Title

Ablation of Chop Transiently Enhances Photoreceptor Survival but Does Not Prevent Retinal Degeneration in Transgenic Mice Expressing Human P23H Rhodopsin

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Chapter 25
Ablation of *Chop* Transiently Enhances Photoreceptor Survival but Does Not Prevent Retinal Degeneration in Transgenic Mice Expressing Human P23H Rhodopsin

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Abstract *RHO* (Rod opsin) encodes a G-protein coupled receptor that is expressed exclusively by rod photoreceptors of the retina and forms the essential photopigment, rhodopsin, when coupled with 11-cis-retinal. Many rod opsin disease mutations cause rod opsin protein misfolding and trigger endoplasmic reticulum (ER) stress, leading to activation of the Unfolded Protein Response (UPR) signal transduction network. *Chop* is a transcriptional activator that is induced by ER stress and promotes cell death in response to chronic ER stress. Here, we examined the role of *Chop* in transgenic mice expressing human P23H rhodopsin (hP23H Rho Tg) that undergo retinal degeneration. With the exception of one time point, we found no significant induction of *Chop* in these animals and no significant change in retinal degeneration by histology and electrophysiology when hP23H Rho Tg animals were bred into a $Chop^{-/-}$ background. Our results indicate that *Chop* does not play a significant causal role during retinal degeneration in these animals. We suggest that other modules of the ER stress-induced UPR signaling network may be involved photoreceptor disease induced by P23H rhodopsin.

Keywords Rhodopsin · P23H · Unfolded protein response · UPR · ER stress · Photoreceptor cell death · Chop · Retinal degeneration · Transgenic mice

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25.1 Introduction

Rhodopsin protein folding begins when *RHO* mRNA is translated into protein at the endoplasmic reticulum (ER) in the photoreceptor (PR) inner segment (IS) ellipsoid region. Many rhodopsin mutations associated with retinal degeneration introduce amino acid substitutions that impair rod opsin's ability to fold properly in the ER (Sung et al. 1991; Kaushal and Khorana 1994). Accumulation of unfolded proteins in the ER triggers ER stress. The Unfolded Protein Response (UPR) is an intracellular signal transduction network that is activated by ER stress and, in turn, activates transcriptional, translational, and post-translational programs that help cells correct the protein misfolding problem that caused ER stress (Walter and Ron 2011). However, if misfolded proteins persist, UPR signaling can activate pro-apoptotic programs leading to cell death (Walter and Ron 2011).

Chop (C/EBP homologous protein) is one genetic component of the UPR and encodes a transcription factor whose mRNA and protein levels are upregulated by the UPR in response to ER stress (Oyadomari and Mori 2004). $Chop^{-/-}$ mouse embryonic fibroblasts are resistant to cell death induced by thapsigargin, an inhibitor of the Ca^{2+} ATPase of the ER, and tunicamycin, which blocks N-linked glycosylation (Zinszner et al. 1998). Akita mice expressing mutant insulin 2 undergo pancreatic β-cell death that was delayed in a $Chop^{-/-}$ background (Oyadomari et al. 2002). Mice expressing mutant myelin protein zero undergo increased Schwann cell death that was delayed by loss of *Chop* (Pennuto 2008). These findings indicate that CHOP contributes to cell death and injury in response to certain types of ER stress. Here, we examined whether *Chop* was induced in transgenic mice expressing

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human P23H rhodopsin, and how retinal degeneration was affected when these animals were bred into a $Chop^{-/-}$ background.

25.2 Materials and Methods

$Chop^{-/-}$ mice were obtained from Jackson Laboratory. Human P23H rhodopsin transgenic (hP23H Rho Tg) mice were generated as previously described (White et al. 2007) and maintained in wild-type rhodopsin ($Rho^{+/+}$) background (C57Bl/6J) for these studies. Histologic studies were performed as previously described (Chiang et al. 2014)

Quantitative PCR analysis of murine *Chop* mRNA levels was performed as previously described (Hiramatsu et al. 2011). Electroretinographic studies were performed on dark-adapted mice as previously described (Gorbatyuk et al. 2010). Studies were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and IACUC guidelines at the University of California, San Francisco and the University of California, San Diego.

25.3 Results

25.3.1 Retinal Degeneration of Human P23H Rhodopsin Transgenic Mice in $Chop^{-/-}$ Background

The outer nuclear layer (ONL) thickness of $Chop^{-/-}$ mice did not differ from wild-type over the first ~9 months of life (Fig. 25.1a). hP23H Rho Tg mice in a $Rho^{+/+}$ background underwent relatively mild retinal degeneration compared to P23H rhodopsin transgenic rats (Pennesi et al. 2008) and P23H rhodopsin knock-in mice (Sakami et al. 2011). At postnatal day (P) 90, the ONL thickness of the hP23H Rho Tg mice was ~25% thinner than the ONL of age-matched wild-type mice (Fig. 25.1b). To investigate the role of *Chop* in photoreceptor cell death induced by P23H rhodopsin, we crossed $Chop^{-/-}$ mice with hP23H Rho Tg mice and measured ONL from P30 to P210. At P60, we found a small, but significant increase in the ONL thickness of retinas from $Chop^{-/-}$ hP23H Rho Tg mice (39.9 ± 0.36 μm) compared to hP23H Rho Tg mice (36.5 ± 0.42 μm) ($P = 0.00124$) (Fig. 25.1b). However, we saw no other improvement of ONL thicknesses in $Chop^{-/-}$ hP23H Rho Tg mice compared to $Chop^{+/-}$ hP23H Rho Tg mice or hP23H Rho Tg mice at any other time points studied (Fig. 25.1b). These data indicated that loss of *Chop* provided a small transient protective effect at P60 but did not significantly alter the eventual loss of photoreceptors in hP23H Rho Tg mice.

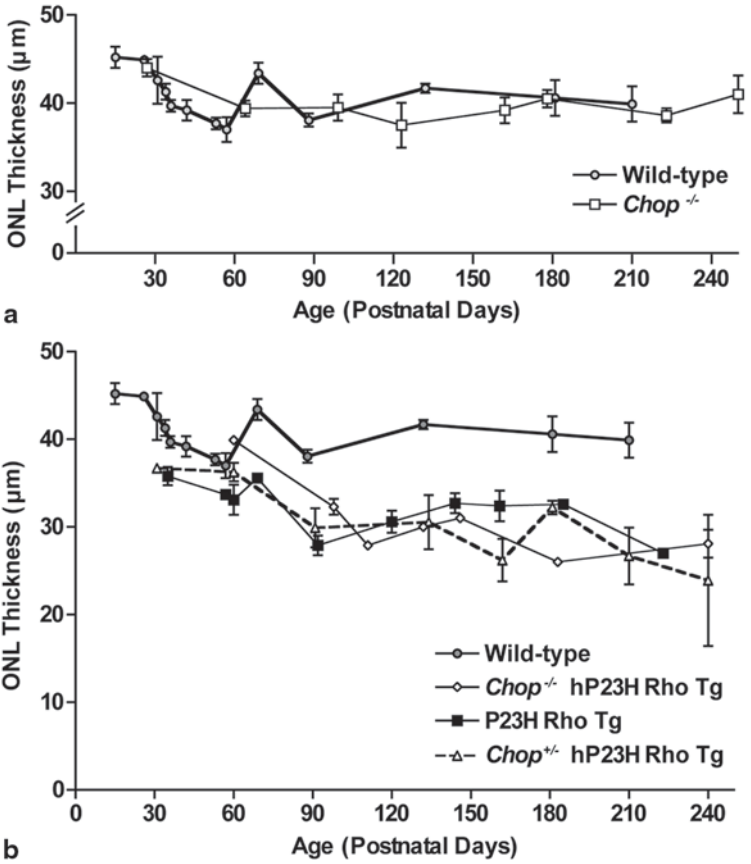

Fig. 25.1 Retinal degeneration in wild-type, hP23H Rho Tg, *Chop*$^{-/-}$, *Chop*$^{-/-}$ hP23H Rho Tg, and *Chop*$^{+/-}$ hP23H Rho Tg mice. **a** Mean ONL thickness of wild-type, and *Chop*$^{-/-}$ mice at the indicated ages. **b** Mean ONL thickness of wild-type, hP23H Rho Tg, *Chop*$^{-/-}$ hP23H Rho Tg, and *Chop*$^{+/-}$ hP23H Rho Tg at the indicated ages. Each value is the mean ± SEM of 2–7 retinas

25.3.2 Expression of Chop in Human P23H Rhodopsin Transgenic Mice

In parallel with our histologic analysis, we measured *Chop* mRNA levels in the retinas of hP23H Rho Tg mice by quantitative RT-PCR from P13 to P118 (Fig. 25.2). *Chop* mRNA levels in hP23H Rho Tg retinas did not differ from age-matched wild-type mice, except at P56 when we observed a modest, but significant, increase of *Chop* expression (1.21 fold increase in *Chop* mRNA levels compared to age-matched wild-types, $P = 0.018$) (Fig. 25.2a and 25.2b). This age of increased *Chop* expression roughly coincided with the rescue in ONL thickness we observed in P60 *Chop*$^{-/-}$ hP23H Rho Tg mice (Fig. 25.1b).

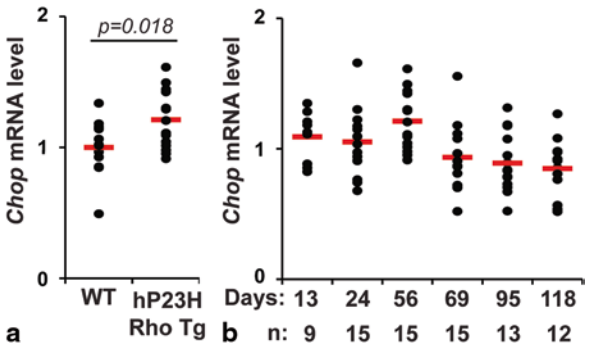

Fig. 25.2 Induction of *Chop* mRNA in retinas of human P23H rhodopsin transgenic mice. **a** Analysis of *Chop* mRNA levels by quantitative PCR using wild-type or hP23H Rho Tg mouse retina samples at postnatal day age 56. Student's two-tailed t-tests were performed to determine *P* values. **b** Analysis of *Chop* mRNA levels in the retinas of hP23H Rho Tg mice by quantitative PCR using mouse retina samples at indicated postnatal day ages. Samples were plotted relative to the average *Chop* mRNA levels at the same age in wild-type control mice. **a**–**b** The mean value at each time point is plotted as a *horizontal line*

25.3.3 Chop Knock-out Did Not Rescue the Function of Retinas of Human P23H Rhodopsin Transgenic Mice

We performed electroretinogram (ERG)) analysis in wild-type and $Chop^{-/-}$ hP23H Rho Tg mice at P95, an age with clear ONL differences between hP23H Rho Tg and wild-type mice. Under scotopic settings, we observed decreased a-wave and b-wave responses in hP23H Rho Tg mice compared to that of the wild-type mice (Fig. 25.3). $Chop^{-/-}$ hP23H Rho Tg mice showed no significant difference in ERG responses compared to hP23H Rho Tg mice or $Chop^{+/-}$ hP23H Rho Tg mice (Fig. 25.3). Together with our ONL measurements (Fig. 25.1), these results show that loss of *Chop* did not significantly alter photoreceptor cell death or retinal function during retinal degeneration in the hP23H Rho Tg mice.

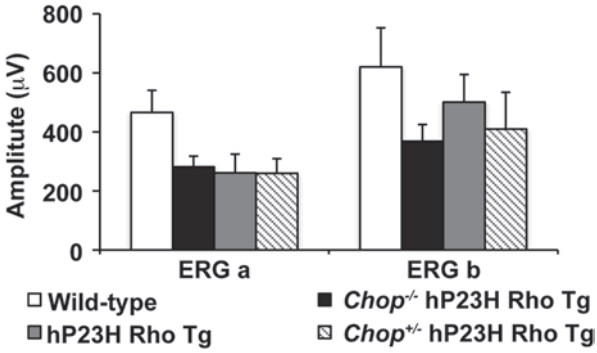

Fig. 25.3 *Chop* deficiency did not rescue the function of photoreceptors in human P23H rhodopsin transgenic mice. ERG a- and b-wave amplitudes were measured with wild-type, hP23H Rho Tg, $Chop^{-/-}$ hP23H Rho Tg, and $Chop^{+/-}$ hP23H Rho Tg mice at postnatal day 95

25.4 Discussion

Many mutations in the human *RHO* causing autosomal dominant retinitis pigmentosa lead to rhodopsin misfolding and activate the UPR signaling network (Mendes et al. 2005; Lin et al. 2007; Gorbatyuk 2010; Chiang et al. 2012). CHOP is one component of the UPR that is potently induced by ER toxins *in vitro* and in some animal models of diabetes and neuropathy; and loss of *Chop* partially prevents cell death in response to these types of ER stress (Zinszner et al. 1998; Oyadomari et al. 2002; Pennuto 2008). Here, we found that transgenic mice expressing human P23H rhodopsin did not induce the expression of *Chop* during retinal degeneration, nor did loss of *Chop* significantly alter retinal degeneration by histology or ERG during the time period we studied, with the exception of an early time point at ~P60, when we saw a mild improvement that did not persist in older animals.

Our findings are similar to prior studies of transgenic mice expressing T17M rhodopsin, transgenic "*GHL*" mice expressing triply mutated V20G, P23H, and P27L rhodopsin, and heterozygous P23H rhodopsin knock-in mice ($Rho^{P23H/+}$) (Nashine et al. 2013; Adekeye et al. 2014; Chiang et al. 2014), where the loss of *Chop* also did not confer significant protection from retinal degeneration in T17M Rho, $Rho^{P23H/+}$, or "*GHL*" mice, except in older *GHL* animals with severe retinal degeneration and then, only in their central retinas. As we did not study hP23H Rho Tg mice beyond 9 months of age, we cannot exclude that *Chop* may play additional roles at more advanced stages of retinal degeneration in older hP23H Rho Tg mice. In summary, our results provide additional evidence that CHOP does not significantly contribute to the photoreceptor cell death associated with rhodopsin mutations. We suggest that photoreceptors expressing mutant rhodopsins may preferentially activate components of the UPR other than CHOP. Given the complexity and diversity of signaling programs activated by ER stress, future studies will determine which components of the UPR signaling network are most important in photoreceptors undergoing misfolded rhodopsin-induced ER stress.

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