Retinal Structure Measurements as Inclusion Criteria for Stem Cell–Based Therapies of Retinal Degenerations

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Purpose. We reviewed and illustrated the most optimal retinal structural measurements to make in stem cell clinical trials.

Methods. Optical coherence tomography (OCT) and autofluorescence (AF) imaging were used to evaluate patients with severe visual loss from nonsyndromic and syndromic retinitis pigmentosa (RP), ABCA4-Stargardt disease, and nonneovascular age-related macular degeneration (AMD). Outer nuclear layer (ONL), rod outer segment (ROS) layer, inner retina, ganglion cell layer (GCL), and nerve fiber layer (NFL) thicknesses were quantified.

Results. All patients had severely reduced visual acuities. Retinitis pigmentosa patients had limited visual fields; maculopathy patients had central scotomas with retained peripheral function. For the forms of RP illustrated, there was detectable albeit severely reduced ONL across the scanned retina, and normal or hyperthick GCL and NFL. Maculopathy patients had no measurable ONL centrally; it became detectable with eccentricity. Some maculopathy patients showed unexpected GCL losses. Autofluorescence imaging illustrated central losses of RPE integrity. A hypothetical scheme to relate patient data with different phases of retinal remodeling in animal models of retinal degeneration was presented.

Conclusions. Stem cell science is advancing, but it is not too early to open the discussion of criteria for patient selection and monitoring. Available clinical tools, such as OCT and AF imaging, can provide inclusion/exclusion criteria and robust objective outcomes. Accepting that early trials may not lead to miraculous cures, we should be prepared to know why—scientifically and clinically—so we can improve subsequent trials. We also must determine if retinal remodeling is an impediment to efficacy.

Keywords: retinitis pigmentosa, Usher syndrome, Stargardt disease, age-related macular degeneration

Inherited retinal degenerations (IRDs) have entered an era from being incurable to having emerging therapeutic options. Many previously discussed therapies are in planning stages, whereas others already are in early phase clinical trials. The expectation of investigators and patients alike is that these efforts will lead to robust and safe methods with predictable outcomes that will improve vision for patients with disabling visual loss.

Among current clinical trials of stem cell transplantation for retinal disease, two types of retinal degenerations are being enrolled. There are a limited number of trials in patients with forms of retinitis pigmentosa (RP), and there are more trials in patients with diseases of the macula, whether IRDs or age-related macular degeneration (AMD). Different sources of stem cells are being used and different delivery methods are represented.

The main inclusion criterion and common to all of the trials is, of course, seriously impaired visual function. Retinal structural inclusion criteria are less well defined than functional criteria, although many trials mention fundus photography, fluorescein angiography, and optical coherence tomography (OCT). The current study illustrates the use of segmented cross-sections of OCT to analyze retinal structural details and en face autofluorescence (AF) imaging to evaluate the integrity of the RPE. Patients representing the two main disease types being targeted for stem cell–based therapies are shown. Retinal remodeling, a longstanding concern to those planning stem cell therapies, also is addressed with illustrations of data from patients. An attempt is made to relate the patient findings to phases of remodeling postulated from studies of animal models. Details of advances in regenerative medicine are not addressed in this article, but are topics covered by others in this symposium issue.

Methods

Subjects

All procedures followed the Declaration of Helsinki and the study was approved by the Institutional Review Board (IRB). Informed consent, assent, and parental permission were obtained, and the work was Health Insurance Portability and Accountability Act (HIPAA)–compliant. Included were patients with forms of nonsyndromic or syndromic RP (n = 10; ages, 3–64), Stargardt disease (n = 2; ages 39 and 42), and nonneovascular AMD (n = 2; ages 67 and 79).

Retinal Imaging

Optical Coherence Tomography. Cross-sectional retinal images were obtained with OCT using methods previously.
Results

Quantifying Retinal Laminae in Forms of Nonsyndromic or Syndromic RP

Among inclusion criteria for stem cell–based trials should be evidence that vision loss is explained by underlying photoreceptor and/or RPE loss. Available modalities of retinal imaging allow this basic tenet to be proved and not simply assumed. Questions also should be asked about postreceptor laminae and RPE losses that would be consistent with the visual disturbances. As for postreceptor inner retinal integrity, however, there is complexity without easy interpretations. Thickenen GCL and/or NFL as in P1 and P3 have been noted previously in forms of RP. The basis of such inner retinopathy is not clear and it remains uncertain whether there would be any effect of such pathology on efficacy of treatments intended to replace photoreceptors.

Quantifying Retinal Laminar Integrity in Maculopathies

Figure 2 illustrates cross-sectional OCT and en face NIR-RAFIn results from patients with ABCA4-Stargardt disease (P4, P5) and with AMD (P6, P7). Patient 4 at age 39 years had a visual acuity of 20/250; kinetic perimetry showed that beyond a central scotoma to the V-4e target of approximately 20° in diameter, the field to this target was within normal limits. A smaller target (I-4e) revealed a relative scotoma of approximately 40° in diameter and the peripheral field was generally reduced in extent. Optic nerve layer thickness was unmeasurable in the foveal area but there was ONL, albeit abnormally reduced in thickness, eccentric to the fovea. Ganglion cell layer thickness was reduced in a relatively wide expanse of central retina but became normal at approximately 10° eccentric to the fovea. The NFL was normal or near normal. NIR-RAFIn showed a bilobular central macular region of demelanization. The same pattern also was apparent on SW-RAFI (not shown). A second Stargardt disease patient (P5, age 42) had a visual acuity of 20/320; the dimensions of the central scotoma and peripheral field (to V-4e and I-4e targets) were very similar to those of P4. Again, the ONL was undetectable, but became measurable, although reduced, only at eccentricities of greater than approximately 7°. Ganglion cell layer and NFL thicknesses, unlike in P4, were within normal limits. NIR-RAFIn showed a large region devoid of signal suggesting RPE atrophy; the latter was consistent with a similar region of signal loss on SW-RAFI (not shown).

Two patients with nonneovascular AMD (Figs. 2D, 2E) and different degrees of ONL loss across the scan length had approximately the same visual acuity (P6, 20/320; P7, 20/250). Patient 6 at age 67 was symptomatic of central visual disturbances for approximately 2 to 3 years. She shows loss of ONL and abnormal IS and OS lamination in the very central retina and there are confluent drusen and drusenoid RPE.
detachment. Outer retinal lamination becomes more normal in appearance at approximately 5° eccentric in the temporal retina and at approximately 7° eccentric nasally. The GCL and NFL fall within normal limits. NIR-RAFIn shows a central region of demelanization and SW-RAFI results are consistent with severe RPE disease or RPE atrophy. Patient 7, age 79, had 24 years of symptomatic central visual disturbances and shows a wide area of central loss of ONL with some confluent drusen, and no discernible IS and OS lamination. There is thinning of the GCL in temporal retina, but not in nasal retina; NFL is within normal limits. NIR-RAFIn shows a large region of demelanization extending across the macula consistent with SW-RAFI results.

Again, inclusion for a central retinal stem cell-based transplant should include a measured loss of central retinal ONL that would be consistent with the visual loss; both types of patients satisfy this criterion. Despite the similarity in age, visual function, and diagnosis of P4 and P5, there is a definite reduction in the GCL in P4, but not in P5. This difference was not predictable and is complicated by the finding that NFL is generally within normal limits for both patients. Patient 7 with AMD has GCL loss in the temporal retina, but both patients show NFL thickness within normal limits.

**FIGURE 1.** Cross-sectional and en face imaging in syndromic and nonsyndromic widespread retinal degenerations. (A) Upper left: OCT cross-sections across the horizontal meridian through the fovea in a normal subject and 3 patients. Outer nuclear layer is highlighted in blue and GCL in orange. Right column of images show NIR-RAFIn in the normal subject and patients. BrM/RPE, Bruch's membrane/RPE. (B) Quantitation of ONL and GCL thickness in the patients (symbols) compared to normal results (shaded areas; mean ± 2 SD). (C) Upper: NFL thickness measurements across the horizontal meridian in patients and normal results. Lower: Polar plots of NFL thickness. N, nasal; S, superior; T, temporal; I, inferior. Inset: Location of circular scan with respect to ONH.
FIGURE 2. Cross-sectional and en face imaging in macular degenerations. (A) Upper left: OCT cross-sections across the horizontal meridian through the fovea in two patients with Stargardt disease due to ABCA4 mutations. Highlighted layers as in Figure 1. Right column: NIR-RAFIN images. (B) Quantitation of ONL and GCL thickness in the same patients compared to normal results (shaded areas; mean ± 2 SD). (C) Upper: NFL thickness measurements across the horizontal meridian in the patients compared to normal results. Lower: Polar plots of NFL thickness. (D) Upper left: OCT cross-sections in two patients with AMD. Highlighted layers, as in Figures 1 and 2A. Right column: NIR-RAFIN images. (E) Quantitation of ONL and GCL thickness in the patients (symbols) compared with normal results (shaded areas; mean ± 2 SD). (F) Upper: NFL thickness measurements across the horizontal meridian in patients and normal results. Lower: Polar plots of NFL thickness.
Retinal Remodeling: Phases and Surrogate Measures From Cross-Sectional Imaging

How the retina changes as a consequence of photoreceptor loss in IRDs has been a topic of scientific study for at least two decades and has included histopathologic studies in human donor tissue as well as in many animal models (e.g., Refs. 10, 27–32). A concern has been whether the many changes in inner retinal structure would alter connectivity in a way that would frustrate attempts to improve vision, such as could occur in stem cell–based transplantation strategies.31

The advent of OCT permitted us to ask in IRD patients whether there was detectable change in inner retinal structure that could represent the effects of remodeling so well demonstrated in animals with retinal degeneration. Our many studies of different forms of IRD have revealed abnormalities in the inner retina associated with photoreceptor loss (e.g., Refs. 18–20, 53, 34) and more recently in Stargardt disease and AMD.35,36 Thickening of the inner nuclear layer (INL) has been notable in retinas with photoreceptor degeneration. Thickening of the GCL and NFL, as in some of the patients illustrated in Figures 1 and 2, also has been observed.31

To our knowledge, there has been no attempt to date to relate the phases of retinal remodeling defined in animal models with observations in human IRDs. Our attempt to do so is presented in Figure 3. In Figure 3A (left), retinal laminar changes across a vertical OCT scan extending from the fovea to 30° in the superior retina are shown for P8, a 3-year-old patient with Usher syndrome 1B due to MYO7A mutations.37,38 Three phases of retinal remodeling have been described in murine models of retinal degeneration.10 Although microscopic details are beyond the resolution of OCT, some general relationships can be drawn between the patient data and the detailed cell biology. Due to the regional variations of severity across the retina in human RP, the phases appear to be definable within this single scan of P8. It is likely that a full study of the phases in a cohort of patients with different severities or in patients followed serially may reveal the different phases as they have been described in animal models. Remodeling phase 1 is considered to occur when there is photoreceptor stress before photoreceptor cell death. In P8, there is a wide zone of ONL that is within normal limits in thickness—extending from the fovea to approximately 17° into the superior retina. In this patient, photoreceptor stress is interpreted as reduced thickness of the outer segment (ROS) layer, that is, shortening of the ROS.

Phase 2 is when there is measurable photoreceptor loss (among other complex changes19) and this is occurring in P8 in a zone superior to the intact ONL. There is loss of ONL and ROS thickness, but the inner retina remains normal in thickness. Phase 3 has been described as the occurrence of neural remodeling, which includes further neural death, Müller cell hypertrophy and migration of cells within the distorted retina.10 In the retinal region from approximately 19° to 30° in P8, there is very reduced, but detectable ONL and no measurable ROS. The inner retina, however, has increased in thickness across the region. The detailed morphologic basis for this thickening is uncertain; we have speculated that it is due to glial cell hypertrophy. In two murine retinal degenerations, rdl6 and rhodopsin T17M models of CEP290-LCA and RHO-adRP, respectively, the reduced ONL tended to merge with the INL, which appeared vacuous and with larger cells, possibly from Müller cell nuclei and processes filling the space previously occupied by lost retinal cells.19-21 There are direct correlations of histopathology with OCT in other retinal degenerations, but the topic of remodeling was not specifically addressed (e.g., Refs. 40–43).

Figure 3A (left) also shows patient data and the detailed cell biology. Due to the regional variations of severity across the retina in human RP, the phases appear to be definable within this single scan of P8. It is likely that a full study of the phases in a cohort of patients with different severities or in patients followed serially may reveal the different phases as they have been described in animal models. Remodeling phase 1 is considered to occur when there is photoreceptor stress before photoreceptor cell death. In P8, there is a wide zone of ONL that is within normal limits in thickness—extending from the fovea to approximately 17° into the superior retina. In this patient, photoreceptor stress is interpreted as reduced thickness of the outer segment (ROS) layer, that is, shortening of the ROS.

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Patient 9, a 39-year-old autosomal dominant (ad) RP patient with a rhodopsin mutation, shows a similar pattern of contiguous retinal regions representing different phases of increasing severity (Fig. 3A, right). Unlike P8, however, P9's expanse of normal ONL but reduced ROS (phase 1) is more limited and closer to the fovea. This corresponded to a more limited visual field than in P8. Other patient scans (Figs. 3B–G) illustrate the diversity of effects in retinal regions with extremely reduced or nondetectable photoreceptors, presumed to be in remodeling phase 3. There could be remarkable thickening (P10 with USH1B; P3 with early-onset RP), to somewhat thinner retina with pigment migration (hyperreflectivities blocking signals distally; arrows in P11 with adRP from RHO mutation and P12 with RP of unknown genotype) to normal thickness of the inner retina despite loss of photoreceptors across most of the scan length (P13 with RP and unknown genotype) to borderline or abnormally thinned retina (P14 with adRP). A sequence of progression from thickening to thinning of inner retina is speculative and awaits longitudinal data collection. If the most thinned inner retina (here illustrated with P14 data) proves to be a later phase of disease, it may be the most "rewired"10 and possibly the least responsive to intervention aiming to restore connectivity and visual function through the retina.

DISCUSSION

Quantifying the Morphologic Basis of Visual Loss

Pre-treatment evaluations in the human gene therapy trials of Leber congenital amaurosis due to RPE65 mutations emphasized that retinal degenerations exist in which there is a disproportionate loss of vision despite retained photoreceptors (e.g., data reported previously17,39,44,45). Retinopathies with this complex pathophysiology at stages when there were retained photoreceptors and RPE, despite severely impaired vision, would not be appropriate candidates for stem cell–based therapies unless other treatment strategies proved not feasible or ineffective. In modern ophthalmic clinics, the tools are available to distinguish these retinopathies from the more common disorders that behave as "simple photoreceptor degenerations." In the latter type of disorder, an OCT measurement of photoreceptor layer thickness and a localized measure of visual sensitivity should relate to each other according to a theoretical model based on photoreceptor quantum catch.17-46 This is the pattern of responding that occurs in most syndromic and nonsyndromic IRDs.17 In other words, there should not only be measures of vision as inclusion criteria for stem cell–based clinical trials, but also measures of retinal structure by OCT (specifically photoreceptor structure and not simply retinal thickness). If the target layer for the transplant is the RPE, the integrity of the RPE can be estimated by melanin- and lipofuscin-based AF imaging and would seem to be a worthy measure at baseline for comparison post treatment.48 The observations of detectable ONL in retinal regions likely corresponding to RPE atrophy (made in the current work) are counterintuitive, but consistent with photoreceptor nuclei found in geographic atrophy in human eyes with AMD.49

Do we know how much retained ONL or RPE warrants consideration for best result of a specific stem cell transplant? One could argue for a measurable amount of both cell populations or no detectable structure, depending on results of studies in direct models (e.g., see the study by MacLaren et al.9). Even if there is no studied answer to this question, it is important to know such parameters so that the field can evolve...
in a more scientific and stepwise manner, and allow negative and positive efficacy to be better interpreted. Currently, the number of variables to be addressed in this field is daunting and if a human trial is deemed unsuccessful or successful by an efficacy outcome, there is a strong need to at least attempt to understand why this occurred from known details of the patient.

Advanced Stages of Human Retinal Degenerations May Not All Be the Same

On the topic of knowing more rather than less details of the patients involved in stem cell-based clinical trials, despite their advanced stages of retinal degeneration, there should be an attempt to determine genotypes, even if not an inclusion or
exclusion criterion. There should be no reluctance to genotype these patients or at least store samples from the patients for later genotyping. It will be argued that statistical significance of the trial outcomes would not be reached even if we do know genotypes, considering the small numbers of patients or single individuals with a specific molecular cause entering early trials. Yet, certain retinal degenerations cannot be assumed to have the same postphotoreceptor retinal connectivity as others. For example, the enhanced S cone syndrome caused by NR2E3 mutations is an IRD that can masquerade as RP, but is a developmental disorder with S cones dominating the photoreceptor mosaic, few if any rods and limited numbers of other cone subtypes from birth.51–55 How this genetic retinal degeneration and its postreceptor retinal connectivity would respond to a form of stem cell therapy is uncertain,56 but it should be known that this was the treated disease; any difference between outcomes compared to other treated patients would provoke a mechanism-based hypothesis to help explain the results. Further, there is an established literature on types of syndromic and nonsyndromic forms of RP that show a negative electroretinogram (ERG) suggesting prominent post-receptor dysfunction (see prior reports20,57–58) or ERGs suggesting an atypical retinal circuitry.59 This would be relevant disease history that could impact outcomes of a stem cell–based therapy in patients who at the time of recruitment would not have a recordable ERG signal.

The Spectre of Retinal Remodeling and Potential Impact of Second-Order Neuron Dysfunction in Stem Cell–Based Therapies

There is an extensive literature on the complex morphologic modifications in postreceptor retina after photoreceptor loss in animal models of RP (e.g., see prior reports27–32) but the current study is the first attempt to relate these observations about phases of retinal remodeling to human inner retinal structural abnormalities. The human work must be extended to determine if these inner retinal laminar changes have implications for efficacy in stem cell treatment trials (or any other photoreceptor-based trials). A feasible step to take is to study patients at late stages of photoreceptor loss, but with some retained photoreceptor laminae. Colocalized measures of visual sensitivity (rod or cone, determined by retinal location) should be made. The same theoretical model described above as an inclusion criterion to confirm that impaired visual loss was not accompanied by disproportionate photoreceptor layer thickness would now be used to define whether residual photoreceptor structure and function are related as expected for a “pure photoreceptor degeneration.” We took the first step toward this goal recently in patients with forms of ABCA4-retinal degeneration.56 Regions with photoreceptor losses and inner retinal laminar abnormalities were compared to those without inner retinal changes; colocalized rod visual function was measured in each of the areas. The model for photoreceptor quantum catch indicated that rod visual loss in the presence of inner retinal laminopathy did not exceed the prediction from photoreceptor loss only. Other retinal degenerations now need to be studied similarly.

Unexpected observations concerning inner retinopathy were made in the maculopathies illustrated in the present study (Fig. 2). These are worthy of mention considering the stem cell trials currently listed in clinicaltrials.gov are most commonly targeting Stargardt disease and AMD patients. In one of the two patients with ABCA4 mutations, the central GCL was abnormally reduced while NFL was essentially within normal limits. Ganglion cell layer measurements may be more a direct and sensitive measure than using NFL as a surrogate.25 There is the possibility that the NFL, in some patients with RP, actually may be thickened but showing normal results and thereby “hiding” a thinning due to GCL losses. The asymmetry of GCL thickness in one of the AMD patients also is somewhat unexpected. Symptoms in this older of the two AMD patients were more longstanding and the extent of the central photoreceptor loss was greater than that in the other patient. There was no evidence of glaucoma or other cause of GCL reduction. Again, the NFL measures did not reveal notable abnormalities. Increases in GCL and NFL thickness in forms of RP (as well the opposite effects) have been noted previously,21,24–26 and attributed to thickened glial cell processes, aberrant neurites, and reactive hyperplasia of astrocytes and possible vascular elements.20,28

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References

Clinical Plans for Stem Cell Therapies


