Editorial

Stem Cell INVESTIGATION

Cone fusion confusion in photoreceptor transplantation

Robert E. MacLaren

Nuffield Laboratory of Ophthalmology, Department of Clinical Neurosciences, University of Oxford & Oxford Eye Hospital, Oxford University NHS Foundation Trust, Oxford, UK

Correspondence to: Robert E. MacLaren. Nuffield Laboratory of Ophthalmology, Department of Clinical Neurosciences, University of Oxford & Oxford Eye Hospital, Oxford University NHS Foundation Trust, Headley Way, Oxford OX3 9DU, UK. Email: enquiries@eye.ox.ac.uk.

Provenance: This is an invited Editorial commissioned by Editor-in-Chief Zhizhuang Joe Zhao (Pathology Graduate Program, University of Oklahoma Health Sciences Center, Oklahoma City, USA).

Comment on: Zhu J, Cifuentes H, Reynolds J, *et al.* Immunosuppression via Loss of IL2rγ Enhances Long-Term Functional Integration of hESC-Derived Photoreceptors in the Mouse Retina. Cell Stem Cell 2017;20:374-84.e5.

Received: 26 July 2017; Accepted: 04 August 2017; Published: 29 August 2017.

doi: 10.21037/sci.2017.08.02

View this article at: http://dx.doi.org/10.21037/sci.2017.08.02

Advances in stem cell biology have highlighted the potential for the eye as an ideal model in which to understand the mechanisms of neuronal repair. It is therefore not entirely surprising that the first reported allograft from one human to another also occurred in the eye, but over 100 years ago, with the successful corneal transplant performed by Eduard Zirm (1). Later in the 20th Century, Peter Medawar and colleagues identified the mechanism of immune rejection that prevented survival of skin grafts and whole organ transplantation (2). That led to the first successful kidney transplant in 1955 but between identical twins, thereby proving that the process was surgically feasible when host and donor tissue were genetically matched (3). Immune rejection did however remain the major obstacle to allograft transplantation for a number of years until the discovery of cyclosporine by Jean-Francois Borel whilst working for Sandoz Laboratories in 1969 (4). Although the commercial applications were at the time limited, Roy Calne and David White in the UK tested the newly discovered cyclosporine and found that it prevented transplanted organ rejection (5). The drug was subsequently approved by the US Food and Drug Administration (FDA) for use in transplant patients in 1983, thereby revolutionising the success of organ transplantation to the point where it has almost become a routine procedure.

Cyclosporine acts primarily on T-cells by reducing expression of Interleukin 2 (IL2), a cytokine that activates a whole range of actions against foreign cells, including CD8 positive T cell and NK cell cytolytic activity (6,7). In the report by Zhu and colleagues (7), the cyclosporine mechanism on immune tolerance is refined further by targeting the IL2- receptor gamma (rγ). Mice deficient of IL2rγ are immune-compromised. Indeed the naturally occurring deficiency of the corresponding gene in humans (IL2RG) leads to X-linked severe combined immunodeficiency (SCID), which is itself a disease target for both haemopoetic stem cell and gene therapies (8). The gamma subunit of IL2 activates the intracellular signalling molecules such as Janus kinase (JAK) 3 and the signal transducer and activator of transcription (STAT) proteins, which drive T-cell stimulation (9). This should in theory provide the ideal host background in which to assess the integration and function of transplanted photoreceptor precursor cells, but without the variable of T-cell mediated graft rejection. Most importantly however, the IL2rγ-null mouse permits assessment of xenographs of human-derived donor cells in an immune-privileged host and provides insight into how human cells might behave in clinical use. It may therefore provide a preclinical model to facilitate future clinical trials of photoreceptor transplantation as a potential treatment for blindness. Although the subretinal space is to some extent immune privileged (10), this is most likely lost in the photoreceptor transplantation paradigm, because there will be focal disruption of the blood-ocular barriers induced by surgical trauma and inflammation resulting from retinal degeneration.

Zhu and colleagues crossed the IL2rγ-null mouse onto the Crx knockout, thereby creating an immune-privileged

host with a severe outer retinal degeneration – similar in the latter case to the human variant of retinitis pigmentosa (RP) (7). They derived human retinal donor cells from human embryonic stem cells (hESCs) using an established protocol based on inhibition of BMP and WNT signalling pathways. The cells were labelled with green fluorescent protein (GFP) using a lentivirus and a strong ubiquitous human elongation factor (hEF) 1α promoter. The cells (500–750,000 per eye) were transplanted subretinally via a transcorneal approach and the retinas were examined at 6 weeks post-injection. They reported that, "*GFP+ donor human retinal cells integrated within the outer nuclear layer (ONL) of the recipient retina and exhibited an unambiguous photoreceptor morphology with properly orientated photoreceptor segments and synaptic endfeet*". In retrospect however this seems implausible, because human cells would not only need to have the capacity to integrate into the host retina by some means of locomotion and without disrupting the surrounding milieu, but they would also need then to adopt the identical morphology of mouse rod photoreceptors. Human photoreceptors are heterogeneous in size (11).

Unfortunately around the time of submission of this otherwise extremely well presented study, cytoplasmic cell fusion was identified as leading to a transplantation artefact in which host cells are labelled with fluorescent markers present in the donor cells. The mechanism whereby cytoplasmic exchange of proteins may occur between transplanted photoreceptors residing in the subretinal space and host photoreceptors in the outer nuclear layer (ONL) was first proposed by Mandeep Singh and colleagues using confetti mice (12). With discordant genetic fluorescent markers in host and donor cells, they showed not only double labelling, but also radial alignment of labelled retinal cells in the ONL with the same colour cell residing in the subretinal space (12). The mechanism of cytoplasmic fusion was subsequently verified and published in three seminal papers in Nature Communications at the end of 2016 (13-15). This observation has now overturned many of the key papers that had set the field of photoreceptor transplantation up to that point.

The report by Zhu and colleagues addressed the question of cell fusion in two ways (7). In the first instance they were unable to identify any cells with double nuclei. Polyploidy was previously identified as the mechanism of haemopoetic cell fusion and transfer of donor cell markers (16), but photoreceptor transplantation is different, because the host and donor cells make contact via the cytoplasm and exchange proteins whilst each maintains a separate nucleus (12-15). In the second instance, they used antibodies to a human stem cell marker STEM121 (SC121) to confirm that the green fluorescent ONL photoreceptor cells were human derived. This too is misleading, because SC121 is a cytoplasmic protein and would therefore diffuse into host cells by the very same mechanism of cytoplasmic exchange. A much better marker would be human nuclear antigen, which is confined to the nucleus and would therefore have a much stronger signal in the host ONL if these were indeed human cells (17). In one of the earliest papers on photoreceptor transplantation, the issue of fusion was addressed by using bromo-deoxyuridine (BrDU) to label mouse donor nuclei, proving that photoreceptors can be transplanted when injected directly into the degenerating ONL (18). In retrospect however the BrDU example shown in that paper differed from the fusion examples in being an isolated single cell rather than a cluster and with a slightly abnormal cell body profile (18). Zhu and colleagues also show green fluorescent cells labelled with cone opsin and PNA, but the nuclei of these cells are at the outer limiting membrane where host cones ordinarily reside (7). Cones are born before rods and are passively displaced to this outer retinal position as a result of the massive rod neurogenesis during post-natal development. Hence, is it confusing to explain how a transplanted cone would know exactly where to locate its nucleus—the only logical explanation is fusion and cytoplasmic labelling of a host cone that is already in place.

It is still plausible that photoreceptors might be transplanted, albeit in smaller numbers than previously believed. Furthermore it is highly likely that transplanted cells will have an abnormal morphology and will not be fully integrated into the host structure. Experiments in hosts that have no ONL—similar to end stage retinal degeneration in humans—are not only less likely to be subject the cell fusion artefact, but also provide more appropriate models of the human disease (17). There are many good examples going back over 25 years on how photoreceptor transplantation might work in this scenario (19-22). The use of immune suppression in these degenerate hosts, as shown in the study by Zhu and colleagues (7), is likely to yield even more helpful information on the behaviour of transplanted human photoreceptor cells as we move into clinical trials.

Acknowledgements

RE MacLaren receives funding by the Oxford NIHR Biomedical Research Centre, the Wellcome Trust, Fight for

Sight, the Medical Research Council and Oxstem Ocular, a subsidiary of Oxstem Ltd.

Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

References

1. Zirm EK. Eine erfolgreiche totale Keratoplastik (A successful total keratoplasty). 1906. Refract Corneal Surg 1989;5:258-61.
2. Billingham RE, Brent L, Medawar PB. Actively acquired tolerance of foreign cells. Nature 1953;172:603-6.
3. Harrison JH, Merrill JP, Murray JE. Renal homotransplantation in identical twins. Surg Forum 1956;6:432-6.
4. Borel JF, Feurer C, Gubler HU, et al. Biological effects of cyclosporin A: a new antilymphocytic agent. Agents Actions 1976;6:468-75.
5. Calne RY, Rolles K, White DJ, et al. Cyclosporin A initially as the only immunosuppressant in 34 recipients of cadaveric organs: 32 kidneys, 2 pancreases, and 2 livers. Lancet 1979;2:1033-6.
6. Prince HE, John JK. Cyclosporine inhibits the expression of receptors for interleukin 2 and transferrin on mitogen-activated human T lymphocytes. Immunol Invest 1986;15:463-72.
7. Zhu J, Cifuentes H, Reynolds J, et al. Immunosuppression via Loss of IL2rγ Enhances Long-Term Functional Integration of hESC-Derived Photoreceptors in the Mouse Retina. Cell Stem Cell 2017;20:374-84.e5.
8. Huston MW, van Til NP, Visser TP, et al. Correction of murine SCID-X1 by lentiviral gene therapy using a codon-optimized IL2RG gene and minimal pretransplant conditioning. Mol Ther 2011;19:1867-77.
9. Chandra A, Zhang F, Gilmour KC, et al. Common variable immunodeficiency and natural killer cell lymphopenia caused by Ets-binding site mutation in the IL-2 receptor γ (IL2RG) gene promoter. J Allergy Clin Immunol 2016;137:940-2.e4.
10. Masli S, Vega JL. Ocular immune privilege sites. Methods Mol Biol 2011;677:449-58.
11. Curcio CA, Sloan KR, Kalina RE, et al. Human photoreceptor topography. J Comp Neurol 1990;292:497-523.
12. Singh MS, Aslam SA, Duncan IL, et al. Cell fusion following photoreceptor transplantation into the non-degenerate retina. Invest Ophthalmol Vis Sci 2014;55:3989.
13. Pearson RA, Gonzalez-Cordero A, West EL, et al. Donor and host photoreceptors engage in material transfer following transplantation of post-mitotic photoreceptor precursors. Nat Commun 2016;7:13029.
14. Santos-Ferreira T, Llonch S, Borsch O, et al. Retinal transplantation of photoreceptors results in donor-host cytoplasmic exchange. Nat Commun 2016;7:13028.
15. Singh MS, Balmer J, Barnard AR, et al. Transplanted photoreceptor precursors transfer proteins to host photoreceptors by a mechanism of cytoplasmic fusion. Nat Commun 2016;7:13537.
16. Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM, et al. Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. Nature 2003;425:968-73.
17. Barnea-Cramer AO, Wang W, Lu SJ, et al. Function of human pluripotent stem cell-derived photoreceptor progenitors in blind mice. Sci Rep 2016;6:29784.
18. MacLaren RE, Pearson RA, MacNeil A, et al. Retinal repair by transplantation of photoreceptor precursors. Nature 2006;444:203-7.
19. Gouras P, Du J, Kjeldbye H, et al. Transplanted photoreceptors identified in dystrophic mouse retina by a transgenic reporter gene. Invest Ophthalmol Vis Sci 1991;32:3167-74.
20. Kwan AS, Wang S, Lund RD. Photoreceptor layer reconstruction in a rodent model of retinal degeneration. Exp Neurol 1999;159:21-33.
21. Arai S, Thomas BB, Seiler MJ, et al. Restoration of visual responses following transplantation of intact retinal sheets in rd mice. Exp Eye Res 2004;79:331-41.
22. Singh MS, Charbel Issa P, Butler R, et al. Reversal of end-stage retinal degeneration and restoration of visual function by photoreceptor transplantation. Proc Natl Acad Sci U S A 2013;110:1101-6.

doi: 10.21037/sci.2017.08.02
Cite this article as: MacLaren RE. Cone fusion confusion in photoreceptor transplantation. Stem Cell Investig 2017;4:71.

