

Researchers set sights on photoreceptor replacement for vision restoration

By Cheryl Guttman
in Fort Lauderdale

PHOTORECEPTOR replacement through retinal stem cell transplantation is being pursued as an attractive strategy for restoring vision in patients afflicted with blinding retinal degenerative diseases.

To date, some encouraging results have been achieved in animal models. However, anatomical and functional restoration has yet to be accomplished, and researchers seem to be facing some difficult challenges in learning to optimally control stem cell development and synapse formation so that transplantation becomes a viable therapeutic approach.

Speaking during a special symposium on "Engineering the Eye" at the annual meeting of the Association for Research in Vision and Ophthalmology, leading scientists in this field shared information on the latest advances in research focusing on use of retinal stem cells for tissue replacement.

Derek van der Kooy PhD, and colleagues in the Regenerative Medicine Program at the McLaughlin Centre for Molecular Medicine, University of Toronto, Ontario, Canada, have been studying mammalian retinal stem cells in vitro and in vivo. After first identifying stem cells in the retina of mouse and human eyes, their work has progressed to a stage where they are now transplanting human retinal stem cell progeny into animal eyes.

Dr van der Kooy told attendees that retinal stem cells could be isolated from the pigmented ciliary margin region of the peripheral retina. The mouse eye was found to contain about 100 stem cells that occurred at a frequency of about 1 in 500 cells. When placed in protein-free media containing no exogenous growth factors, single pigmented cells derived from the ciliary margin zone of the adult mouse retina proliferated to form clonal sphere colonies of cells. Within one week, each sphere would contain approximately 10,000 cells.

Determination that those cells were stem cells was based on their demonstration of the two cardinal properties of stem cells: 1) self-renewal – single spheres gave rise to one or more new spheres with subsequent passaging, and 2) multipotentiality – the cells from the spheres

produced all of the different cell types of the neural retina, including photoreceptors, bipolar neurons, and Muller glia.

Turning to the human eye bank eyes, Dr van der Kooy and colleagues also found that cells isolated from the ciliary margin zone could be used to generate retinal stem cells. The human eye was found to contain about

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10,000 retinal stem cells regardless of donor age, and the cells shared similar properties with the mouse stem cells, including with respect to their ability to rapidly proliferate.

"The finding that human and mouse retinal cells share the same rapid proliferation kinetics came as a surprise because human brain neural stem cells grown in the lab have cell cycle times that are three to four times longer than mouse cells. The remarkable conservation of mouse and human retinal stem cell properties, however, bodes well for the eventual therapeutic potential of human retinal stem cells," Dr van der Kooy said.

Gene transfer increases photoreceptor production

Photoreceptors represent only about 10 to 30% of the progeny of the stem cells. However, using gene transfection methods, Dr van der Kooy and colleagues have been able to drive the retinal stem cell progeny down the photoreceptor lineage. He reported that using a combination of several genes, it was possible to increase photoreceptor development to a level of about 70%.

In vitro, the photoreceptors that were differentiated from retinal stem cells did not assume normal morphology. However, when human retinal stem cell clones were injected into the retina of early postnatal immunodeficient mice, the photoreceptor progeny took on a photoreceptor morphology with inner and outer segments and

were found to express the major rod photoreceptor protein, Rom1, in the outer segment.

"These results are encouraging because they suggest human retinal stem cells placed into the retinal environment will derive endogenous signals and differentiate into normal photoreceptors," Dr van der Kooy said.

Ongoing studies are attempting to transplant the human retinal stem cells into the eyes of blind mice to see if the progeny can functionally integrate into the host retina and even perhaps gain visual function. In addition, Dr van der Kooy and colleagues are focusing on trying to identify extrinsic and intrinsic genes that regulate retinal stem cell development and bias those cells toward specific fates.

Controlling stem cell differentiation

In his research, Ruben Adler MD, Arnall Patz Distinguished Professor of Ophthalmology, Johns Hopkins University, Baltimore, MD, has also been concentrating on identifying strategies for influencing photoreceptor differentiation and synaptogenesis that are necessary to enable use of retinal stem cells for the treatment of retinal degenerative diseases. He is approaching this problem by taking a developmental view and turning to the embryo itself as a teacher to identify the cellular and molecular changes that drive normal photoreceptor development.

"When I consider how we can learn to induce stem cells to recapitulate their embryonic development so that they differentiate into fully mature, synaptically integrated photoreceptors when transplanted into the abnormal microenvironment of the eye with degenerative disease, the answer in my mind comes from the words of the late Viktor

Hamburger. He said, 'our real teacher has been and still is the embryo, who is, incidentally, the only teacher who is always right,'" Dr Adler stated.

Three important challenges remain

Considering the process of embryonic development, Dr Adler identified three basic principles that will be important in research with retinal stem cells:

First, the embryo does things in a programmed sequence so that attempts to bypass normal developmental stages with use of transcription or growth factors may be resisted.

Second, photoreceptor development in the embryo involves a complex interplay of combinations of signaling molecules and transcription factors, suggesting that researchers need to find the "magic bullet" that will stimulate a stem cell to differentiate into the desired cell type.

Third, signaling molecules and growth factors may be "recycled" by the embryo so that it will be important to recognise that individual factors may play a different role at different stages in the development process.

Retinas undergo complex maturation process

Dr Adler noted there is general agreement that some early event in development tells the retinal stem cell to become a photoreceptor progenitor. However, while there appears to be some early genetic program that controls the establishment of the photoreceptor phenotype, a series of additional inductive signals are needed for the process of the progenitor cell to develop into a mature photoreceptor.

"If cell differentiations were a cell autonomous phenomenon and the genetic program alone drove the cell to become a mature photoreceptor, then our problem would be relatively simple because we would only have to find this initial inductive signal. Unfortunately, that is not the way development happens, and our task is to find the induction signal for a retinal stem cell to become a photoreceptor as well as the signals driving cell maturation," Dr Adler said.

Retinal environment different in embryos

That concept is supported by studies performed in a variety of species that show there is a period of days or weeks between the earliest phase of photoreceptor generation and the development of visual pigment expression, formation of outer segments, and synaptogenesis. During that time, the inner retina is differentiating and making contacts with the brain, creating an environment characterised by spontaneous electrical activity that might be influencing photoreceptor differentiation.

"Since the degenerating retina has extensive pathologic changes in the inner retina, the microenvironment in which retinal stem cells are transplanted to restore vision is unlikely to be similar to that present during embryologic development," Dr Adler said.

Multi-purpose signalling molecules

During the later stages of photoreceptor differentiation, the photoreceptor specific genes are independently regulated by a variety of extracellular signalling molecules and intracellular transcription factors. Those will need to be identified and characterised with respect to their sources and potentially complex roles in cell development.

So far, a host of extracellular signaling molecules have been reported by various laboratories to influence photoreceptor differentiation. The limitation of those studies, however, is that they have generally worked with individual factors in an isolated fashion, and so neglect an important lesson taught by the embryo.

"The classical view of neurotrophic factors and other signaling molecules is that they are very cell specific such that each type of neuron would respond to a single factor and that the source of each factor was very restricted. The contemporary view is much more complicated. We now recognise that each factor can act on multiple neurons and other types of cells, that each cell type has receptors for many factors, and that the factors are derived from multiple sources including postsynaptic cells, glial cells, and presynaptic cells."

That view underlies a key notion, which is that all of these factors surrounding the cell are integrated into a homeostatic system that will be altered if perturbed in any way. Therefore, the challenge in the laboratory is to find a way to investigate these homeostatic networks rather than studying the effects of single factors one at a time, Dr Adler said.

Thanks to recent advances in genetic research tools, a number of transcription factors involved in photoreceptor development have also been identified. However, that list is not complete and several important challenges remain in delineating their roles as well.

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“While we can profile differentiated cells, profiling of stem cells and progenitor cells is difficult because they are morphologically undifferentiated, and so we cannot distinguish cells that will develop into photoreceptors versus other types of cells. Furthermore, while we can analyse gain and loss of function with current technology, we can only do those experiments in black-and-white where function of one or just a few genes are completely blocked or introduced at a time. What is needed is the capability to do these experiments in shades of grey where the expression of multiple genes is modulated at varying levels,” Dr Adler said.

Generation of synapses

Finally, researchers are facing the challenge of inducing transplanted stem cells to make synaptic contacts with host cells. Failure to date in achieving synapse formation between grafted cells and host tissue is not surprising considering the extreme complexity of photoreceptor synapses, Dr Adler said.

“At the molecular level, the number of presynaptic and postsynaptic molecules and cell adhesion molecules necessary to bring these two compartments together is huge, and so it is doubtful that synaptogenesis is going to happen spontaneously, particularly in a degenerative host environment,” he explained.

Looking again to the embryo for clues, Dr Adler and colleagues have been analysing synaptogenic

molecules expressed in developing retina isolated from a chick embryo. Without yet completing their analyses, they have already identified 60 molecules related to synaptogenesis. Consistent with the lessons from the embryo, their studies also indicate that the various factors are expressed in a precise sequence.

“Nature does not throw all of these molecules at the cell at one time, and so we need to determine how they are assembled and then do functional analysis to find which are critical for what events so that we can eventually determine how to manipulate transplanted stem cells in the human host,” Dr Adler said.

After reviewing all of the challenges researchers face, he concluded his talk on a note of cautious optimism.

“We know that it is possible to get retinal stem cells to make photoreceptors. After all, the embryo does it all the time, and while we have not yet been able to induce transplanted stem cells to make mature, synaptically-connected photoreceptors, my guess is that too can be achieved if we listen to lessons from the embryo.”

“How long it will be before we can cure blindness through stem cell transplantation is of course unknown. From the scientific perspective, the challenge is tough, and there are also many nonscientific considerations that may delay progress. However, as articulated in the ARVO statement on stem cell research, there are important reasons to aim for the sooner rather than the later because stem cell research seems to offer unique opportunities for treating degenerative retinal diseases and alleviating their associated disability, suffering, and economic loss,” Dr Adler said.

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