ARVO 2014: A Report from the Annual Meeting

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The annual meeting of the Association for Research in Vision and Ophthalmology (ARVO) is like no other in the field of eye and vision research. Thousands of researchers from around the world convene at a US location to learn about the latest developments that help explain the eye in health and disease. They share ideas, build collaborations, and expand their thinking in ways that lead to further ideas that they then flesh out in their laboratories and clinics until the next ARVO annual meeting where the cycle repeats. This year's meeting was particularly exciting with gains being reported in many areas related to the retina.

The information below will give readers an appreciation of some of the new areas of development and discovery that were highlights of the ARVO 2014 meeting, from stem cells through drug treatments.

Stem Cells

Growing retinal cells in the laboratory would provide scientists with a ready model system for studying the impact of drug and gene treatments on these cells, and for producing ample quantities of new cells for transplantation to replace damaged retinal tissue. In hopes of achieving these goals, several groups of scientists are working to understand biological influences on retinal cell development. For example, researchers at ARVO 2014 reported that they had induced undifferentiated cells (stem cells) to express photoreceptor and retinal pigment epithelial (RPE) cell properties. Other researchers showed the influence of microRNAs (miRNAs) on the differentiation of retinal ganglion cells from human Muller glia with stem cell characteristics. (A miRNA is a small RNA molecule involved in the regulation of gene expression.)

The results are early stage developments, yet some are likely to enrich the understanding of the visual system and ultimately lead to new approaches for treatments, cures, and preventions of vision disorders.

1. **Human insulin-like growth factor (IGF) Coaxes Cells to Develop Eye Characteristics**

   The approach used by Carla B. Mellough and her colleagues at Newcastle University, United Kingdom, adds a protein called human **insulin-like growth factor (IGF)** to the chemical medium in which they incubate stem cells. The researchers report that supplementing the system with IGF increases the frequency of development of ocular structures (compared to cells grown under control conditions with no IGF), including cells with properties of the neural retina, RPE, lens, and corneal epithelium. The IGF-treated cells organize into a recognizable pattern and establish synaptic connections.

   Their research contributes to a growing body of knowledge about factors that control the development and function of cells of the retina, which someday could be exploited to produce quantities of cells for study and for replacing retinal tissue affected by disease or injury.

   C Mellough, JF Collin, M Khazim, E Sernagor, et al.

2. **Skin Cells for Screening Chemical Compounds for Fighting JNCL**

   Another group of scientists, from the University of Iowa, have forced **skin cells** (fibroblasts) from patients with a blinding genetic disease (juvenile neuronal ceroid lipofuscinosis, JNCL) to regress and then develop in the laboratory into retinal neurons containing a specific marker of cell death—capsase-sensitive fluorescent apoptosis reporter construct, Apoliner—for the purpose of rapidly **screening chemical compounds** that might slow down this progressive disease that also leads to cognitive deficits and premature death.

   LA Wiley, K Anfinson, EM Stone, BA Tucker, et al.

3. **Working to Capture the Signal that Restores Retinal Ganglion Cells in Fish and Amphibians**

Yet other scientists are working to explain the molecular biology behind the transformation of human **Muller glia with stem cell characteristics** (hMSCs) into **retinal ganglion cells** in fish and amphibians with damaged retinas. Researchers are looking at the role of **microRNA**s (miRNAs) in the silencing of genes in hMSCs that might contribute to the development (differentiation), or not, of retinal ganglion cells from hMSCs. *In vitro*, while directing the transformation of hMSCs toward a retinal ganglion cell fate, the researchers compared miRNA in cells that differentiated and miRNAs in cells that did not. The data revealed 19 miRNAs that are upregulated (increased) in differentiated cells compared to undifferentiated cells. The activated miRNAs include several involved in an important cell signaling pathway (the NOTCH signaling pathway) related to cell development, cell maintenance, and the growth of neuronal axons and dendrites. The findings could provide an entree to transforming hMSCs into retinal ganglion cells in humans. Retinal ganglion cells form the axons that carry visual signals from the photoreceptor cells of the retina to the brain via the optic nerve. Loss of vision in certain medical conditions, including diabetes and glaucoma, is associated with a loss of retinal ganglion cells.

H Jayaram, MF Jones, D Frampton, GA Limb, et al.

4. Nanosheets as a Subretinal Delivery System for Cultured RPE Cell

Laboratory-generated retinal cells would be only as good as the delivery system that keeps them viable during retinal implantation. At ARVO 2014, researchers from Tohoku University and Waseda University in Japan described a system they are developing for delivering **replacement retinal pigment epithelial (RPE) cells to the subretinal space** of the eye, ultimately for use in patients with age-related macular degeneration (AMD). Their system involves preparation of micropatterned nanosheets of biodegradable poly(lactic-co-glycolic acid) (PGLA) upon which they culture RPE cells. The researchers used a viability staining assay to establish the viability of the cells and then injected the nanosheet (1 mm in diameter) via a 24G intravenous catheter into the subretinal space in *ex vivo* swine ocular globes. A significant portion of the RPE cells retained their viability.

H Kaji, T Fujie, N Nagai, TAbe

Nanotechnology and Regenerative Medicine

Nanotechnology is a field of bioengineering where atoms and molecule of cells are altered through manipulation. This includes the manipulation of genetic material of cells. Regenerative medicine refers to the field of medicine seeking to replace, engineer, or regenerate human cells in order to restore normal functioning to cells, tissues, and organs. Researchers in ophthalmology are using nanotechnology in many ways including as a means of triggering the functioning of cells that have lost their normal stimuli; to affect the functioning of therapeutic interventions; and for assessing gene replacement therapy, to name a few. (#4 above uses nanotechnology.)

5. **Chemical Photoswitches for Restoring Visual Function in Blind Mice**

   A hallmark of retinitis pigmentosa (RP) and age-related macular degeneration (AMD) is visual impairment related to the loss of retinal photoreceptor cells (rods and cones) for capturing light from the visual field. Optogenetics and surgically-implanted electronic retinal prosthetic devices are two areas of research that are showing promise for stimulating small areas of remaining photoreceptors or other retinal cells (i.e., retinal ganglion cells) to provide some visual perception. Another technology using "**photoswitches**" is also being investigated.

   The primary investigators of the photoswitch technology—led by Richard H. Kramer of the University of California at Berkeley—are from laboratories at Berkeley, Ludwig-Maximilians-Universitat Munchen, and University of Washington. At ARVO 2013, they had described their early results using a photoswitchable potassium channel marker named AAQ for photosensitizing the retina and restoring light-elicited behavior in blind mice. But AAQ diffuses quickly, they found, and has to be injected daily, into the vitreous, for an ongoing effect—an untenable therapeutic model. At ARVO 2014 the researchers described similar compounds they have developed—**DENAQ and BENAQ**—that **bestow light responses on retinal ganglion cells** in mouse models of retinitis pigmentosa for weeks after a single intravitreal injection. The compounds also have the advantage over other potential therapies in allowing for responses from the entire retina and not just in regions being stimulated by a device or a regionally-applied treatment. Further, the compounds are selective for diseased tissue and easily reversible in the event of complications. The researchers even suggest the possibility of a slow-release formulation for longer-lasting

effects. Their current goal is to demonstrate safety and efficacy of the compounds in larger animal models in order to obtain FDA approval for testing in human patients.

I Tochitsky, A Polosukhina, V Degtyar, R Kramer, et al.

6. An Azobenzene Photoswitch

Another group of researchers report success using a different compound as a photoswitch for triggering the activation of retinal ganglion cells in degenerating mouse retina and also in a dog model of retinitis pigmentosa. They designed a system that uses an **azobenzene photoswitch** to activate a light-gated ionotropic glutamate receptor (LiGluR) on retinal ganglion cells **to restore light sensitivity**. Treated mice displayed visually-evoked potentials in the visual cortex of the brain and behavioral changes indicative of light detection. Responses to light stimulation were also recorded from retinal cells of a dog model of RP.

B Gaub, M Berry, G Aguirre, J Flannery, E Isacof, et al.

7. Nanoceria + a Second Antioxidant = Slower Retinal Degeneration in Mice

Oxidative stress is linked to neurodegenerative disease progression, including the death of photoreceptors in retinitis pigmentosa. Researchers have shown that **nanoceria** (nanoparticles of a cerium oxide, a catalytic scavengers of reactive oxygen species) can delay photoreceptor cell death in rodents, but the impact has lasted only 2 or 3 weeks. Now, researchers at ARVO 2014 reported that adding an ER stress regulator (**GRP78/Bip**) to nanoceria treatment synergistically or additively prevents retinal degeneration in a mouse model of inherited retinal disease. They had previously shown that ER stress is involved in retinal degeneration in this mouse.

ER stress refers to a misfolding of protein within a cell organelle called the endoplasmic reticulum (ER). Nanoceria and GRP78/Bip are both antioxidants but with different mechanisms of action.

The researchers report that sub-retinally injected GRP78/Bip (AAV5-hGrp78/Bip) is picked up by photoreceptor cells and, when combined with nanoceria, has an effect that is more than it would have been with either alone. The evidence was in the form of activation of certain visual system proteins and genes and in the maintenance of retinal structures.

The scientists are now investigating the reason for the effect. They believe this work could indicate that a combination of therapeutic agents will be beneficial in treating RP and other disorders caused by oxidative stress.

X Cail, MS Gorbatyuk, AS Lewin, WW Hauswirth, JF McGinnis, et al.

8. *Ex vivo* Genetic Modification of Photoreceptor Precursors Could Lead to Early Efficacy Assessments

The first photoreceptor cells of the retina to die in retinitis pigmentosa are the rods and then, secondarily, the cones. Researchers are investigating ways to grow replacement rods in the laboratory using a patient's own cells, thereby preserving both rod and cone function. They seek to induce pluripotent stem cells (iPSc) to become rods for transplantation. But first, of course, the RP-causing mutations in a patient's own cells would need repair, which a group at ARVO 2014 reported they are showing is possible. Researchers, from University of Oxford, United Kingdom, are developing ***ex vivo* gene therapy for cell replacement in RP**. *Ex vivo* gene therapy means that the cells are taken directly from the body, transduced with a gene *in vitro* (a process whereby a foreign gene is introduced into a cell via a viral vector), and then returned to the body.

Their work is proceeding using rod pluripotent stem cells from a mouse model with a genetic variant related to rod photoreceptor dysfunction. They successfully transfected the cultured cells with a human rhodopsin gene, allowed the cells to multiply in culture, and then transplanted the cells into the subretinal space of adult mice with retinal degeneration. Their development of a method where genetically-modified *ex vivo* cells and transplanted rod cells survive demonstrates the viability of carrying out an important step in human gene therapy—assessing the efficacy of a gene therapy on a patient's pluripotent stem cells before a gene therapy or cell transplantation is performed.

AO Cramer, MS Singh, M McClements, RE MacLaren

9. Choroideremia: Looking for Signs of Improvement Related to Gene Therapy

Choroideremia is a recessive chorioretinal dystrophy affecting approximately 1 person out of every 50,000. It is caused by mutations in the CHM gene on the long arm of the X chromosome (Xq21.2). The gene defect leads to the absence of an important protein for

sustaining retinal cell health, progressive loss of retinal cells, and reduced vision. Researchers at ARVO 2014 reported on anatomical features of the retina (e.g., fundus autofluorescence (FAF), retinal thickness, structural abnormalities) in choroideremia patients, which they plan to use for comparison in patients undergoing gene therapy to replace the missing gene and its associated protein. As for now, they believe that FAF will be a useful long-term indicator of outcomes.

M Moosajee, SC Ramsden, GC Black, AT Moore, A Webster

(Note: The first clinical trial for choroideremia is being led by Professor Robert MacLaren of the Nuffield Laboratory of Ophthalmology at University of Oxford. The trial involves delivering normal versions of the gene to the cells of the retina—by injection into the space beneath the retina—where the genes will instruct the cells to make the missing protein. The trial began in 2011 and results from the first six patients were recently reported. Tests of best-corrected visual acuity and retinal sensitivity show that all six choroideremia patients, including those with the most profound vision loss, had improvement in their visual acuity and an increase in retinal sensitivity. Both are signs of improved photoreceptor cell function. The optimal treatment dose is yet to be determined.)

Other

10. Therapeutic Efficacy of Unoprostone Isopropyl in Patients with RP

Unoprostone isopropyl is being studied for its potential therapeutic effect in patients with retinitis pigmentosa. The eye drop compound is traditionally used in the management of open-angle glaucoma and ocular hypertension, under the name Rescula. Researchers at the 2011 ARVO annual meeting reported positive effects of the drug in a Phase 2 clinical trial (for efficacy and safety of different drug doses) in Japan of patients with mid- to late-stage RP. The researchers reported promising results; RP patients receiving the highest doses experienced an improvement in the sensitivity of their central vision and less reduction in their vision compared to patients receiving placebo treatment. Newer reports confirm the findings: In a study using 0.12% topical unoprostone twice daily, researchers report finding preserved visual acuity and visual field measurements in the treated eye of RP patients, and also in the fellow untreated eye. The researchers also saw improvements in macular sensitivity, especially in the treated eye. They believe the effect is possibly due to improved blood flow related to topical unoprostone treatment or perhaps a direct neuroprotective effect of the drug on the retinal

cells. A Phase 3 trial has been launched in Japan. No serious side effects have been reported. The study is expected to be completed by the end of 2014.

In animal studies, unoprostone has been shown to protect against retinal cell death, reactive oxygen species, and apoptosis in a model of retinitis pigmentosa. (*J Chakrabarti, J Cuppoletti*)

Researchers are testing a transscleral drug delivery system that would release unoprostone isopropyl continuously rather than by eye drops. (*N Nagai, H Kaji, AK Nexhad, et al*)

Unoprostone isopropyl received orphan drug designation for retinitis pigmentosa in Europe by the EMA and in the United States by the FDA. It is also being studied for its possible therapeutic effect in patients with the dry form of age-related macular degeneration.

11. GI Serious Adverse Events: Comparison of Bevacizumab and Ramibizumab for AMD

Gastrointestinal (GI) side effects related to systemic administration of bevacizumab (Avastin) are known to occur. Complaints of serious GI disturbances have also been made by patients receiving intraocular injections of bevacizumab, and its related compound ranibizumab (Lucentis), for treatment of age-related macular degeneration. Researchers from several institutions in the United Kingdom collaborated on a study comparing the reports of serious GI side effects in patients using one drug or the other as participants in randomized clinical trials of the two compounds. The scientists performed a meta-analysis—using data from the CATT, IVAN, Gefal, MANTA, and LUCAS studies—to quantify the incidence and risk of the GI side effects. In all, 1496 patients had received bevacizumab and 1533 had received ranibizumab.

Pooled results showed that GI serious side effects were reported by 46 (3.1%) patients randomized to bevacizumab and 24 (1.6%) patients receiving ranibizumab. This was a statistically significant difference and showed an almost double risk of GI serious adverse events from bevacizumab. The side effects included vomiting, abdominal pain, intestinal obstruction, and intestinal perforation, of varying severity.

L Scott, U Chakravarthy, R Nash, et al.

12. High Dose Docosahexaenoic Acid (DHA) Supplementation in Patients with X-linked Retinitis Pigmentosa (XLRP): A 4-Year Randomized Clinical Trial

Researchers from the Retina Foundation of the Southwest and University of Texas Southwestern Medical Center collaborated on a study looking at the effect of high doses of the

omega-3 fatty acid docosahexaenoic acid (DHA) on cone photoreceptor function, night vision, letter acuity, and rate of decline of visual fields in patients with X-linked RP. DHA is found in the diet especially in salmon, soybeans, and walnuts. It concentrates naturally in retinal photoreceptor cells and is important for normal functioning of the retina. In a 4-year long placebo-controlled clinical trial, the researchers tested whether high daily doses of DHA would slow vision loss in these patients.

At ARVO 2014 the scientists reported that a limited effect of high doses of DHA in slowing the rate of disease progression. They found no difference between patients receiving placebo and those receiving DHA-supplementation on the electrical activity of cone photoreceptors or on night-vision or letter acuity. The only benefits they found of DHA supplementation was in slowing the rate of decline of peripheral visual fields.

They also looked for signs of adverse events related to high doses of DHA. Adverse events were transient and not considered severe (e.g., slight gastrointestinal irritability, blood chemistry alterations, cholesterol/lipid panel deviations).

D Hoffman, DK Wheaton, D Birch et al.

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