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Pathophysiological Mechanism and Treatment Strategies for Leber Congenital Amaurosis

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Abstract

Mutations in retinoid isomerase, RPE65, or lecithin-retinol acyltransferase (LRAT) disrupt 11-*cis*-retinal recycling and cause Leber congenital amaurosis (LCA), the most severe retinal dystrophy in early childhood. We used *Lrat*^{-/-}, a murine model for LCA, to investigate the mechanism of rapid cone degeneration. We found that mislocalized M-opsin was degraded whereas mislocalized S-opsin accumulated in *Lrat*^{-/-} cones before the onset of massive ventral/central cone degeneration. Since the ventral and central retina expresses higher levels of S-opsin than the dorsal retina in mice, our results may explain why ventral and central cones degenerate more rapidly than dorsal cones in *Rpe65*^{-/-} and *Lrat*^{-/-} LCA models. In addition, human blue opsin and mouse S-opsin, but not mouse M-opsin or human red/green opsins, aggregated to form cytoplasmic inclusions in transfected cells, which may explain why blue cone function is lost earlier than red/green-cone function in LCA patients. The aggregation of short-wavelength opsins likely caused rapid cone degenerations through an ER stress pathway as demonstrated in both the *Lrat*^{-/-} retina and transfected cells. Based on this mechanism, we designed a new therapy of LCA by reducing ER stress. We found that systemic injection of an ER chemical chaperone, tauroursodeoxycholic acid (TUDCA), is effective in reducing ER stress, preventing apoptosis, and preserving cones in *Lrat*^{-/-} mice.

Keywords

aggregation; RPE65; LRAT; Leber congenital amaurosis (LCA); Short-wavelength sensitive opsins (SWS); Medium/long-wavelength sensitive opsins (M/LWS); Cone degeneration

99.1 Introduction

Both Retinoid isomerase (RPE65) and lecithin-retinol acyltransferase (LRAT) are required for 11-*cis*-retinal recycling in the RPE. Mutations in *RPE65* or *LRAT* cause Leber congenital amaurosis (LCA) [1]. Two mouse models, *Rpe65*^{-/-} and *Lrat*^{-/-}, capture many salient pathologic features of human LCA [2–4]. Both rod and cone function are severely compromised due to the lack of 11-*cis*-retinal [2, 3]. The cone opsins (S-opsin and M-opsin)

fail to traffic from the cone inner segment to the cone outer segment and the cone photoreceptors in the central/ventral regions degenerate rapidly (< 4 weeks). Early loss of foveal cones also occur in RPE65-deficient patients [5]. The mechanisms responsible for early cone death in both mouse models and human patients are not well understood. Specifically it is unclear why ventral and central cones in mouse models die much more rapidly than dorsal cones. Similarly, it is unclear why blue cone function is lost early in patients [5, 6]. Here, we used the *Lrat*^{-/-} mouse, a model for LCA, to investigate the mechanism and treatment for cone photoreceptor degeneration.

99.2 Materials and Methods

99.2.1 Animals and TUDCA Injection

Lrat^{-/-} mice were generated and described previously [3]. WT (*C57BL/6J*) mice were purchased from Jackson Laboratory. All animal experiments were approved by the Institutional Animal Care and Use Committees (IACUC) at the University of Utah and were performed in accordance with the ARVO Statement for the Use of Animal in Ophthalmic and Vision Research. Mice were reared under cyclic light (12 h light/12 h dark). *Lrat*^{-/-} and WT mice were treated with TUDCA (TCI America) or vehicle (0.15 M NaHCO₃, pH 7.0) following a published procedure [7].

99.2.2 Statistics

Data were presented as mean ± SEM, and the differences were analyzed with unpaired two-sample Student's *t*-test. *P* values < 0.05 were considered statistically significant.

99.3 Results

99.3.1 Different Protein Stability of M and S Opsins in the *Lrat*^{-/-} Retina

By immunohistochemistry, we found that *Lrat*^{-/-} cones accumulated more mislocalized S-opsin than M-opsin [8]. We proceeded to verify this finding by western blot at three stages of cone degeneration: (1) P14, pre-degeneration [9]; (2) P18, early-stage degeneration; (3) P30, late-stage degeneration. In P14 *Lrat*^{-/-}, the protein levels of both M-opsin and S-opsin were similar to those in WT (Fig. 99.1a) although their mRNA levels were slightly reduced [8]. However, in P18 *Lrat*^{-/-} retina, the M-opsin protein was markedly reduced (~ 1.9 times) whereas the S-opsin level remained the same, as compared with WT (Fig. 99.1a). After we normalized the protein levels of cone opsins against their mRNA levels (i.e. protein/mRNA), this ratio almost doubled (~ 1.9 times) for S-opsin in P18 *Lrat*^{-/-} compared to WT (Fig. 99.1b). In contrast, the protein/mRNA ratio of M-opsin in P18 *Lrat*^{-/-} was similar to that in WT. Assuming the protein synthesis for cone opsins is minimally affected in the early stage of *Lrat*^{-/-} cone degeneration, our results suggest that mislocalized S-opsin was more resistant to proteasome degradation than mislocalized M-opsin. Since the ventral and central retina express higher levels of S-opsin than the dorsal retina in mice [10], our results suggest that accumulation of mislocalized S-opsin may underlie the fast ventral/central cone degeneration in *Lrat*^{-/-} mice. Cones at the far-dorsal region, which express more M-opsin than S-opsin, degenerated much more slowly, corresponding to less accumulation of mislocalized M-opsin. In P30 *Lrat*^{-/-}, both the protein (Fig. 99.1a) and mRNA [8] levels of

S-opsin were drastically reduced, due to significant loss of ventral and central cones [4, 9] (Fig. 99.1c).

99.3.2 Mouse and Human SW Opsins Aggregate and Cause ER Stress in Transfected Cells

By using a cell culture system (COS-7), we found that mouse S-opsin and human blue opsin showed prominent aggregations manifesting as green dots of varying sizes in the perinuclear region (Fig. 99.2a). This was not observed for mouse M-opsin and human red/green opsins. To examine the possibility that the aggregation of SW opsins may induce UPR and ER stress, COS-7 cells were co-transfected with various opsins and a CHOP-GFP reporter plasmid. CHOP (C/EBP homology protein) is a well characterized UPR (unfolded protein response) target gene and an ER stress marker associated with apoptosis. It has been shown that CHOP-GFP can track the expression of endogenous CHOP [11]. The expression of mouse S and human blue opsins induced significant CHOP activation compared with the expression of rhodopsin, mouse M-opsin or human red/green opsins (Fig. 99.2b, $p < 0.001$). Consistent with the cell culture result, the CHOP signal was markedly increased in the ONL of the ventral retina of P18 *Lrat*^{-/-}, coinciding with the maximal S-opsin accumulation and the early stage of cone degeneration [8]. The CHOP signal was lower in the ventral retina of P14 *Lrat*^{-/-} when S-opsin accumulation was much less. In the far-dorsal retina of *Lrat*^{-/-} (P14 and P18) that mainly expresses M-opsin, no CHOP upregulation was observed [8].

99.3.3 TUDCA Significantly Slows Down Cone Degeneration in *Lrat*^{-/-} Mice

To determine whether TUDCA can prevent or slow down this rapid cone degeneration, *Lrat*^{-/-} mice were injected subcutaneously once every 3 days starting at P9. Treated mice were euthanized at P28 and cone cell densities were determined at different retinal regions from fluorescence images of cone-arrestin stained retinal flatmounts. There was significant cone degeneration in the ventral and central regions of retina in vehicle injected *Lrat*^{-/-} mice (Fig. 99.3). TUDCA treatment resulted in a significant increase (~ 3-fold, $p < 0.001$) in cone density in the ventral and central retina compared with the vehicle (Fig. 99.1). Cone cell morphology was greatly improved as evidenced by the presence of intact cone structures [12]. TUDCA treatment also increased cone numbers (25 %) in the dorsal retina although the difference was not statistically significant ($p = 0.057$). Although TUDCA provided significant protection of central and ventral cones of *Lrat*^{-/-} mice, the cone densities are still lower than those of WT (54.5 and 62.7 % lower for central cones and ventral cones, respectively). We observed a substantial reduction of CHOP in the ventral retina of TUDCA-treated *Lrat*^{-/-} mice compared to that in vehicle-treated littermates at P18 [12]. Furthermore, TUDCA injection virtually eliminated apoptotic signals in the ONL of *Lrat*^{-/-} mice judged by caspase-3 activation [12]. Our data suggest that TUDCA slows down cone degeneration by reducing ER stress and preventing apoptosis.

99.4 Discussion

Our studies showed that M and S opsins face very different fates in *Lrat*^{-/-} mice: A significant amount of mislocalized M-opsin is degraded whereas S-opsin is resistant to proteasome degradation, resulting in far more toxic aggregation of S-opsin in the ventral and central cones than that of M-opsin in the dorsal cones. In addition, we found that

aggregation of S-opsin activated the unfolded protein response (UPR) and caused ER stress. It is likely that the UPR in cones copes with mislocalized M-opsin more effectively than with mislocalized S-opsin. Thus, M-opsin is significantly degraded by the ER-associated degradation (ERAD) pathway, which relieves ER stress. On the other hand, S-opsin is resistant to ERAD resulting in aggregation/ accumulation, which induces apoptosis. As the ventral and central retina express higher levels of S-opsin than the dorsal retina in mice [10], our results explain the region-specific cone degeneration pattern in *Lrat*^{-/-} and *Rpe65*^{-/-} retinas. In addition, we demonstrated that systemic injection of TUDCA is effective in reducing ER stress, preventing apoptosis, and preserving cones in *Lrat*^{-/-} mice. TUDCA has the potential to lead to the development of a new class of therapeutic drugs for treating LCA.

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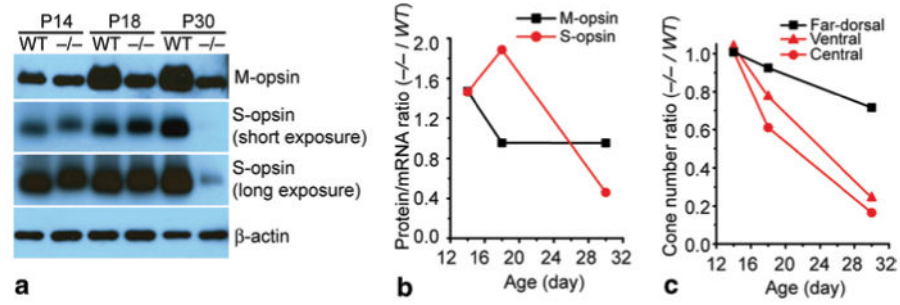


Fig. 99.1.

Expression of M and S opsins in *Lrat*^{-/-} and WT retinas. **a** Western blot analysis of M and S opsins in the retinas of *Lrat*^{-/-} and WT mice at P14, P18, and P30. Equal loading was indicated by β -actin. **b** The average protein/mRNA ratios of M and S opsins of *Lrat*^{-/-} were normalized against those of WT. **c** The average cone numbers from ventral, central, and far-dorsal retina of *Lrat*^{-/-} were normalized against those of WT. (Data were from Fig. 2 of [8]. Copyright National Academy of Sciences, USA)

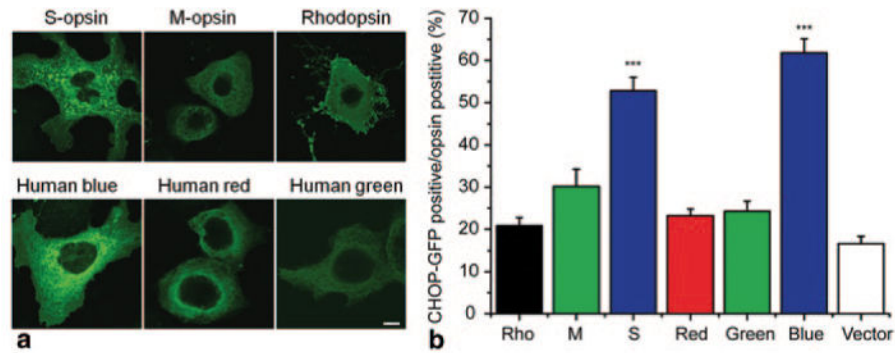


Fig. 99.2.

Mouse *S-opsin* and human blue opsin aggregated and induced *CHOP* activation in COS-7 cells. **a** COS-7 cells were transfected with plasmids encoding various opsins, and were labeled with opsin antibodies (*green*). Scale bar, 10 μ m. **b** Quantification of *CHOP-GFP* positive cells as percentages of opsin expressing cells. COS-7 cells were co-transfected with plasmids encoding *CHOP-GFP* and various opsins (or control vector pRK5 and pmCherry-N1), and were labeled with opsin antibodies. The numbers of vector transfected cells were estimated from mCherry positive cells. $N = 10$ for Rho; $N = 11$ for M, *red*, *blue*; $N = 12$ for S and *green* opsins; $N = 6$ for *vector* control. Both mouse *S-opsin* (S) and human blue opsin induced significant more CHOP activation than mouse *M-opsin* (M), human red/green opsins, bovine rhodopsin, and vector control. ***, $p < 0.001$. (Data were from Figs. 5 and 6 of [8]. Copyright National Academy of Sciences, USA)

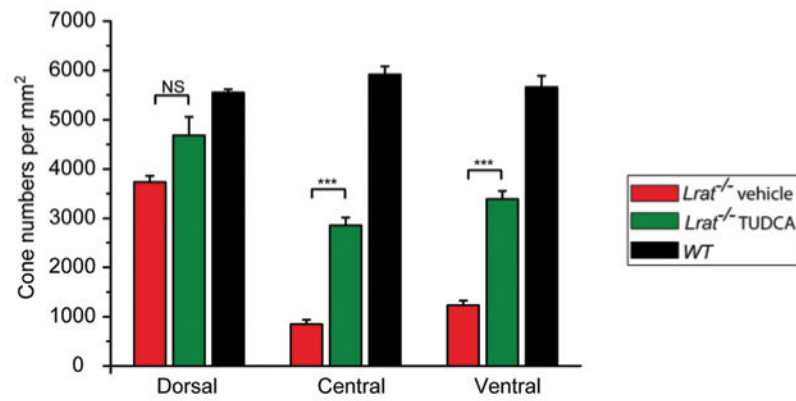


Fig. 99.3.

Quantification on the protective effect of TUDCA on *Lrat*^{-/-} cones. Cone photoreceptors were counted in the *ventral*, *central*, and *dorsal* sections in TUDCA injected *Lrat*^{-/-} ($n = 8$), vehicle injected *Lrat*^{-/-} ($n = 6$), and untreated WT ($n = 5$). Data were expressed as the average numbers of cones per mm² (mean \pm SEM). Untreated WT mice were included as controls to evaluate the efficacy of TUDCA. *** $p < 0.001$, NS, not significant. (From Fig. 1 of [12]. Copyright Association for Research in Vision and Ophthalmology)