Regina Hanson

Regina Hanson grew up on the beaches of Santa Barbara, CA. The growing campus of the valley inspired her to attend the University of California, Merced after high school. Regina is now a fourth year Biology major emphasizing her undergraduate degree in Microbiology and Immunology. After graduating she plans to attend Optometry School and pursue a career in eye care. Regina's career goals influenced her review of degenerative eye diseases and the research being done to prevent and possibly reverse vision loss.

Retinal Regeneration Using Stem Cells for Regenerative Treatment Therapy

University of California, Merced

Abstract

Researchers have been studying different forms of stem cells and associate proteins in order to find possible restorative treatments for vision loss. Current research and available treatments provide ways to prevent and stall progressive vision loss, but there are no treatments available to reverse the damage caused by diseases like age-related macular degeneration (ARMD). This review focuses on three main aspects in the search for applicable treatments for degenerated vision that not only treat but also repair visual cells. One area that holds promise is based on different cell associated proteins that regulate the signaling pathways of retinal pluripotent stem cells (RPCs) and their evolutionary role to repair the retinal pigment epithelium (RPE). Another approach is finding the most beneficial types of stem cells for replacing and restoring progressive vision loss. Finally I review, the most important aspect in the field: implantation methods and which ones are most successful to fully regenerate retinal cells.

Introduction

Regenerative cellular therapy is a promising area in the search for cures of various chronic and degenerative diseases, including age-related macular degeneration (ARMD), which is the leading cause of blindness in the world (CDC, 2009). This and other degenerative retinal disorders attack the inner ocular tissues causing vision loss when the cells within the layers of the retinal pigment epithelium (RPE) lose their ability to provide the photoreceptors the support and nourishment necessary for healthy vision. Stem cell research was already in high gear when researchers discovered viable adult retinal stem cells and began to investigate how to manipulate these cells (Tropepe et al., 2000). Further research conducted on invertebrates like zebra fish; found that endogenous repair is possible in damaged retinal tissue. This was an important breakthrough: not only could we halt ARMD but we could also repair damaged RPE . Researchers also found that cell growth in damaged retina is continuous from the germinal zone at the outer edges, and that cells migrate and replace damaged tissue throughout the life span of these animals. Moreover, two key points that caused a jump-start in this research field were finding a proper source of malleable cells and also a real-life model of endogenous repair of retinal cells (Nakamura, et al., 2007).

Although there are currently therapeutic and preventive methods to protect patients from further progressive vision loss, there are no available cures to reverse the damage to the RPE, retina, or the photoreceptors. Moreover, there is also a lack of viable implantation techniques and no one has successfully integrated replacement cells into existing RPE. Regenerative medicine has had many recent gradual, small successes, with each new finding building on the ones before it, but regenerative retinal therapies that use stem cells can replenish damaged tissues when properly induced.

This review explores current research on the cellular pathways and specific types of cells that will lead us deeper into discovering endogenous repair mechanisms for the human body. Finding the most viable and attainable form of stem cells and growth media are the first steps in regenerative retinal therapy. Another key piece of research is defining the cellular signaling pathways necessary for successfully growing retinal tissue. Some studies have investigated various associate proteins and their differentiation processes involved in RPE regeneration which has provided more evidence towards retinal cellular replacement therapy as a possible cure for ARMD.

Important Cell Types and their Associate Proteins

Since its inception, research has focused on stem cells because of their endless possibilities of differentiating into any other bodily tissue. Bone marrow-derived stem cells (BMC) including, hematopoietic stem cells (HSC) not only allow researchers to avoid the controversial and political battle of embryonic stem cells, but are also easily transplanted allowing for relatively non-invasive method of implantation. HSCs are a derivative of BMCs that are undifferentiated and uncommitted. These cells are stored within the bone marrow which then migrate alongside facilitating cells (FC) to damaged areas including degenerated retinal pigment epithelium to stimulate endogenous repair or differentiating into specific repair tissue (Atmaca-Sonmez, et al., 2006). The strong partnership between HSCs and FCs highlights the importance of successful migration and aide HSC survival along the journey to the damaged RPE. Ultimately, research on HSCs with laboratory mice provided that cells were successful in traveling to damaged retinal tissue, and differentiating and expressing RPE-65, a specific associative protein marker for RPE tissue.

Retinal progenitor cells (RPC) are another form of stem cell that were found in the ciliary marginal zone (CMZ) in adult mice and targeted for their capability to evolve into adult retinal cells (Schmitt, et al., 2009). Inducing the evolution of RPCs into healthy RPE tissue, or other types of photoreceptors, creates the endogenous regeneration once thought impossible. Regeneration of RPCs and their further stimulation is initiated by retinal homeobox genes (Rx gene, CHX10) which are essential for inner ocular development (Martinez -De Luna, et al., 2011). The Rx gene is a common marker used to identify RPCs and provides other major functions to the cells like specification and maintenance. Isolation experiments on the Rx genes by Martinez-De Luna and associates confirmed that RPCs were dependant on the presence of Rx genes. With their absence, RPE cell population is completely disorganized and would form premature, deformed retinal tissue cells.

The discovery of cellular identification genes and associate proteins led to further experimentation of their specific functions. These proteins, in partnership with different stem cells, have the ability to initiate or inhibit differentiation and proliferation processes within RPCs. Transduction signaling causes proteins to be initiated causing a specific order cascade which is essential to finding successful tissue regeneration pathways. When signaled, HSCs begin to express the RPE-65 gene which signals other transcription factors and causes differentiation to photoreceptors or retinal cells. After full migration of BMCs the majority (over 85%) of HSCs in damaged retina of adult mice were expressing RPE-65 and further differentiating and regenerating damaged retinal tissue (Atmaca-Sonmez, et al., 2006).

One of the master control genes, Pax6, found during the development of retinal progenitor cells is initiated by the presence of RPE-65 ; this cellular marker is essential for further differentiation of healthy RPE. Pax6 along with homeobox gene, CHX10 provide successful localization of differentiating RPCs (Ahmadieh, et al., 2012). Notch-1 is another specific transcription factor turned on by the presence of RPE-65 that has a strong influence to the signaling pathways of retinal cell regeneration (Nakamura, Chikafumi, 2007). Studies conducted on both embryonic and adult retinas in mice provided evidence that the presence of Notch-1 was a key determining factor of proper retinal growth and the timing of specific growth patterns (Nakamura, Chika-

fumi, 2007). Properly inducing regenerative retinal growth from damaged tissue thrives on the successful communication between associate proteins, transcription factors and the RPE as a whole, complete unit.

Cell Source Locations and Laboratory Advancements

Although regeneration of individual visual cells has occurred successfully outside of the retina, localizing cells properly and finding the precise locations of stem cells to induce regeneration is still a problem. Stem cells must be presented where cellular markers will cause replacement tissue to adhere and continue to proliferate. RPCs were originally found in lower invertebrates, newts and zebra fish, within a specialized subsection of the eye called the ciliary marginal zone (CMZ). The RPCs within the CMZ migrate throughout the eye to replenish dead retinal cell and also when damage occurs. Cells located here are easily accessible because the region is located in the central part of the retina and once induced by specific amounts of damage; they begin to differentiate and localize to repair endogenously (Martinez-De Luna, et al., 2011). Researchers discovered a homologous cell site storing RPCs called the pigmented ciliary margin, leading them to believe endogenous retinal repair is attainable for humans (Ballios, et al., 2010). Gaining direct access to RPCs, and inducing proper migration from CMZ and pigmented ciliary margin provides a localizing strategy to initiate endogenous repair, which is the ultimate goal in using stem cells for regenerative retinal therapy.

Different media houses for stem cell differentiation have been studied. Using different media like amniotic fluid is another way RPCs can be influenced to promote successful differentiation (Ahmadieh, et al., 2012). Experimentation done between amniotic fluid (AF), FBS, and DMEM/F12 used as a control, provided hard evidence that undifferentiated RPCs that were pre-cultured between the different media were successfully induced in the AF because of the many associative proteins and enzymes present. Specific AF proteins were linked to influence RPC factors like overall cell homeostasis by albumin, serotransferrin and ceruloplasmin. The presence of transforming growth factor-beta (TGF-β) in AF increased effectiveness of cell migration to damaged retinal areas and further induced signaling pathways which promoted differentiation to healthy RPE tissue. Another major protein association found linked to the AF was complement C3 (C3), which plays an important role in initiating proliferation at the retinal damage site (Ahmadieh, et al,. 2012).

Another pathway of cell differentiation in damaged retinal patches is the idea of transdifferentiation. Transdifferentiation is the idea where cells are spontaneously converted from one cell type to another (*Theory of Transdifferentiation,* 2006). This idea has only been witnessed in several situations including the regeneration of RPE in adult and embryonic newts. The RPE cells and iris pigment epithelial cells showed this capacity of transdifferentiation when transplanted into the damaged retinal tissue (Nakamura, et al., 2007). Localized associative proteins in the damaged area, like RPE-65, were able to initiate transformation of the foreign cells and cause them to further differentiate and proliferate in damaged retinal areas. Since the parameters where transdifferentiation is successfully initiated among retinal cells is still unknown, transplanting RPE tissue cells seems like the most promising treatment method in order to reverse progressive vision loss.

Clinical Treatment Application and Implantation to Integration

Although most of this research has explored and has been successful with lower vertebrate and invertebrate models, the most essential aspiration of regenerative medicine is to find a simple implantation method to reverse progressive vision loss in humans. Researchers have attempted indirect implantation, for example using the bone marrow pathway, which is ideal because it is the least invasive, therefore, making it easier to administer and obtain treatments (Schmitt, et al., 2009). Direct implantation has shown more signs of success; however, more invasive treatments like this one come with greater risks (Atmaca-Sonmez, et al., 2006). Both transplant methods have provided evidence that once stem cells are implanted properly, healthy retinal tissue will begin to proliferate.

Integration is the main measure of success in regenerative therapy. It is necessary not only to transport cells capable of regeneration but for them to be claimed by the host's retinal tissue and proliferate other healthy visual cells. Damaged retinal tissues alone cause RPCs and HSCs to be recruited and their signaling pathways are initiated, but damaged tissue cannot provide the signals to induce productive differentiation on their own (Ballios, et al., 2010). Recently, there have been advances where researchers have found a minimally invasive and biodegradable transport system to deliver adult RSCs to the inner ocular retinal layers. This specific system has revealed possible benefits of implantation methods, but has not provided continual integration

(Ballios, et al., 2006, 2). Discovery of a successful direct implantation method has taken the field leaps and bounds closer to finding a cure for degenerative retinal disorders.

Conclusion

Overall, there is a well-rounded foundation of research in regenerative medicine that could lead to novel retinal therapies. Researchers have identified specific stem cell types like BMCs and RPCs that provide the cellular material for regenerating retinal tissue, and also inducing proliferation of RPE. Other functions of these stem cells include recruiting and initiating associative proteins and transcription factors that are essential to regulating regeneration of damaged retinal cells. Localizing both stem cells and associated proteins to damaged retinal tissue is essential to creating successful retinal treatments. Outer influential factors like AF, cellular media, and the possibility of transdifferentiation have provided alternate avenues of possibility that will lead to potential cures. The most important aspect of this research is finding the best method for the implanting regenerative retinal tissue. Direct and indirect methods have both been explored and demonstrated signs of success in initiating healthy regenerating cells. Implantation is an important initial aspect of finding a cure but proper integration of therapeutic cells is avenue of further research left to explore. Learning to manipulate these cellular pathways is still an obstacle that researchers are looking to overcome. Once researchers are able to successfully control the therapeutic cells and how they integrate into the host's retina, we will finally have a cure for progressive vision loss.

References

Ahmadieh, Hamid , Fatemeh Sanie-Jahromi, Zahra-Soheila Soheili, Maliheh Davari, Shima Ghaderi, Mozhagan R Kanavi, Shahram Samiei, Abdolkhalegh Deezagi, Jalil Pakravesh, and Abouzar Bagheri. "Enhanced generation of retinal progenitor cells from human retinal pigment epithelial cells induced by amniotic fluid." *BMC Research Notes* 5 (2012): n. pag. *BioMed Central*. Web. 14 Oct. 2012.

Atmaca-Sonmez, Pelin, et al. "Systemically Transferred Hematopoietic Stem Cells Home to the Subretinal Space and Express RPE-65 in a Mouse Model of Retinal Pigment Epithelium Damage." *Experimental eye research* 83.5 (2006): 1295-302. Web.

Ballios, Brian G., and Derek van der Kooy. "Biology and Therapeutic Potential of Adult Retinal Stem Cells." *Canadian Journal of Ophthalmology / Journal Canadien d'Ophtalmologie* 45.4 (2010): 342-51. Web.

Ballios, BG, MJ Cooke, D van der Kooy, and MS Shoichet. "A Hydrogel-based Stem Cell Delivery System to Treat Retinal Degenerative Diseases."*Biomaterials* 31.9 (2010): 2555-64. Print.

Centers for Disease Control and Prevention (CDC). "Common Eye Disorders." *Centers for Disease Control and Prevention*. Centers for Disease Control and Prevention, 31 July 2009. Web. 14 Oct. 2012. <http://www.cdc.gov/visionhealth/basic_information/eye_disorders.htm>.

Martinez-De Luna, Reyna I., Lisa E. Kelly, and Heithem M. El-Hodiri. "The Retinal Homeobox (Rx) Gene is Necessary for Retinal Regeneration." *Developmental biology* 353.1 (2011): 10-8. Web. "The Retinal Homeobox (Rx) Gene is Necessary for Retinal Regeneration." *Developmental biology* 353.1 (2011): 10 -8. Web.

Nakamura, Kenta, and Chikafumi Chiba. "Evidence for Notch Signaling Involvement in Retinal Regeneration of Adult Newt." *Brain research* 1136.0 (2007): 28-42. Web.

Schmitt, Scott, Unber Aftab, Caihui Jiang, Stephen Rednti, Henry Klassen, Erik Mijan, John Sinden, and Michael Young. "Molecular Characterization of Human Retinal Progenitor Cells." *Investigative opthal-*

mology and visual science 50.12 (2009): 5901-8. *IVOS*. Web. 27 Oct. 2012.

Tropepe, V, BL Coles, BJ Chiasson, DJ Horsford, AJ Elia, RR McInnes, and D van der Kooy. "Retinal Stem Cells in Adult Mammalian Eye." *Science.* 287.5460 (2000): 2032-6. Print.

"Theory of Transdifferentiation."*Transdifferentiation.com*. University of Bath, United Kingdom, 2 May 2006. Web. 28 Oct. 2012. <http://www.transdifferentiation.com/theory.htm