Stem Cell Plasticity, Neuroprotection and Regeneration in Human Eye Diseases

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Abstract: Regeneration and plasticity refer to the ability of certain progenitor cells to produce cell lineages with specific morphological and functional settings. The pathway from a less delineated or immature phenotype to a mature or specialized one follows intricate routes where a monumental array of molecular elements, basically transcription factors and epigenetic regulators that turn off or on a specific phenotypic change, play a fundamental role. Nature itself offers procedures to healing strategies. Therapy approaches to pathologies in the realm of ophthalmology may benefit from the knowledge of the properties and mechanisms of activation of different routes controlling the pathways of cell definition and differentiation. Specification of cell identity, not only in terms of phenotypic traits, but also regarding the mechanisms of gene expression and epigenetic regulation, will provide new tools to manipulating cell fates and status, both forward and backwards. In the human eye, two main locations shelter stem cells: the *limbus,* which is situated in the limit of the cornea and the conjuctiva, and the ciliary body *pars plana*. Transplantation of limbal cells is currently used in certain pathologies where corneal epithelium is damaged. Therapeutic applications of retina progenitors are not yet fully developed due to the complexity of the cellular components of the multilayer retinal architecture. Animal models of Retinitis pigmentosa or Glaucoma offer an interesting approach to validate certain techniques, such as the direct injection of progenitors into the vitreal compartment, aimed to restoring retinal function.

Key words: Ciliary body, *limbus*, plasticity, photoreceptors, regeneration, retinal ganglion cells, retina, stem cell.

INTRODUCTION

In this review we analyze recent advances in the use of stem cells in therapies related to corneal and retinal diseases. Also, the concepts of "stemness", plasticity and regeneration are revised in the context of eye diseases and therapy action.

A large number of eye diseases are, today, far from being included in a frame of effective treatment. In most cases this is due to the difficulties posed by the extensive and serious damage caused either by external agents such as trauma, infections, chemical and physical agents, or by genetic disorders, among others. It is, hence, necessary to implement new therapies designated to repair the visual function when conventional treatments are not efficacious.

The recognition and observation of regeneration sinks in the poorly defined borders of Greek mythology and positive analysis of nature (*physis*). Heracles tried to destroy the multi-headed Hydra but, in spite of the efforts to cut off the heads, Hydra would grow two for each one severed. Approximately three centuries BC, the Greek philosopher Aristotle observed that the eyes of the swallow-chick regenerated. During the eighteenth and nineteenth centuries numerous reports showed the capacity of different organisms to regenerate limbs, heads, tails, horns, eyes…etc. The amazement reached both the scientists and the general public [1]. Today, stem cell therapy and research attract the attention of many and stimulates fierce debates in some forum, not always based on objective and reliable information.

Regeneration, i.e. the recovery of a damaged tissue or organ to a complete normal structure, requires progenitor silent stem cells that need to be activated or primed in order to replace a cell population missing or dead. In some cases, cells can be reprogrammed to the progenitor state from a differentiated state [2, 3]. Furthermore, certain cells may switch from a given differentiated state to a different differentiated state. For example, anuran amphibians can regenerate the retina through, among other mechanisms, transdifferentiation of the retinal pigmented epithelium and obtain a new lens from dorsal iris pigmented epithelium [4]. For a long time it has been accepted that differentiation was a one-way path: once the process started, cellular mechanisms would hinder the way back. However, this model does not explain differentiation in all its forms [2, 5].

Stem cells are characterized by its quiescent state and by its high differentiation potential, should certain conditions occur [6]. The term "stemness" is vague and ill-defined. It includes different types of potencies. For example, the fertilized oocyte is a totipotent cell able to produce all cells of the

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future organism, including the trophoblast. Embryonic stem cells are named pluripotent because they give rise to all types of cells of the organism, except the cells forming the trophoblast. Furthermore, multipotent postnatal stem cells generate cells only in the tissue where they dwell [6, 7]. Stem cells are able to both self-renew and also bring about cells that differentiate [8]. The fate is resolved by the type of division. A stem cell may divide symmetrically generating twin cells. These cells may conserve its "stemness" or may render a progeny that differentiates. Alternatively, an asymmetrical division may originate one stem cell that replenishes the stem cell pool and one cell that differentiates [6, 8]. Thus, the regenerative capacity of tissues relies on the optional use of symmetric and asymmetric divisions. The ability of the cells to switch between different modes of division, which depend both on developmental and environmental mechanisms, most unknown, increases their capacity for repair [8].

Maintenance or loss of "stemness" depends significantly on the control of transcriptional mechanisms and key molecular modulators responding to external and internal cues [9, 10]. New technologies and strategies will facilitate the possibility of characterizing more precisely the molecular mechanisms defining the identity of the cells, their plasticity and potency. This knowledge is fundamental to manipulate and reprogram their fates and, consequently, to suit their properties to tailored therapies [5].

Advances in the control and definition of the properties and applications of stem cell populations, both residing in eye niches and outside the organ, will provide new ways to tackle numerous eye diseases with idiopathic, hereditary, inflammatory, degenerative, dystrophic, oxidative, malignant, traumatic or iatrogenic origin that ultimately produce blindness [11].

THE INVISIBILITY OF THE CORNEA ENSURES VISION

A transparent tissue and its adequate refractive shape are requisites that permit an undisturbed entrance of light into the eye and the processing of the stimulus by the retina and the brain with the result of conscious vision.

The cornea represents a peerless highly organized tissue of ectoderm origin that preserves, jointly with the sclera, the integrity of the eye against external agents and maintains the spherical structure of the eye. Avascularity, together with other features, is necessary to preserve its transparency. In many organisms, including humans, the cornea is formed by three strata: epithelium, stroma and endothelium. The stroma, where keratocytes reside, is separated by two covers, the Bowman membrane, situated between the epithelium and the stroma, made of collagen, and the Descemet membrane, which separates the stroma and the endothelium (a single layer of endothelial cells that extracts water form the stroma) and is also composed of collagen [12, 13]. Collagen fibres have an order in the space in a way that permits direct pass of light through the tissue and eliminates light scattering. Fourier analysis, that establishes the main frequencies in a set of waves or other repeating phenomena, results very useful to analyze corneal transparency [14].

As a consequence of many insults and injuries, the cornea loses its clearness and vision might be blurred or disappears. Among many therapy approaches to treat corneal disfunction, including corneal transplant alternatives, from penetrating keratoplasty to Descemet´s stripping endothelial keratoplasty (DSEK) [15-17] or application of artificial cornea, have provided successful solutions to repairing damaged tissue. However, other alternatives, sometimes a combination of strategies, should be considered since tissue availability, graft rejection or administration of immunosuppressors may make the treatment difficult or cumbersome [18]. Both corneal and conjunctival cells may be useful candidates for repairing [19].

ADULT STEM CELLS RESIDENT IN THE CORNEA

Tissue turnover in the cornea and other epithelia such as the epidermis, lung epithelium or intestinal epithelium is essential to maintain tissue homeostasis and to supply new cells when damage occurs [20]. The corneal epithelium is continually renewed through desquamation (Z component), centripetal cell migration [Y component] and proliferation of basal cells (X component). The XYZ hypothesis establishes that corneal maintenance follows the expression X+Y=Z [21]. This model is bound to the principal role of the limbus, a limited area situated in the boundaries between the cornea, the conjunctiva and the sclera as the main cell feeding source. The limbus is a vascularised transition tissue, where two different epithelia meet, with an essential role in the metabolism of the cornea. It accommodates a group of cells, called limbal epithelial stem cells (LESC or LSCs), described forty years ago [22]. Their potency is restricted to the production of differentiated corneal epithelial cells. The pathway of differentiation has intermediate cellular entities, namely transient amplifying cells (TAC), post mitotic cells and fully differentiated corneal epithelial cells [23]. Distinguishing stem cells and isolating them from other residents in the limbal dwellings continues to be a challenge for researches in spite of successful application of limbal grafts to human corneal alterations. Several markers, such as ABCG2 protein or cytokeratin 19 have been postulated useful to identify and isolate LESC [24]. Also, the proliferation potential, and other morphological characteristics, including cell size, may benefit an appropriate isolation of this slow cycling population [23, 25]. On the other hand, the utilization of microarrays has provided abundant information that may help to establish biological profiles, attending to the expression of different genes, and signature markers of LESC identity [26, 27]. The definition of "stemness" based on specific molecular profile appears as a monumental task since this profile may change without loosing the capacity to produce differentiated cell populations. Therefore, today, it is not possible to conclusively identify these cells with definitive markers, either present or absent [28]. The combination of different markers and morphological features may help the identification of these stem cells [29]. The continuity of this population of cells lies in asymmetrical divisions that give rise to a cell population that stay as LESC or that differentiate from the state of transient amplifying cell [18]. Apparently, LESC are not the only adult stem cells living in the cornea. It has been reported that oligopotent stem cells reside throughout the corneal epithelium in different mammals

[19]. The same authors suggest that corneal and conjunctival epithelia meet at the limbus and any disruption between these two epithelia, induced for instance by an alteration in the cornea, causes migration of the cells to repair the wounded area. Interestingly, the limbus appears enriched of stem cells because they accumulate by the play of different forces [19] that cause the displacement to the boundaries situated between the cornea and the conjunctiva. The stem cells studied have the capacity of generating both corneal and conjunctival adult cells. The results highlight the fact that the cornea is not different from other squamous epithelia where stem cells are distributed throughout the tissue. This model, however, has been the subject of recent debate and discussion [30].

Interestingly, the limbal rim does not show a regular population of stem cells. Within the limbal circumference, a higher yield of stems cells is obtained from superior and inferior enclaves [29, 31]. Also, the analysis of the transcript profile combined with morphological and immunohistochemical studies of limbal cells harvested from different localizations have shown that no differences in transcription or phenotype were observed. However, cells acquired from the superior region exhibited higher outgrowth and generated a thicker epithelium when cultivated *ex vivo* [32]. This ability of the cells from the superior limbus may be related to the richer presence of limbal crypts and focal stromal projections [13, 31].

Dua H *et al.* [33] have shown that in patients diagnosed of limbal deficiency the epithelium from the central regions of the cornea remained unchanged for long periods and claim that limbal cells may not be as important, as it is broadly believed, in maintaining corneal epithelium if it remains intact. However, they may have an important role when the cornea suffers trauma. After induced damage the central corneal epithelium responds rapidly by repairing the injured tissue whereas the onset of response from LESC is delayed several hours after the central epithelium acted [34]. These results suggest that the response of LESC depends on a certain threshold of damage. Hence, the physiological maintenance of the corneal epithelium and the repairing responses may follow different paths and initiate different mechanisms able to counteract lesions.

THERAPEUTICAL APPLICATIONS OF LESC

Trauma or disease may produce a condition, called stem cell deficiency, where limbal anatomy is lost, the conjunctiva penetrates the cornea and the corneal epithelium loses volume and appears disorganized. Also, the conjunctivalized cornea is vascularized [33, 35]. Derived consequences of LESC deficiency are opacity, fragility of the corneal surface and serious discomfort and pain. It is broadly accepted that many ocular pathologies are related to the decline of corneal regenerative capacity, based on the supply from stem cells niches. However, until a precise morpho-molecular definition of LESC is achieved and the role of the influence of the neighbour stromal cells understood, we can not classify LESC deficiency as a delimited entity in all cases where repairing of the corneal surface fails [36].

If the condition is unilateral, the healthy contralateral limbal annulus may serve to replace the altered epithelium (autologous transplant). Direct grafting of uninjured contralateral eye limbal fragments was first performed in patients suffering serious unilateral corneal insult [37]. In some cases of severe ocular surface disorders, cadaver allografts have been applied to eliminate corneal opacity [38]. Cultivation of LESC, extracted from a healthy contralateral limbus, has launched new avenues to treat corneal deficiencies. The group of Graziella Pellegrini reported in 1997 [39] the use of autologous continuous corneal epithelial sheets, grown *in vitro*, to repair wounded corneas in two patients bearing unilateral corneal lesions caused by alkali burns. To obtain *ex vivo* good expansion this technique needs suited conditions that include the use of scaffold substrates such as collagen, amniotic membranes, synthetic polymers, fibrin, fibroin, etc [13, 18, 40]. Stimuli form other cells that provide growth signals may accelerate differentiation processes since cocultivation of LESC together with feeder cells, such as limbal fibroblasts, has proven to be a good strategy to attain epithelial coats in good condition for transplant [41]. To better understand the influence of the microenvironment on the fate and development of stem cells, new approaches regarding the role of exogenous regulators and 3-dimensional cell culture procedures need to be achieved [42, 43]. The appropriate combination of biomaterials and cells of different origins may result in new applications to treat specific corneal damages [44].

When both eyes are affected, external sources, from cadavers, living donors or *ex vivo* multiplication of limbal stem cells, should be sought after in order to regenerate healthy tissue. However, to assure success, the use of allograft transplants needs the administration of immunosuppressor treatments to secure the survival of the transplanted cells. Cell viability and cure may be compromised after immunosuppression [45, 46]. Unfortunately, in most cases, it has been reported that cadaver grafts do not last for more than five years [47]. Another alternative that should be considered in case of bilateral limbal cell deficiency is the use of cells from other localizations. For example, oral mucosa, is a good candidate as a source of cells to use in allogenic transplant [48-50].

But many factors, related to the cause of damage, the clinical condition of the patient and the integrity and cell survival of the tissue transplanted, influence successful treatments [51].

THERAPEUTICAL APPLICATIONS OF OTHER CELLS TO CORNEAL PATHOLOGIES

Mesenchymal stem cells have de capacity of differentiate into diverse adult cells, including osteoblasts, adipocytes, fibroblasts or epithelial cells, among others [52]. The corneal epithelium is the external cover of the eye exposed to damage in the first line. However, the stroma and the epithelium can also suffer from trauma or disease. Although we have focused our attention to the capacity of the epithelium to heal, and the possibilities to externally apply epithelial stem cells to recover corneal transparency, the internal layers house a number of cells that may have a potential role in treating corneal diseases. For instance, a population of mes-

enchymal cells that can differentiate to adipocytes and osteoblasts has been identified in mouse corneal stroma in a recent study [53]. This finding provides new information on a source of cells suitable to use in the future in corneal therapy and regeneration.

The cornea is not the only tissue that produces "therapeutic" cells. Other localizations are been looked after in order to obtain cells adequate for use in corneal therapy. For example, epithelial cells extracted from the oral mucosa or from the conjunctiva, cultivated on amniotic membranes, offer new possibilities in patients bearing seriously scarred ocular surfaces in both eyes [46, 54]. However, some bias, such as the risk of vascularisation of the grafted cells, must be overcome [54]. Due to the similarity with the corneal epithelium, rectal, nasal, esophageal, anal or vaginal squamous epithelial cells should be pondered for autologous transplant as well [46]. Also, the use of human umbilical cord blood (hUCB) cells [55] or adipose-derived stem cells (ASCs) [56] should be considered.

It is known that limbal transplantation is contraindicated in severe dry eye [47]. A valuable approach in such cases is the application of osteo-odonto keratoprothesis (OOKP), made of autologous osteoalveolar tissue, together with synthetic material. It has been applied with relative good results in patients bearing corneal deficiencies associated with dry eye [57].

CELL ENGINEERING AND REPROGRAMMING

Differentiated cells can be reprogrammed to induced pluripotent stem (iPS) cells by exogenous addition of specific transcription factors [58] or by altering the microenvironment to influence the expression of endogenous genes [59]. The obtainment, by different methods, of iPS cells offers a new way to produce individual-specific cells, showing the capacity to differentiate to the three germ layers, which can be applied to numerous deficiencies. This novel approach eases the many problems posed by the use of human embryonic stem cells with therapeutic or research purposes [60]. However, it is necessary to point that many questions related to cell reprogramming need convincing answers. As Cox and Rizzino report [60] we need to know with more detail the mechanics of the factors and the molecular relationships that influence reprogramming. Also, the reprogramming process can be controlled to get cells in intermediate states of "stemness" which can be more appropriately applied to a specific regenerative therapy. The control of the epigenetic influence on the function and viability of the reprogrammed cells needs to be addressed too.

Replacement of corneal epithelium after an insult requires the onset of many molecular mechanisms which direct the differentiation of stem cells to the epithelial phenotype and also stimulate the migration of the cells to the appropriated localizations. For instance, it has been reported the participation of Slug, a transcription factor, member of the Snail family, in epithelial cell migration related to corneal wound healing [61]. Among all molecular participants, efforts should be directed to define principal molecules. Indubitably, the control and modulation of some of those key molecular elements that participate in cell differentiation and cell migration, and the existence of epigenetic mechanisms taking control of the expression of prominent regulatory genes [62] may have clinical relevance and application in therapy.

Further studies focused on the morphological, developmental and molecular characterization of limbal stem cells, as well as other epithelial cells, and on the definition of biochemical mechanisms underlying differentiation pathways, the maintenance of quiescence and the role of environmental cues of the natural niches, are needed to appropriately and efficiently enforce these cells in repairing treatments applied to serious corneal disturbances. It is also noteworthy that corneal deficiencies require tailored treatments and hence, many different approaches should be available. Fig. (**1**) summarizes the main therapy procedures to restore corneal function by using stem cells.

RETINAL DISSEASES AND STEM CELL THERAPY

Visual disability as a result of retinal diseases is suffered by millions of people worldwide. Inherited and acquired retinal degenerations are frequent causes of visual impairment originated either by the loss of photoreceptor or by the loss of photoreceptors and the adjacent retinal pigment epithelium, causing age-related macular degeneration (DMAE), which frequently renders patients legally blind. The disease with the highest incidence of untreatable blindness worldwide is glaucoma [63]. Visual field changes in glaucoma are believed to be caused by the damage of retinal ganglion cells (RGCs). The decay of retinal neurons (photoreceptors or ganglion cells) caused by different diseases is one of the main causes of blindness. For that reason, using stem cells to develop replacement therapies could represent an important approach to restore visual function.

The complexity of the retinal structure makes the procedures of cell replacement or regenerative medicine very difficult indeed. The retina is a very sophisticated laminar structure formed during development with the creation of particular circuits, the disappearance of some cells and the refinement of excitatory and inhibitory synapses. Neurons resident in the retina are very sophisticated due to, either their specific function like photo-transduction (photoreceptors), interconnectivity between different cell types with excitatory and inhibitory synapses (horizontal and amacrine cells) or the complexity of long distance transmission through axons connecting with the brain in a specific retinotopic manner, as is the case of retinal ganglion cells. These difficulties are, among others, the reason why the advances in the use of stem cells as a method to repair the retina have proven particularly difficult to design and generate. Nevertheless, some advantages for stem cell therapies within the retina are the optimal combination of ease surgical access and the ability to observe transplanted cells directly through the clear ocular media [64]. It is noteworthy that the eye represents a partially immune-privileged site and appears to eventually reject allogenic cells transplanted to the sub-retinal space. This may be of concern for long-term retinal repair by using cell transplantation [65].

The retina is a complex neural structure with many specific connections established between the different cell types

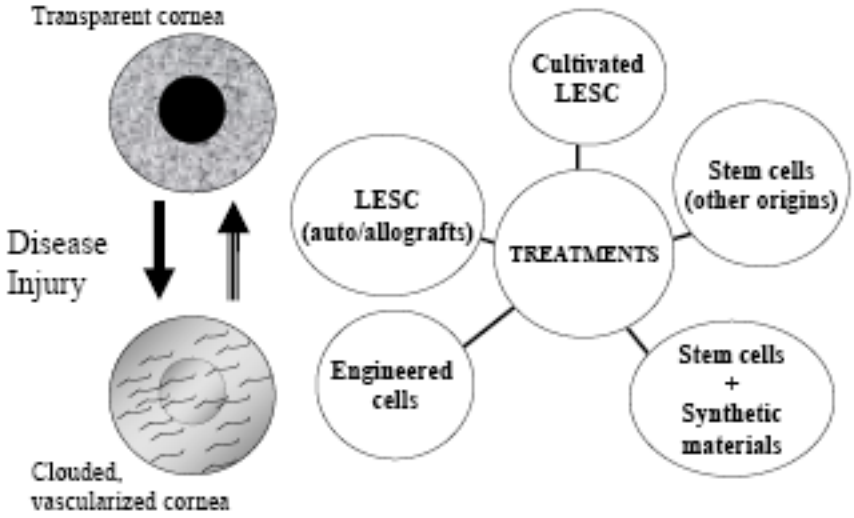

Fig. (1). Some therapeutical approaches to restore tissue transparency and vision by using stem cells. LESC: Limbal Epithelial Stem Cells.

that are organized in layers. In humans, the retina has a limited capacity of self-repair while in non-mammal vertebrates it has the capacity of growing and regenerating through their entire life. Fish, amphibians and, to a limited extent birds, replace lost neurons by the dedifferentiation of Müller glia to a progenitor state followed by the replication of these neuronal progenitor cells. The eye retains significant regenerative abilities and new retinal neurons are added to the adult retina as they grow [66-68]. These cells are added at the peripheral edge, the ciliary margin zone, in a manner that is thought to recapitulate embryonic retinal cell development [69]. The presence of a ciliary margin zone has not been detected in mouse [70]. However, a population of quiescent cells isolated from the ciliary body of the mammalian retina was discovered to proliferate *in vitro*. Moreover, they were able to express immature retinal markers and, upon differentiation, expressed markers of mature retinal cell types [71, 72].

The extensive morphological and functional diversity exhibited by the retinal neurons presents challenging questions regarding the processes of cell fate determination and differentiation [73]. Retinal progenitor cells have been shown to be multipotent throughout development and their molecular characterization has been done *in vitro*. Retinal progenitor cells can produce restricted subsets of rod and cone photoreceptors [74], horizontal cells [75] or retinal ganglion cells [76] among other types of cells. In the last four years, the scientific advance in the stem cells research has been evident, going from the ES (Embryonic Stem) cell based replacement therapy in the retina to the use of ES-like iPS specific retinal neurons. This advance will circumvent the intricate immune rejection, the ethical debate concerning the use of ES and also a reliable and renewable source of donor cells.

K. Takahashi and colleagues published that iPS cells could be generated from somatic cells by known transcription factors in mouse and in human [77, 78]. Two years after, several reports indicated that iPS can be differentiated into neurons [79-81] among other specific cell types. Presently, enriched populations of human photoreceptors [82] as well as retinal ganglion cells-like [83] can derive from iPS cells. However, the reprogramming is still open to research directed to answer many questions, especially those concerning the epigenetic influence in differentiation, as well as the investigation of strategies to beat barriers present in the degenerate neural retina and to improve retinal cell integration.

PHOTORECEPTORS AND STEM CELL THERAPY

Photoreceptor loss causes irreversible blindness in many retinal diseases. Repair of such damage by cell transplantation is one of the most feasible types of central nervous system repair; photoreceptor degeneration initially leaves the inner retinal circuitry almost intact and to contribute to the retinotopic map new photoreceptors need only make single, short synaptic connections.

Differentiation into neural and retinal pigment epithelium cell types has been relatively facile but achieving a convincing photoreceptor phenotype has been more elusive. Takahashi and colleagues demonstrated that transplanted neuronal progenitor cells could integrate into the developing retina and assume the morphological features of local cell types, including photoreceptors [84]. Transplantation studies using immature cells derived from the neural retina demonstrated the expression of the functionally important marker rhodopsin. However, the importance of the ontogenetic stage of donor cells for successful rod photoreceptor transplantation was demonstrated in the coming years. The hypothesis was that progenitor or precursor cells have a higher probability of success upon transplantation at later ontogenetic stages. Thus, donor cells can integrate into the adult or degenerating retina if they are taken from the developing retina at a time coincident with the peak of rod genesis. These transplanted cells integrate, differentiate into rod photoreceptors, form synaptic connections and improve visual function [85]. It was also shown that the sub-retinal environment could pro-

mote the differentiation of human ES cell-derived neural precursors into a limited number of cells expressing photoreceptor markers [86]. However, it was very difficult to obtain cells that could express photoreceptor markers *in vitro*. Initially, only co-cultivation of ES with other retinal cells could give rise to cells expressing photoreceptor markers such as recoverin [87, 88]. Several studies have since demonstrated mature photoreceptor morphology of integrated precursor cells injected into adult retinas [89, 90]. Recent studies on ES cells have established defined culture conditions to differentiate ES cells into photoreceptors [74].

A recent advance in stem cell biology has been the reprogramming of adult human fibroblasts, by retroviral transduction, to generate induced pluripotent stem (iPS) cells. Three independent studies used various combinations of four transcription factors, known to be required for pluripotency in ES cells and to induce pluripotent characteristics in adult cells [78, 91, 92]. Furthermore, very recent studies have shown that iPS obtained from human fibroblasts can produce retinal progenitor fate competent to generate photoreceptors. The photoreceptors derived from the iPS cells can be purified using fluorescence activated cell sorting (FACS) after labeling photoreceptors with a lentivirus driving green fluorescent protein (GFP). Afterwards, these cells can be transplanted, integrate into a normal mouse retina and express photoreceptor markers [82, 88]. However, the use of virus as a vector results in multiple random insertions of the transgene which can also lead to oncogenesis in certain circumstances. Therefore, further investigation of this cell population is required to improve the efficiency of the methods used and establish virus-free protocols of induction that would be less oncogenic and offer greater viability for therapeutic applications.

RETINAL GANGLION CELLS AND GLAUCOMA TREATMENT

Retinal ganglion cell death in glaucoma give rise to irreversible loss of vision. Neuroprotective strategies are effective at early stages of the disease. In advanced damage, cell replacement therapy may be a potential treatment for restoration of visual function. However, the retinal ganglion cells have an additional difficulty to be replaced. Their cell body is located in the retina while the terminal axons are in the brain forming a retinotopic map of connections built up during development. Despite the above, the potential use of retinal ganglion cells progenitors to replace damaged cells has been investigated during the last years.

Embryonic stem cells (ES) are capable of self-renewal and hold pluripotency to generate all specialized cell types which have been shown valuable as donor cells in retinal neuroregeneration [93, 94]. ES cells are able to generate RGC-like cells upon differentiation by basic fibroblast growth factor (bFGF) and are capable of integrating into the host retina [76]. The major barrier to retinal integration of intravitreally transplanted stem cells is the inner limiting membrane. Pluripotent bone marrow-derived mesenchymal stem cells (MSCs) have been used to test the capacity of penetration of the vitreal surface using collagenase or modulating the glial cell reactivity with α-aminoadipic acid (AAA) and the results demonstrated that the extracellular matrix of the inner basal lamina is neither necessary nor sufficient to prevent migration of transplanted cells into the neural retina. In contrast, glial reactivity was associated with poor graft migration since targeted disruption of glial reactivity dramatically improved the structural integration of intravitreally transplanted cells [95]. The methodology used to generate retinal RGC-like from reprogrammed mouse fibroblasts [83] is one more step further in the complicated cell therapy research field that is still very far from its application in humans.

INTEGRATION OF THE STEM CELLS IN THE RETINA: PERMISIVE ENVIROMENT AND IMMUNE REJECTION

Transplanted photoreceptor precursors are required to migrate and integrate into the degenerated outer nuclear layer of the retina. While the limited number of integrated photoreceptor precursor cells, demonstrated in the adult neural retina, is sufficient to restore the pupil light reflex, only a relatively small number of transplanted cells integrate. Greater numbers of integrated cells would be required in order to improve visual acuity in degenerate models. However, the ability of transplanted cells to integrate within the host retina has been shown to decline with host maturation [96]. Moreover, it has been demonstrated that injury-induced cues play a significant role in promoting the incorporation of ocular stem cells or progenitors, regardless of their origin or their differentiation, along specific retinal sublineage. Therefore, traumatized or diseased retina may support cell replacement by providing a local milieu suitable for the incorporation and differentiation of exogenous ocular stem cells. Furthermore, the transplanted cells expressed markers specific to cells of the lamina in which they were incorporated, suggesting that the cues that regulate lamina-specific differentiation are localized within the inner and outer retina [97]. Experiments carried out with a mouse model for autoimmune studies, which show elevated matrix metalloproteinase expression and decreased levels of scar-related inhibitory molecules, demonstrated that a more permissive environment facilitates cell integration within the retina [98]. Müller cell processes have also been shown to form glial barriers along the outer edge of the retina after retinal detachment [99]. Similar barriers to cell transplantation, such as the outer limiting membrane and glial scarring, have been reported to limit the integration of retinal sheets within the host retina [100]. Further to this, activated Müller cells and microglia are thought to produce increased extracellular matrix components, which have been shown to limit axon extension in the brain [101]. Outer limiting membrane of the retina disruption caused by the administration of the glial toxin α-aminoadipic acid (AAA), at the time of cell transplantation, was shown to correspond with increased photoreceptor precursor cell integration [89]. These studies demonstrated that a permissive regenerative environment is crucial for the correct integration of the cells, but we do not know all the precise clues yet.

The eye represents a partially immune-privileged site and appears to eventually reject allogenic cells transplanted to the sub-retinal space. This may be of concern for long-term retinal repair by using cell transplantation. It remains to be seen whether a homogenous population of cultured photore-

ceptor precursor cells would elicit immune rejection following transplantation to the neural retina. Cultured neural progenitors have been shown to be less immunogenic compared with freshly dissociated neural progenitors. The most likely explanation for this is the lack of donor-derived microglia in the cultured cell population [102, 103]. One of the problems concerning cell-based therapies is that recipients may require immunosuppressors to prevent rejection of the transplanted cells. One way around this complication is the utilization of cells derived from closely related or HLA-matched individuals or even the patients themselves, using induced pluripotent stem cells (iPS). However, the reprogramming is still open to research that must answer many questions, especially in relation to the role of epigenetic influences in differentiation, as well as to the investigation of strategies directed to overcome barriers present in the degenerate neural retina and the improvement of retinal cell integration.

Even when there are more questions open to the use of stem cells in the retina than positive results, some treatments have started in humans, especially in photoreceptor-related diseases like Retinitis pigmentosa. Even some institutions offer treatments with stem cells for diseases like glaucoma or DMAE. For medical and ethical reasons, we should be cautious in the use of stem cell therapy in humans and not recommend or practice these treatments until we are sure about the safety and efficacy of the therapy in experimental laboratory animals.

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