Chapter XX

Title: Gliosis can impede integration following photoreceptor transplantation into the diseased retina.

Authors: Claire Hippert*, Anna B. Graca and Rachael A. Pearson

Author affiliation: Department of Genetics, University College London Institute of Ophthalmology, 11-43 Bath Street, London, EC1V 9EL.

* Correspondence should be sent to Dr Claire Hippert, Department of Genetics, University College London Institute of Ophthalmology, 11-43 Bath Street, London, EC1V 9EL, +44 (0) 2076086981, e-mail: c.hippert@ucl.ac.uk

Key Words:

Gliosis, Müller glia, Intermediate filament, GFAP, CSPG, Photoreceptor transplantation, Barrier modulation

Abstract: Retinal degenerations leading to the loss of photoreceptor (PR) cells are a major cause of vision impairment and untreatable blindness. There are few clinical treatments and none can reverse the loss of vision. With the rapid advances in stem cell biology and techniques in cell transplantation, PR replacement by transplantation represents a broad treatment strategy applicable to many types of degeneration. The number of donor cells that integrate into the recipient retina determines transplantation success, yet the degenerating retinae presents a number of barriers that can impede effective integration. Here, we briefly review recent advances in the field of PR transplantation. We then describe how different aspects of gliosis may impact on cell integration efficiency.

XX1. Introduction:

Despite very different aetiologies and pathogenesis, retinal neurodegenerative diseases like age-related macular degeneration, retinitis pigmentosa (RP), glaucoma and diabetic retinopathy culminate in the loss of light-sensing PR cells and the subsequent loss of vision. Currently, there are few effective therapeutic approaches to treat PR loss, and none of them can reverse the loss of vision. Innovative medical therapies such as electronic retinal implants (Stingl and Zrenner 2013), or gene and cell therapy (Cuenca et al. 2014) are attractive approaches for the treatment of retinal disease. Gene therapy for the treatment of inherited retinal disorders has yielded very exciting and promising results (Smith et al. 2012), however this therapeutic strategy can only be applied in the early stages of retinal degeneration as it relies on the presence of the endogenous PR cells, offering limited help for advanced disease. Cell replacement therapy is of particular interest in this particular circumstance as it offers a direct replacement of the lost tissue and can potentially restore visual function. Over the past decade, we have seen a considerable progress in using this approach to repair the degenerating retina (Cuenca et al. 2014). However, it has been shown that although it is

possible to treat some forms of end stage (Kwan et al. 1999; Singh et al. 2013), the precise nature and characteristics of the degeneration arising from a given disease-causing defect is important in determining transplantation outcome. As degeneration progresses the retinal microenvironment undergoes a number of significant changes that are potentially hostile to therapeutic interventions. A number of studies have indicated that a major determinant of successful retinal transplantation is the extent of reactive gliosis within the recipient retina, which acts as both a physical and chemical barrier to migrating cells (Pearson et al. 2014).

XX2. Advances in the field of PR transplantation:

In recent years, one of the most extensively studied therapeutic strategies has been the transplantation of dissociated PRs and their precursor cells. MacLaren et al. demonstrated that integration and appropriate differentiation of donor PR cells is achievable if the transplanted cells are at an appropriate developmental stage at the time of transplantation (MacLaren et al. 2006). The use of a genetic marker, *Nrl*, a transcription factor first expressed in immature rods shortly after terminal mitosis (Akimoto et al. 2006), demonstrated that post-mitotic rod precursor cells taken from postnatal retinae were optimal for transplant and led to better integration than donor cells from earlier or later stages in development. These transplanted PR precursors were able to migrate from the site of transplantation, the subretinal space, into the recipient outer nuclear layer (ONL), where they settled in an appropriate place. The new PRs continue to mature and form inner and outer segments and synaptic connections with the remaining neurons within the retina (Warre-Cornish et al. 2013). Moreover, these new PRs are light sensitive and can transmit visual information to the brain, leading to restoration of visual function in a murine model of stationary night blindness (Pearson et al. 2012). Recent advances in stem cell technology have demonstrated the potential to generate renewable sources of donor cells from embryonic (ES) and induced

pluripotent stem cells. Gonzalez-Cordero et al. have shown that ES-derived rod precursors can migrate and integrate into the recipient retina in a manner very similar to precursors derived from the developing retina (Eiraku et al. 2011; Gonzalez-Cordero et al. 2013).

Much of the research into PR transplantation has been performed in wild-type or isolated models of RP. This raises the fundamental question as whether PR transplantation is equally able to treat a wide spectrum of inherited retinopathies. It is well known that during disease progression the retina undergoes structural remodeling, including changes in neuronal connections, gliosis and changes in outer limiting membrane integrity (OLM). These changes may then have a positive or negative influence on the outcome of PR precursor cell transplantation. Barber et al. performed the first comprehensive study of rod PR transplantation in murine models of slow, moderate and fast PR degeneration. Importantly, they found that PR transplantation was feasible in all examined animals; however disease type had a significant impact on both the number of integrated cells and their morphology. This study identified two key determinants of transplant success; the extent of glial scarring and the integrity of OLM. Both factors can impede the migration of donor cells from the subretinal space and their successful integration within the recipient retina. Below, we focus on gliosis and its impact on cell transplantation.

XX3. Gliosis a potential barrier to photoreceptor transplantation:

Gliosis is the term given to the process in which the glial cells become activated. When these cells are activated, they upregulate the glial intermediate filament (IF) proteins vimentin and glial fibrillary acidic protein (GFAP), their apical terminal processes may undergo hypertrophy and a concomitant increase in the deposition of inhibitory extracellular matrix (ECM) molecules, such as chondroitin sulphate proteoglycans (CSPGs) can be observed.

These changes represent physical and biochemical barriers, respectively, which may prevent transplanted PRs from reaching the recipient retina.

XX3.1. Glial cell hypertrophy may act as a physical barrier:

In the retina, Müller glia (MG) span the entire thickness of the vertebrate retina and represent the major type of glial cells. They are responsible for the structural stabilization of the retina, support the functioning and metabolism of retinal neurons and are active players in normal retinal function as well as in virtually all types of retinal degeneration where they undergo reactive gliosis (Bringmann et al. 2006). Gliosis in the retina can be induced by mechanical insult (Lewis et al. 2010), retinal degeneration (Zhang et al. 2003), inflammation (Dinet et al. 2012) and/or ageing (Kim et al. 2004). It includes morphological, biochemical and physiological changes, which can vary with the type and severity of the insult. One of the readily detectable responses to retinal diseases and injuries, which is often used as a universal early cellular marker for retinal injury, is the upregulation of the IF protein, GFAP (Dahl 1979). In a healthy retina expression of $GFAP^{+ve}$ IF is largely restricted to astrocytes with only a few $GFAP^{+ve}$ Müller glial processes detected in the inner retina. In the diseased retina, GFAP is increased in both activated cell types. The level and localisation of GFAP IF expression in the MG processes is disease specific (Hippert et al., unpublished data). The increased expression of IFs is thought to help stabilize the newly formed terminal processes of MG and provide resistance to mechanical stress (Verardo et al. 2008). At first, gliosis seems to represent a cellular attempt to protect the tissue from further damage to promote repair and to limit neuronal remodeling. However, MG activation can also be exacerbated and lead to the hypertrophy of the MG end-feet processes, which fill in the gaps where PRs die (Bringmann et al. 2006). This contributes to the formation of a glial scar in the subretinal space which may impair neurite outgrowth and act as a barrier to regenerating and/or

transplanted cells. Supporting this view are the findings that transgenic animals lacking both GFAP and vimentin in MG shown a more permissive environment for the grafted cells as shown by better integration and differentiation of transplanted cells as well as a higher neurite outgrowth than in wild-type recipients (Kinouchi et al. 2003). In line with this, Barber et al. (2013) reported that transplantation outcome of rod precursor cells in different models of inherited blindness is broadly inversely correlated with the extent of GFAP expression.

XX3.2. The extracellular matrix changes may act as a chemical barrier:

The retinal environment, like elsewhere in the CNS, is enriched in CSPGs. These include a variety of core proteins each carrying chondroitin sulphate glycosaminoglycans (GAG) chains. CSPGs bind many different ECM proteins and growth factors making them important players in a variety of regulatory processes including cell adhesion, migration and differentiation (Ichijo 2004). In the CNS, CSPGs are upregulated after injury and participate in the inhibition of axon regeneration mainly through their GAG side chains. Application of the bacterial enzyme chondroitinase ABC (ChABC), which degrades GAG chains into disaccharides, promotes functional recovery in the injured CNS (Bradbury et al. 2002). In retinal degeneration our understanding of the role of CSPGs is surprisingly limited. In the healthy retina, CSPGs are found in several regions including the optic nerve, inner and outer plexiform layer, the interphotoreceptor matrix and in the ganglion cell layer (Inatani and Tanihara 2002). When using a broad spectrum CSPG antibody in murine models of RP, we have observed marked variations in the level of expression of CSPGs (Hippert et al., unpublished data). Numerous studies with both stem cell and PR precursor transplants demonstrated that treatment with ChABC prior the transplantation increased the number and survival of integrated donor cells (Singhal et al. 2008; Ma et al. 2011; Barber et al. 2013). An improvement of viral vector diffusion and transduction has also been described when

applying this enzyme in conjunction with lentiviral vector to the sub-retinal space (Grüter et al. 2005).

XX4. Conclusion, Importance of characterizing retinal environment changes:

Dependent on the ocular disease type, different changes occur in the retina which lead to altered retinal microenvironments. A better understanding and characterization of these changes is essential for the development of new therapeutic approaches. To our knowledge no drugs have been able to show an efficient removal of IF proteins to overcome the glial scar barrier. We are using RNA interference to modulate the expression of GFAP in conjunction with PR precursor transplantation, to establish the precise role of GFAP in impeding donor cell integration (unpublished data). Currently, local treatment with ChABC is the major strategy to override the inhibitory effect of CSPGs on cell-based therapies. However, ChABC presents some disadvantages in using it as a therapeutic treatment in patients, including the potential for inflammatory reaction due to its bacterial origin (Lee et al. 2010). A more detailed characterization of the major changes in ECM composition may enable the identification of specific CSPGs that undergo potentially disease-specific changes. This may enable targeted breakdown of specific CSPGs and enhance cell transplantation efficiency. Our focus here has been gliosis as a barrier to cell transplantation, however other barriers exist. Different studies reported that the OLM may also act as a physical barrier to cell transplantation (West et al. 2008; Pearson et al. 2010). Finally, combining cell transplantation with the manipulation of two or more barriers will be another interesting approach to investigate. We recently combined OLM disruption and CSPG degradation with encouraging results (Barber et al. 2013), while others have combined ChABC with growth factors (IGF-1)(Ma et al. 2011).

In summary, significant progress has been made in the field of PR transplantation therapy but achieving high numbers of new integrated PRs in the diseased retina remains a major challenge. A better understanding of the microenvironmental changes in the degenerating retina should help to overcome this.

References

Akimoto M, Cheng H, Zhu D et al (2006) Targeting of GFP to newborn rods by Nrl promoter and temporal expression profiling of flow-sorted photoreceptors. Proceedings of the National Academy of Sciences of the United States of America 103:3890-3895

Barber AC, Hippert C, Duran Y et al (2013) Repair of the degenerate retina by photoreceptor transplantation. Proceedings of the National Academy of Sciences 110:354-359

Bradbury EJ, Moon LD, Popat RJ et al (2002) Chondroitinase ABC promotes functional recovery after spinal cord injury. Nature 416:636-640

Bringmann A, Pannicke T, Grosche J et al (2006) Müller cells in the healthy and diseased retina. Progress in retinal and eye research 25:397-424

Cuenca N, Fernández-Sánchez L, Campello L et al (2014) Cellular responses following retinal injuries and therapeutic approaches for neurodegenerative diseases. Progress in retinal and eye research

Dahl D (1979) The radial glia of Müller in the rat retina and their response to injury. An immunofluorescence study with antibodies to the glial fibrillary acidic (GFA) protein. Experimental eye research 28:63-69

Dinet V, Bruban J, Chalour N et al (2012) Distinct effects of inflammation on gliosis, osmohomeostasis, and vascular integrity during amyloid beta-induced retinal degeneration. Aging cell 11:683-693

Eiraku M, Takata N, Ishibashi H et al (2011) Self-organizing optic-cup morphogenesis in three-dimensional culture. Nature 472:51-56

Gonzalez-Cordero A, West EL, Pearson RA et al (2013) Photoreceptor precursors derived from three-dimensional embryonic stem cell cultures integrate and mature within adult degenerate retina. Nature biotechnology 31:741-747

Grüter O, Kostic C, Crippa S et al (2005) Lentiviral vector-mediated gene transfer in adult mouse photoreceptors is impaired by the presence of a physical barrier. Gene therapy 12:942-947

Ichijo H (2004) Proteoglycans as cues for axonal guidance in formation of retinotectal or retinocollicular projections. Molecular neurobiology 30:23-33

Inatani M, Tanihara H (2002) Proteoglycans in retina. Progress in retinal and eye research 21:429-447

Kim K-Y, Ju W-K, Neufeld AH (2004) Neuronal susceptibility to damage: comparison of the retinas of young, old and old/caloric restricted rats before and after transient ischemia. Neurobiology of aging 25:491-500

Kinouchi R, Takeda M, Yang L et al (2003) Robust neural integration from retinal transplants in mice deficient in GFAP and vimentin. Nature neuroscience 6:863-868

Kwan AS, Wang S, Lund RD (1999) Photoreceptor layer reconstruction in a rodent model of retinal degeneration. Experimental neurology 159:21-33

Lee H, McKeon RJ, Bellamkonda RV (2010) Sustained delivery of thermostabilized chABC enhances axonal sprouting and functional recovery after spinal cord injury. Proceedings of the National Academy of Sciences 107:3340-3345

Lewis GP, Chapin EA, Luna G et al (2010) The fate of Müller's glia following experimental retinal detachment: nuclear migration, cell division, and subretinal glial scar formation.

Ma J, Kabiel M, Tucker BA et al (2011) Combining chondroitinase ABC and growth factors promotes the integration of murine retinal progenitor cells transplanted into Rho−/− mice. Molecular vision 17:1759

MacLaren RE, Pearson R, MacNeil A et al (2006) Retinal repair by transplantation of photoreceptor precursors. Nature 444:203-207

Pearson R, Barber A, West E et al (2010) Targeted disruption of outer limiting membrane junctional proteins (Crb1 and ZO-1) increases integration of transplanted photoreceptor precursors into the adult wild-type and degenerating retina. Cell transplantation 19:487

Pearson RA, Hippert C, Graca AB et al (2014) Photoreceptor replacement therapy: Challenges presented by the diseased recipient retinal environment. Vis Neurosci:1-12

Singh MS, Charbel Issa P, Butler R et al (2013) Reversal of end-stage retinal degeneration and restoration of visual function by photoreceptor transplantation. Proceedings of the National Academy of Sciences of the United States of America 110:1101-1106

Singhal S, Lawrence JM, Bhatia B et al (2008) Chondroitin sulfate proteoglycans and microglia prevent migration and integration of grafted Müller stem cells into degenerating retina. Stem Cells 26:1074-1082

Smith AJ, Bainbridge JW, Ali RR (2012) Gene supplementation therapy for recessive forms of inherited retinal dystrophies. Gene Ther 19:154-161

Stingl K, Zrenner E (2013) Electronic Approaches to Restitute Vision in Patients with Neurodegenerative Diseases of the Retina. Ophthalmic research 50:215-220

Verardo MR, Lewis GP, Takeda M et al (2008) Abnormal reactivity of Müller cells after retinal detachment in mice deficient in GFAP and vimentin. Investigative ophthalmology & visual science 49:3659-3665

Warre-Cornish K, Barber AC, Sowden JC et al (2013) Migration, Integration and Maturation of Photoreceptor Precursors Following Transplantation in the Mouse Retina. Stem cells and development 23:941-954

West E, Pearson R, Tschernutter M et al (2008) Pharmacological disruption of the outer limiting membrane leads to increased retinal integration of transplanted photoreceptor precursors. Experimental eye research 86:601-611

Zhang Y, Caffé AR, Azadi S et al (2003) Neuronal integration in an abutting-retinas culture system. Investigative ophthalmology & visual science 44:4936-4946