are the last cells to be born, and this needs to be reassessed in the context of Müller functions and retinal regeneration. Although extensive work has been so far undertaken *in vitro* towards the generation of photoreceptors from retinal organoids, very few studies have explored the development and role of Müller glia within these organoids. A recent single cell RNA sequencing analysis showed the resemblance of human retinal organoids with retinal development *in vivo*. The study showed that a progenitor population gives rise to RGCs first, followed by cones, rods and finally Müller glia, which was reported to be first observed at around day 60 of organoid development, followed by an increase at day 200 (Collin et al., 2019). Other studies have also reported the appearance of Müller glia at late stages of organoid development (Chen et al., 2016, Fligor et al., 2018). Contrasting with previous reports of the late appearance of Müller cells during retinal organoid growth, recent data have shown that Müller glia are observed as early as day 30-40 in hPSC derived retinal organoids and that these cells can also be isolated and propagated at this early stage of

organoid differentiation (Eastlake et al., 2019), thus highlighting the early appearance of human Müller glia during retinal development *in vitro* (Fig. 3). These observations are more in agreement with early studies of retinal development in vertebrates published in the 1900s, which showed that immediately after the invagination of the primary optic vesicle to form the optic cup, formation of Müller glia could be clearly observed in amphibians and chicks (Mann, 1928). Later electron microscopy studies of the rabbit retina during development clearly demonstrated that whilst Muller glia are formed during the fourteenth day of gestation, morphological changes occur after birth, including changes in the elongation of microvillous processes, lateral cytoplasmic extensions, and rearrangement of their nuclei (Uga and Smelser, 1973). It is the authors' view, that despite the current contradictory evidence on the timing of Müller appearance within the organoid, it is evident that there remain some mitotically active Müller cells in the developed retina and this is observed in the retinal organoids at late stages of development *in vitro.*

5.3 Derivation and transplantation of Müller glia from retinal organoids formed by hPSC

High quality retinal organoid production gives rise to specimens which display a distinctive retinal morphology with Müller glia expanding across the neural layers as that seen *in vivo.* Optimization of this generation process *in vitro* has been recently demonstrated (Eastlake et al., 2019). This has facilitated the isolation of pure populations of Müller glia that can be expanded in culture over several passages, and exhibit a characteristic phenotypic and genotypic profile that are comparable to published Müller cell lines derived from human cadaveric donors, including the established human MIO-M1 Müller cell line (Limb et al., 2002). Capitalizing on the Müller cell expression of CD29, a β1 integrin receptor and ligand of fibronectin, cells dissociated from retinal organoids, were adhered onto culture plates coated with fibronectin to selectively enrich Müller glia. Following propagation in standard culture medium, adherent cells were shown to express mRNA and protein coding for well-known Müller glia markers including glutamine synthetase, nestin, and vimentin, as well as neural progenitor markers including Notch1, Pax6 and Sox 9. Flow cytometry analysis of the CD29/CD44 positive population showed a Müller glia purity >97%, which indicated the purity of the cell preparations, therefore avoiding the need for further purification. This straightforward selection of enriched Müller cell populations by selective culture on fibronectin may also be aided by the fact that retinal neurons do not survive for long periods

in culture without the addition of factors to promote their survival (Meyer-Franke et al., 1995, Bardy et al., 2015). Based on these observations that Müller glia can be easily isolated from human PSC-derived retinal organoids, and that they can be propagated *in vitro* whilst conserving their phenotypic and gene profiles, the use of these cells in cell based therapies to treat retinal disease provides an attractive option.

Intravitreal injection of Müller glia isolated from hPSC- derived retinal organoids has been performed in a rat model of RGC depletion by NMDA (Eastlake et al., 2019). As previously observed when Müller isolated from the adult human retina were transplanted into this model, the results showed that at 4 weeks after intravitreal transplantation of these cells into the RGC depleted rat, there was a partial but significant restoration of visual function in these animals, as demonstrated by an increase in the nSTR of the ERG (Eastlake et al., 2019). Although the transplanted cells appeared to have strongly adhered to the inner retinal surface, there was no evidence of these cells establishing synaptic connections or sending axon projections towards the optic nerve, suggesting that like in previous transplantation studies using Müller glia, the effect was due to the release of neuroprotective factors by these cells. This is in agreement with previous demonstrations of RGC cell survival upon the influence of neurotrophic factors (Goldberg et al., 2002).

6. Müller glia as a source of neuroprotective factors to promote neuronal survival and repair

Observations that partial restoration of visual function upon Müller cell transplantation in animal models of retina degeneration can be achieved without significant integration of these cells or replacement of affected neurons (Becker et al., 2016, Eastlake et al., 2019, Jayaram et al., 2014) suggest that the synthesis, release, and delivery of neuroprotective molecules by Müller glia should be considered key to their efficacy for the development of human therapies. Due to their strategic location and functions within the retina, Müller cells regulate most aspects of retinal function and neuronal activity. Because of their close interactions with all retinal neurons in the retina (MacDonald et al., 2015, de Melo Reis et al., 2008), it is likely that the production and release of neurotrophic factors is a major component of the support and regulation that Müller glial cells provide to the neural retina. One of the well-established mechanisms underlying the pathogenesis of RGC loss in

glaucoma is the breakdown in transport of neurotrophic factors along axons at the optic nerve head (Knox et al., 2007, Fahy et al., 2016). The dimensions of RGCs are such that axons are limited in terms of proteins and organelles required for cell survival and metabolic balance (Nuschke et al., 2015). As a result, the majority of these components are manufactured in the cell body and transported in an anterograde manner towards the distal synapse by a series of kinesin-dependent mechanisms. Meanwhile, retrograde transport allows for the clearance of waste metabolites and misfolded or aggregated proteins, as well as the delivery of trophic signals from the distal axon to the cell body. Available evidence shows that blockage and obstruction of axonal transport by ischaemia or axotomy in experimental animals halts axon transport of Brain derived growth factor (BDNF) and its receptor, TrkB, leading to RGC death (Iwabe et al., 2007, Chou et al., 2013). Additional support for the protective effect of neurotrophin supplementation in animal models of glaucoma has been widely given by demonstrations that BDNF, Ciliary neurotrophic factor (CNTF) and Glial cell derived neurotrophic factor (GDNF), significantly attenuate the degeneration of RGC following optic nerve damage (Yan et al., 1999, Johnson et al., 2011). Strong indication of the paracrine neurotrophic signalling mediated by Müller-glia has been provided by *in vitro* studies in which RGC from adult pig retinae co-cultured with Müller glia significantly enhance their survival and axonal outgrowth (García et al., 2002). In these studies, the neuroprotective effect observed appeared to be mediated through both, direct cell-to-cell contact, as well as by soluble factors released by Müller cells. In similar studies, purified RGCs co-cultured with Müller glia were found to be resistant to the excitotoxic effect of glutamate, even when there was no direct contact between the two cell types (Kawasaki et al., 2000). This neuroprotective ability of Müller glia has been also demonstrated *in vivo* as confirmed by studies in which conditioned medium from purified chick Müller cells obtained at embryonic day 11, supports the survival of chick embryonic day 10 sympathetic neurons (Reis et al., 2002). In addition, Müller glia derived neurotrophins have been shown to play an important role during retinal development and maturation of RGC, as demonstrated by the increased survival of rat RGC isolated from 17-day embryos when cultured with supernatants of Müller glia (Armson et al., 1987). Furthermore, evidence for the dependence of RGCs on neurotrophic factors is evident during optic nerve development, where RGC extend neurites along the visual pathway towards neurotrophin-secreted targets in the brain (Meyer-Franke et al., 1995). Although

Müller glia supernatants can promote survival of neonatal rat RGCs, extensive neurite outgrowth from RGC have been observed when they are cultured on glial monolayers (Raju and Bennett, 1986).

As that seen with RGC, photoreceptor neurons are also dependent on the neurotrophic and metabolic support of Müller glia, as shown by several studies in experimental models of inherited retinal degenerations, known to cause blindness due to photoreceptor apoptosis. Investigations in various experimental models of photoreceptor degeneration have shown that neurotrophins promote the survival and function of these neurons (Abed et al., 2015). Intraocular injection of Adeno-associated virus expressing the CNTF gene was seen to significantly reduce the loss of photoreceptors in normal mice and in the Prph2Rd2/Rd2 mouse model of retinal degeneration (Schlichtenbrede et al., 2003), whilst RCS rat eyes treated with adenoviral-CNTF displayed better retinal function than the untreated contralateral eyes, indicating that adenoviral CNTF efficiently rescues degenerating photoreceptors in this model (Huang et al., 2004). Furthermore, Simian lentiviral vector-mediated retinal gene transfer of PEDF showed to preserve the photoreceptor cell layer and retinal function in the dystrophic Royal College of Surgeons rat, as determined by immuno-histological studies of the retina at the site of injection (Miyazaki et al., 2003). Furthermore, transplantation of Schwann cell line clones secreting GDNF or BDNF into the retinas of RCS rats was shown to have a greater effect on photoreceptor survival than control cells or sham surgery (Lawrence et al., 2004).

Several observations have highlighted the importance of neurotrophin production by Müller glia as a key feature of the neuroprotective role of these cells in the neural retina. It has been suggested that Müller glia may act as an endogenous source of BDNF in the retina, as they readily secrete high levels of this factor in culture (Seki et al., 2005). Interestingly, following visual deprivation in rats, BDNF immunoreactivity is significantly diminished in RGC, but not in Müller cells, which remain unaffected (Seki et al., 2003). This suggests that Müller glia, which are highly active metabolically, produce this factor to protect retinal neurons. Rat Müller glia express nerve growth factor (NGF) mRNA and its corresponding protein, as well as the receptors for this neurotrophin (Chakrabarti et al., 1990, Garcia et al., 2014). In addition to producing NGF, Müller cells can also be protected by this neurotrophin

as demonstrated by observations that binding of NGF to its TrkA receptors inhibit their osmotic swelling, causing release of bFGF, and opening of K^+ and Cl^- channels (Garcia et al, 2014). Glial derived neurotrophic factor (GDNF), which is not only produced by Müller glia but also by astrocytes within the retina, promotes photoreceptor cell survival during retinal degeneration (Harada et al., 2003), whilst neurotrophin-3 (NT-3), which has also been detected in Müller cells (García et al., 2003), is highly upregulated in these cells together with neurotrophin-4 (NT-4), BDNF, NGF, and GDNF in response to glutamate (Taylor et al., 2003).

Different approaches investigating the direct delivery of neurotrophins to the retina *in vivo* have been evaluated. Intravitreal injection of recombinant GDNF, BDNF and CNTF have all proved particularly efficient at promoting RGC survival following axotomy (Koeberle and Ball, 2002), as has both viral and non-viral vector-based gene delivery (Rodger et al., 2012, Wilson and Di Polo, 2012). A strategy involving encapsulation of cells engineered to over-express CNTF within semipermeable biomaterials has also been used to facilitate sustained transportation of these therapeutic molecules into the vitreous cavity (Zanin et al., 2012). However, despite some encouraging results, injection of neurotrophins have proved to be short lived, for which there are still challenges to overcome before neurotrophins can be therapeutically delivered directly into the eye to enhance or promote neural cell survival during retinal degeneration. Stem cells delivered into the vitreous as a source of neuroprotective molecules have also been investigated, and have included mesenchymal stem cells (MSCs) (Johnson et al., 2010, Mead et al., 2016) to promote RGC survival in rat models of glaucoma, and umbilical tissue-derived stem cells to preserve photoreceptor survival and visual function in the royal college of surgeons (RCS) rat (Koh et al., 2018). Gene-engineering based approaches using MSCs aimed at enhancing their production of BDNF, GDNF and VEGF to protect the neural retina after optic nerve transection have also been developed (Levkovitch-Verbin et al., 2010). Although these modified MSCs have shown to promote neuronal survival, no significant difference in their efficacy have been observed when compared to wild-type MSCs secreting low levels of these factors, suggesting that even small amounts of neurotrophic factors can be neuroprotective when present in combination. In this context, a Müller cell transplant-based strategy may offer a good therapeutic alternative since the neuroprotective signals produced by these cells are many and varied,

and may not only include neurotrophins, but also antioxidants and neuroprotective cytokines (Eastlake et al., 2018, Grosche et al., 2016). Although there is evidence that Müller glia secrete BDNF, NGF, NT-3, NT-4, and GDNF, as well as CNTF, identification of the precise combination of neurotrophins responsible for the therapeutic effect of these cells upon transplantation is not known. A recent study on the proteomic content of supernatants from porcine Müller glia has identified two proteins, osteopontin and basigin which showed to significantly enhance RGC survival *in vitro* (Ruzafa et al., 2018), suggesting that Müller glia may potentially produce many neuroprotective factors not yet identified.

7. Potential neuroprotective role of extracellular vesicles (EVs) released by Müller glia

Considering the extensive neuroprotective functions exerted by Müller cells within the neural retina, these effects could not only be attributed to their release of neurotrophic factors, which by nature can be short-lived, but potentially, to the release of exosomes controlling neuronal cell functions such as apoptosis and survival. These secreted cell nano-organelles are believed to mediate the paracrine benefits of stem cell transplantation and have recently elicited much research interest. Although previously considered a cellular waste-disposal system, secreted EVs comprising of cytosol enclosed within a lipid bilayer membrane have been identified as a previously underappreciated mode of intercellular communication. Nano-sized EVs are released by all types of eukaryotic cells, and contain protein and nucleic acid cargos that are capable of initiating phenotypic changes when internalised by recipient cells within a given tissue (Valadi et al., 2007, Yuan et al., 2017). Generally, EVs are produced either through blebbing of the external cellular membrane (often called “microvesicles”), or through inward budding of the endosomal membrane, which produces vesicle filled compartments known as multi-vesiculated bodies. These ultimately fuse with the cell membrane, releasing their vesicle contents (commonly referred to as “exosomes”) into the extracellular environment (Théry et al., 2002). It has been proposed that EV-mediated communication represents a hitherto underappreciated mode of glial cell function in the retina. The recognition of the importance of EVs for developing therapeutic modalities can be traced back to a 1996 publication showing that vesicles derived from the endocytic compartments of B lymphocytes presented MHC class II molecules and were capable of inducing an immune response (Raposo et al., 1996). Shortly afterwards, the first evidence of EVs as a potential therapeutic agent was published, showing that vesicles derived from

dendritic cells and presenting MHC I and II proteins were capable of priming cytotoxic T lymphocytes for the growth inhibition of murine tumours in vivo (Zitvogel et al., 1998). These studies led to the first phase I clinical trials of EV-based therapeutics in 2005, where metastatic melanoma patients received four doses of autologous dendritic cell-derived EVs, but unfortunately, no clinical outcomes were reported (Escudier et al., 2005). EVs have been increasingly explored as potential therapeutic agents not only for tissue regeneration, but also for immune modulation and as potential alternatives to stem cell therapies (Wiklander et al., 2019). Although some studies have explored their use for experimental treatment of retinal degenerative disease, no clinical trials arising from these studies have yet emerged. In the broader CNS field, a growing body of literature reports functional regulation of microglia by EVs secreted by neurons, as illustrated by observations that neuron-derived EVs can facilitate removal of degenerating neurites, by inducing up-regulation of the C3 complement factor in microglia (Bahrini et al., 2015), whilst neuronal EVs containing amyloid-β have can be internalised by microglia for degradation (Yuyama et al., 2012) . Our most recent data shows that Müller glia in culture secrete large number of EVs into their supernatants, which can be fractioned by ultracentrifugation to yield enriched preparations of small (sEVs), measuring 50-250 nm diameter, and large (lEVs), measuring 200-600nm diameter (Fig.4). Whereas lEVs were shown to contain small amounts of protein and RNAs coding for the neurotrophins BDNF, NGF and GDNF, sEVs mainly contained miRNAs (Fig.5) Unpublished data from our laboratory indicates that EVs released by Müller glia can be readily internalised by both neural, and non-neural cells in primary retinal cell cultures, supporting their role as a mediator of neuron-glia communication in the retina.

Key to the potential neuroprotective functions of EVs are observations that their molecular cargos are reflective of their cellular origin, and stem cell derived EVs appear to represent a significant component of the paracrine neuroprotective effect of these cells. Data have started to emerge on the evaluation of the potential of stem cells derived EVs as a stand-alone therapy, or as an adjunct to cell transplantation (Kim and Kim, 2019). Bone marrow mesenchymal stem cell (BMSC)- derived small EVs pre-loaded with fluorescent label have been shown to deliver their cargo to RGCs following transplantation into the vitreous, as demonstrated by detection of the fluorescent signal in the nerve fibre layer (Mead and Tomarev, 2017). Furthermore, this study showed that EVs conferred significant

neuroprotection and preservation of RGC function in the rat following optic nerve crush, and interestingly, no neuroprotective benefit was observed when EVs produced by fibroblasts were substituted by EVs produced by BMSC, suggesting that the therapeutic benefits observed were related to specific molecules contained within BMSC, rather than the action of EV internalisation. These observations have been confirmed by similar studies by others which show that transplantation of small EVs derived from umbilical cord MSC results in improved survival of RGCs after optic nerve crush in the rat (Pan et al., 2019). Surprisingly, a study utilizing fibroblast EVs in an *in vitro* experiment conducted on cortical neurons did report significant axon regeneration, although no neuroprotective effect was specifically identified (Tassew et al., 2017). In an effort to more closely examine the protective effect of EVs during glaucomatous RGC damage, a recent study administered BMSC- derived EVs into the vitreous of rats undergoing IOP elevation, reporting significant neuroprotection of RGC while preventing degenerative thinning of the nerve fibre layer (Mead et al., 2018b). The same effect was observed when analogous experiments were conducted in the DBA/2J mouse model of glaucoma, in which multiple injections of EVs preserved RGC numbers and reduced axonal degeneration (Mead et al., 2018a). Investigations on the use of EVs as a potential therapeutic tool to treat retinal diseases have also been undertaken in experimental model of photoreceptor degeneration, and studies have shown that injection of mouse neural progenitor cell-derived EVs delayed photoreceptor degeneration in the RCS rat, whilst preserving visual function and decreasing photoreceptor apoptosis (Bian et al., 2020). In addition, intravenous injection of MSC-derived EVs in a mice model of autoimmune disease was shown to effectively prevent the onset of experimental autoimmune uveitis (Shigemoto-Kuroda et al., 2017).

7.1 Neuroprotective ability of micro-RNAs contained in EVs

EVs are well recognized to act as neuroprotective cargos (Lemaire et al., 2019), and the most well studied molecules in these organelles are miRNAs (Liu et al., 2019). These are small regulatory RNAs that are processed from stem-loop regions of longer RNA transcripts and have distinctive but varied expression patterns. They post-transcriptionally control gene expression via binding to complementary sequences in target mRNAs, leading to translational inhibition or degradation (Hwang and Mendell, 2006, Li et al., 2017, Cai et al., 2009). Within the miRNA pathway, primary miRNA (pri-miRNAs) transcripts are cleaved by a

microprocessor complex composed of the ribonuclease III enzyme 'Drosha', and its co-factor 'DiGeorge syndrome critical region gene 8' (DGCR8). The processed products, called precursor miRNAs (pre-miRNAs), are transferred into the cytoplasm, where the pre-miRNA stem-loop is processed by another RNase III, known as 'Dicer', generating the mature miRNA. Mature miRNAs form the RNA-induced silencing complex (miRISC) with the AGO2 protein and other proteins, which is capable of recognising and repressing target gene expression (Kobayashi and Tomari, 2016).

A number of studies have been aimed at establishing the function and expression profile of specific miRNAs in the retina and the CNS (Andreeva and Cooper, 2014), and there is evidence that miR24a is a regulator of the pro-apoptotic factors caspase9 and apaf1 and is required for correct eye morphogenesis in Xenopus. Inhibition of miR-24a during development causes a reduction in eye size due to a significant increase in apoptosis in the developing neural retina (Walker and Harland, 2009), whilst knockout of the miR-183/96/182 cluster in mice leads to retinal degeneration (Lumayag et al., 2013). We have recently sequenced the microRNA profile of sEVs released from Müller glia cells in culture (manuscript in preparation) and have observed that the master inhibitor of the AKT/mTOR pathway phosphatase and tensin homolog (PTEN) was the target of several of the most abundant miRNA transcripts identified in our EV libraries: miR-21-5p, miR-148a-3p, miR-221-3p, and miR-29b-3p (Fig, 5). Therefore, from current evidence and our data it may be possible to suggest that miRNAs delivered by EVs released by Müller glia may be responsible for regulating the well-known functions of these cells within the retina.

7.2 microRNAs and retinal degeneration

It has been hypothesized that EV-miRNA may target genes associated with either the initiation of apoptosis, or signal transduction pathways governing cell growth and survival. Studies have shown that intravitreal delivery of miR-141-3P into a mouse model of glutamate-induced excitotoxicity resulted in a significant increase in RGC survival. This effect was attributed to miR-141-3p inhibition of the apoptotic signalling pathways Bax and caspase-3 via targeting of docking protein 5 (Zhang et al., 2019). Similarly, it has been recognised that miR-93-5p is downregulated in the retina following injection of NMDA, and that delivery of the same miRNA to RGCs in culture protects these neurons from NMDA-induced cell death (Li et al., 2018). Furthermore, retinal miR-200a has been shown to be significantly downregulated in a

mouse model of glaucoma, whilst retinal supplementation with a miR-200a mimic preserved the thickness of the nerve fibre layer, as an indication of providing significant neuroprotection to RGCs (Pan et al., 2019). Since knockdown or silencing of PTEN is known to initiate robust regeneration of RGCs in experimental models (Li et al., 2018, Li et al., 2015, Mak et al., 2020) it is possible that a proportion of the efficacy of Müller cell extracellular signalling could be attributed to these miRNAs and needs further investigations.

Although it is not clear how long EVs remain in the extracellular compartment after administration into biological tissues, a potential advantage is their use as vehicles for the transfer of therapeutic molecules including miRNAs, aided by the fact that their bilayered lipid membrane is able to protect their cargo from degradation. While retinal therapies based upon direct delivery of neurotrophic factors, or RNA-based molecules like siRNA to the vitreous have been shown to have a beneficial response, they are often characterized by rapid clearance and degradation and require frequent repeat injections which are poorly tolerated (Del Amo et al., 2017). On this basis, it is possible that EVs may be more effective in modifying cell functions if they deliver specific mRNA or miRNAs that can modify the recipient cell protein production and gene expression (Valadi et al., 2007).

In the experimental glaucoma field, studies have demonstrated that exosomes derived from BMSC are able to protect RGCs from death in a mice model of glaucoma. In addition, intravitreal injection of EVs isolated from BMSCs into a rat model of optic nerve crush, causes a reduction in the thinning of the nerve fibre layer and promote significant neuroprotection (Mead and Tomarev, 2017). Other reports have shown that knockdown of Argonaute-2 (AGO2), a protein critical for miRNA packaging and function, significantly attenuate the neuroprotective effects of BMSCs in the same glaucoma model, suggesting that RGC neuroprotection conferred by EVs may be likely to be mediated by miRNAs (Mead et al., 2018b). Given that Müller glia are the structural cell of the neural retina, and in view of their multiple metabolic, neuroprotective and regenerative potential, it is plausible that miRNAs produced by these cells play an important role in the mediation of these functions. Various miRNAs have been shown to mediate metabolic and survival functions in retinal neurons and Müller glia (Zuzic et al., 2019), and elucidating the miRNA components of Müller derived EVs would be important for their potential therapeutic applications.

8. Müller glia as a target to promote endogenous regeneration of the human retina

Experimental transplantation of retinal neurons or Müller glia derived from adult retina or PSC has been primarily explored as a therapeutic tool to treat retinal degenerative diseases. However, evidence presented in recent years that Müller glia in the mammalian eye exhibit neurogenic potential, opens new avenues to develop new retinal therapies based on the activation of endogenous repair mechanisms. When considering the need for large and costly resources to establish cell transplantation therapies as a treatment for people affected by retinal diseases, inducing self-repair by intraocular injections would constitute a preferable option by patients and health care systems.

Whilst the endogenous regenerative ability of Müller glia in the zebrafish retina is thought to be conserved throughout life (Hitchcock et al., 2004), in chicken (Fischer and Reh, 2001) and other lower vertebrates regeneration occurs mainly in early postnatal life (Moshiri and Reh, 2004, Löffler et al., 2015). Interestingly, it has been shown that the endogenous Müller cell regenerative ability is more limited in young Xenopus when compared with older animals, perhaps suggesting variabilities amongst species (Langhe et al., 2017). In the adult Royal College of Surgeons (RCS) rat, an animal model of retinitis pigmentosa, it was observed that significantly more proliferating cell in the marginal retinal region of RCS are found *in vivo* from birth until post-natal day 90 (PN90), when compared with wild type Long-Evan's rats (Jian et al., 2009). In addition, the proportion of cells co-expressing vimentin and Chx-10 in these rats significantly increased at PN30 whilst gradually decreasing from PND3 to PND15. These observations further support the view that proliferating marginal regions in the mammalian retina have the potential to regenerate retinal neurons upon degeneration (Jian et al., 2009). In this context, it is of special interest that the ciliary epithelium in the retinal margin of adult primates has been shown to preserve some neurogenic potential (Fischer et al., 2001), and that a ciliary margin-like zone harbouring cells with neural progenitors markers has been identified in the adult human retina (Bhatia et al., 2009). However, until recently, these observations had not been considered to have any functional significance for the promotion of endogenous retinal regeneration in the adult mammalian eye, but with additional knowledge gained during recent years on the potential neurogenic

ability of adult Müller glia, more research in this field is now being pursued by several groups.

Unlike that seen in fish and amphibians, the adult mammalian retina does not readily support neurogenesis, for which various strategies to induce *in situ* proliferation and neural differentiation of retinal Müller glia have been investigated. Studies have indicated that EGF receptor (EGFR) is necessary for the maintenance of normal levels of progenitors and Müller glia in mice *in vivo*, as confirmed by observations that deletion of EGFR decreases cell proliferation in late retinal neurogenesis. In addition, EGF-induced proliferation observed in early postnatal life is reduced to two weeks after birth and correlates with the reduction of this receptor. Nevertheless, light induced damage to the retina in these animals reverts the decline in EGFR expression, suggesting that retinal injury can promote neurogenesis in the developed eye (Close et al., 2006). Several studies in the adult rat retina have demonstrated that Müller glia can be stimulated to proliferate in response to toxic injury and produce bipolar cells and rod photoreceptors (Osakada et al., 2007, Wan et al., 2008, Das et al., 2006, Wan et al., 2007). Furthermore, treatment of NMDA treated Sprague–Dawley rats with retinoic acid (RA) or upregulation by viral transfection of basic helix-loop-helix and homeobox, Math3, NeuroD and Pax6 or Crx and NeuroD1 genes, revealed an increase in the number of proliferating Müller cells expressing markers of bipolar, amacrine cells, and rod photoreceptor cells (Ooto et al., 2004). This ability of Müller glia to re-acquire their neurogenic capacity has been also observed in cells isolated from the adult rat retina, that when cultured *in vitro* with neurotoxins in the presence of FGF2 and insulin, they proliferate and express markers of retinal neurons as well as of neural progenitors, including Sox2, Nestin and Musashi1 (Das et al., 2006). Importantly, this effect has been attributed to the activation of the Notch and Wnt pathways. Confirmation of the involvement of Wnt signalling in the activation of the regenerative ability of Müller glia was shown in later studies which demonstrated that Wnt (wingless-type MMTV integration site family)/beta-catenin signalling promotes proliferation of Müller glia-derived retinal progenitors and neural regeneration upon retinal damage in adult rats and mice (Osakada et al., 2007). In these studies Wnt3a treatment was shown to increase proliferation and dedifferentiation of Müller glia in the photoreceptor-damaged mouse retina, whilst retinoic acid supplementation induced differentiation of these cells into Crx (cone rod homeobox)-

positive and rhodopsin-positive photoreceptors. This was accompanied by nuclear accumulation of beta-catenin, cyclin D1 upregulation, and Wnt/beta-catenin reporter activity (Osakada et al., 2007). The involvement of Sonic hedgehog (Shh) in neurogenesis mediated by Müller glia has also been highlighted by observations that induction of Shh signalling in rat Müller glia in culture, causes these cells to proliferate and dedifferentiate by expressing neural progenitor-specific markers, followed by the adoption of the rod photoreceptor cell fate (Wan et al., 2007). Other investigations into the potential of Müller glia to generate new neurons in the mouse retina depleted of RGC and amacrine cells, showed that in response to EGF and FGF, Müller glia not only proliferated *in situ*, but also dedifferentiated into amacrine cells, as defined by the expression of amacrine cell-specific markers Calretinin, NeuN, Prox1, and GAD67-GFP (Karl et al., 2008). In adult mice, Müller glia overexpression of the progenitor marker Ascl1 was shown to promote the transition of these cells to a neurogenic state upon cytotoxic damage with NMDA. In the belief that epigenetic factors limit retinal regeneration by Müller glia, this process was further facilitated by treatment with a histone deacetylase inhibitor, which enabled Müller glia to generate new neurons expressing markers of inner retinal neurons, synapsing with host retinal neurons, and responding to light (Jorstad et al., 2017).

In humans, despite the retina harbouring Müller glia with stem cell characteristics (Lawrence et al., 2007, Bhatia et al., 2009), there is no evidence that regeneration occurs after disease or injury. Because the physiological functions of Müller glia are well conserved throughout species, it is conceivable that Müller glia could be induced to proliferate and differentiate *in situ* to repair the degenerated retina. It is also important to recapitulate the features of neurogenesis that may help us to understand developmental signals that ceased to operate upon retinal maturation. To be able to identify molecules that could be activated to promote endogenous regeneration of the human retina, we need to consider the complex factor interaction that governs retinal neurogenesis in lower vertebrates. A combination of cell-cell signalling, growth factor expression and transcription factor regulation is required for retinal progenitor differentiation and the promotion of glial cell fate during vertebrate development (Gao et al., 2021, Langhe et al., 2017). Therefore, a better understanding of these signals may aid in the identification of molecules that could be used to target the promotion or suppression of the regenerative ability of Müller glia,

and this constitutes a major subject of research for the developing of retinal regenerative therapies.

It has been suggested that the neurogenic property of Müller cells is evolutionarily conserved and that it may be activated for the regeneration of the mammalian retina (Del Debbio et al., 2010). This is supported by evidence that a number of signalling factors known to play an important role during retinal development, can be reactivated to generate new neurons in the mature retina of both mammalian and lower vertebrate species. On this account, Notch and Wnt signalling has been shown to activate Müller cells in the postnatal rat and mice retina and to induce their differentiation along the rod photoreceptor lineage in the outer nuclear layer (Del Debbio et al., 2010). Notch signalling has been shown to facilitate re-entry of postnatal rat Müller glia into the cell cycle by inhibiting p27Kip1 expression transcriptionally and post-translationally (Del Debbio et al., 2016). In the zebrafish, it has been demonstrated that signalling by each of the four Notch proteins expressed by Müller glia function in a complex manner during retinal regeneration, and that each of these proteins specifically regulates the expression of different downstream targets (Campbell et al., 2021). This was suggested by observations that downregulation of Notch3 in response to light damage, is accompanied by Müller glia proliferation, whilst specific knockdown of Notch1a/b or Notch2 reduces the number of proliferating Müller glia in the light injured retina, suggesting that Notch signalling is also required for proliferation during retinal regeneration (Campbell et al., 2021).

Other transcription factors of neural progenitors known to promote Müller glia cell survival in the mature retina include Sox9 and Sox2. Sox9 is essential for the differentiation and survival of postnatal Müller glial cells, persists into adulthood, and is downregulated in these cells upon their neural differentiation (Poche et al., 2008). Sox2 is expressed by Müller glia and a subset of amacrine cells within the inner nuclear layer of the mature mammalian (Lin et al., 2009) and zebrafish retina (Gorsuch et al., 2017). Studies in transgenic mice indicate that Sox2 expression in the postnatal retina is required to maintain Müller glia quiescence and to prevent cell cycle exit, whilst ablation of Sox2 in these animals causes aberrant Müller glia morphology and laminar disorganisation, leading to retinal degeneration accompanied by a downregulation of the glial markers Sox9 and Pax6 (Surzenko et al.,

2013). The involvement of Sox2 in the proliferation and neural differentiation of Müller glia in the mature retina has been further confirmed in the zebrafish, in which loss of Sox2 expression prevents complete regeneration of cone photoreceptors (Gorsuch et al., 2017). Based on the above evidence that neurogenic genes expressed by Müller glia are necessary for the postnatal proliferation and neurogenic ability of these cells in lower vertebrates, it is of special importance that most of these factors have been identified in foetal human retina (Schmitt et al., 2009) as well as in Müller cells isolated from the adult human eye (Lawrence et al., 2007), thus supporting the belief that these cells could potentially be reactivated to promote neurogenesis in the degenerated human retina.

In contrast to observations that in response to injury Müller glia in the adult zebrafish undergo 'de-differentiation'- characterized by downregulation of markers and upregulation of retinal progenitor genes-, Müller glia isolated from the adult human eye, do not show evidence of 'de-differentiation'. However, upon isolation from the retina, they co-express markers of mature Müller cells as well as markers of neural progenitor cells, including Sox2, Pax6, Chx10, Notch and Nestin. These characteristic markers can be observed is situ in sections of cadaveric retina, and are maintained indefinitely under normal culture conditions in the absence of growth or differentiation factors (Lawrence et al., 2007, Bhatia et al., 2009). Significantly, and of relevance to the potential regenerative ability of the human retina, cells present in the anterior-non laminated retina (referred to as ciliary margin-like zone), as well in the inner nuclear layer of the laminated retina (where the soma of Müller glia reside), proliferate in response to EGF (Bhatia et al., 2009) (Fig. 6). In addition, Müller glia that express neural progenitor markers, form neurospheres when cultured in the presence of FGF2, resembling the neurospheres formed by Müller glia with progenitor characteristics in other mammalian species (Das et al., 2006). Müller glia derived from adult donor retina have been shown to differentiate and express markers of RGC precursors *in vitro* upon culture with FGF2 and gamma-secretase inhibitors (Singhal et al., 2012), whereas culture with FGF2, taurine, retinoic acid and IGF has shown to promote their expression of markers of photoreceptor precursors (Jayaram et al., 2014), suggestive of a neurogenic potential of Müller glia in the adult human retina. Given the similarities between neural progenitors and Müller glia in lower vertebrate species, and the presence in the adult human retina of Müller glia with stem cell/progenitor characteristics, it is highly conceivable

that the quiescent neurogenic potential of these cells can be activated to induce endogenous retinal regeneration. Current knowledge on human retinal development derives from anatomical and histological examinations of foetal and adult cadaveric retina, and although attempts to recreate human retina development using PSC-derived retinal organoids may give us some clues on potential approaches to induce endogenous regeneration, much research will be needed before such therapies can be established.

It is important to consider that in a similar fashion to the mature retina of lower mammals, human retina respond to injury by reactive gliosis. This is characterized by biochemical and physiological changes occurring in the retina that often lead to Müller glia proliferation and hypertrophy and is a feature of many neuro-degenerative and inflammatory diseases (Bringmann et al., 2006). A well- known feature of reactive gliosis is the increased production of the intermediate filament protein GFAP by Müller glia (Taylor et al., 2015), which is thought to develop as a protective mechanism to prevent further damage and to promote tissue repair. Characteristic of their response to injury, human Müller glia also produce pro-inflammatory cytokines such as tumour necrosis factor α (TNFα), Tumour growth factor ß (TGFß), interleukin 6 (IL-6) and interleukin 1 ß (IL-1ß) (Eastlake et al., 2016, Guérin et al., 2001, Franks et al., 1992). Although these cytokines are characteristic of inflammatory responses and may promote pathological damage, they might also be aimed to function as pro-regenerative factors, as suggested by studies in the zebrafish, in which some of these cytokines are known to stimulate the reprogramming of Müller glia during retina regeneration' (Kassen et al., 2009, Zhao et al., 2014). In the zebrafish, TNFα is the first cytokine produced by dying retinal neurons and is necessary to induce Müller glia to proliferate. It increases Ascl1a and Stat3 expression, which are necessary for TNFα expression in Müller glia. Suppression of TNFα production results in fewer proliferating Müller glia, suggesting that this cytokine may induce these cells to re-enter the cell cycle (Nelson et al., 2013). Other growth factors and extracellular signalling mechanisms contribute to the activation of the downstream signalling events that lead to regeneration in the zebrafish retina. For example, Heparin-binding EGF-like growth factor (HB-EGF) is induced in Müller glia at the injury site, and can also stimulate these cells to become multipotent in the uninjured retina (Wan et al., 2012). In addition, down-regulation of Smad2/3-mediated TGFß signalling by the transcriptional corepressors Tgif1 and Six3b is

necessary for the injury-induced proliferative response of Müller glia (Lenkowski et al., 2013). That inflammation plays an important role in the regeneration of the injured zebrafish retina is further supported by findings that in the absence of microglia-Müller cell signalling, regeneration does not occur (Conedera et al., 2019). Therefore, based on these observations in the zebrafish, it is not understood why similar cytokines produced by human Müller glia or microglia reactivity observed during gliosis, are not able to promote regenerative signals, and this is a subject for further investigations.

The development of omics techniques, has facilitated the examination of the proteomic profile of retinectomy specimens from eyes with proliferative vitreoretinopathy, and to identify the contribution of Müller glia to this profile. Our recent studies have identified that the most highly abundant cytokines and growth factors found upregulated in the gliotic retina constitute major proteins expressed by Müller glia (Eastlake et al., 2018), as indicated by observations that 80% of the cytokines found significantly upregulated in the gliotic retinal tissue were also identified in Müller glia in culture. These findings strongly suggest that Müller glia are an important source of cytokines and growth factors associated with retinal gliosis and investigations aimed at controlling the expression of these proteins may aid in the identification of factors that could be targeted to promote endogenous regeneration of the adult human retina upon disease or injury.

9. Conclusions and future directions

Since the description of Müller glia first made in the early 1850's by Heinrich Müller, much has been learnt about the important metabolic functions and neural support that these cells exert within the retina (Bringmann et al., 2006, Pannicke et al., 2017). However, the quiescent stem cell properties of these cells in the adult mammalian retina have only been recognized during the past 20 years, when we have gained a better understanding of the potential regenerative ability of these cells in fish and Xenopus species (Blackshaw et al., 2004, Jadhav et al., 2009, Garcia-Garcia et al., 2020, Langhe et al., 2017). Based on our current knowledge of Müller glia in the human retina, there are many avenues that further deserve exploration and which may potentially lead to the application of these cells in retina regenerative therapies (Fig 7). Transplantation of Müller glia in experimental rodent models of RGC and photoreceptor degeneration was shown to partially restore visual function without clear evidence of cell integration into the retinal circuitry (Singhal et al., 2012,

Becker et al., 2016, Jayaram et al., 2014), although some migration into the retina was observed when transplanted cells had been induced to acquire markers of retinal neurons. However, when cells attached to the retina, they appeared to remain viable for a considerable period of time, suggesting that neuroprotective molecules produced by Müller glia were the factors responsible for the recovery of retinal function observed after experimental transplantation of these cells. These observations provide the basis for future development and refining of Müller glia transplantation techniques for neuroprotective approaches in the clinical setting. Recent advances in the stem cell field and the availability of pluripotent stem cell lines produced under GMP conditions, has made possible the generation of Müller glia derived from retinal organoids for potential use in human therapies. Like any other transplantation approach, Müller glia can trigger allogenic immune responses, but availability of stem cell banks may facilitate the derivation of cells that can be match to HLA antigens of potential transplant recipients. Since Müller glia exhibit the same phenotypic and function as progenitor cells across all species (Bringmann et al., 2006, Limb et al., 2002), and are responsible for the regeneration observed in zebrafish and neonate vertebrates after injury (Bernardos et al., 2007, Thummel et al., 2008, Löffler et al., 2015), it might be plausible to develop approaches to promote the endogenous regenerative ability of these cells without the need for cell transplantation. This is supported by current knowledge that Müller glia express progenitor genes that can be reactivated to induce neurogenicity (Campbell et al., 2021, Gorsuch et al., 2017), and that they produce neurotrophins (Harada et al., 2002, Eastlake et al., 2020, Harada et al., 2000) and antioxidant metabolites (Arai-Gaun et al., 2004, Deliyanti et al., 2016) which can confer neuroprotection and promote axonal regeneration. Current approaches to directly deliver purified neurotrophins into the eye have proved to only have a transient effect. In this context, Müller glia may be more efficient at providing neuroprotection as neurotrophins are not the only protective molecules produced by these cells and merits exploration. Finally, it is possible that extracellular vesicles produced by Müller glia may be explored for use in neuroprotective therapies due to their rich miRNA content, which is known to regulate apoptosis and to control neural cell functions. To conclude, further investigations on the application of Müller cell in regenerative therapies, either by transplantation, neuroprotection or induction of endogenous regeneration, provide exciting possibilities for the development of retinal regenerative therapies in the non-distant future.

Acknowledgements

The study was supported by the Medical Research Council (MRC grants Refs. MR/P01660X/1 and MR/K008722/1); Fight for Sight (through two private donations), UK; Moorfields Eye Charity, UK; and NIHR Biomedical Research Centre at Moorfields Eye Hospital and UCL Institute of Ophthalmology, London, UK. The views expressed are those of the authors and not of the NIHR.

10. Figure legends

Fig 1. Müller glia present in the adult human retina express stem cell markers. Confocal microscopy images showing: **A.** Distribution of Müller glia within the laminated mid/posterior retina displaying their characteristic expression of Vimentin. Only a small proportion of Müller cells co-express the neuronal marker Nestin. Scale bar: 200µm **B.** Images show individual Müller cells within the retina *in situ* co-expressing Nestin and Sox2 or Nestin and Chx10. Scale bar: 25µm. **C.** Characteristic appearance of Müller glia isolated from adult human retina upon *in vitro* culture**.** Scale bar: 25µm. ONL, outer nuclear layer; INL, inner nuclear layer; RGC, retinal ganglion cell layer.

Fig 2. Evidence for the presence of a ciliary margin-like zone in the adult human retina. A. Montage of retinal sections illustrating the distribution of Nestin positive cells within the ciliary body and the neural retina. White arrow indicates the point at which the ciliary body joins the neural retina (ora serrata) Scale bar: 200µm **B.** Corresponding region of the ciliary body shown in A (indicated by segmented arrow), and the most anterior neural retina under Nomarski illumination. The lack of lamination (ie, retinal cell layers) in this region is clearly observed. Scale bar: 200µm **C.** Fluorescent micrograph corresponding to image above (segmented arrow), showing that Vimentin is only expressed in the non-pigmented ciliary epithelium, and that neither Vimentin nor Nestin are expressed in the ciliary body. PE, pigmented epithelium, NPE, non-pigmented epithelium. **D.** In situ expression of the neural progenitor markers Sox2, Chx10 and vimentin by cells of the non-laminated marginal region of the human retina, resembling the ciliary marginal zone of lower vertebrate species (a ciliary margin-like zone). Scale bar: 40µm

Fig 3. Derivation of Müller glia from retinal organoids formed by pluripotent stem cells (PSC) in vitro. A. Development of retinal organoids from PSC is initiated by induced cellular aggregation of PSC to resemble embryoid bodies, which upon exposure to growth and differentiation factors develop a characteristic mantle (arrow head) resembling retina at around 20-25 days after initiation of differentiation. Dissected mantles can be maintained in culture for over 200 days prior to Müller cell isolation from the organoids. **B.** Retinal organoids are dissociated using papain to obtain a single cell suspension. These cells are

then grown and expanded on fibronectin coated plates to give high yields over a short period of time. **C.** Appearance of retinal organoid formation, showing a characteristic mantle after 20-25 days in culture, with retinal morphology clearly observed under light inverted microscopy after 60-65 days. **D.** Confocal images of a mature retinal organoid showing expression of the Müller glia-specific markers Nestin, Vimentin, Chx10 and glutamine synthetase. Scale bars: 20µm **E.** Confocal images of Müller glia isolated from retinal organoids showing co-expression of Nestin and CD29. Scale bar: 50µm

Fig 4. Müller glia are a rich source of microvesicles and exosomes. A. Scanning electron micrograph (SEM) shows a Müller glial cell in culture. Large microvesicles blebbing directly from the plasma membrane can be seen on the cell surface (arrows). Scale bar: 50µm **B.** Transmission electron micrograph (TEM) shows a MIO-M1 cell in cross-section. Indicated with by black arrows are microvesicles shedding directly from the cell surface membrane. White arrows identify the presence of multi-vesiculated late endosomes (MVBs). Scale bar: 500 nm **C.** Schematic diagram of enriched miRNAs present in EVs isolated from Müller glia supernatants (unpublished data). MicroRNAs present in EVs are known to induce translational repression of phosphatase and tensin homologue (PTEN) mRNA, leading to activation of the PI3K/AKT pathway that regulates neuronal growth and axon regeneration (Fleming et al., 2019). Scale bar: 200nm **D.** Bands of RT-PCR gels showing expression of RNA coding for BDNF, NGF and GDNF in large EVs (lEVs) but not in small EVs (sEVs) isolated from Müller glia in culture.

Fig 5. Selected miRNAs found to be differentially enriched within sEVs isolated from Müller glia are neuroprotective. miRNAs shown in red box, identified in sEVs isolated from Müller glia are known to induce translational repression of phosphatase and tensin homologue (PTEN) mRNA, leading to activation of the PI3K/AKT pathway that regulates neuronal growth and axon regeneration (Fleming et al., 2019). Unpublished data

Fig 6. In situ induced cell proliferation within explants of neural retina.

A. Section of human retinal explant (ciliary margin-like zone) cultured for 5 days in the presence of EGF and stained for Nestin (green) and the proliferating cell marker Ki67 (red). Proliferating cells as defined by nuclear immuno-reactivity for Ki67 and intense

immunostaining for Nestin were observed in marginal region (non-laminated) retina. Bottom micrograph showing staining for Dapi and Ki67 of the image above. Scale bar: 50µm **B.** Laminated neural retina cultured for 3 days in the presence or absence of EGF and stained for Nestin (green) and Ki67 (red). In the absence of EGF (control) only a small number of proliferating cells can be observed. In contrast, a higher number of cells expressing Ki67 were observed in retina cultured with EGF, with the majority of proliferating cells localized to the INL, where the nuclei of Müller glia are primarily located. Scale bar: 50µm; ONL, outer nuclear layer; INL, inner nuclear layer; RGC, retinal ganglion cell layer.

Fig 7. Potential strategies for retinal repair and regeneration using Müller glia. Diagram summarizing the potential application of human Müller glia in regenerative therapies.

11. REFERENCES

AASEN, T., RAYA, A., BARRERO, M. J., GARRETA, E., CONSIGLIO, A., GONZALEZ, F., VASSENA, R., BILIĆ, J., PEKARIK, V., TISCORNIA, G., EDEL, M., BOUÉ, S. & IZPISÚA BELMONTE, J. C. 2008. Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. *Nat Biotechnol,* 26**,** 1276-84.

ABED, E., CORBO, G. & FALSINI, B. 2015. Neurotrophin family members as neuroprotectants in retinal degenerations. *BioDrugs,* 29**,** 1-13.

AHMAD, I., DOOLEY, C. M., THORESON, W. B., ROGERS, J. A. & AFIAT, S. 1999. In vitro analysis of a mammalian retinal progenitor that gives rise to neurons and glia. *Brain Res,* 831**,** 1-10.

AHMAD, I., TANG, L. & PHAM, H. 2000. Identification of neural progenitors in the adult mammalian eye. *Biochem Biophys Res Commun,* 270**,** 517-21.

ARAI-GAUN, S., KATAI, N., KIKUCHI, T., KUROKAWA, T., OHTA, K. & YOSHIMURA, N. 2004. Heme oxygenase-1 induced in muller cells plays a protective role in retinal ischemia-reperfusion injury in rats. *Invest Ophthalmol Vis Sci,* 45**,** 4226-32.

ARAMANT, R. & SEILER, M. 1991. Cryopreservation and transplantation of immature rat retina into adult rat retina. *Brain Res Dev Brain Res,* 61**,** 151-9.

ARMSON, P. F., BENNETT, M. R. & RAJU, T. R. 1987. Retinal ganglion cell survival and neurite regeneration requirements: the change from Muller cell dependence to superior colliculi dependence during development. *Brain Res,* 429**,** 207-16.

ASHER, R. A., MORGENSTERN, D. A., MOON, L. D. & FAWCETT, J. W. 2001. Chondroitin sulphate proteoglycans: inhibitory components of the glial scar. *Prog Brain Res,* 132**,** 611-9.

BAHRINI, I., SONG, J. H., DIEZ, D. & HANAYAMA, R. 2015. Neuronal exosomes facilitate synaptic pruning by up-regulating complement factors in microglia. *Sci Rep,* 5**,** 7989.

BANIN, E., OBOLENSKY, A., IDELSON, M., HEMO, I., REINHARDTZ, E., PIKARSKY, E., BEN-HUR, T. & REUBINOFF, B. 2006. Retinal incorporation and differentiation of neural precursors derived from human embryonic stem cells. *Stem Cells,* 24**,** 246-57.

BARDY, C., VAN DEN HURK, M., EAMES, T., MARCHAND, C., HERNANDEZ, R. V., KELLOGG, M., GORRIS, M., GALET, B., PALOMARES, V., BROWN, J., BANG, A. G., MERTENS, J., BÖHNKE, L., BOYER, L., SIMON, S. & GAGE, F. H. 2015. Neuronal medium that supports basic synaptic functions and activity of human neurons in vitro. *Proc Natl Acad Sci U S A,* 112**,** E2725-34.

BASSETT, E. A. & WALLACE, V. A. 2012. Cell fate determination in the vertebrate retina. *Trends Neurosci,* 35**,** 565-73.

BECKER, S., EASTLAKE, K., JAYARAM, H., JONES, M. F., BROWN, R. A., MCLELLAN, G. J., CHARTERIS, D. G., KHAW, P. T. & LIMB, G. A. 2016. Allogeneic Transplantation of Müller-Derived Retinal Ganglion Cells Improves Retinal Function in a Feline Model of Ganglion Cell Depletion. *Stem Cells Transl Med,* 5**,** 192-205.

BECKER, S., SINGHAL, S., JONES, M. F., EASTLAKE, K., COTTRILL, P. B., JAYARAM, H. & LIMB, G. A. 2013. Acquisition of RGC phenotype in human Müller glia with stem cell characteristics is accompanied by upregulation of functional nicotinic acetylcholine receptors. *Mol Vis,* 19**,** 1925-36.

BERNARDOS, R. L., BARTHEL, L. K., MEYERS, J. R. & RAYMOND, P. A. 2007. Late-stage neuronal progenitors in the retina are radial Müller glia that function as retinal stem cells. *J Neurosci,* 27**,** 7028-40.

BHATIA, B., SINGHAL, S., LAWRENCE, J. M., KHAW, P. T. & LIMB, G. A. 2009. Distribution of Müller stem cells within the neural retina: evidence for the existence of a ciliary margin-like zone in the adult human eye. *Exp Eye Res,* 89**,** 373-82.

BHUTTO, I. & LUTTY, G. 2012. Understanding age-related macular degeneration (AMD): relationships between the photoreceptor/retinal pigment epithelium/Bruch's membrane/choriocapillaris complex. *Mol Aspects Med,* 33**,** 295-317.

BIAN, B., ZHAO, C., HE, X., GONG, Y., REN, C., GE, L., ZENG, Y., LI, Q., CHEN, M., WENG, C., HE, J., FANG, Y., XU, H. & YIN, Z. Q. 2020. Exosomes derived from neural progenitor cells preserve photoreceptors during retinal degeneration by inactivating microglia. *J Extracell Vesicles,* 9**,** 1748931.

BLACKSHAW, S., HARPAVAT, S., TRIMARCHI, J., CAI, L., HUANG, H., KUO, W. P., WEBER, G., LEE, K., FRAIOLI, R. E., CHO, S. H., YUNG, R., ASCH, E., OHNO-MACHADO, L., WONG, W. H. & CEPKO, C. L. 2004. Genomic analysis of mouse retinal development. *PLoS Biol,* 2**,** E247.

BONGSO, A. & TAN, S. 2005. Human blastocyst culture and derivation of embryonic stem cell lines. *Stem Cell Rev,* 1**,** 87-98.

BRADBURY, E. J., MOON, L. D., POPAT, R. J., KING, V. R., BENNETT, G. S., PATEL, P. N., FAWCETT, J. W. & MCMAHON, S. B. 2002. Chondroitinase ABC promotes functional recovery after spinal cord injury. *Nature,* 416**,** 636-40.

BRINGMANN, A., PANNICKE, T., GROSCHE, J., FRANCKE, M., WIEDEMANN, P., SKATCHKOV, S. N., OSBORNE, N. N. & REICHENBACH, A. 2006. Müller cells in the healthy and diseased retina. *Prog Retin Eye Res,* 25**,** 397-424.

CAI, Y., YU, X., HU, S. & YU, J. 2009. A brief review on the mechanisms of miRNA regulation. *Genomics Proteomics Bioinformatics,* 7**,** 147-54.

CAMPBELL, L. J., HOBGOOD, J. S., JIA, M., BOYD, P., HIPP, R. I. & HYDE, D. R. 2021. Notch3 and DeltaB maintain Muller glia quiescence and act as negative regulators of regeneration in the light-damaged zebrafish retina. *Glia,* 69**,** 546-566.

CHACKO, D. M., DAS, A. V., ZHAO, X., JAMES, J., BHATTACHARYA, S. & AHMAD, I. 2003. Transplantation of ocular stem cells: the role of injury in incorporation and differentiation of grafted cells in the retina. *Vision Res,* 43**,** 937-46.

CHAKRABARTI, S., SIMA, A. A., LEE, J., BRACHET, P. & DICOU, E. 1990. Nerve growth factor (NGF), proNGF and NGF receptor-like immunoreactivity in BB rat retina. *Brain Res,* 523**,** 11-5.

CHANG, K. C., SUN, C., CAMERON, E. G., MADAAN, A., WU, S., XIA, X., ZHANG, X., TENERELLI, K., NAHMOU, M., KNASEL, C. M., RUSSANO, K. R., HERTZ, J. & GOLDBERG, J. L. 2019. Opposing Effects of Growth and Differentiation Factors in Cell-Fate Specification. *Curr Biol,* 29**,** 1963-1975.e5.

CHAO, J. R., LAMBA, D. A., KLESERT, T. R., TORRE, A., HOSHINO, A., TAYLOR, R. J., JAYABALU, A., ENGEL, A. L., KHUU, T. H., WANG, R. K., NEITZ, M., NEITZ, J. & REH, T. A. 2017. Transplantation of Human Embryonic Stem Cell-Derived Retinal Cells into the Subretinal Space of a Non-Human Primate. *Transl Vis Sci Technol,* 6**,** 4.

CHAVALI, V. R. M., HAIDER, N., RATHI, S., VRATHASHA, V., ALAPATI, T., HE, J., GILL, K., NIKONOV, R., DUONG, T. T., MCDOUGALD, D. S., NIKONOV, S., O'BRIEN, J. & MILLS, J. A. 2020. Dual SMAD inhibition and Wnt inhibition enable efficient and reproducible differentiations of induced pluripotent stem cells into retinal ganglion cells. *Sci Rep,* 10**,** 11828.

CHEN, H. Y., KAYA, K. D., DONG, L. & SWAROOP, A. 2016. Three-dimensional retinal organoids from mouse pluripotent stem cells mimic in vivo development with enhanced stratification and rod photoreceptor differentiation. *Mol Vis,* 22**,** 1077-1094.

CHHABRA, A. 2017. Inherent Immunogenicity or Lack Thereof of Pluripotent Stem Cells: Implications for Cell Replacement Therapy. *Front Immunol,* 8**,** 993.

CHO, A. K., SAMPATH, A. P. & WEILAND, J. D. 2011. Physiological response of mouse retinal ganglion cells to electrical stimulation: effect of soma size. *Annu Int Conf IEEE Eng Med Biol Soc,* 2011**,** 1081-4.

CHOU, T. H., PARK, K. K., LUO, X. & PORCIATTI, V. 2013. Retrograde signaling in the optic nerve is necessary for electrical responsiveness of retinal ganglion cells. *Invest Ophthalmol Vis Sci,* 54**,** 1236-43.

CICERO, S. A., JOHNSON, D., REYNTJENS, S., FRASE, S., CONNELL, S., CHOW, L. M., BAKER, S. J., SORRENTINO, B. P. & DYER, M. A. 2009. Cells previously identified as retinal stem cells are pigmented ciliary epithelial cells. *Proc Natl Acad Sci U S A,* 106**,** 6685-90.

CLARK, B. S., STEIN-O'BRIEN, G. L., SHIAU, F., CANNON, G. H., DAVIS-MARCISAK, E., SHERMAN, T., SANTIAGO, C. P., HOANG, T. V., RAJAII, F., JAMES-ESPOSITO, R. E., GRONOSTAJSKI, R. M., FERTIG, E. J., GOFF, L. A. & BLACKSHAW, S. 2019. Single-Cell RNA-Seq Analysis of Retinal Development Identifies NFI Factors as Regulating Mitotic Exit and Late-Born Cell Specification. *Neuron,* 102**,** 1111-1126.e5.

CLOSE, J. L., LIU, J., GUMUSCU, B. & REH, T. A. 2006. Epidermal growth factor receptor expression regulates proliferation in the postnatal rat retina. *Glia,* 54**,** 94-104.

COLES, B. L., ANGÉNIEUX, B., INOUE, T., DEL RIO-TSONIS, K., SPENCE, J. R., MCINNES, R. R., ARSENIJEVIC, Y. & VAN DER KOOY, D. 2004. Facile isolation and the characterization of human retinal stem cells. *Proc Natl Acad Sci U S A,* 101**,** 15772-7.

COLLIN, J., QUEEN, R., ZERTI, D., DORGAU, B., HUSSAIN, R., COXHEAD, J., COCKELL, S. & LAKO, M. 2019. Deconstructing Retinal Organoids: Single Cell RNA-Seq Reveals the Cellular Components of Human Pluripotent Stem Cell-Derived Retina. *Stem Cells,* 37**,** 593-598.

CONEDERA, F. M., POUSA, A. M. Q., MERCADER, N., TSCHOPP, M. & ENZMANN, V. 2019. Retinal microglia signaling affects Muller cell behavior in the zebrafish following laser injury induction. *Glia,* 67**,** 1150-1166.

CUEVAS, E., PARMAR, P. & SOWDEN, J. C. 2019. Restoring Vision Using Stem Cells and Transplantation. *Adv Exp Med Biol,* 1185**,** 563-567.

DAS, A. V., MALLYA, K. B., ZHAO, X., AHMAD, F., BHATTACHARYA, S., THORESON, W. B., HEGDE, G. V. & AHMAD, I. 2006. Neural stem cell properties of Müller glia in the mammalian retina: regulation by Notch and Wnt signaling. *Dev Biol,* 299**,** 283-302.

DE MELO REIS, R. A., VENTURA, A. L., SCHITINE, C. S., DE MELLO, M. C. & DE MELLO, F. G. 2008. Müller glia as an active compartment modulating nervous activity in the vertebrate retina: neurotransmitters and trophic factors. *Neurochem Res,* 33**,** 1466-74.

DE MIGUEL-BERIAIN, I. 2015. The ethics of stem cells revisited. *Adv Drug Deliv Rev,* 82-83**,** 176-80.

DE SOUSA, P. A., DOWNIE, J. M., TYE, B. J., BRUCE, K., DAND, P., DHANJAL, S., SERHAL, P., HARPER, J., TURNER, M. & BATEMAN, M. 2016a. Development and production of good manufacturing practice grade human embryonic stem cell lines as source material for clinical application. *Stem Cell Res,* 17**,** 379-390.

DE SOUSA, P. A., TYE, B. J., BRUCE, K., DAND, P., RUSSELL, G., COLLINS, D. M., GREENSHIELDS, A., MCDONALD, K., BRADBURN, H., CANHAM, M. A., KUNATH, T., DOWNIE, J. M., BATEMAN, M. & COURTNEY, A. 2016b. Derivation of the clinical grade human embryonic stem cell line RCe013-A (RC-9). *Stem Cell Res,* 17**,** 36-41.

DECEMBRINI, S., MARTIN, C., SENNLAUB, F., CHEMTOB, S., BIEL, M., SAMARDZIJA, M., MOULIN, A., BEHAR-COHEN, F. & ARSENIJEVIC, Y. 2017. Cone Genesis Tracing by the Chrnb4-EGFP Mouse Line: Evidences of Cellular Material Fusion after Cone Precursor Transplantation. *Mol Ther,* 25**,** 634-653.

DEL AMO, E. M., RIMPELÄ, A. K., HEIKKINEN, E., KARI, O. K., RAMSAY, E., LAJUNEN, T., SCHMITT, M., PELKONEN, L., BHATTACHARYA, M., RICHARDSON, D., SUBRIZI, A., TURUNEN, T., REINISALO, M., ITKONEN, J., TOROPAINEN, E., CASTELEIJN, M., KIDRON, H., ANTOPOLSKY, M., VELLONEN, K. S., RUPONEN, M. & URTTI, A. 2017. Pharmacokinetic aspects of retinal drug delivery. *Prog Retin Eye Res,* 57**,** 134-185.

DEL CERRO, M., NOTTER, M. F., DEL CERRO, C., WIEGAND, S. J., GROVER, D. A. & LAZAR, E. 1989. Intraretinal transplantation for rod-cell replacement in light-damaged retinas. *J Neural Transplant,* 1**,** 1-10.

DEL DEBBIO, C. B., BALASUBRAMANIAN, S., PARAMESWARAN, S., CHAUDHURI, A., QIU, F. & AHMAD, I. 2010. Notch and Wnt signaling mediated rod photoreceptor regeneration by Muller cells in adult mammalian retina. *PLoS One,* 5**,** e12425.

DEL DEBBIO, C. B., MIR, Q., PARAMESWARAN, S., MATHEWS, S., XIA, X., ZHENG, L., NEVILLE, A. J. & AHMAD, I. 2016. Notch Signaling Activates Stem Cell Properties of Müller Glia through

Transcriptional Regulation and Skp2-mediated Degradation of p27Kip1. *PLoS One,* 11**,** e0152025.
DELIYANTI, D., LEE, J. Y., PETRATOS, S., MEYER, C. J., WARD, K. W., WILKINSON-BERKA, J. L. & DE HAAN, J. B. 2016. A potent Nrf2 activator, dh404, bolsters antioxidant capacity in glial cells and attenuates ischaemic retinopathy. *Clin Sci (Lond),* 130**,** 1375-87.
DRAPER, J. S., MOORE, H. D., RUBAN, L. N., GOKHALE, P. J. & ANDREWS, P. W. 2004. Culture and characterization of human embryonic stem cells. *Stem Cells Dev,* 13**,** 325-36.
EASTLAKE, K., BANERJEE, P. J., ANGBOHANG, A., CHARTERIS, D. G., KHAW, P. T. & LIMB, G. A. 2016. Müller glia as an important source of cytokines and inflammatory factors present in the gliotic retina during proliferative vitreoretinopathy. *Glia,* 64**,** 495-506.
EASTLAKE, K., HEYWOOD, W. E., BANERJEE, P., BLISS, E., MILLS, K., KHAW, P. T., CHARTERIS, D. & LIMB, G. A. 2018. Comparative proteomic analysis of normal and gliotic PVR retina and contribution of Müller glia to this profile. *Exp Eye Res,* 177**,** 197-207.
EASTLAKE, K., LUIS, J. & LIMB, G. A. 2020. Potential of Müller Glia for Retina Neuroprotection. *Curr Eye Res,* 45**,** 339-348.
EASTLAKE, K., WANG, W., JAYARAM, H., MURRAY-DUNNING, C., CARR, A. J. F., RAMSDEN, C. M., VUGLER, A., GORE, K., CLEMO, N., STEWART, M., COFFEY, P., KHAW, P. T. & LIMB, G. A. 2019. Phenotypic and Functional Characterization of Müller Glia Isolated from Induced Pluripotent Stem Cell-Derived Retinal Organoids: Improvement of Retinal Ganglion Cell Function upon Transplantation. *Stem Cells Transl Med,* 8**,** 775-784.
ELDRED, K. C., HADYNIAK, S. E., HUSSEY, K. A., BRENERMAN, B., ZHANG, P. W., CHAMLING, X., SLUCH, V. M., WELSBIE, D. S., HATTAR, S., TAYLOR, J., WAHLIN, K., ZACK, D. J. & JOHNSTON, R. J., JR. 2018. Thyroid hormone signaling specifies cone subtypes in human retinal organoids. *Science,* 362.
EMINLI, S., FOUDI, A., STADTFELD, M., MAHERALI, N., AHFELDT, T., MOSTOSLAVSKY, G., HOCK, H. & HOCHEDLINGER, K. 2009. Differentiation stage determines potential of hematopoietic cells for reprogramming into induced pluripotent stem cells. *Nat Genet,* 41**,** 968-76.
ESCUDIER, B., DORVAL, T., CHAPUT, N., ANDRÉ, F., CABY, M. P., NOVAULT, S., FLAMENT, C., LEBOULAIRE, C., BORG, C., AMIGORENA, S., BOCCACCIO, C., BONNEROT, C., DHELLIN, O., MOVASSAGH, M., PIPERNO, S., ROBERT, C., SERRA, V., VALENTE, N., LE PECQ, J. B., SPATZ, A., LANTZ, O., TURSZ, T., ANGEVIN, E. & ZITVOGEL, L. 2005. Vaccination of metastatic melanoma patients with autologous dendritic cell (DC) derived-exosomes: results of thefirst phase I clinical trial. *J Transl Med,* 3**,** 10.
EVANS, M. J. & KAUFMAN, M. H. 1981. Establishment in culture of pluripotential cells from mouse embryos. *Nature,* 292**,** 154-6.
FAHY, E. T., CHRYSOSTOMOU, V. & CROWSTON, J. G. 2016. Mini-Review: Impaired Axonal Transport and Glaucoma. *Curr Eye Res,* 41**,** 273-83.
FANG, I. M., YANG, C. H., CHIOU, S. H. & YANG, C. M. 2014. Induced pluripotent stem cells without c-Myc ameliorate retinal oxidative damage via paracrine effects and reduced oxidative stress in rats. *J Ocul Pharmacol Ther,* 30**,** 757-70.
FERRARI, S., DI IORIO, E., BARBARO, V., PONZIN, D., SORRENTINO, F. S. & PARMEGGIANI, F. 2011. Retinitis pigmentosa: genes and disease mechanisms. *Curr Genomics,* 12**,** 238-49.
FISCHER, A. J., HENDRICKSON, A. & REH, T. A. 2001. Immunocytochemical characterization of cysts in the peripheral retina and pars plana of the adult primate. *Invest Ophthalmol Vis Sci,* 42**,** 3256-63.
FISCHER, A. J. & REH, T. A. 2001. Müller glia are a potential source of neural regeneration in the postnatal chicken retina. *Nat Neurosci,* 4**,** 247-52.
FISCHER, A. J. & REH, T. A. 2003. Growth factors induce neurogenesis in the ciliary body. *Dev Biol,* 259**,** 225-40.
FLAXMAN, S. R., BOURNE, R. R. A., RESNIKOFF, S., ACKLAND, P., BRAITHWAITE, T., CICINELLI, M. V., DAS, A., JONAS, J. B., KEEFFE, J., KEMPEN, J. H., LEASHER, J., LIMBURG, H., NAIDOO, K.,

PESUDOVS, K., SILVESTER, A., STEVENS, G. A., TAHHAN, N., WONG, T. Y. & TAYLOR, H. R. 2017. Global causes of blindness and distance vision impairment 1990-2020: a systematic review and meta-analysis. *Lancet Glob Health,* 5**,** e1221-e1234.

FLEMING, T., BALDERAS-MARQUEZ, J. E., EPARDO, D., AVILA-MENDOZA, J., CARRANZA, M., LUNA, M., HARVEY, S., ARAMBURO, C. & MARTINEZ-MORENO, C. G. 2019. Growth Hormone Neuroprotection Against Kainate Excitotoxicity in the Retina is Mediated by Notch/PTEN/Akt Signaling. *Invest Ophthalmol Vis Sci,* 60**,** 4532-4547.

FLIGOR, C. M., LANGER, K. B., SRIDHAR, A., REN, Y., SHIELDS, P. K., EDLER, M. C., OHLEMACHER, S. K., SLUCH, V. M., ZACK, D. J., ZHANG, C., SUTER, D. M. & MEYER, J. S. 2018. Three-Dimensional Retinal Organoids Facilitate the Investigation of Retinal Ganglion Cell Development, Organization and Neurite Outgrowth from Human Pluripotent Stem Cells. *Sci Rep,* 8**,** 14520.

FRANKS, W. A., LIMB, G. A., STANFORD, M. R., OGILVIE, J., WOLSTENCROFT, R. A., CHIGNELL, A. H. & DUMONDE, D. C. 1992. Cytokines in human intraocular inflammation. *Curr Eye Res,* 11 Suppl**,** 187-91.

FRØEN, R., JOHNSEN, E. O., NICOLAISSEN, B., FACSKÓ, A., PETROVSKI, G. & MOE, M. C. 2013. Does the adult human ciliary body epithelium contain "true" retinal stem cells? *Biomed Res Int,* 2013**,** 531579.

GAO, H., A, L., HUANG, X., CHEN, X. & XU, H. 2021. Muller Glia-Mediated Retinal Regeneration. *Mol Neurobiol*.

GARCIA-GARCIA, D., LOCKER, M. & PERRON, M. 2020. Update on Muller glia regenerative potential for retinal repair. *Curr Opin Genet Dev,* 64**,** 52-59.

GARCÍA, M., FORSTER, V., HICKS, D. & VECINO, E. 2002. Effects of müller glia on cell survival and neuritogenesis in adult porcine retina in vitro. *Invest Ophthalmol Vis Sci,* 43**,** 3735-43.

GARCÍA, M., FORSTER, V., HICKS, D. & VECINO, E. 2003. In vivo expression of neurotrophins and neurotrophin receptors is conserved in adult porcine retina in vitro. *Invest Ophthalmol Vis Sci,* 44**,** 4532-41.

GARCIA, T. B., PANNICKE, T., VOGLER, S., BERK, B. A., GROSCHE, A., WIEDEMANN, P., SEEGER, J., REICHENBACH, A., HERCULANO, A. M. & BRINGMANN, A. 2014. Nerve growth factor inhibits osmotic swelling of rat retinal glial (Muller) and bipolar cells by inducing glial cytokine release. *J Neurochem,* 131**,** 303-13.

GARDNER, T. W., ABCOUWER, S. F., BARBER, A. J. & JACKSON, G. R. 2011. An integrated approach to diabetic retinopathy research. *Arch Ophthalmol,* 129**,** 230-5.

GASPARINI, S. J., LLONCH, S., BORSCH, O. & ADER, M. 2019. Transplantation of photoreceptors into the degenerative retina: Current state and future perspectives. *Prog Retin Eye Res,* 69**,** 1-37.

GIANNELLI, S. G., DEMONTIS, G. C., PERTILE, G., RAMA, P. & BROCCOLI, V. 2011. Adult human Müller glia cells are a highly efficient source of rod photoreceptors. *Stem Cells,* 29**,** 344-56.

GOLDBERG, J. L., ESPINOSA, J. S., XU, Y., DAVIDSON, N., KOVACS, G. T. & BARRES, B. A. 2002. Retinal ganglion cells do not extend axons by default: promotion by neurotrophic signaling and electrical activity. *Neuron,* 33**,** 689-702.

GONZALEZ-CORDERO, A., KRUCZEK, K., NAEEM, A., FERNANDO, M., KLOC, M., RIBEIRO, J., GOH, D., DURAN, Y., BLACKFORD, S. J. I., ABELLEIRA-HERVAS, L., SAMPSON, R. D., SHUM, I. O., BRANCH, M. J., GARDNER, P. J., SOWDEN, J. C., BAINBRIDGE, J. W. B., SMITH, A. J., WEST, E. L., PEARSON, R. A. & ALI, R. R. 2017. Recapitulation of Human Retinal Development from Human Pluripotent Stem Cells Generates Transplantable Populations of Cone Photoreceptors. *Stem Cell Reports,* 9**,** 820-837.

GONZALEZ-CORDERO, A., WEST, E. L., PEARSON, R. A., DURAN, Y., CARVALHO, L. S., CHU, C. J., NAEEM, A., BLACKFORD, S. J. I., GEORGIADIS, A., LAKOWSKI, J., HUBANK, M., SMITH, A. J., BAINBRIDGE, J. W. B., SOWDEN, J. C. & ALI, R. R. 2013. Photoreceptor precursors derived from three-dimensional embryonic stem cell cultures integrate and mature within adult degenerate retina. *Nat Biotechnol,* 31**,** 741-7.

GORSUCH, R. A., LAHNE, M., YARKA, C. E., PETRAVICK, M. E., LI, J. & HYDE, D. R. 2017. Sox2 regulates Muller glia reprogramming and proliferation in the regenerating zebrafish retina via Lin28 and Ascl1a. *Exp Eye Res,* 161**,** 174-192.

GROSCHE, A., HAUSER, A., LEPPER, M. F., MAYO, R., VON TOERNE, C., MERL-PHAM, J. & HAUCK, S. M. 2016. The Proteome of Native Adult Muller Glial Cells From Murine Retina. *Mol Cell Proteomics,* 15**,** 462-80.

GUALDONI, S., BARON, M., LAKOWSKI, J., DECEMBRINI, S., SMITH, A. J., PEARSON, R. A., ALI, R. R. & SOWDEN, J. C. 2010. Adult ciliary epithelial cells, previously identified as retinal stem cells with potential for retinal repair, fail to differentiate into new rod photoreceptors. *Stem Cells,* 28**,** 1048-59.

GUÉRIN, C. J., HU, L., SCICLI, G. & SCICLI, A. G. 2001. Transforming growth factor beta in experimentally detached retina and periretinal membranes. *Exp Eye Res,* 73**,** 753-64.

HALLAM, D., HILGEN, G., DORGAU, B., ZHU, L., YU, M., BOJIC, S., HEWITT, P., SCHMITT, M., UTENG, M., KUSTERMANN, S., STEEL, D., NICHOLDS, M., THOMAS, R., TREUMANN, A., PORTER, A., SERNAGOR, E., ARMSTRONG, L. & LAKO, M. 2018. Human-Induced Pluripotent Stem Cells Generate Light Responsive Retinal Organoids with Variable and Nutrient-Dependent Efficiency. *Stem Cells,* 36**,** 1535-1551.

HANKIN, M. H. & LUND, R. D. 1987. Specific target-directed axonal outgrowth from transplanted embryonic rodent retinae into neonatal rat superior colliculus. *Brain Res,* 408**,** 344-8.

HANKIN, M. H. & LUND, R. D. 1990. Directed early axonal outgrowth from retinal transplants into host rat brains. *J Neurobiol,* 21**,** 1202-18.

HARADA, C., HARADA, T., QUAH, H. M., MAEKAWA, F., YOSHIDA, K., OHNO, S., WADA, K., PARADA, L. F. & TANAKA, K. 2003. Potential role of glial cell line-derived neurotrophic factor receptors in Müller glial cells during light-induced retinal degeneration. *Neuroscience,* 122**,** 229-35.

HARADA, T., HARADA, C., KOHSAKA, S., WADA, E., YOSHIDA, K., OHNO, S., MAMADA, H., TANAKA, K., PARADA, L. F. & WADA, K. 2002. Microglia-Müller glia cell interactions control neurotrophic factor production during light-induced retinal degeneration. *J Neurosci,* 22**,** 9228-36.

HARADA, T., HARADA, C., NAKAYAMA, N., OKUYAMA, S., YOSHIDA, K., KOHSAKA, S., MATSUDA, H. & WADA, K. 2000. Modification of glial-neuronal cell interactions prevents photoreceptor apoptosis during light-induced retinal degeneration. *Neuron,* 26**,** 533-41.

HARRIS, W. A. & PERRON, M. 1998. Molecular recapitulation: the growth of the vertebrate retina. *Int J Dev Biol,* 42**,** 299-304.

HEMMATI-BRIVANLOU, A. & MELTON, D. 1997. Vertebrate embryonic cells will become nerve cells unless told otherwise. *Cell,* 88**,** 13-7.

HITCHCOCK, P., OCHOCINSKA, M., SIEH, A. & OTTESON, D. 2004. Persistent and injury-induced neurogenesis in the vertebrate retina. *Prog Retin Eye Res,* 23**,** 183-94.

HOLLYFIELD, J. G. 1968. Differential addition of cells to the retina in Rana pipiens tadpoles. *Dev Biol,* 18**,** 163-79.

HOMMA, K., OKAMOTO, S., MANDAI, M., GOTOH, N., RAJASIMHA, H. K., CHANG, Y. S., CHEN, S., LI, W., COGLIATI, T., SWAROOP, A. & TAKAHASHI, M. 2013. Developing rods transplanted into the degenerating retina of Crx-knockout mice exhibit neural activity similar to native photoreceptors. *Stem Cells,* 31**,** 1149-59.

HU, Z. L., LI, N., WEI, X., TANG, L., WANG, T. H. & CHEN, X. M. 2017. Neuroprotective effects of BDNF and GDNF in intravitreally transplanted mesenchymal stem cells after optic nerve crush in mice. *Int J Ophthalmol,* 10**,** 35-42.

HUANG, S. P., LIN, P. K., LIU, J. H., KHOR, C. N. & LEE, Y. J. 2004. Intraocular gene transfer of ciliary neurotrophic factor rescues photoreceptor degeneration in RCS rats. *J Biomed Sci,* 11**,** 37-48.

HWANG, H. W. & MENDELL, J. T. 2006. MicroRNAs in cell proliferation, cell death, and tumorigenesis. *Br J Cancer,* 94**,** 776-80.

INOUE, Y., IRIYAMA, A., UENO, S., TAKAHASHI, H., KONDO, M., TAMAKI, Y., ARAIE, M. & YANAGI, Y. 2007. Subretinal transplantation of bone marrow mesenchymal stem cells delays retinal degeneration in the RCS rat model of retinal degeneration. *Exp Eye Res,* 85**,** 234-41.

IWABE, S., MORENO-MENDOZA, N. A., TRIGO-TAVERA, F., CROWDER, C. & GARCÍA-SÁNCHEZ, G. A. 2007. Retrograde axonal transport obstruction of brain-derived neurotrophic factor (BDNF) and its TrkB receptor in the retina and optic nerve of American Cocker Spaniel dogs with spontaneous glaucoma. *Vet Ophthalmol,* 10 Suppl 1**,** 12-9.

JADHAV, A. P., ROESCH, K. & CEPKO, C. L. 2009. Development and neurogenic potential of Müller glial cells in the vertebrate retina. *Prog Retin Eye Res,* 28**,** 249-62.

JAGATHA, B., DIVYA, M. S., SANALKUMAR, R., INDULEKHA, C. L., VIDYANAND, S., DIVYA, T. S., DAS, A. V. & JAMES, J. 2009. In vitro differentiation of retinal ganglion-like cells from embryonic stem cell derived neural progenitors. *Biochem Biophys Res Commun,* 380**,** 230-5.

JANSSEN, P., NASKAR, R., MOORE, S., THANOS, S. & THIEL, H. J. 1996. Evidence for glaucoma-induced horizontal cell alterations in the human retina. *Ger J Ophthalmol,* 5**,** 378-85.

JAYARAM, H., JONES, M. F., EASTLAKE, K., COTTRILL, P. B., BECKER, S., WISEMAN, J., KHAW, P. T. & LIMB, G. A. 2014. Transplantation of photoreceptors derived from human Muller glia restore rod function in the P23H rat. *Stem Cells Transl Med,* 3**,** 323-33.

JIAN, Q., XU, H., XIE, H., TIAN, C., ZHAO, T. & YIN, Z. 2009. Activation of retinal stem cells in the proliferating marginal region of RCS rats during development of retinitis pigmentosa. *Neurosci Lett,* 465**,** 41-4.

JOHNSON, T. V., BULL, N. D., HUNT, D. P., MARINA, N., TOMAREV, S. I. & MARTIN, K. R. 2010. Neuroprotective effects of intravitreal mesenchymal stem cell transplantation in experimental glaucoma. *Invest Ophthalmol Vis Sci,* 51**,** 2051-9.

JOHNSON, T. V., BULL, N. D. & MARTIN, K. R. 2011. Neurotrophic factor delivery as a protective treatment for glaucoma. *Exp Eye Res,* 93**,** 196-203.

JONES, B. W., PFEIFFER, R. L., FERRELL, W. D., WATT, C. B., MARMOR, M. & MARC, R. E. 2016. Retinal remodeling in human retinitis pigmentosa. *Exp Eye Res,* 150**,** 149-65.

JONES, B. W., WATT, C. B., FREDERICK, J. M., BAEHR, W., CHEN, C. K., LEVINE, E. M., MILAM, A. H., LAVAIL, M. M. & MARC, R. E. 2003a. Retinal remodeling triggered by photoreceptor degenerations. *J Comp Neurol,* 464**,** 1-16.

JONES, L. L., MARGOLIS, R. U. & TUSZYNSKI, M. H. 2003b. The chondroitin sulfate proteoglycans neurocan, brevican, phosphacan, and versican are differentially regulated following spinal cord injury. *Exp Neurol,* 182**,** 399-411.

JONES, L. L., SAJED, D. & TUSZYNSKI, M. H. 2003c. Axonal regeneration through regions of chondroitin sulfate proteoglycan deposition after spinal cord injury: a balance of permissiveness and inhibition. *J Neurosci,* 23**,** 9276-88.

JORSTAD, N. L., WILKEN, M. S., GRIMES, W. N., WOHL, S. G., VANDENBOSCH, L. S., YOSHIMATSU, T., WONG, R. O., RIEKE, F. & REH, T. A. 2017. Stimulation of functional neuronal regeneration from Muller glia in adult mice. *Nature,* 548**,** 103-107.

KAMAO, H., MANDAI, M., OKAMOTO, S., SAKAI, N., SUGA, A., SUGITA, S., KIRYU, J. & TAKAHASHI, M. 2014. Characterization of human induced pluripotent stem cell-derived retinal pigment epithelium cell sheets aiming for clinical application. *Stem Cell Reports,* 2**,** 205-18.

KANEMURA, H., GO, M. J., SHIKAMURA, M., NISHISHITA, N., SAKAI, N., KAMAO, H., MANDAI, M., MORINAGA, C., TAKAHASHI, M. & KAWAMATA, S. 2014. Tumorigenicity studies of induced pluripotent stem cell (iPSC)-derived retinal pigment epithelium (RPE) for the treatment of age-related macular degeneration. *PLoS One,* 9**,** e85336.

KARL, M. O., HAYES, S., NELSON, B. R., TAN, K., BUCKINGHAM, B. & REH, T. A. 2008. Stimulation of neural regeneration in the mouse retina. *Proc Natl Acad Sci U S A,* 105**,** 19508-13.

KASSEN, S. C., THUMMEL, R., CAMPOCHIARO, L. A., HARDING, M. J., BENNETT, N. A. & HYDE, D. R. 2009. CNTF induces photoreceptor neuroprotection and Müller glial cell proliferation

through two different signaling pathways in the adult zebrafish retina. *Exp Eye Res,* 88**,** 1051-64.
KAUPPINEN, A., PATERNO, J. J., BLASIAK, J., SALMINEN, A. & KAARNIRANTA, K. 2016. Inflammation and its role in age-related macular degeneration. *Cell Mol Life Sci,* 73**,** 1765-86.
KAWAMATA, S., KANEMURA, H., SAKAI, N., TAKAHASHI, M. & GO, M. J. 2015. Design of a Tumorigenicity Test for Induced Pluripotent Stem Cell (iPSC)-Derived Cell Products. *J Clin Med,* 4**,** 159-71.
KAWASAKI, A., OTORI, Y. & BARNSTABLE, C. J. 2000. Müller cell protection of rat retinal ganglion cells from glutamate and nitric oxide neurotoxicity. *Invest Ophthalmol Vis Sci,* 41**,** 3444-50.
KELLEY, M. W., TURNER, J. K. & REH, T. A. 1995. Regulation of proliferation and photoreceptor differentiation in fetal human retinal cell cultures. *Invest Ophthalmol Vis Sci,* 36**,** 1280-9.
KIM, B. G., DAI, H. N., LYNSKEY, J. V., MCATEE, M. & BREGMAN, B. S. 2006. Degradation of chondroitin sulfate proteoglycans potentiates transplant-mediated axonal remodeling and functional recovery after spinal cord injury in adult rats. *J Comp Neurol,* 497**,** 182-98.
KIM, S. & KIM, T. M. 2019. Generation of mesenchymal stem-like cells for producing extracellular vesicles. *World J Stem Cells,* 11**,** 270-280.
KIM, S., LOWE, A., DHARMAT, R., LEE, S., OWEN, L. A., WANG, J., SHAKOOR, A., LI, Y., MORGAN, D. J., HEJAZI, A. A., CVEKL, A., DEANGELIS, M. M., ZHOU, Z. J., CHEN, R. & LIU, W. 2019. Generation, transcriptome profiling, and functional validation of cone-rich human retinal organoids. *Proc Natl Acad Sci U S A,* 116**,** 10824-10833.
KLASSEN, H. J., NG, T. F., KURIMOTO, Y., KIROV, I., SHATOS, M., COFFEY, P. & YOUNG, M. J. 2004. Multipotent retinal progenitors express developmental markers, differentiate into retinal neurons, and preserve light-mediated behavior. *Invest Ophthalmol Vis Sci,* 45**,** 4167-73.
KNOX, D. L., EAGLE, R. C., JR. & GREEN, W. R. 2007. Optic nerve hydropic axonal degeneration and blocked retrograde axoplasmic transport: histopathologic features in human high-pressure secondary glaucoma. *Arch Ophthalmol,* 125**,** 347-53.
KOBAYASHI, H. & TOMARI, Y. 2016. RISC assembly: Coordination between small RNAs and Argonaute proteins. *Biochim Biophys Acta,* 1859**,** 71-81.
KOBAYASHI, W., ONISHI, A., TU, H. Y., TAKIHARA, Y., MATSUMURA, M., TSUJIMOTO, K., INATANI, M., NAKAZAWA, T. & TAKAHASHI, M. 2018. Culture Systems of Dissociated Mouse and Human Pluripotent Stem Cell-Derived Retinal Ganglion Cells Purified by Two-Step Immunopanning. *Invest Ophthalmol Vis Sci,* 59**,** 776-787.
KOEBERLE, P. D. & 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**,** 555-67.
KOH, S., CHEN, W. J., DEJNEKA, N. S., HARRIS, I. R., LU, B., GIRMAN, S., SAYLOR, J., WANG, S. & EROGLU, C. 2018. Subretinal Human Umbilical Tissue-Derived Cell Transplantation Preserves Retinal Synaptic Connectivity and Attenuates Müller Glial Reactivity. *J Neurosci,* 38**,** 2923-2943.
LAKOWSKI, J., WELBY, E., BUDINGER, D., DI MARCO, F., DI FOGGIA, V., BAINBRIDGE, J. W. B., WALLACE, K., GAMM, D. M., ALI, R. R. & SOWDEN, J. C. 2018. Isolation of Human Photoreceptor Precursors via a Cell Surface Marker Panel from Stem Cell-Derived Retinal Organoids and Fetal Retinae. *Stem Cells,* 36**,** 709-722.
LAMBA, D. A., GUST, J. & REH, T. A. 2009. Transplantation of human embryonic stem cell-derived photoreceptors restores some visual function in Crx-deficient mice. *Cell Stem Cell,* 4**,** 73-9.
LANGHE, R., CHESNEAU, A., COLOZZA, G., HIDALGO, M., AIL, D., LOCKER, M. & PERRON, M. 2017. Muller glial cell reactivation in Xenopus models of retinal degeneration. *Glia,* 65**,** 1333-1349.
LAWRENCE, J. M., KEEGAN, D. J., MUIR, E. M., COFFEY, P. J., ROGERS, J. H., WILBY, M. J., FAWCETT, J. W. & LUND, R. D. 2004. Transplantation of Schwann cell line clones secreting GDNF or BDNF into the retinas of dystrophic Royal College of Surgeons rats. *Invest Ophthalmol Vis Sci,* 45**,** 267-74.

LAWRENCE, J. M., SINGHAL, S., BHATIA, B., KEEGAN, D. J., REH, T. A., LUTHERT, P. J., KHAW, P. T. & LIMB, G. A. 2007. MIO-M1 cells and similar muller glial cell lines derived from adult human retina exhibit neural stem cell characteristics. *Stem Cells,* 25**,** 2033-43.

LEMAIRE, Q., RAFFO-ROMERO, A., ARAB, T., VAN CAMP, C., DRAGO, F., FORTE, S., GIMENO, J. P., BEGARD, S., COLIN, M., VIZIOLI, J., SAUTIERE, P. E., SALZET, M. & LEFEBVRE, C. 2019. Isolation of microglia-derived extracellular vesicles: towards miRNA signatures and neuroprotection. *J Nanobiotechnology,* 17**,** 119.

LENGNER, C. J. 2010. iPS cell technology in regenerative medicine. *Ann N Y Acad Sci,* 1192**,** 38-44.

LENKOWSKI, J. R., QIN, Z., SIFUENTES, C. J., THUMMEL, R., SOTO, C. M., MOENS, C. B. & RAYMOND, P. A. 2013. Retinal regeneration in adult zebrafish requires regulation of TGFβ signaling. *Glia,* 61**,** 1687-97.

LEVKOVITCH-VERBIN, H., SADAN, O., VANDER, S., ROSNER, M., BARHUM, Y., MELAMED, E., OFFEN, D. & MELAMED, S. 2010. Intravitreal injections of neurotrophic factors secreting mesenchymal stem cells are neuroprotective in rat eyes following optic nerve transection. *Invest Ophthalmol Vis Sci,* 51**,** 6394-400.

LEWIS, G. P. & 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**,** 263-90.

LEWIS, G. P., MATSUMOTO, B. & FISHER, S. K. 1995. Changes in the organization and expression of cytoskeletal proteins during retinal degeneration induced by retinal detachment. *Invest Ophthalmol Vis Sci,* 36**,** 2404-16.

LI, H. P., KOMUTA, Y., KIMURA-KURODA, J., VAN KUPPEVELT, T. H. & KAWANO, H. 2013. Roles of chondroitin sulfate and dermatan sulfate in the formation of a lesion scar and axonal regeneration after traumatic injury of the mouse brain. *J Neurotrauma,* 30**,** 413-25.

LI, R., JIN, Y., LI, Q., SUN, X., ZHU, H. & CUI, H. 2018. MiR-93-5p targeting PTEN regulates the NMDA-induced autophagy of retinal ganglion cells via AKT/mTOR pathway in glaucoma. *Biomed Pharmacother,* 100**,** 1-7.

LI, S., HE, Q., WANG, H., TANG, X., HO, K. W., GAO, X., ZHANG, Q., SHEN, Y., CHEUNG, A., WONG, F., WONG, Y. H., IP, N. Y., JIANG, L., YUNG, W. H. & LIU, K. 2015. Injured adult retinal axons with Pten and Socs3 co-deletion reform active synapses with suprachiasmatic neurons. *Neurobiol Dis,* 73**,** 366-76.

LI, X. J., REN, Z. J., TANG, J. H. & YU, Q. 2017. Exosomal MicroRNA MiR-1246 Promotes Cell Proliferation, Invasion and Drug Resistance by Targeting CCNG2 in Breast Cancer. *Cell Physiol Biochem,* 44**,** 1741-1748.

LIMB, G. A., SALT, T. E., MUNRO, P. M., MOSS, S. E. & KHAW, P. T. 2002. In vitro characterization of a spontaneously immortalized human Müller cell line (MIO-M1). *Invest Ophthalmol Vis Sci,* 43**,** 864-9.

LIN, Y. P., OUCHI, Y., SATOH, S. & WATANABE, S. 2009. Sox2 plays a role in the induction of amacrine and Muller glial cells in mouse retinal progenitor cells. *Invest Ophthalmol Vis Sci,* 50**,** 68-74.

LIU, T., ZHANG, Q., ZHANG, J., LI, C., MIAO, Y. R., LEI, Q., LI, Q. & GUO, A. Y. 2019. EVmiRNA: a database of miRNA profiling in extracellular vesicles. *Nucleic Acids Res,* 47**,** D89-D93.

LÖFFLER, K., SCHÄFER, P., VÖLKNER, M., HOLDT, T. & KARL, M. O. 2015. Age-dependent Müller glia neurogenic competence in the mouse retina. *Glia,* 63**,** 1809-24.

LOWRY, W. E., RICHTER, L., YACHECHKO, R., PYLE, A. D., TCHIEU, J., SRIDHARAN, R., CLARK, A. T. & PLATH, K. 2008. Generation of human induced pluripotent stem cells from dermal fibroblasts. *Proc Natl Acad Sci U S A,* 105**,** 2883-8.

LU, A. Q. & BARNSTABLE, C. J. 2018. Generation of Photoreceptor Precursors from Mouse Embryonic Stem Cells. *Stem Cell Rev Rep,* 14**,** 247-261.

MACDONALD, R. B., RANDLETT, O., OSWALD, J., YOSHIMATSU, T., FRANZE, K. & HARRIS, W. A. 2015. Müller glia provide essential tensile strength to the developing retina. *J Cell Biol,* 210**,** 1075-83.

MACLAREN, R. E. & PEARSON, R. A. 2007. Stem cell therapy and the retina. *Eye (Lond),* 21**,** 1352-9.
MACLAREN, R. E., PEARSON, R. A., MACNEIL, A., DOUGLAS, R. H., SALT, T. E., AKIMOTO, M., SWAROOP, A., SOWDEN, J. C. & ALI, R. R. 2006. Retinal repair by transplantation of photoreceptor precursors. *Nature,* 444**,** 203-7.
MAK, H. K., NG, S. H., REN, T., YE, C. & LEUNG, C. K. 2020. Impact of PTEN/SOCS3 deletion on amelioration of dendritic shrinkage of retinal ganglion cells after optic nerve injury. *Exp Eye Res,* 192**,** 107938.
MANN, I. C. 1928. The Process of Differentiation of the Retinal Layers in Vertebrates. *Br J Ophthalmol,* 12**,** 449-78.
MANTEL, C., GUO, Y., LEE, M. R., KIM, M. K., HAN, M. K., SHIBAYAMA, H., FUKUDA, S., YODER, M. C., PELUS, L. M., KIM, K. S. & BROXMEYER, H. E. 2007. Checkpoint-apoptosis uncoupling in human and mouse embryonic stem cells: a source of karyotpic instability. *Blood,* 109**,** 4518-27.
MARC, R. E., JONES, B. W., WATT, C. B. & STRETTOI, E. 2003. Neural remodeling in retinal degeneration. *Prog Retin Eye Res,* 22**,** 607-55.
MARKUS, A., SHAMUL, A., CHEMLA, Y., FARAH, N., SHAHAM, L., GOLDSTEIN, R. S. & MANDEL, Y. 2019. An optimized protocol for generating labeled and transplantable photoreceptor precursors from human embryonic stem cells. *Exp Eye Res,* 180**,** 29-38.
MCGRATH, M., TAM, E., SLADKOVA, M., ALMANAIE, A., ZIMMER, M. & DE PEPPO, G. M. 2019. GMP-compatible and xeno-free cultivation of mesenchymal progenitors derived from human-induced pluripotent stem cells. *Stem Cell Res Ther,* 10**,** 11.
MCHUGH, K. J., SAINT-GENIEZ, M. & TAO, S. L. 2013. Topographical control of ocular cell types for tissue engineering. *J Biomed Mater Res B Appl Biomater,* 101**,** 1571-84.
MEAD, B., AHMED, Z. & TOMAREV, S. 2018a. Mesenchymal Stem Cell-Derived Small Extracellular Vesicles Promote Neuroprotection in a Genetic DBA/2J Mouse Model of Glaucoma. *Invest Ophthalmol Vis Sci,* 59**,** 5473-5480.
MEAD, B., AMARAL, J. & TOMAREV, S. 2018b. Mesenchymal Stem Cell-Derived Small Extracellular Vesicles Promote Neuroprotection in Rodent Models of Glaucoma. *Invest Ophthalmol Vis Sci,* 59**,** 702-714.
MEAD, B., HILL, L. J., BLANCH, R. J., WARD, K., LOGAN, A., BERRY, M., LEADBEATER, W. & SCHEVEN, B. A. 2016. Mesenchymal stromal cell-mediated neuroprotection and functional preservation of retinal ganglion cells in a rodent model of glaucoma. *Cytotherapy,* 18**,** 487-96.
MEAD, B. & TOMAREV, S. 2017. Bone Marrow-Derived Mesenchymal Stem Cells-Derived Exosomes Promote Survival of Retinal Ganglion Cells Through miRNA-Dependent Mechanisms. *Stem Cells Transl Med,* 6**,** 1273-1285.
MEYER-FRANKE, A., KAPLAN, M. R., PFRIEGER, F. W. & BARRES, B. A. 1995. Characterization of the signaling interactions that promote the survival and growth of developing retinal ganglion cells in culture. *Neuron,* 15**,** 805-19.
MEYER, J. S., SHEARER, R. L., CAPOWSKI, E. E., WRIGHT, L. S., WALLACE, K. A., MCMILLAN, E. L., ZHANG, S. C. & GAMM, D. M. 2009. Modeling early retinal development with human embryonic and induced pluripotent stem cells. *Proc Natl Acad Sci U S A,* 106**,** 16698-703.
MIYAZAKI, M., IKEDA, Y., YONEMITSU, Y., GOTO, Y., SAKAMOTO, T., TABATA, T., UEDA, Y., HASEGAWA, M., TOBIMATSU, S., ISHIBASHI, T. & SUEISHI, K. 2003. Simian lentiviral vector-mediated retinal gene transfer of pigment epithelium-derived factor protects retinal degeneration and electrical defect in Royal College of Surgeons rats. *Gene Ther,* 10**,** 1503-11.
MOON, S. H., KIM, J. S., PARK, S. J., LIM, J. J., LEE, H. J., LEE, S. M. & CHUNG, H. M. 2011. Effect of chromosome instability on the maintenance and differentiation of human embryonic stem cells in vitro and in vivo. *Stem Cell Res,* 6**,** 50-9.
MOSHIRI, A. & REH, T. A. 2004. Persistent progenitors at the retinal margin of ptc+/- mice. *J Neurosci,* 24**,** 229-37.

NAKAGAWA, M., TAKIZAWA, N., NARITA, M., ICHISAKA, T. & YAMANAKA, S. 2010. Promotion of direct reprogramming by transformation-deficient Myc. *Proc Natl Acad Sci U S A,* 107**,** 14152-7.

NAKANO, T., ANDO, S., TAKATA, N., KAWADA, M., MUGURUMA, K., SEKIGUCHI, K., SAITO, K., YONEMURA, S., EIRAKU, M. & SASAI, Y. 2012. Self-formation of optic cups and storable stratified neural retina from human ESCs. *Cell Stem Cell,* 10**,** 771-785.

NELSON, C. M., ACKERMAN, K. M., O'HAYER, P., BAILEY, T. J., GORSUCH, R. A. & HYDE, D. R. 2013. Tumor necrosis factor-alpha is produced by dying retinal neurons and is required for Muller glia proliferation during zebrafish retinal regeneration. *J Neurosci,* 33**,** 6524-39.

NEWMAN, E. A. & ZAHS, K. R. 1998. Modulation of neuronal activity by glial cells in the retina. *J Neurosci,* 18**,** 4022-8.

NISHIGUCHI, K. M., KANEKO, H., NAKAMURA, M., KACHI, S. & TERASAKI, H. 2009. Generation of immature retinal neurons from proliferating cells in the pars plana after retinal histogenesis in mice with retinal degeneration. *Mol Vis,* 15**,** 187-99.

NUSCHKE, A. C., FARRELL, S. R., LEVESQUE, J. M. & CHAUHAN, B. C. 2015. Assessment of retinal ganglion cell damage in glaucomatous optic neuropathy: Axon transport, injury and soma loss. *Exp Eye Res,* 141**,** 111-24.

OKITA, K., NAKAGAWA, M., HYENJONG, H., ICHISAKA, T. & YAMANAKA, S. 2008. Generation of mouse induced pluripotent stem cells without viral vectors. *Science,* 322**,** 949-53.

OOTO, S., AKAGI, T., KAGEYAMA, R., AKITA, J., MANDAI, M., HONDA, Y. & TAKAHASHI, M. 2004. Potential for neural regeneration after neurotoxic injury in the adult mammalian retina. *Proc Natl Acad Sci U S A,* 101**,** 13654-9.

ORTIN-MARTINEZ, A., TSAI, E. L., NICKERSON, P. E., BERGERET, M., LU, Y., SMILEY, S., COMANITA, L. & WALLACE, V. A. 2017. A Reinterpretation of Cell Transplantation: GFP Transfer From Donor to Host Photoreceptors. *Stem Cells,* 35**,** 932-939.

OSAKADA, F., IKEDA, H., MANDAI, M., WATAYA, T., WATANABE, K., YOSHIMURA, N., AKAIKE, A., SASAI, Y. & TAKAHASHI, M. 2008. Toward the generation of rod and cone photoreceptors from mouse, monkey and human embryonic stem cells. *Nat Biotechnol,* 26**,** 215-24.

OSAKADA, F., OOTO, S., AKAGI, T., MANDAI, M., AKAIKE, A. & TAKAHASHI, M. 2007. Wnt signaling promotes regeneration in the retina of adult mammals. *J Neurosci,* 27**,** 4210-9.

OSWALD, J. & BARANOV, P. 2018. Regenerative medicine in the retina: from stem cells to cell replacement therapy. *Ther Adv Ophthalmol,* 10**,** 2515841418774433.

OVANDO-ROCHE, P., WEST, E. L., BRANCH, M. J., SAMPSON, R. D., FERNANDO, M., MUNRO, P., GEORGIADIS, A., RIZZI, M., KLOC, M., NAEEM, A., RIBEIRO, J., SMITH, A. J., GONZALEZ-CORDERO, A. & ALI, R. R. 2018. Use of bioreactors for culturing human retinal organoids improves photoreceptor yields. *Stem Cell Res Ther,* 9**,** 156.

OZAWA, Y., KURIHARA, T., SASAKI, M., BAN, N., YUKI, K., KUBOTA, S. & TSUBOTA, K. 2011. Neural degeneration in the retina of the streptozotocin-induced type 1 diabetes model. *Exp Diabetes Res,* 2011**,** 108328.

PAN, D., CHANG, X., XU, M., ZHANG, M., ZHANG, S., WANG, Y., LUO, X., XU, J., YANG, X. & SUN, X. 2019. UMSC-derived exosomes promote retinal ganglion cells survival in a rat model of optic nerve crush. *J Chem Neuroanat,* 96**,** 134-139.

PANNICKE, T., IVO CHAO, T., REISENHOFER, M., FRANCKE, M. & REICHENBACH, A. 2017. Comparative electrophysiology of retinal Müller glial cells-A survey on vertebrate species. *Glia,* 65**,** 533-568.

PARK, S. C., DE MORAES, C. G., TENG, C. C., TELLO, C., LIEBMANN, J. M. & RITCH, R. 2012. Enhanced depth imaging optical coherence tomography of deep optic nerve complex structures in glaucoma. *Ophthalmology,* 119**,** 3-9.

PEARSON, R. A., GONZALEZ-CORDERO, A., WEST, E. L., RIBEIRO, J. R., AGHAIZU, N., GOH, D., SAMPSON, R. D., GEORGIADIS, A., WALDRON, P. V., DURAN, Y., NAEEM, A., KLOC, M., CRISTANTE, E., KRUCZEK, K., WARRE-CORNISH, K., SOWDEN, J. C., SMITH, A. J. & ALI, R. R.

2016. Donor and host photoreceptors engage in material transfer following transplantation of post-mitotic photoreceptor precursors. *Nat Commun,* 7**,** 13029.

POCHE, R. A., FURUTA, Y., CHABOISSIER, M. C., SCHEDL, A. & BEHRINGER, R. R. 2008. Sox9 is expressed in mouse multipotent retinal progenitor cells and functions in Muller glial cell development. *J Comp Neurol,* 510**,** 237-50.

RAHMAN, F., ZEKITE, A., BUNCE, C., JAYARAM, H. & FLANAGAN, D. 2020. Recent trends in vision impairment certifications in England and Wales. *Eye (Lond),* 34**,** 1271-1278.

RAJU, T. R. & BENNETT, M. R. 1986. Retinal ganglion cell survival requirements: a major but transient dependence on Müller glia during development. *Brain Res,* 383**,** 165-76.

RAPOSO, G., NIJMAN, H. W., STOORVOGEL, W., LIEJENDEKKER, R., HARDING, C. V., MELIEF, C. J. & GEUZE, H. J. 1996. B lymphocytes secrete antigen-presenting vesicles. *J Exp Med,* 183**,** 1161-72.

RAYMOND, P. A., BARTHEL, L. K., BERNARDOS, R. L. & PERKOWSKI, J. J. 2006. Molecular characterization of retinal stem cells and their niches in adult zebrafish. *BMC Dev Biol,* 6**,** 36.

REICHENBACH, A. & BRINGMANN, A. 2013. New functions of Muller cells. *Glia,* 61**,** 651-78.

REICHENBACH, A., SCHNITZER, J., REICHELT, E., OSBORNE, N. N., FRITZSCHE, B., PULS, A., RICHTER, U., FRIEDRICH, A., KNOTHE, A. K., SCHOBER, W. & ET AL. 1993. Development of the rabbit retina, III: Differential retinal growth, and density of projection neurons and interneurons. *Vis Neurosci,* 10**,** 479-98.

REICHENBACH, A., WURM, A., PANNICKE, T., IANDIEV, I., WIEDEMANN, P. & BRINGMANN, A. 2007. Müller cells as players in retinal degeneration and edema. *Graefes Arch Clin Exp Ophthalmol,* 245**,** 627-36.

REINHARD, J., RENNER, M., WIEMANN, S., SHAKOOR, D. A., STUTE, G., DICK, H. B., FAISSNER, A. & JOACHIM, S. C. 2017. Ischemic injury leads to extracellular matrix alterations in retina and optic nerve. *Sci Rep,* 7**,** 43470.

REIS, R. A., CABRAL DA SILVA, M. C., LOUREIRO DOS SANTOS, N. E., BAMPTON, E., TAYLOR, J. S., DE MELLO, F. G. & LINDEN, R. 2002. Sympathetic neuronal survival induced by retinal trophic factors. *J Neurobiol,* 50**,** 13-23.

ROCHE, S. L., WYSE-JACKSON, A. C., BYRNE, A. M., RUIZ-LOPEZ, A. M. & COTTER, T. G. 2016. Alterations to retinal architecture prior to photoreceptor loss in a mouse model of retinitis pigmentosa. *Int J Dev Biol,* 60**,** 127-39.

RODGER, J., DRUMMOND, E. S., HELLSTRÖM, M., ROBERTSON, D. & HARVEY, A. R. 2012. Long-term gene therapy causes transgene-specific changes in the morphology of regenerating retinal ganglion cells. *PLoS One,* 7**,** e31061.

ROESCH, K., JADHAV, A. P., TRIMARCHI, J. M., STADLER, M. B., ROSKA, B., SUN, B. B. & CEPKO, C. L. 2008. The transcriptome of retinal Müller glial cells. *J Comp Neurol,* 509**,** 225-38.

ROQUE, R. S., IMPERIAL, C. J. & CALDWELL, R. B. 1996. Microglial cells invade the outer retina as photoreceptors degenerate in Royal College of Surgeons rats. *Invest Ophthalmol Vis Sci,* 37**,** 196-203.

RUZAFA, N., PEREIRO, X., LEPPER, M. F., HAUCK, S. M. & VECINO, E. 2018. A Proteomics Approach to Identify Candidate Proteins Secreted by Muller Glia that Protect Ganglion Cells in the Retina. *Proteomics,* 18**,** e1700321.

SANTOS-FERREIRA, T., LLONCH, S., BORSCH, O., POSTEL, K., HAAS, J. & ADER, M. 2016. Retinal transplantation of photoreceptors results in donor-host cytoplasmic exchange. *Nat Commun,* 7**,** 13028.

SARTHY, V. P., BRODJIAN, S. J., DUTT, K., KENNEDY, B. N., FRENCH, R. P. & CRABB, J. W. 1998. Establishment and characterization of a retinal Müller cell line. *Invest Ophthalmol Vis Sci,* 39**,** 212-6.

SCHLICHTENBREDE, F. C., DA CRUZ, L., STEPHENS, C., SMITH, A. J., GEORGIADIS, A., THRASHER, A. J., BAINBRIDGE, J. W., SEELIGER, M. W. & ALI, R. R. 2003. Long-term evaluation of retinal

function in Prph2Rd2/Rd2 mice following AAV-mediated gene replacement therapy. *J Gene Med,* 5**,** 757-64.

SCHMITT, S., AFTAB, U., JIANG, C., REDENTI, S., KLASSEN, H., MILJAN, E., SINDEN, J. & YOUNG, M. 2009. Molecular characterization of human retinal progenitor cells. *Invest Ophthalmol Vis Sci,* 50**,** 5901-8.

SEKI, M., NAWA, H., FUKUCHI, T., ABE, H. & TAKEI, N. 2003. BDNF is upregulated by postnatal development and visual experience: quantitative and immunohistochemical analyses of BDNF in the rat retina. *Invest Ophthalmol Vis Sci,* 44**,** 3211-8.

SEKI, M., TANAKA, T., SAKAI, Y., FUKUCHI, T., ABE, H., NAWA, H. & TAKEI, N. 2005. Müller Cells as a source of brain-derived neurotrophic factor in the retina: noradrenaline upregulates brain-derived neurotrophic factor levels in cultured rat Müller cells. *Neurochem Res,* 30**,** 1163-70.

SELLÉS-NAVARRO, I., ELLEZAM, B., FAJARDO, R., LATOUR, M. & MCKERRACHER, L. 2001. Retinal ganglion cell and nonneuronal cell responses to a microcrush lesion of adult rat optic nerve. *Exp Neurol,* 167**,** 282-9.

SHIGEMOTO-KURODA, T., OH, J. Y., KIM, D. K., JEONG, H. J., PARK, S. Y., LEE, H. J., PARK, J. W., KIM, T. W., AN, S. Y., PROCKOP, D. J. & LEE, R. H. 2017. MSC-derived Extracellular Vesicles Attenuate Immune Responses in Two Autoimmune Murine Models: Type 1 Diabetes and Uveoretinitis. *Stem Cell Reports,* 8**,** 1214-1225.

SINGHAL, S., BHATIA, B., JAYARAM, H., BECKER, S., JONES, M. F., COTTRILL, P. B., KHAW, P. T., SALT, T. E. & LIMB, G. A. 2012. Human Müller 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**,** 188-99.

SINGHAL, S., LAWRENCE, J. M., BHATIA, B., ELLIS, J. S., KWAN, A. S., MACNEIL, A., LUTHERT, P. J., FAWCETT, J. W., PEREZ, M. T., KHAW, P. T. & LIMB, G. A. 2008. Chondroitin sulfate proteoglycans and microglia prevent migration and integration of grafted Müller stem cells into degenerating retina. *Stem Cells,* 26**,** 1074-82.

SODHA, S., WALL, K., REDENTI, S., KLASSEN, H., YOUNG, M. J. & TAO, S. L. 2011. Microfabrication of a three-dimensional polycaprolactone thin-film scaffold for retinal progenitor cell encapsulation. *J Biomater Sci Polym Ed,* 22**,** 443-56.

SURZENKO, N., CROWL, T., BACHLEDA, A., LANGER, L. & PEVNY, L. 2013. SOX2 maintains the quiescent progenitor cell state of postnatal retinal Muller glia. *Development,* 140**,** 1445-56.

TASSEW, N. G., CHARISH, J., SHABANZADEH, A. P., LUGA, V., HARADA, H., FARHANI, N., D'ONOFRIO, P., CHOI, B., ELLABBAN, A., NICKERSON, P. E. B., WALLACE, V. A., KOEBERLE, P. D., WRANA, J. L. & MONNIER, P. P. 2017. Exosomes Mediate Mobilization of Autocrine Wnt10b to Promote Axonal Regeneration in the Injured CNS. *Cell Rep,* 20**,** 99-111.

TAYLOR, C. J., BOLTON, E. M., POCOCK, S., SHARPLES, L. D., PEDERSEN, R. A. & BRADLEY, J. A. 2005. Banking on human embryonic stem cells: estimating the number of donor cell lines needed for HLA matching. *Lancet,* 366**,** 2019-25.

TAYLOR, C. J., PEACOCK, S., CHAUDHRY, A. N., BRADLEY, J. A. & BOLTON, E. M. 2012. Generating an iPSC bank for HLA-matched tissue transplantation based on known donor and recipient HLA types. *Cell Stem Cell,* 11**,** 147-52.

TAYLOR, L., ARNÉR, K. & GHOSH, F. 2015. First responders: dynamics of pre-gliotic Müller cell responses in the isolated adult rat retina. *Curr Eye Res,* 40**,** 1245-60.

TAYLOR, S., SRINIVASAN, B., WORDINGER, R. J. & ROQUE, R. S. 2003. Glutamate stimulates neurotrophin expression in cultured Müller cells. *Brain Res Mol Brain Res,* 111**,** 189-97.

THÉRY, C., ZITVOGEL, L. & AMIGORENA, S. 2002. Exosomes: composition, biogenesis and function. *Nat Rev Immunol,* 2**,** 569-79.

THUMMEL, R., KASSEN, S. C., ENRIGHT, J. M., NELSON, C. M., MONTGOMERY, J. E. & HYDE, D. R. 2008. Characterization of Müller glia and neuronal progenitors during adult zebrafish retinal regeneration. *Exp Eye Res,* 87**,** 433-44.

TOMITA, M., LAVIK, E., KLASSEN, H., ZAHIR, T., LANGER, R. & YOUNG, M. J. 2005. Biodegradable polymer composite grafts promote the survival and differentiation of retinal progenitor cells. *Stem Cells,* 23**,** 1579-88.

TROPEPE, V., COLES, B. L., CHIASSON, B. J., HORSFORD, D. J., ELIA, A. J., MCINNES, R. R. & VAN DER KOOY, D. 2000. Retinal stem cells in the adult mammalian eye. *Science,* 287**,** 2032-6.

TURNER, D. L. & CEPKO, C. L. 1987. A common progenitor for neurons and glia persists in rat retina late in development. *Nature,* 328**,** 131-6.

UGA, S. & SMELSER, G. K. 1973. Electron microscopic study of the development of retinal Mullerian cells. *Invest Ophthalmol,* 12**,** 295-307.

VALADI, H., EKSTRÖM, K., BOSSIOS, A., SJÖSTRAND, M., LEE, J. J. & LÖTVALL, J. O. 2007. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol,* 9**,** 654-9.

VILLEGAS-PÉREZ, M. P., LAWRENCE, J. M., VIDAL-SANZ, M., LAVAIL, M. M. & LUND, R. D. 1998. Ganglion cell loss in RCS rat retina: a result of compression of axons by contracting intraretinal vessels linked to the pigment epithelium. *J Comp Neurol,* 392**,** 58-77.

VINCENT, A., SHETTY, R., DEVI, S. A., KURIAN, M. K., BALU, R. & SHETTY, B. 2010. Functional involvement of cone photoreceptors in advanced glaucoma: a multifocal electroretinogram study. *Doc Ophthalmol,* 121**,** 21-7.

VÖLKNER, M., ZSCHÄTZSCH, M., ROSTOVSKAYA, M., OVERALL, R. W., BUSSKAMP, V., ANASTASSIADIS, K. & KARL, M. O. 2016. Retinal Organoids from Pluripotent Stem Cells Efficiently Recapitulate Retinogenesis. *Stem Cell Reports,* 6**,** 525-538.

VRABEC, J. P. & LEVIN, L. A. 2007. The neurobiology of cell death in glaucoma. *Eye (Lond),* 21 Suppl 1**,** S11-4.

WAN, J., RAMACHANDRAN, R. & GOLDMAN, D. 2012. HB-EGF is necessary and sufficient for Müller glia dedifferentiation and retina regeneration. *Dev Cell,* 22**,** 334-47.

WAN, J., ZHENG, H., CHEN, Z. L., XIAO, H. L., SHEN, Z. J. & ZHOU, G. M. 2008. Preferential regeneration of photoreceptor from Müller glia after retinal degeneration in adult rat. *Vision Res,* 48**,** 223-34.

WAN, J., ZHENG, H., XIAO, H. L., SHE, Z. J. & ZHOU, G. M. 2007. Sonic hedgehog promotes stem-cell potential of Müller glia in the mammalian retina. *Biochem Biophys Res Commun,* 363**,** 347-54.

WEINREB, R. N. & KHAW, P. T. 2004. Primary open-angle glaucoma. *Lancet,* 363**,** 1711-20.

WERNIG, M., MEISSNER, A., FOREMAN, R., BRAMBRINK, T., KU, M., HOCHEDLINGER, K., BERNSTEIN, B. E. & JAENISCH, R. 2007. In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature,* 448**,** 318-24.

WEST, E. L., GONZALEZ-CORDERO, A., HIPPERT, C., OSAKADA, F., MARTINEZ-BARBERA, J. P., PEARSON, R. A., SOWDEN, J. C., TAKAHASHI, M. & ALI, R. R. 2012. Defining the integration capacity of embryonic stem cell-derived photoreceptor precursors. *Stem Cells,* 30**,** 1424-35.

WEST, E. L., PEARSON, R. A., TSCHERNUTTER, M., SOWDEN, J. C., MACLAREN, R. E. & ALI, R. R. 2008. Pharmacological disruption of the outer limiting membrane leads to increased retinal integration of transplanted photoreceptor precursors. *Exp Eye Res,* 86**,** 601-11.

WIKLANDER, O. P. B., BRENNAN, M., LÖTVALL, J., BREAKEFIELD, X. O. & EL ANDALOUSSI, S. 2019. Advances in therapeutic applications of extracellular vesicles. *Sci Transl Med,* 11.

WILSON, A. M. & DI POLO, A. 2012. Gene therapy for retinal ganglion cell neuroprotection in glaucoma. *Gene Ther,* 19**,** 127-36.

YAN, Q., WANG, J., MATHESON, C. R. & URICH, J. L. 1999. Glial cell line-derived neurotrophic factor (GDNF) promotes the survival of axotomized retinal ganglion cells in adult rats: comparison to and combination with brain-derived neurotrophic factor (BDNF). *J Neurobiol,* 38**,** 382-90.

YOUNG, M. J., RAY, J., WHITELEY, S. J., KLASSEN, H. & GAGE, F. H. 2000. Neuronal differentiation and morphological integration of hippocampal progenitor cells transplanted to the retina of immature and mature dystrophic rats. *Mol Cell Neurosci,* 16**,** 197-205.

YUAN, D., ZHAO, Y., BANKS, W. A., BULLOCK, K. M., HANEY, M., BATRAKOVA, E. & KABANOV, A. V. 2017. Macrophage exosomes as natural nanocarriers for protein delivery to inflamed brain. *Biomaterials,* 142**,** 1-12.

YUYAMA, K., SUN, H., MITSUTAKE, S. & IGARASHI, Y. 2012. Sphingolipid-modulated exosome secretion promotes clearance of amyloid-β by microglia. *J Biol Chem,* 287**,** 10977-89.

ZANIN, M. P., PETTINGILL, L. N., HARVEY, A. R., EMERICH, D. F., THANOS, C. G. & SHEPHERD, R. K. 2012. The development of encapsulated cell technologies as therapies for neurological and sensory diseases. *J Control Release,* 160**,** 3-13.

ZHANG, L. Q., CUI, H., YU, Y. B., SHI, H. Q., ZHOU, Y. & LIU, M. J. 2019. MicroRNA-141-3p inhibits retinal neovascularization and retinal ganglion cell apoptosis in glaucoma mice through the inactivation of Docking protein 5-dependent mitogen-activated protein kinase signaling pathway. *J Cell Physiol,* 234**,** 8873-8887.

ZHAO, T., LI, Y., TANG, L., LI, Y., FAN, F. & JIANG, B. 2011. Protective effects of human umbilical cord blood stem cell intravitreal transplantation against optic nerve injury in rats. *Graefes Arch Clin Exp Ophthalmol,* 249**,** 1021-8.

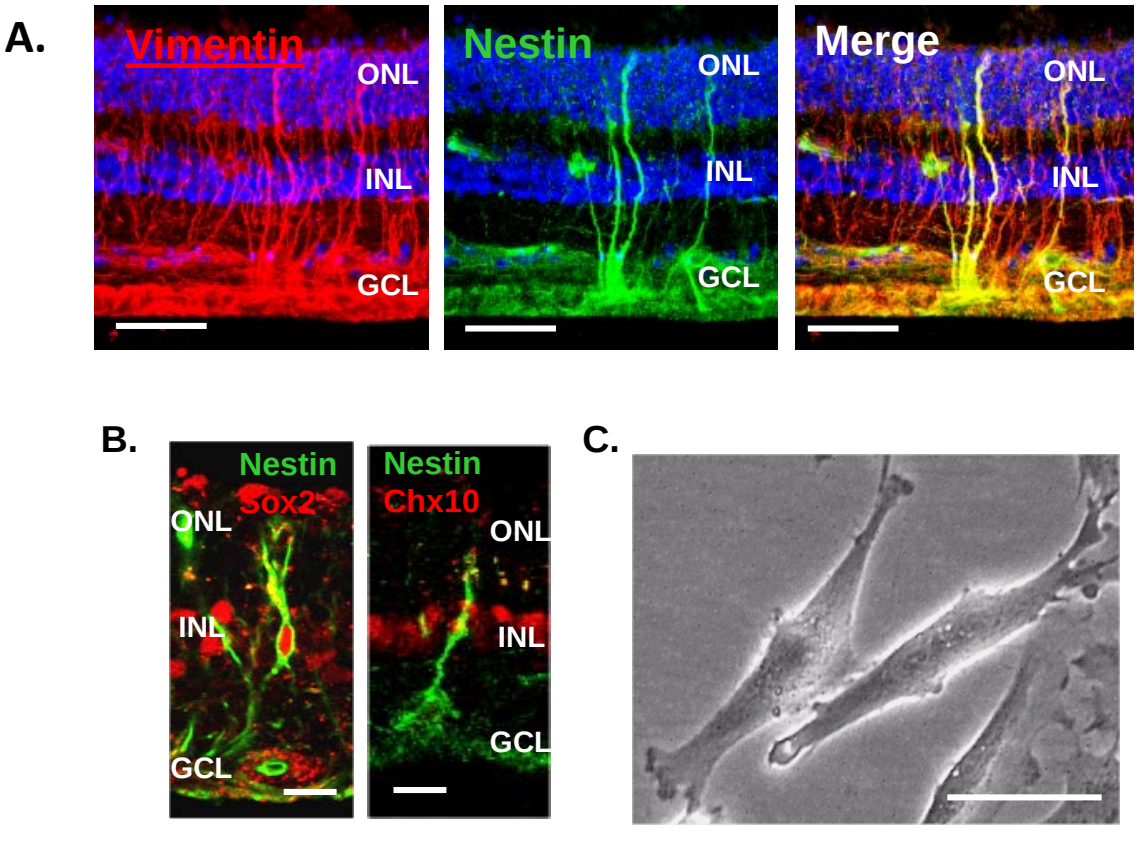
ZHAO, X. F., WAN, J., POWELL, C., RAMACHANDRAN, R., MYERS, M. G., JR. & GOLDMAN, D. 2014. Leptin and IL-6 family cytokines synergize to stimulate Müller glia reprogramming and retina regeneration. *Cell Rep,* 9**,** 272-284.

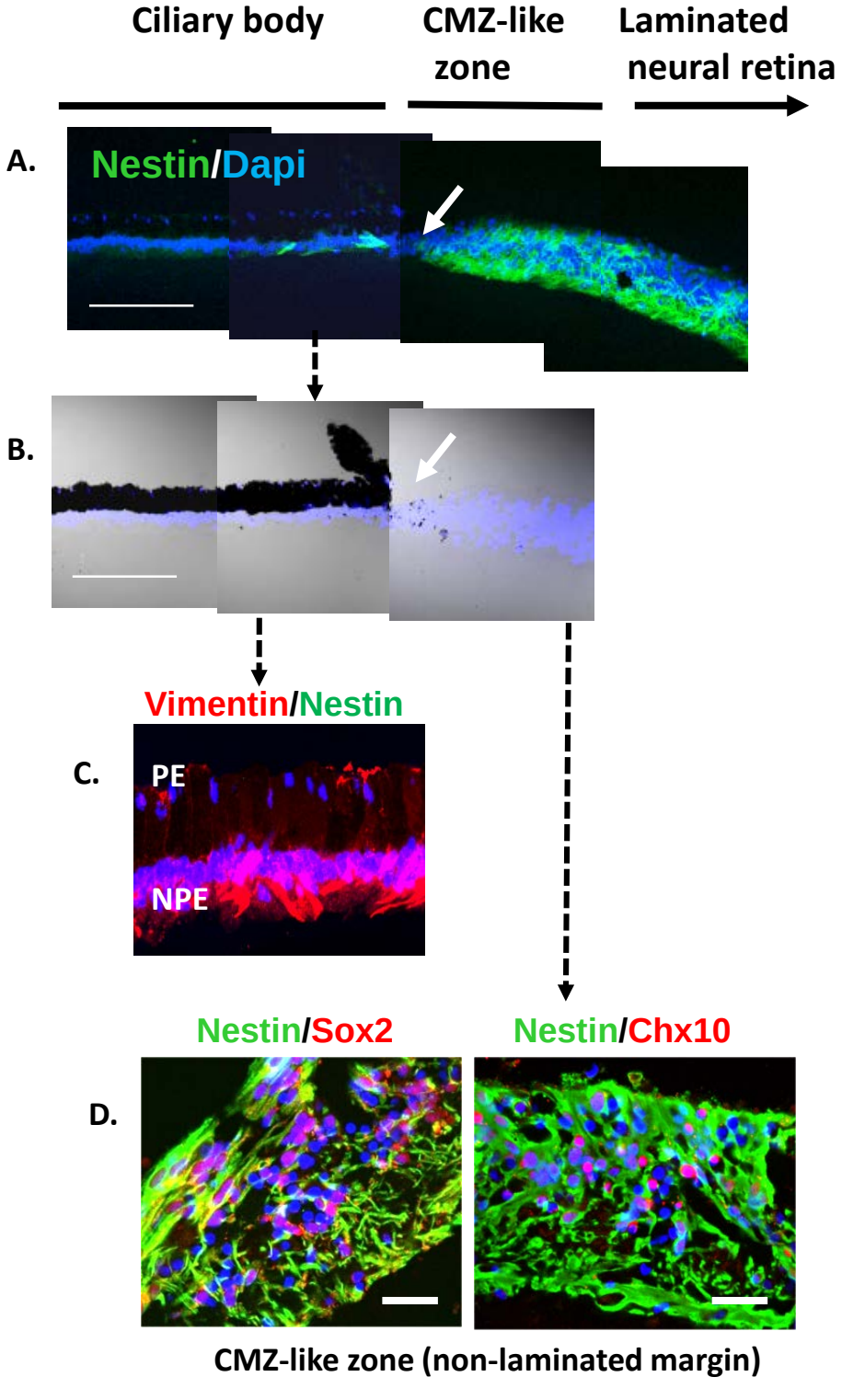
ZHU, J., CIFUENTES, H., REYNOLDS, J. & LAMBA, D. A. 2017. Immunosuppression via Loss of IL2rγ Enhances Long-Term Functional Integration of hESC-Derived Photoreceptors in the Mouse Retina. *Cell Stem Cell,* 20**,** 374-384.e5.

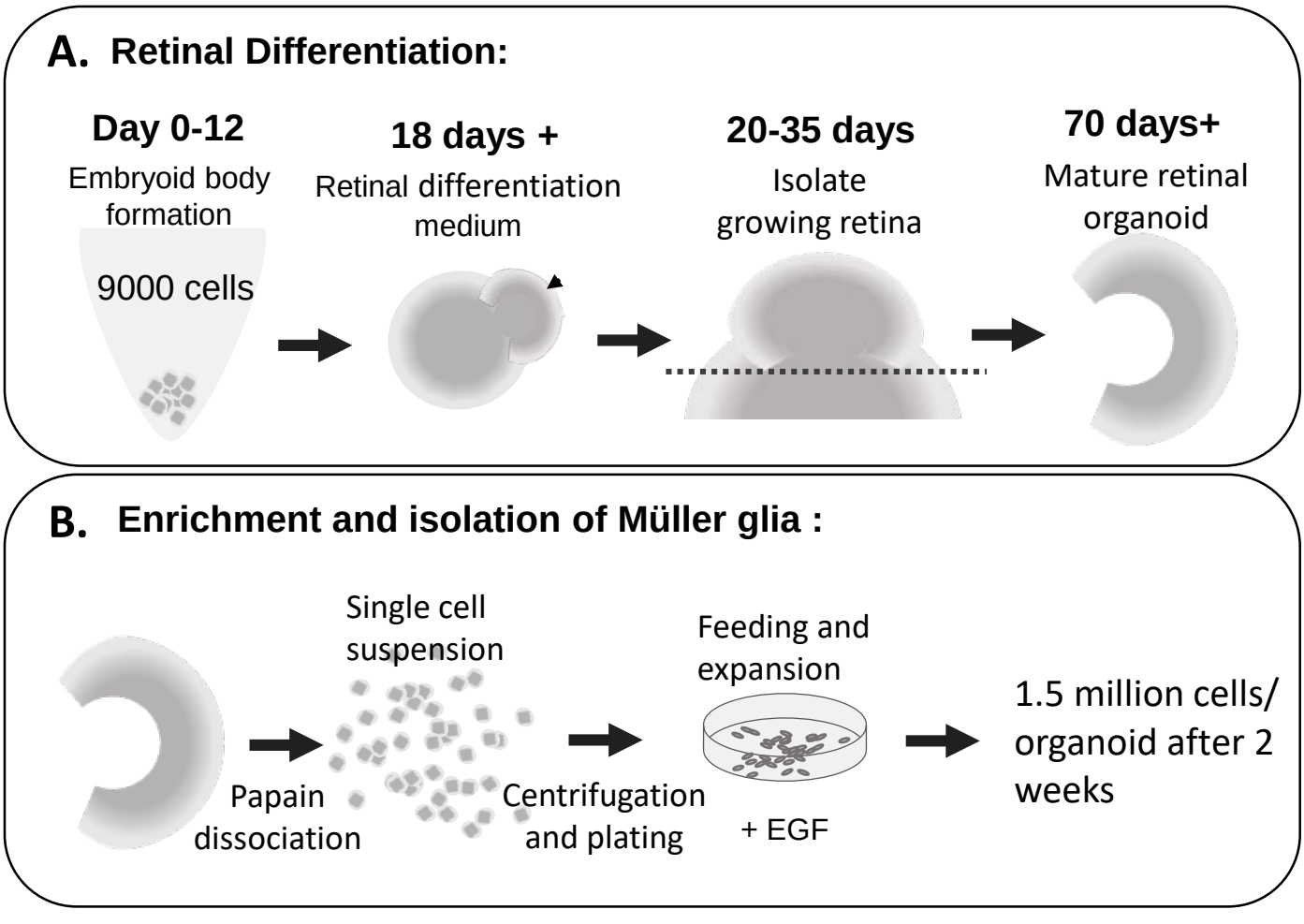
ZITVOGEL, L., REGNAULT, A., LOZIER, A., WOLFERS, J., FLAMENT, C., TENZA, D., RICCIARDI-CASTAGNOLI, P., RAPOSO, G. & AMIGORENA, S. 1998. Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. *Nat Med,* 4**,** 594-600.

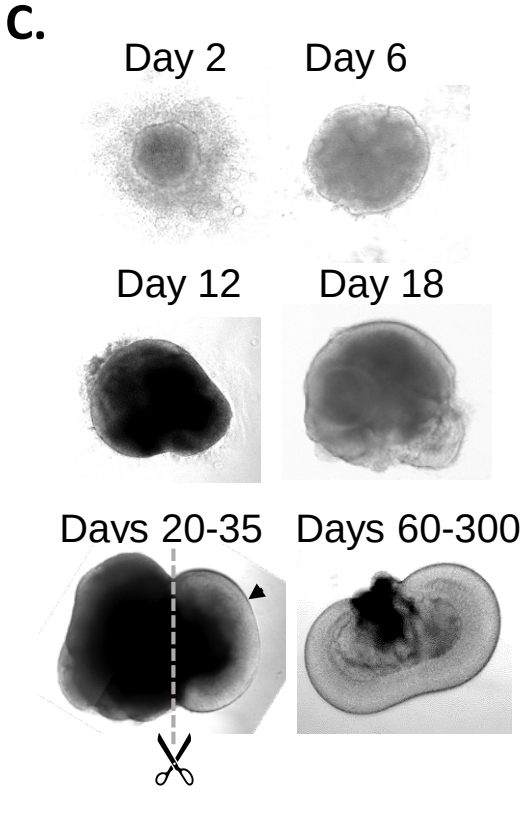
ZUZIC, M., ROJO ARIAS, J. E., WOHL, S. G. & BUSSKAMP, V. 2019. Retinal miRNA Functions in Health and Disease. *Genes (Basel),* 10.

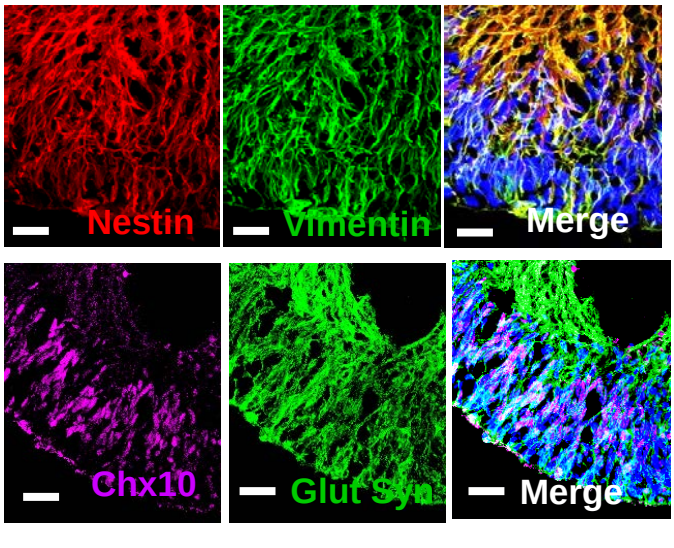
Fig. 1

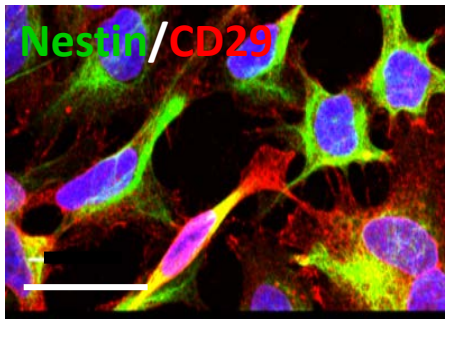

A.
Vimentin
ONL
INL
GCL
Nestin
ONL
INL
GCL
Merge
ONL
INL
GCL
B.
Nestin
Sox2
ONL
INL
GCL
Nestin
Chx10
ONL
INL
GCL
C.


Ciliary body
CMZ-like zone
Laminated neural retina
A.
Nestin/Dapi
B.
Vimentin/Nestin
C.
PE
NPE
Nestin/Sox2
Nestin/Chx10
D.
CMZ-like zone (non-laminated margin)

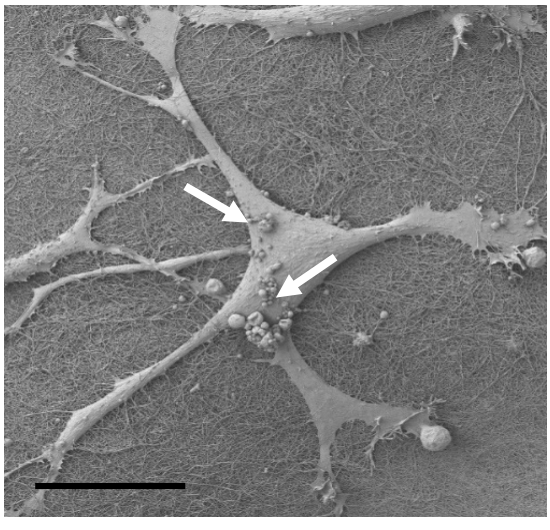

A. Retinal Differentiation:
Day 0-12
Embryoid body formation
9000 cells
18 days +
Retinal differentiation medium
20-35 days
Isolate growing retina
70 days+
Mature retinal organoid
B. Enrichment and isolation of Müller glia :
Papain dissociation
Single cell suspension
Centrifugation and plating
Feeding and expansion
+ EGF
1.5 million cells/ organoid after 2 weeks


C.
Day 2
Day 6
Day 12
Day 18
Days 20-35
Days 60-300

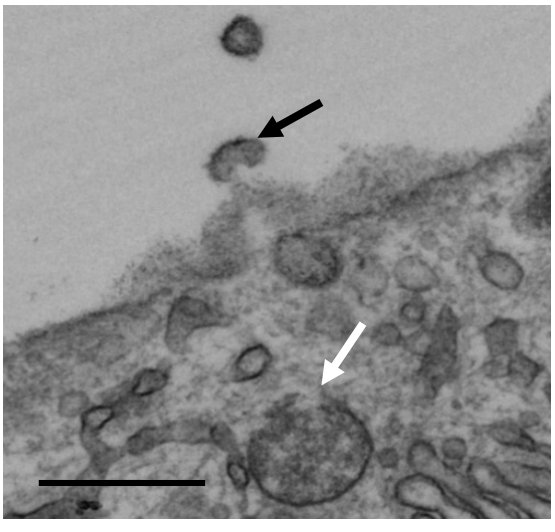

D.
Nestin
Vimentin
Merge
Chx10
Glut Syn
Merge


E.
Nestin/CD29

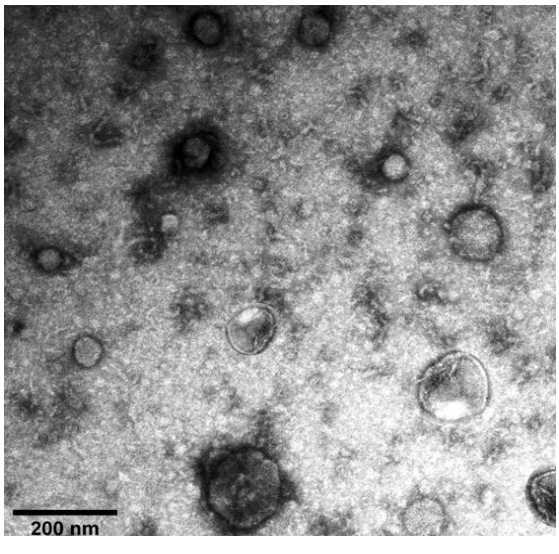
A.



B.



C.



D.

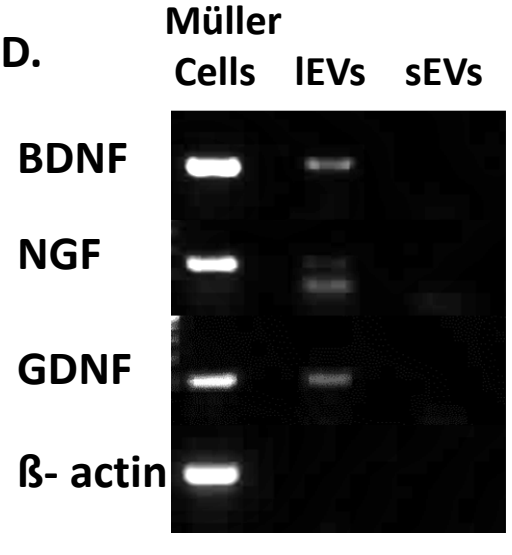


Fig 5

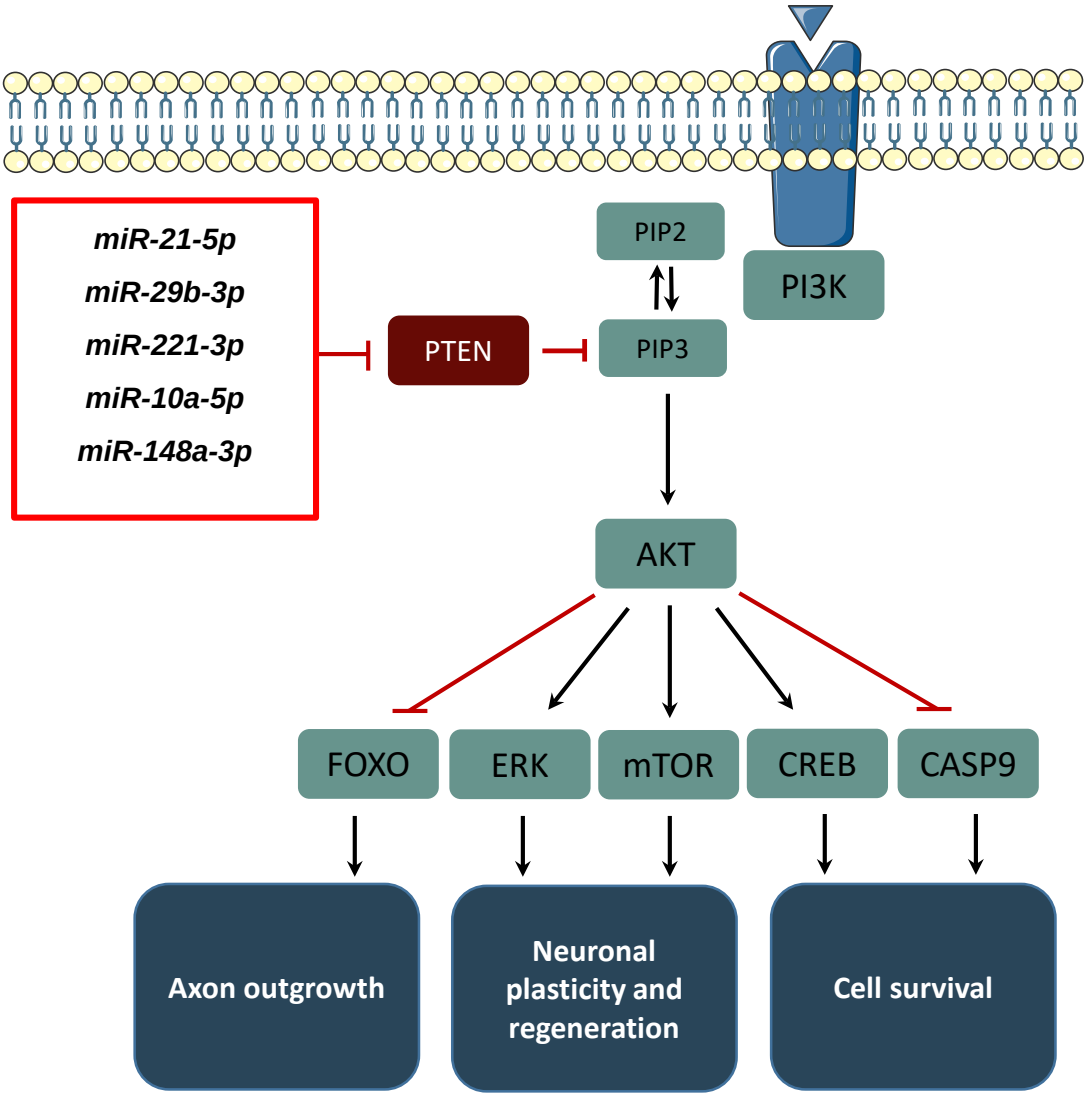

miR-21-5p
miR-29b-3p
miR-221-3p
miR-10a-5p
miR-148a-3p
PTEN
PIP2
PIP3
PI3K
AKT
FOXO
ERK
mTOR
CREB
CASP9
Axon outgrowth
Neuronal plasticity and regeneration
Cell survival

Fig.6

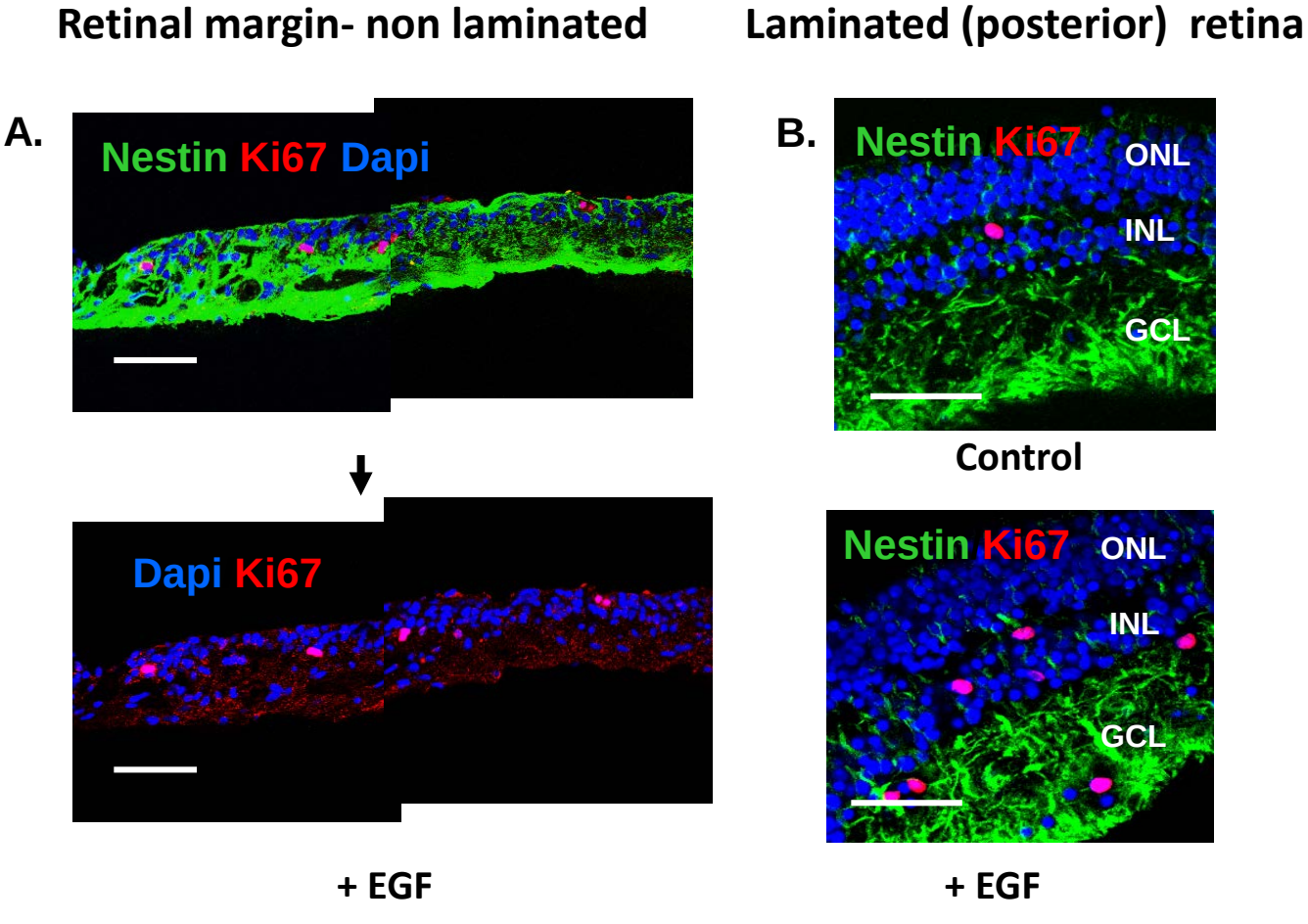
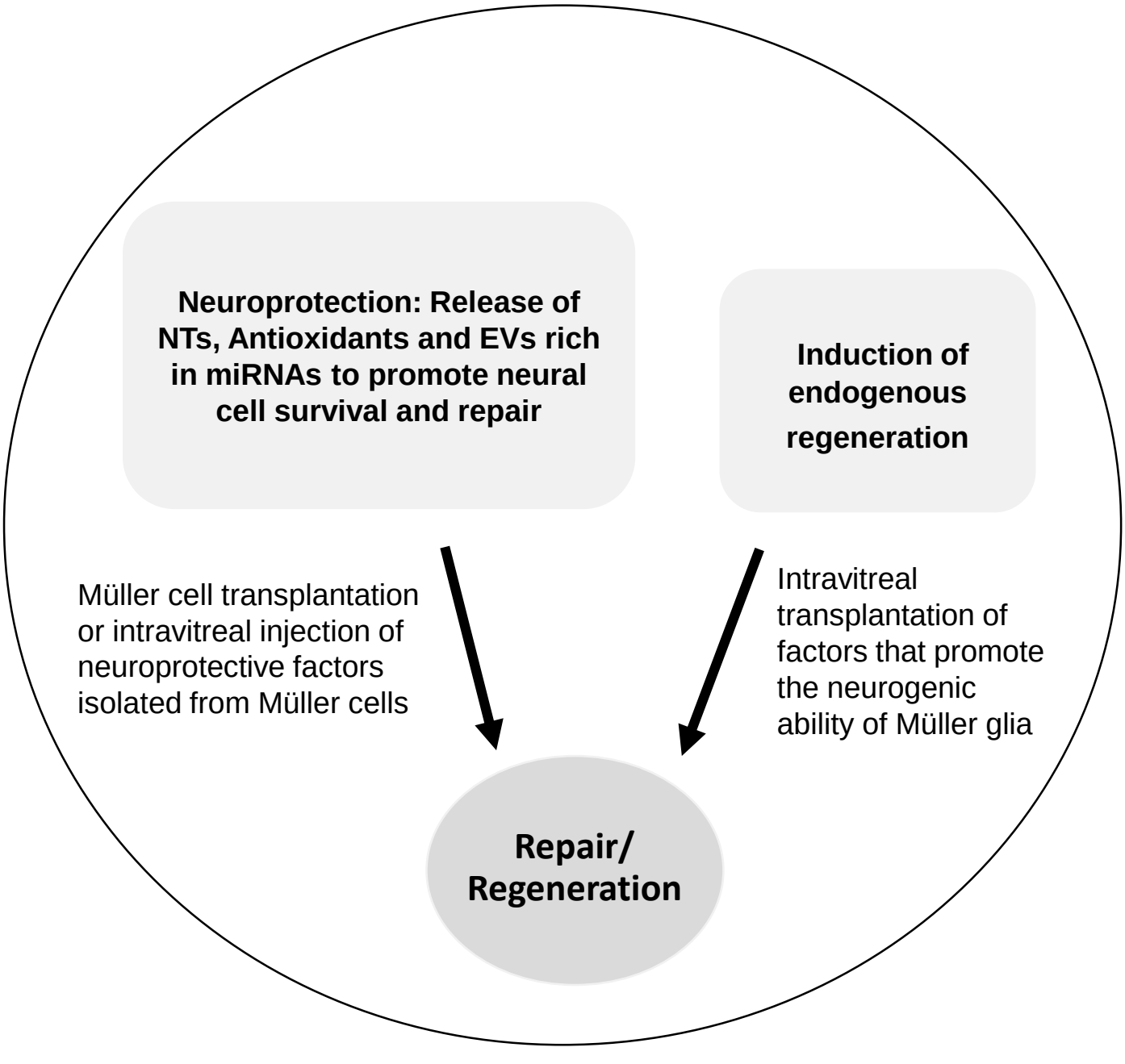

Retinal margin- non laminated
Laminated (posterior) retina
A.
Nestin Ki67 Dapi
Dapi Ki67
+ EGF
B.
Nestin Ki67
ONL
INL
GCL
Control
Nestin Ki67
ONL
INL
GCL
+ EGF

Fig.7


Neuroprotection: Release of NTs, Antioxidants and EVs rich in miRNAs to promote neural cell survival and repair
Induction of endogenous regeneration
Müller cell transplantation or intravitreal injection of neuroprotective factors isolated from Müller cells
Intravitreal transplantation of factors that promote the neurogenic ability of Müller glia
Repair/ Regeneration