

Stem Cells of the Eye – An Illuminating Viewpoint

By A/Prof Donald TH Tan & Dr Leonard PK Ang

Sight is arguably among the most important of the five senses in the human body. Two prerequisites are essential for good vision. The first is a clear optical focusing system. The main components of this (the cornea, lens and intraocular fluids) are designed to bring visual images to a focus on the retina, with the cornea contributing most of the refractive power of the eye. The second is an intact neural system, which detects and transmits images in a coherent fashion from the retina, through an intricate series of neural pathways, to the visual cortex, where they are perceived and interpreted.

It is no wonder that the focus of an immense amount of research has been on the two parts of the eye that are essential for clear vision: the cornea and the retina, and it is therefore not surprising that recent interests in ocular stem cell research have concentrated wholly on these two major components of the ocular system.

OCULAR SURFACE STEM CELLS

The greatest advances in ocular stem cell biology and treatment have been in relation to the cornea, which forms part of the ocular surface. The ocular surface is an ideal region to study epithelial stem cell biology, because of the unique spatial arrangement of stem cells and their daughter cells, which are called "transient amplifying cells". Corneal epithelial stem cells reside in the basal region of a narrow 1.5mm band of tissue that straddles the cornea and conjunctiva, known as the limbus. Following various perturbations (e.g. injury), these stem cells are recruited to divide repeatedly, giving rise to transient amplifying cells that are the workhorses of the regenerating epithelium. These transient amplifying cells further divide rapidly to form terminally differentiated cells that take on the final characteristics of the tissue.

The compartmentalization of the corneal epithelial stem cells within the limbus provides us with a valuable opportunity to study the behaviour of these cells, and the mechanisms that modulate their growth and differentiation. It is no wonder that many stem cell biologists interested in epithelial tissues have resorted to studying this unique area, in the hope of better understanding the characteristics of stem cells, as well as the niche environment.

Our knowledge of ocular surface stem cells has led to significant improvements in the treatment of severe scarring conditions that were previously not amenable to conventional medical or surgical therapy, for example Stevens Johnson syndrome and severe chemical injury. Over the past

decade a better understanding of ocular surface stem cells and their role in wound healing has allowed us to better manage these conditions. Limbal stem cell transplantation has revolutionised the management of these difficult cases by replacing the depleted stem cell population. In cases of bilateral limbal stem cell deficiency, cadaveric limbal allografts are the only treatment option, while for unilateral disease, autologous tissue from the fellow eye may be used.

The use of allogeneic tissue, however, creates the attendant problem of tissue rejection. At present, long-term survival outcomes of limbal allografts are only in the region of 50% 5-year survival, despite the fact that patients are often on long-term topical and systemic immunosuppression regimes. In recent years, this problem has been partially circumvented by the ex-vivo expansion of autologous adult limbal epithelial stem cells on substrates, forming tissue-equivalents that could be transplanted back to the diseased eye. This novel method of treatment has allowed us to carry out stem cell transplantation, without compromising the donor eye. The additional advantage of using autologous tissue is that transmission of infection and graft rejection is minimised.

The other component of the ocular surface is the conjunctiva, which is the translucent, vascularised sheet covering the globe. The conjunctiva is extremely important in supporting the normal milieu of the ocular surface, and in fact a healthy functional conjunctiva is an essential support structure to the continued clarity and survival of the corneal epithelium and stroma. Blindness from ocular surface disorders such as Stevens Johnson syndrome, ocular cicatricial pemphigoid and severe chemical burns, may be directly due to corneal damage, but this in turn is only secondary to a primary disease process which initially affects the conjunctiva. Conjunctival stem cells involved in the regeneration of the conjunctiva are believed to be concentrated in the conjunctival fornices, which are superior and inferior recesses formed by the lid and globe.

The presence of healthy conjunctiva is important in maintaining the homeostasis of the ocular surface and its regeneration. A new technique that we have developed is the cultivation of autologous adult conjunctival epithelial cells on amniotic membranes to form conjunctival equivalents. These are transplanted back into diseased eyes that require ocular surface reconstruction. We recently used this technique in a 9-year-old Indian girl with an extensive conjunctival naevus that covered almost $\frac{2}{3}$ of the visible conjunctival surface. Excision of this naevus would result in a large epithelial defect. Conventional surgical reconstruction would have required obtaining a conjunctival graft from the

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fellow eye to resurface the bare defect, or leaving the defect bare to heal by secondary intention. Both of these options had their disadvantages, in view of the iatrogenic injury that would be caused to the previously healthy fellow eye, or the significant scarring that could result in the operated eye if the wound were left bare. Instead, we used our technique of creating a conjunctival equivalent, by the ex-vivo expansion of a small quantity of conjunctival epithelial cells, taken from the fellow eye, to form a large epithelial sheet over an amniotic membrane substrate. This conjunctival equivalent was then used in the reconstruction of the ocular surface, following complete excision of the naevus. The good cosmetic and functional result that followed gives hope to the possibility of this technique being employed in future for other conditions of the eye where normal healthy tissue is deficient.

Another breakthrough has been the ability to propagate ocular surface cells for clinical transplantation using serum-free media, without the need for animal feeder cells (these are traditionally required to support the proliferation of cells). Because animal products are eliminated from the culture system, this minimizes the spread of infection or xenograft rejection. Although long-term results have yet to be determined, the use of conjunctival equivalents show great promise in the treatment of conditions where the conjunctiva is deficient or diseased, for instance, in cicatrizing diseases or following extensive ocular surgery.

THE POSTERIOR SEGMENT – RETINAL STEM CELLS

The eye is a highly specialized sensory organ that can be viewed as an extension of the nervous system. This is supported by the fact that much of the eye is derived from neural elements during embryogenesis. The pigmented ciliary body and the peripheral margin of the retina in the adult mammalian eyes are believed to harbor neural progenitors that display stem cell properties and have the capacity to give rise to retinal neurons. They have been shown to be multipotential and have the ability to self-renew. Interestingly enough, despite our conventional understanding that neural cells are highly specialized and highly differentiated cells, some degree of plasticity has been demonstrated – investigators have shown that human neural stem cells possessed the potential to differentiate into retinal cells, if given the appropriate stimulus.

The field of retinal stem cell transplantation is comparatively in its infancy. Retinal stem cells transplanted into the eyes of rats were able to survive and differentiate into cells of photoreceptor lineage. Others have attempted transplanting neural or glial precursors, and showed that they could undergo differentiation and survive for long periods. If successful, this may be the only hope for sufferers of retinal degenerative disorders, where no treatment currently exists.

FUTURE AREAS OF RESEARCH

Our understanding of the characteristics and behavior of stem cells has thus far been limited by the absence of markers that

can distinguish stem cells from their daughter cells. Much of our knowledge regarding stem cell physiology has been through indirect means. Various putative stem cell markers have been proposed, but to date, no exclusive stem cell marker has been identified. Finding a specific stem cell marker will have tremendous impact in increasing our knowledge and understanding of the normal homeostatic mechanisms that regulate the proliferation and maintenance of tissues in the body.

Factors responsible for maintaining the “stemness” of stem cells have also yet to be determined. It is believed that both intrinsic factors (inherent to the cell), and extrinsic factors (environmental factors surrounding the cell) are involved in the regulation and maintenance of the stem cell “niche” or stem cell microenvironment. Although much of the research in the area of stem cell biology has been in understanding the physiology and homeostasis of these cells, understanding the “niche” microenvironment where stem cells reside is equally important. It is currently believed that this microenvironment maintains the stem cells in its undifferentiated state at rest, while at times of injury, it supplies the necessary cytokine-mediated or neural-mediated signals, to recruit these cells to undergo multiple cell divisions. Understanding the factors that maintain and preserve the stem cell population may provide us with new treatment options that help to prevent stem cell loss in ocular diseases.

All current work on ocular stem cells has to date focused on adult stem cells and research has progressed to the reasonably advanced stage of adult stem cell expansion and transplantation. We need to turn our attention to embryonic stem cells of the ocular system. Embryonic stem cells are touted to be the ultimate “mother-of-all stem cells”, as they represent the most primitive of all progenitor cells. These cells are pluripotent, and their daughter cells are able to develop into all the tissues and organs in the body. Harnessing the immense potential of these cells would have tremendous impact, as this would allow whole organs to be engineered. The biggest hurdle at present is to direct these primitive cells along specific cell lines. Some headway has been made in this area. Investigators have shown that embryonic stem cell-derived neural progenitors are able to acquire retinal phenotypes. Other sources of multipotent stem cells that have been studied for use in the eye include bone marrow derived stem cells and mesenchymal stem cells. The challenge is to be able to induce these to differentiate into specific cell lineages under strict conditions in-vitro. Much of the work in this area is at present preliminary, and it will be many more years before the full potential of these multipotent stem cells can be realised.

With the advent of what essentially is the first stage of ocular tissue engineering, harnessing the power of stem cells, we certainly hope that, in the not too distant future, we will be able to harness the full potential of these cells, thereby providing a cure for many blinding conditions of the eye.

We will be keeping a close *eye* on this, and can only hope that new research endeavours *stemming* from this line of research will lead us to a better insight into our sight. ■