Article

Report on the National Eye Institute Audacious Goals Initiative: Photoreceptor Regeneration and Integration Workshop

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The National Eye Institute (NEI) hosted a workshop on May 2, 2015, as part of the Audacious Goals Initiative (AGI) to foster a concerted effort to develop novel therapies for outer retinal diseases. The central goal of this initiative is to "demonstrate by 2025 the restoration of usable vision in humans through the regeneration of neurons and neural connections in the eye and visual system." More specifically, the AGI identified two neural retinal cell classes—ganglion cells and photoreceptors—as challenging, high impact targets for these efforts. A prior workshop and subsequent white paper provided a foundation to begin addressing issues regarding optic nerve regeneration, whereas the major objective of the May 2015 workshop was to review progress toward photoreceptor replacement and identify research gaps and barriers that are limiting advancement of the field. The present report summarizes that discussion and input, which was gathered from a panel of distinguished basic science and clinical investigators with diverse technical expertise and experience with different model systems. Four broad discussion categories were put forth during the workshop, each addressing a critical area of need in the pursuit of functional photoreceptor regeneration: (1) cell sources for photoreceptor regeneration, (2) cell delivery and/or integration, (3) outcome assessment, and (4) preclinical models and target patient populations. For each category, multiple challenges and opportunities for research discovery and tool production were identified and vetted. The present report summarizes the dialogue that took place and seeks to encourage continued interactions within the vision science community on this topic. It also serves as a guide for funding to support the pursuit of cell and circuit repair in diseases leading to photoreceptor degeneration.

Introduction

It has long been a goal of vision science to restore sight in patients blinded by the physical loss of photoreceptors, either as a result of degenerative disease or injury. However, until recently the tools and knowledge required to achieve this goal have been limited. With the advent of pluripotent stem cell technology and in situ reprogramming techniques, as well as devices to assess cell structure and function in vivo, we now have an opportunity to devise and test photoreceptor regeneration strategies in a more directed and rigorous manner. In addition, an increasing willingness to share expertise and resources among scientists across disciplines has provided an environment that favors research acceleration over individual accomplishment. Such integrated research efforts should lead to the elucidation of cell and molecular mechanisms that promote or hinder photoreceptor regeneration, allowing us to build upon successes and overcome failures in an expedited manner.

When contemplating the prospect of photoreceptor regeneration, it is helpful to start with a set of basic assumptions. The first, and perhaps most fundamental, is that meaningful restoration of vision via photoreceptor regeneration is attainable in humans. However, defining "meaningful" vision im-

provement will require careful and inclusive debate in order to set realistic expectations and avoid misunderstandings among scientists, clinicians, patients, and other vested individuals when assessing the progress and impact of the Audacious Goals Initiative (AGI). Second, additional knowledge and technological advancements are still needed to confidently proceed with photoreceptor regeneration therapies, although it can be argued that such confidence may only come with carefully designed human clinical trials. Other safe assumptions include: no single therapeutic approach will succeed under all conditions of photoreceptor loss, treatment effects will likely be modest (at least initially), and even successful strategies will need refinement beyond the 2025 goal for the AGI. Lastly, paramount to all other aspects of this initiative is the need to maximize patient safety. With these thoughts in mind, workshop participants sought to delineate the research challenges and knowledge gaps that need to be addressed to accomplish the goal of photoreceptor regeneration in humans.

Cell Sources for Photoreceptor Regeneration

Photoreceptor replacement can theoretically be pursued using either exogenous or endogenous cell sources, with both approaches potentially employing a variety of donor cell types. For exogenous photoreceptor replacement, possible donor sources include human embryonic or induced pluripotent stem cells (hESCs or hiPSCs), prenatal retinal cells, cultured adult tissue stem cells, or transdifferentiated cell cultures. Endogenous repair strategies could target host Müller glia, retinal pigment epithelium (RPE), or perhaps ciliary margin cells, although the last option was felt to have limited appeal in part due to their localization to the far retinal periphery.

Human pluripotent stem cells were felt to be the most promising donor source for exogenous photoreceptor replacement given their capacity to produce cone and rod precursors as well as more mature photoreceptor progeny.[1–14] Furthermore, evidence suggests that photoreceptor lineage cells derived from pluripotent stem cell sources can integrate and restore visual function in at least some mouse models of photoreceptor dysfunction.[13–17] Identified challenges to the use of hESCs and hiPSCs as sources for exogenous photoreceptor regeneration include: (1) the inherent heterogeneity of differentiating neural retinal cell populations, (2) the potential for donor cell rejection, (3) difficulties adapting lab-based protocols to current Good Manufacturing Practice (cGMP)

guidelines, (4) the variability of photoreceptor cell production across lines, (5) enrichment of photoreceptor lineage cells, (6) the high cost and lengthy time requirements of cell manufacture, and (7) assuring patient safety. Between hESCs and hiPSCs, the latter was noted to have an advantage with regard to widespread ethical acceptance and the theoretical potential to circumvent immune rejection via autologous cell transplantation. That being said, autologous therapies carry a huge cost and time burden and might not be necessary, particularly considering the future availability of banks of homozygous HLA "super donor" hiPSC lines[18–22] or genetically engineered "immuno-neutral" hESCs.

Advantages and disadvantages of 2D versus 3D hESC and hiPSC retinal differentiation protocols were discussed, the former being more conducive to scale up but perhaps less capable or efficient at producing integration-competent photoreceptor precursors.[16,17] Participants acknowledged the need to enrich for hESC- and hiPSC-derived photoreceptor cells prior to transplantation, but it was unclear what level of purity was optimal or necessary to promote survival and integration. Enrichment can be achieved in mouse ESC cultures using cell surface markers to select integration-competent photoreceptor precursors[23]; however, this strategy must be confirmed with hESCs and hiPSCs. Also imperative are studies to better understand the diversity of photoreceptor cell types and maturation states in these cultures, which is essential for the interpretation and subsequent refinement of transplantation strategies. The specific need to concentrate on the production of cones was voiced by numerous workshop participants, owing to their unique importance to human visual function. Lastly, concerns regarding the potential for teratoma formation from residual pluripotent cell contamination in donor cell populations were raised, although it was also pointed out that methods to detect even minute amounts of unwanted cell types are available.

The use of primary prenatal tissue and the potential to transdifferentiate nonphotoreceptor cells into cone and/or rods (or rods into cones) were also discussed. It was acknowledged that these approaches carry some benefits, including less time required to generate donor cells and likely lower costs as well. However, ethical concerns surrounding the former cell source and the degree of authenticity achievable with transdifferentiation were among issues that reduced enthusiasm for these options.

Numerous advancements in endogenous replacement of neural retinal cell types have been made

recently, building on decades of ground-breaking research performed in fish, amphibians, and chicks, which display varying amounts of retinal regenerative capacity.[24–28] Conversion of Müller glia to retinal interneurons in damaged mammalian retina has been demonstrated via introduction of the proneural transcription factor Ascl1,[29] and newts display robust endogenous retinal repair originating from RPE cells following injury.[30] The existence of a quiescent population of RPE stem cells in adult human retina offers another population of endogenous cell types that theoretically could be commandeered to trans-differentiate and replace lost photoreceptor cells.[31,32] However, major challenges to endogenous photoreceptor regeneration remain to be addressed, most notably the fact that significant production of photoreceptors has not yet been demonstrated using this approach. Furthermore, the conversion of large numbers of Müller glia to retinal neurons could overdeplete this important cell type. Even when this hurdle is overcome, it is unclear whether the newly converted photoreceptors will be appropriately positioned within the retina, or if their location even matters as long as they are properly connected within the retinal circuitry. At the end of this discussion, it was largely felt by workshop attendees that, although endogenous photoreceptor regeneration has a high ceiling in terms of restoration of visual function, exogenous strategies are further along at present and more likely to move toward clinical trials in the near term.

Delivery and Integration

Workshop participants were asked to discuss the key steps that are required to optimize the introduction of donor cells into the host retina. Two areas were identified as particularly challenging: (1) improving the survival rate of photoreceptor transplantation, and (2) promoting synaptogenesis of transplanted cells to restore light-driven signals.

There are currently three major strategies for cell delivery: subretinal injections of dissociated cells, retinal tissue transplant, and introduction of cells seeded onto engineered scaffolds. Discussants agreed that progress has been made for all approaches, but it is clear that no single approach has as yet overcome all of its challenges.[16,33–38] For example, whereas cell survival rates are better in tissue transplants, subretinal delivery of dissociated photoreceptors has led to more consistent functional integration.[39] Overall, attendees favored exogenous photoreceptor delivery using stem-cell derived donor sources, but clear arguments were made for endogenous production of photoreceptors via delivery of reprogramming vectors to host cell targets such as Müller glial or RPE. Regardless of the strategy, it was the consensus of the group that all delivery methods need to be improved in order to provide the number of integrated or reprogrammed cells necessary for significant vision restoration.

The discussion then focused on the important issue of cone replacement, for which little information is currently available. Survival and integration rates of rod photoreceptors in rodent models have improved, and levels of cell and circuit function can be achieved to the extent that visual behavior is partially regained using this photoreceptor subtype.[16,33,40–42] In contrast, attempts at cone photoreceptor replacement therapy has thus far only achieved low levels of integrated cones.[43,44] What factors underlie this discrepancy? First, it remains challenging to produce and deliver large quantities of cones from donor tissue or stem cell cultures, where they constitute a minority photoreceptor subpopulation. A second issue is the apparent poor long-term survival of transplanted cones, even after they integrate into the host retina.[44] Lastly, the diverse and complex circuits formed between cones and their postsynaptic cells make it more demanding to obtain an accurate assessment of the extent of cone integration at the circuit level. Discussants raised the need to improve our understanding of cone photoreceptor biology, function, and repair, particularly in relation to the fovea in humans and nonhuman primates.

A second major area that requires considerable attention is the promotion of synaptogenesis between transplanted photoreceptors and host bipolar cells in the degenerating retina. It is widely recognized that gliosis and remodeling of the diseased retina seriously impair photoreceptor integration. However, an understanding of how these disruptive events are triggered and subsequently evolve during disease progression is still lacking. Increasing this knowledge is critical for overcoming hurdles imposed by a hostile host environment that undoubtedly imposes limits to photoreceptor survival and integration.

Outcome Assessment

Workshop participants were asked the following questions regarding outcome assessment: (1) How can transplanted photoreceptors be distinguished from host cells? (2) What technology (not currently available) is most needed to ensure photoreceptor replacement and functional integration in patients?

(3) What are relevant anatomical and functional outcomes?

To date, these questions have been addressed in animal models using a large battery of in vitro and in vivo assays, ranging from morphological analyses of transplanted photoreceptors and their synapses to functional recordings including electrophysiology, electroretinography (ERG), and measurements of pupillary light reflexes and light responses in higher visual centers.[13,39] Moreover, several behavioral assays have been devised to test restoration of motion and contrast detection in rodents.[39] Workshop participants indicated that one of the roadblocks that most severely limits translation of work in animal models to humans is a general lack of imaging tools for assessing photoreceptor survival and integration in living patients. Much of the discussion centered on the need to develop such technology specifically for humans. The importance of standardizing assays across patient populations and test sites was also raised.

With regard to outcome assessments in human patients, participants felt there was a need to develop new tools to assess visual function for photoreceptor regeneration trials. Such a need has been underscored in *RPE65* gene therapy trials for Leber congenital amaurosis, in which patients showed significant improvements in visual behavior without documented changes in vision assessment metrics traditionally used in clinical trials (e.g., Snellen acuity).[45,46]

Preclinical Models and Target Patient Populations

Numerous options for preclinical model systems are available to investigators, including small and large animals, nonhuman primates, and even cell culture systems. Critical questions regarding preclinical safety and efficacy studies for photoreceptor regeneration include: (1) What will the Food and Drug Administration (FDA) require? (2) What are the expectations of the scientific and clinical communities and the public? (3) What useful information can we hope to obtain prior to human clinical trials? To date, the vast majority of in vivo experiments pertaining to exogenous photoreceptor regeneration have been performed in mice using allografts. Furthermore, numerous mouse models of photoreceptor degeneration have been employed with variable results. These important efforts have revealed that certain mouse models are more amenable to donor photoreceptor integration than others, likely

due to the relative absence of physical barriers within the recipient tissue (e.g., gliotic scar, intact outer limiting membrane).[40] Therefore, it is possible that that the structural state of the host retina, and not necessarily the cause of the disease or injury, is the primary dictator of the success or failure of photoreceptor replacement therapies. Mice have also been used to show proof of concept for endogenous retinal neuron regeneration in mammals. Similar to exogenous replacement approaches, endogenous photoreceptor regeneration may ultimately be limited by host retinal structure and the degree of scar formation and remodeling present at the time of intervention.

A major concern set forth by the discussants for both exogenous and endogenous regeneration strategies was the relative reliance of the field on mouse models to demonstrate safety and efficacy. Human cells in general show very poor survival as xenografts, and the behavior of human donor photoreceptor cells in mouse (or other animal models) may inadequately inform or perhaps even mislead investigators or regulatory agencies in their quest to predict patient responses to therapeutic interventions. One proposed solution was to move more quickly toward nonhuman primate studies. However, the use of macaques and larger monkeys is costly, time consuming, and not practical for researchers at most institutions. Thus, there was a perceived need to develop and provide access to colonies of smaller monkey species to investigators, and to devise means to simulate conditions of photoreceptor loss in these animals.

Finally, workshop participants discussed factors that might go into choosing a first indication for photoreceptor regeneration therapies. Monogenetic, early-onset photoreceptor degenerative processes offer a more predictable disease course than most late-onset disorders with complex genetics, but candidates in the former category are rare, and industry partners may not be as interested in supporting orphan disease trials. Interestingly, it was felt by many that the stage of disease and/or the structural condition of the retina was as important (or more important) in choosing patients than the underlying cause of the photoreceptor loss for two major reasons. First, the early and mid-stages of disease in which a significant number of functional photoreceptors still remain are unlikely to be first targets for cell regeneration therapies. Indeed, at these stages, initial therapeutic efforts are probably better directed toward cell preservation or gene replacement, if feasible. Eventually, as the risk/benefit ratio of photoreceptor regeneration approaches becomes clearer, intervention at earlier disease stages

may be deemed appropriate. Second, very late stages of disease or injury may be accompanied by pervasive host retinal remodeling and gliosis, creating a "point of no return" for regeneration of a single retinal cell type. As a result, optimizing the timing of photoreceptor replacement is almost assuredly going to require advanced imaging and functional testing pre-operatively to predict the degree of "meaningful" vision restoration possible in a given patient candidate.

Gaps in Scientific Knowledge and Barriers to Progress

Category 1: Cell Sources for Photoreceptor Regeneration

Topics discussed under this category included (1) exogenous versus endogenous photoreceptor regeneration strategies, (2) rod versus cone production, (3) demonstration of donor cell authenticity, and (4) safety considerations.

Exogenous Versus Endogenous Cell Sources

Two major approaches to photoreceptor regeneration were evaluated: (1) photoreceptor replacement via transplantation of exogenous donor cells, and (2) activation and/or reprogramming of endogenous retinal cell types to produce new photoreceptors. A major gap identified for the exogenous approach was a relative lack of detailed investigation into the dynamic and heterogeneous composition of retinal (and nonretinal) cells present in differentiating hESCs and hiPSCs, as well as primary and expanded human prenatal retinal cultures. Such knowledge would provide a basis for evaluating why and how donor cell integration occurs, given current evidence that only a subset of photoreceptor subtypes and differentiation stages may be competent to migrate into host retina and establish functional synapses. For the endogenous regeneration approach, identification of conditions and factors specifically necessary for conversion of Müller glia and RPE (and perhaps other neural retinal cell classes) to photoreceptors—and cones in particular—is a high priority. In addition, methods to control the localization of newly reprogrammed cells and encourage their synaptic integration are necessary to move the research toward clinical trials. To address these issues, the group identified efforts aimed at understanding mechanisms of endogenous regeneration in fish, amphibians, and chick as being highly important.

Rod Versus Cone Production

Conditions favoring rod precursor integration have been examined in considerable detail in the mouse, but efforts to promote cone replacement have been much less successful to date. Given that our most critical visual functions rely on cones, the pursuit of methods to enrich for their production from exogenous and endogenous sources (with an emphasis on medium and long wavelength cones) was thought to be a high priority area for the AGI. However, rod replacement may be more attainable in the short term given current animal model data and the preponderance of rod production over cones in normal human retinogenesis. Another advantage of pursuing rod replacement is that earlier disease stages can be targeted, which may lead to improved rod integration and function while also serving to protect remaining foveal cones. However, rod production currently takes an exceptionally long time in hESC and hiPSC cultures, and much greater cell quantities and host coverage areas would likely be needed when compared to cone replacement strategies. Regardless of the preferred donor photoreceptor subtype, current production protocols yield a mixture of photoreceptor cells with varying maturation states, along with nonphotoreceptor cell types. As such, developing methods to modulate cell composition and align differentiation states in vitro was also considered necessary to accelerate progress.

Demonstration of New Photoreceptor Authenticity and Functional Capacity

In addition to probing the composition of cell types produced from pluripotent stem cells, prenatal tissue, or reprogrammed cells, efforts to understand photoreceptor differentiation and transdifferentiation mechanisms and to overcome limitations regarding their functional potential were deemed critical for future study, for reasons discussed previously.

Understanding the Role of Müller Glia in Health and Disease

Members of the workshop pointed out the importance of Müller glia in maintaining a healthy environment for new photoreceptors, as well as their opposing potential to create significant physical barriers to successful photoreceptor regeneration. Further work was encouraged to define the triggers that tilt the balance toward induction of deleterious Müller glia responses in an effort to mitigate or reverse these effects. Successful pursuit of this goal would aid both exogenous and endogenous regeneration approaches, perhaps more so in the latter since

Müller glia are a major target for endogenous reprogramming. Studies to determine whether distinct subclasses of Müller glia cells exist that are more or less capable of transdifferentiation or gliosis was also identified as a worthwhile component of the AGI. Of note, such a situation exists in adult human RPE, which is known to possess a subpopulation that retains quiescent stem-like properties that predisposes it to transdifferentiation.[31,32] Thus, investigation into the unique characteristics and capabilities of this adult "RPE stem cell" was also deemed important in order to determine whether it can be harnessed for photoreceptor protection and/or regeneration purposes.

Safety Considerations

Safety issues for exogenous photoreceptor replacement therapies that workshop participants felt needed to be addressed included induction of inflammation and gliosis, host immune rejection, dedifferentiation of donor cells in vivo, and the potential for prolonged or recurrent retinal detachment. Endogenous photoreceptor regeneration strategies raised concerns for the possibility of differentitation to unwanted cell types, enhancement of gliosis due to Müller glia activation, and deleterious or short-lived effects of viral vectors. For both approaches there were concerns for enhanced degeneration of host retina, tumorigenicity, establishment of inappropriate synaptic connections, and poor long-term viability and function of new photoreceptors within a diseased, damaged, and/or toxic host tissue environment.

Category 2: Delivery and Integration

The discussion centered on two major areas: (1) how to improve the number of photoreceptors that survive and integrate, and (2) how to promote their synaptogenesis to recreate circuits that can restore motion detection, visual acuity, contrast sensitivity, and color vision.

Improving the Number of Photoreceptors That Survive and Integrate Into Host Retina

It was suggested that an immediate goal should be to ascertain how many photoreceptors (rods or cones) are needed to integrate successfully in order to restore visual function. In mice, rod delivery and integration appear sufficient to restore visual behavior, but cone photoreceptor replacement and synaptic integration remains immensely challenging. Several discussants suggested that one way to overcome limitations in cone replacement therapy is to better understand the

cellular and molecular factors that specify cone photoreceptor fate during development, especially in the human retina. This knowledge could then be used to promote cone generation either in vitro or in situ. However, it was also recognized that cones can vary in morphology and possibly function across the retina. Thus, defining differences in the morphology, molecular make-up, and connectivity of foveal versus peripheral cones in humans and nonhuman primates requires further investigation. Finally, current approaches for photoreceptor replacement center largely on rodent models, which are not ideal for investigating cone replacement therapies. Thus, one important consideration for the future is to determine which small and large animal model systems are best suited for testing strategies for cone replacement therapy.

Photoreceptor Synaptogenesis and Wiring Specificity

Ultrastructural studies to date demonstrate that transplanted rod photoreceptors connect with rod bipolar cells. There is evidence for cone synaptogenesis in mouse models of cone replacement, but the specificity of these connections remains unknown. The discussants felt that the field lacks a basic knowledge of the molecular cues and cellular interactions that regulate specificity in the synaptic connectivity of both rods and cones during normal development. Filling this knowledge gap could help identify factors that could be employed to promote appropriate wiring of transplanted rod or cone photoreceptors. Moreover, knowledge of the mechanisms underlying photoreceptor circuit development could facilitate the design of strategies to redirect connections of surviving cells and restore vision via novel circuits. For example, the discussants raised the possibility that cone-driven signals could be restored by manipulating opsin expression in surviving rod photoreceptors (i.e., turn rods into cones). However, there was debate as to whether this approach would work since the downstream signaling cascades are different for rods and cones.

Remodeling of the Diseased Environment

There was general agreement that a major roadblock in rebuilding circuitry in the damaged retina is the aberrant remodeling that occurs after photoreceptor loss.[47,48] We currently do not know how to prevent such remodeling, since the factors and cellular interactions that cause retinal neurons to alter their position, morphology, connectivity, and function have remained elusive. It is clear, however, that reactive gliosis (especially gliosis involving Müller glial cells) can present a major physical barrier to

photoreceptor integration and play a role in the remodeling process. Thus, increasing our understanding of the intrinsic and extrinsic controls over gliosis is immensely important. The discussants emphasized a need to discover strategies to prevent gliosis as well as to direct glial cells along paths that could actually be beneficial, such as production of trophic factors or as targets for endogenous repair. Furthering knowledge of the factors that control reprogramming in zebrafish,[24,25] which leads to the production of both Müller glial cells and neuronal progenitors, would also help inform the field how to replenish lost photoreceptors without excessively depleting the retina of these glia. There was also consensus that we need to better understand the role of microglia in the remodeling process, as well as the innate immune response.

Because remodeling is a progressive process within the diseased retina, the group discussed the importance of intervening at early stages of disease to take advantage of the more conducive environment for photoreceptor integration and circuit rewiring. Since remodeling appears to accelerate with cone degeneration, one unconventional approach that was suggested was to transplant cones specifically to halt or slow down retinal remodeling, thereby providing more time to develop a more definitive treatment.

Where Do We Set the Bar for Photoreceptor Integration?

It was also recognized that the field should prioritize its goals—for example, should we aim for restoring high acuity vision, a large visual field, and/or cone-mediated vision? It was argued that patients without any visual function would benefit immensely from the restoration of any visual function. Because rods may integrate more readily than cones, it was suggested that even placing rods in the fovea might provide some improvement in visual function if they succeeded in connecting with cone bipolar cells. Finally, further thought should be given to combining photoreceptor replacement therapy with retinal prosthetic technology to enhance the outcome of the latter.

Category 3: Outcome Assessment

Currently, one of the most significant challenges for photoreceptor regeneration is the lack of technology to enable visualization of transplanted photoreceptors in patients. Devising such tools is critical for monitoring the survival, location, and functional impact of cell integration in clinical trials. Much progress has been made in the development of

imaging methods that use adaptive optics to visualize fluorescently tagged cells in nonhuman primates. Discussants felt that new fluorescent tags are needed that label transplanted photoreceptors without modifying their differentiation and function. It was also noted that novel split detector technology is available to visualize inner segments of photoreceptors in humans, thereby bypassing the need to label the cells.[49,50] However, there is concern that such imaging approaches will not work in severely degenerated retinas where the orderly arrangement of photoreceptors is disrupted. Thus, there is a pressing need to develop innovative approaches to visualize and monitor photoreceptor transplants, and to test them first in nonhuman primate to assess their utility in future clinical trials.

Category 4: Preclinical Models and Target Patient Populations

Knowledge gaps and hurdles pertaining to this discussion category were prevalent and challenging for participants to devise solutions to overcome. It was also noted that choices regarding preclinical models and target patient populations cannot be made in isolation, since they require knowledge of the donor cell population and other variables presented in the preceding sections. However, notable difficulties surrounding preclinical model selection include (1) limited confidence in extrapolating safety and efficacy results from mouse models to future human clinical trials, (2) a relative lack of animal models of selective cone loss, and (3) a poor understanding of FDA requirements for proceeding with phase I trials for cell replacement therapies. While nonhuman primates were felt to be anatomically far superior as a model system for cone and rod replacement, no such models of degenerative photoreceptor diseases are available, limiting their utility. The excessive cost and restricted availability of nonhuman primates also poses a considerable hurdle to their widespread use.

Challenges to selecting initial disease targets for photoreceptor regeneration therapies emanate from limitations in our understanding of genetic, epigenetic, and biological changes that occur during retinal disease and injury. Further hindrances come from difficulties assessing host retinal structure and function on a cellular level in vivo, which is needed to estimate the presence and degree of glial scarring and inner retinal remodeling. Better visualization techniques are also needed to define the course and impact of host inflammation on donor cell survival and

integration. The workshop participants acknowledged the recent AGI awards directed toward advancing ocular imaging technology as taking a significant step toward addressing these gaps. Further investment in the study of the longitudinal courses of outer retinal degenerative diseases will also be helpful in this regard.

Areas for Open-Ended, Non-Hypothesis-Driven Discovery Research

Cell Sources

- For exogenous photoreceptor regeneration efforts to advance and succeed, knowledge of the composition and maturation states of differentiating donor cell populations (particularly photoreceptor subtypes) is required. Methods to modulate cellular heterogeneity and enrich for particular photoreceptor cell types are also of high value.
- New cell surface markers or other tools are needed to distinguish and separate human photoreceptors from other retinal cell classes (or enrich for cone subtypes versus rods).
- In the pursuit of endogenous photoreceptor regeneration, research is needed to understand mammalian Müller glia development and probe their potential to revert to a progenitor state. Such efforts would benefit from continued examination of classic lower vertebrate models capable of regenerating photoreceptors from Müller glia, in order to understand why that capacity is lost in mammals. Similarly, projects aimed at probing the potential for mammalian RPE or other retinal cell types (e.g., bipolar cells) to transdifferentiate and regenerate photoreceptors are of interest. Also, the possibility that specific subpopulations of Müller glia and RPE cells exist that have quiescent multipotent retinal progenitor properties should be examined. Lastly, efforts to bring about endogenous photoreceptor regeneration in mammals would be greatly aided by a better understanding of retinal repair mechanisms exhibited by teleosts, urodeles, and chicks.
- Both exogenous and endogenous photoreceptor repair strategies would benefit from an improved understanding of how retinal progenitor cells differentiate into neural retinal cell classes, including photoreceptor subtypes. This is particularly important to investigate in donor cell populations such as hESCs and hiPSCs.

Reassembling Photoreceptor Circuits

- Discovery science aimed at increasing our understanding of the molecular and cellular mechanisms that control photoreceptor fate and differentiation during normal development should be encouraged. Especially important is increasing this knowledge for cone photoreceptors, particularly in humans and nonhuman primates.
- Innovative approaches that prevent or slow down degeneration, gliosis, and remodeling are needed to create a more conducive environment for synaptogenesis of transplanted photoreceptors. As such, factors that control the responses of Müller glial cells and microglia cells upon injury and in early stages of the diseases need to be determined.
- Increasing our understanding of the cellular and molecular mechanisms that regulate photoreceptor synaptogenesis and circuit development in vivo also needs to be advanced. Such knowledge will greatly facilitate the design of knowledge-based strategies that drive photoreceptor circuit repair in animal models and eventually, in patients.

Assessing Outcomes

- It remains challenging to track the survival of transplants in a human eye and to monitor the neural signals generated by their connectivity. To overcome these hurdles, we need to devise better ways of introducing markers into the human eye. Innovations in optical coherence tomography (OCT), adaptive optics, and techniques that integrate existing approaches are rapidly advancing, but as yet there is no effective approach to evaluate the in vivo performance of transplanted cells. Thus, an important goal in this category is to develop methods to distinguish newly introduced cells and surviving host cells.
- While fluorescent markers have been used successfully in animal models and remain a viable option to track donor cells, the field should also invest in discovering label-free imaging techniques for use in humans.
- Better methods are needed for assessing the functional response of integrated cells and identifying whether these cells have made meaningful connections that directly lead to improved visual behavior in patients.

Preclinical Models and Target Patient Populations

- Development of affordable and convenient nonhuman primate models is a worthwhile goal in order

A. Cell Sources for Photoreceptor Regeneration

1. What is the true diversity of cell types present in differentiating hESCs, hiPSCs, and prenatal retinal cultures?

2. Are there ways to enrich for photoreceptors or otherwise reduce heterogeneity of neural retinal donor cell populations? Can such enrichment select for specific cone subtypes and/or maturation states?

3. How can the production of desired photoreceptor cell subtypes (e.g., medium and long wavelength cones) be augmented from donor cell sources?

4. How can the efficiency of photoreceptor production from mammalian Müller glia, RPE, or other retinal cells be improved?

5. What potential dangers do we face in trying to regenerate photoreceptors via exogenous or endogenous means?

6. Why do Müller glia and RPE lose their capacity to regenerate photoreceptors in mammals?

7. How are photoreceptors (and in particular, foveal cones) generated during normal human retinal development?

8. For exogenous photoreceptor replacement strategies, how can we ensure that the most healthy and integration-competent photoreceptor donor cells are used for transplantation?

9. How can health and maturation states of donor cell populations be assessed prior to transplantation?

B. Delivery and Integration

1. How can we promote maximal donor cell survival and integration into diseased or injured host retina?

2. At what stage of maturation should cells be transplanted to ensure optimal integration?

3. What combinations of molecules/factors might improve donor cell delivery and integration?

4. What are the underlying differences between foveal and peripheral cones, and how is cone fate specified during development?

5. What model systems are best suited for developing and testing cone replacement strategies?

6. What are the developmental cues that regulate specificity in rod- and cone-driven circuits?

7. How well can transplanted cones form synaptic circuits?

8. How can we prevent remodeling of the diseased or damaged retina?

9. Can we direct Müller glial cells away from gliosis and towards desired cell lineages without depleting the glia population (i.e., what can we learn from non-mammalian models)?

10. What level of visual function should the field strive for in designing strategies for photoreceptor replacement therapy?

C. Outcome Assessment

1. What technology is needed to visualize and monitor photoreceptor transplants in patients?

2. How can photoreceptor replacement be distinguished from rescue?

3. What is 'meaningful' vision restoration? How is it defined by the many groups of individuals that have a stake in this initiative?

4. What assessments of visual function should be employed (or developed) for use in future photoreceptor regeneration therapies? What are the expectations for visual improvement in initial trials?

D. Preclinical Models and Target Patient Populations

1. What are the most appropriate animal models for testing exogenous photoreceptor replacement and/or endogenous photoreceptor regeneration?

2. Can a practical non-human primate model be developed to test photoreceptor regeneration?

3. Can an adequate animal model of age-related macular degeneration be created?

4. How can the host retinal environment be optimized to improve donor photoreceptor survival and functional integration? For example, can retinal scarring and remodeling be prevented or overcome?

5. What critical evidence is needed to proceed to clinical trials for photoreceptor regeneration?

6. What are the necessary steps to assure safety and/or demonstrate efficacy prior to proceeding with human clinical trials? What will the FDA require?

7. What types and stages of retinal disease (or injury) would represent the best first indication for photoreceptor regeneration?

E. General considerations

1. What is the best mechanism to encourage data sharing and build collaborative research teams?

2. How do we promote rapid communication of both positive and negative results?

3. How can critical tools developed through the AGI be made widely available to researchers in need of them?

4. Can early phase testing in human patients be safely pursued in order to accelerate progress?

Box 1. Critical questions to address in order to accelerate progress toward functional photoreceptor regeneration.

to more accurately assess regeneration and/or replacement of cones within the macula and fovea. In addition, effective and reproducible means of inducing localized photoreceptor degeneration in such models is important to provide a platform for assessing restoration of cell circuitry and visual function.

- Critical evaluation of the utility and predictive power of widely employed mammalian animal models of retinal degeneration (e.g., mouse models) is required to assure their appropriate selection and use. Special attention should be given to developing better age-related macular degeneration models.

- The possibility of making in vitro models of normal and diseased human retina from hESCs, hiPSCs, and/or prenatal tissue should be investigated. For example, hiPSCs could be used to create three-dimensional disease-in-a-dish models to test functional photoreceptor replacement and/or regeneration (e.g., examination of Müller glia plasticity or photoreceptor synaptogenesis).

- Further investigation of lower vertebrate models of adult photoreceptor regeneration is warranted to gain further insights into the biology of regeneration and ascertain why mammalian Müller glia and RPE cells lose this capacity.

- Studies should be solicited to determine what types and stages of diseases would be best to target in initial photoreceptor regeneration trials.

- Involvement of ocular imaging specialists is needed to ensure that future clinical trials will have a high likelihood of yielding reliable structure/function information in living patients.

Summary

Regeneration of photoreceptors in patients with severe vision loss due to outer retinal degeneration is a truly audacious goal, but one that is now conceivable owing to recent scientific advancements superimposed on over a century of research progress. The eye is uniquely suited to test strategies to replace neurons, and photoreceptors are particularly attractive as donor cells given their short-ranged connections and the surgical accessibility of the outer retina. Still, much work is still required to realize this goal, both in terms of acquisition of basic scientific knowledge and development and refinement of critical technology. Such advancements should improve our ability to generate "replacement" photoreceptors and assess their ability to integrate and restore host retinal circuitry and function. Team-based research and open sharing of results will aid these efforts and help overcome the formidable hurdles that lie ahead. Managing expectations is also important, since results from early patient trials are likely to be modest due to a necessary focus on safety. However, improvements in clinical trial design should allow us to learn from our successes and failures and continuously improve strategies to regenerate photoreceptors, and in so doing provide tangible results to a wide range of patients who currently have no treatment options.

Acknowledgments

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