Editorial

Stem Cell INVESTIGATION

Stem cells with a view: a look inside a retinal ciliopathy

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Ciliopathies are a group of severe developmental disorders that are clinically and genetically heterogeneous. They occur due to the dysfunction of cilia, which are microtubule-based extensions of the plasma membrane. The ciliary membrane acts as a hub for transmembrane receptors and specific lipids and proteins that assist in the detection and downstream signaling of extrinsic cues. They carry out diverse signaling cascades, such as sonic hedgehog signaling and sensory perceptions, including olfaction, chemosensation and photoreception (1,2). Not surprisingly, ciliary dysfunction results in severe developmental and systemic disorders, including Meckel-Gruber Syndrome, Joubert Syndrome, Bardet-Biedl Syndrome, and Senior-Loken Syndrome (1).

Retinal degeneration due to ciliary dysfunction is commonly observed in ciliopathies (3). It is predominantly caused due to the dysfunction and degeneration of the ciliary outer segment of the photoreceptors, the light-sensing neurons in the retina. Surprisingly however, photoreceptor degeneration due to ciliary dysfunction can also manifest as an isolated case with no systemic manifestations. An example of such an observation is mutations in the ciliary protein-encoding gene *CEP290*. In 2006, the *CEP290* gene was cloned as a causative gene for Joubert Syndrome (4,5). Subsequently, *CEP290* mutations were identified in a wide range of ciliopathies and were associated with a spectrum of systemic manifestations, including mental retardation, polydactyly, renal failure and retinal dystrophies (6-10). Simultaneous studies also identified a spontaneously occurring mouse mutant of *Cep290*, *Cep290*^{rd16} (retinal degeneration 16) (11). The *Cep290*^{rd16} mouse carries an in-frame deletion of exons 16–19 and interestingly, predominantly exhibits early onset severe retinal degeneration. No other systemic manifestations were observed. Such a retina-restricted phenotype was attributed to the expression of a partially functional deleted variant of CEP290, in the *Cep290*^{rd16} mouse, which might spare other cell types but was detrimental to photoreceptors.

Building upon these observations, *CEP290* mutations, particularly a deep intronic homozygous *CEP290* mutation, c.2991 + 1665A>G, were reported in isolated cases of childhood blindness disorder, Leber congenital amaurosis (LCA) (12). This mutation creates a cryptic exon between exons 26 and 27 with a premature stop codon in the *CEP290* gene. Curiously, patient lymphocytes carrying this intronic mutation express, albeit at lower level the wild type *CEP290* transcript as well (12). Thus, it was hypothesized that non-retinal cell types may escape the splice defect more efficiently and result in the production of near-normal normal levels of the full-length *CEP290* transcript; however, such a mechanism might not be functional in the photoreceptors. Investigating such mechanisms is key to our understanding of the molecular underpinnings of the manifestation of retina-specific disease due to *CEP290* mutations.

In a recent study, Parfitt *et al*. (13) took on this challenging question. They resorted to induced pluripotent stem cells (iPSCs) to not only investigate the disease mechanisms but also to develop rational therapeutic modalities. They generated iPSCs from fibroblasts (14,15) of control subjects or from *CEP290*-LCA patients carrying the deep intronic mutation c.2991 + 1665A>G. Using previously published procedures they differentiated the iPSCs into optic cups and the RPE (16). The authors then compared the effect of the mutation on the splicing of the *CEP290* gene in the iPSCs as well as in the differentiated 3-dimensional (3D) optic cups (to induce retina and photoreceptor formation) and the RPE. The

authors first found that the three cell types derived from control fibroblasts exhibited differential regulation of the *CEP290* transcription levels. This observation led them to hypothesize that differential expression of the *CEP290* gene may underlie a tissue-specific effect of some *CEP290* mutations. Commensurately, they found that such regulation is altered due to the deep intronic mutation. It resulted in aberrant splicing of the *CEP290* gene and concomitantly reduced CEP290 protein levels in the mutant iPSCs as compared to controls. The mutant iPSCs derived retinas and photoreceptors exhibited relatively severe deregulation of *CEP290* expression; they exhibited fewer cilia predominantly in the photoreceptors and defective cilia formation and localization of retinitis pigmentosa GTPase regulator (RPGR), a CEP290-interacting retinal ciliopathy protein (17,18) and small GTPase RAB8A.

Antisense oligonucleotide (AON)-mediated splice modulation is a powerful tool to correct splice site defects in disease genes. In fact, AON-mediated correction of the c.2991 + 1665A > G *CEP290* mutation in patient-derived fibroblasts was demonstrated in two independent studies (19,20). The corrected gene exhibited improved transcription of the wild type transcript and restoration of cilia formation in the patient fibroblasts. Building upon the success of these studies, Parfitt *et al*., designed a 25-bp antisense morpholino (MO) against the c.2991 + 1665A>G CEP290 mutation and tested its efficacy. They found that the *CEP290*-MO reduced the levels of the cryptic exon inclusion and resulted in a concomitant increase in the normal transcript levels in LCA fibroblasts. The *CEP290*-MO treatment also restored ciliation defects and ciliary protein trafficking of RPGR, and of opsins in the 3D-optic cup derived photoreceptors (13).

The differentiated retinal tissue has provided an excellent platform to also assess the efficacy of other gene correction strategies, such as genome editing. However, such approaches will have to be continuously designed and modified to fit the patient mutation. Future studies should also focus on utilizing the differentiated tissues from patients to explore the efficacy of mutation-independent approaches. Previous studies using the $Cep290^{rd16}$ mouse and the zebrafish have identified potential pathway intermediates that can be exploited to mitigate or delay photoreceptor degeneration due to the loss of CEP290 (21,22). Such approaches can also be readily tested in the differentiated optic cups. Taken together, the results of Parfitt *et al*. provide a valuable clue to our understanding of the tissue-enriched or tissue-specific disease manifestations in several clinically heterogeneous ciliopathies. In addition, this study has opened new and exciting avenues to design and test novel therapeutic modalities for several blinding diseases.

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Footnote

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