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Modulation of the rate of retinal degeneration in T17M RHO mice by reprogramming the Unfolded Protein Response

Shreyasi Choudhury[1], **Sonali Nashine**[1], **Yogesh Bhootada**[2], **Mansi Motiwale Kunte**[1], **Oleg Gorbatyuk**[3], **Alfred S. Lewin**[3], and **Marina Gorbatyuk**[2]

[1]Department of Cell Biology and Anatomy, University of North Texas Health Science Center, Fort Worth, TX

[2]Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

[3]Department of Vision Sciences, University of Alabama at Birmingham, Birmingham, AL.

Abstract

The goal of this study is to validate whether reprogramming of the UPR via modulation of pro-apoptotic caspase-7 and CHOP proteins could be an effective approach to slow down the rate of retinal degeneration in ADRP mice. In order to pursue our goal we created the T17M *RHO* CASP7 and T17M *RHO* CHOP mice to study the impact of the CASP7 or CHOP ablations in T17M *RHO* retina by ERG, SD-OCT, histology and western blot analysis. The scotopic ERG demonstrated that the ablation of the CASP7 in T17M *RHO* retina leads to significant preservation of the function of photoreceptors compared to control. Surprisingly, the ablation of pro-apoptotic CHOP protein in T17M *RHO* mice led to a more severe form of retinal degeneration. Results of the SD-OCT and histology were in agreement with the ERG data. The further analysis demonstrated that the preservation of the structure and function or the acceleration of the onset of the T17M *RHO* photoreceptor degeneration occurred via reprogramming of the UPR. In addition, the CASP7 ablation leads to the inhibition of cJUN mediated apoptosis, while the ablation of CHOP induces an increase in the HDAC. Thus, manipulation with the UPR requires careful examination in order to achieve a therapeutic effect.

Keywords

ADRP; UPR; Caspase-7; CHOP; apoptosis

58.1 Introduction

The T17M mutation within Rhodopsin (RHO) gene affects the assembly of the opsin protein with 11-cis-retinal [1] and presumably impairs protein stability, folding and trafficking [1, 2] leading to a severe form of retinal degeneration known as autosomal dominant retinitis pigmentosa (ADRP). Recently, we have shown that the ER stress associated caspase-7 and the pro-apoptotic CHOP protein are elevated in ADRP retina [3-5]. However, no direct

Corresponding author: Marina Gorbatyuk, 1670 University Blvd., Birmingham, AL, 35294; Phone: (2205) 934-6762; mgortk@uab.edu.

evidence of the important role of the caspase-7 and CHOP proteins in the mechanism of ADRP progression has been found so far. Therefore, our goal is to verify whether the genetic manipulation with pro-apoptotic UPR-associated caspase-7 and CHOP proteins is beneficial for ADRP photoreceptors.

58.2 Materials and Methods

58.2.1 Animal Models

C57BL/6 (wild type, WT), Caspase 7−/− (CSP7) and CHOP−/− (CHOP) were purchased from the Jackson Laboratory. The T17M CSP7 and T17M CHOP mice were obtained from the breeding of knockout mice with T17M RHO (T17M) mice. All mice were raised under a 12-hour light/12-hour dark cycle.

58.2.2. Electroretinography

The scotopic ERG with dark-adapted (12 h) and anesthetized mice at postnatal day (P) 30, 60 and 90 was performed using LKC Technologies as previously described [3].

58.2.3. Spectra-Domain Optical Coherent Tomography (SD-OCT)

The SD-OCT was performed in P30 mice using the SDOIS as previously described [3]. The thickness of the outer nuclear layer (ONL) was determined by averaging 10 measurements within 100, 200, 300 and 400 μm of the optic nerve head in the superior and inferior hemispheres of the retina.

58.2.4. H&E staining

The histological analysis and H&E staining in WT, T17M, T17M CSP7 and T17M CHOP mice was conducted as previously described [6].

58.2.5. Western Blot

Protein extracts from P30 retinas were obtained and analyzed as previously described [4]. Antibodies detected the pATF6, peIf2α, ATF4, spliced XBP1 (sXBP1) and β-actin proteins were purchased from Imgenex (1:1000), Abcam (1:1000) and Sigma-Aldrich (1:1000).

58.3. Results

58.3.1. Both the CSP-7 and CHOP Ablations Modulate the Loss of Vision in T17M retina

The a and b-waves of the ERG were measured in mice at P30, P60 and P90 (Figure 1). The a-wave amplitudes in T17M CSP7 retina were significantly increased by 138%, 233% and 422% at P30, P60 and P90, respectively whereas the T17M CHOP mice showed a significant decrease in the a-wave amplitudes by 57% at P30 and no difference at P60 and P90 compared to T17M mice. The b-wave amplitudes in T17M CSP7 mice were also significantly elevated by 154%, 187% and 179% at P30, P60 and P90, respectively the T17M CHOP mice had 26% lower the b-wave amplitude at P30 than T17M mice and no difference at P60 and P90.

58.3.2. Both the CSP7 and CHOP Ablations Alter the Retinal Structure in T17M Mice

The thickness of the ONL of the inferior and superior in P30 T17M CSP7 retina was significantly increased by 166%, whereas the T17M CHOP mice demonstrated 25% reduction (Figure 2). The histological analysis confirmed the OCT results and revealed that the number of the nuclei is higher by 30% in T17M CSP7 retina and is lower by 55% in T17M CHOP retina compared to T17M mice.

58.3.3. Both the CSP7 and CHOP Ablations Reprogram the UPR in T17M Retina

Both ablations reprogram the UPR in transgenic retina (Figure 3). The PERK pathway was modified in T17M CSP7 and T17M CHOP mice. For example, in T17M CSP7 retina the ATF4 protein was decreased by 55% compared to T17M retina. In T17M CHOP mice the peIF2α protein was dramatically (over 10-fold) increased compared to T17M mice. The ATF6 signaling was modified in T17M CSP7 mice as well. The pATF6(50) was decreased by 57% compared to T17M mice and was no different compared to wt retina. The IRE1 signaling was not altered in T17M CSP7 retina. However, in T17M CHOP retina the sXbp1 protein was 30% lower compared to T17M mice.

58.3.4. Both the CSP7 and CHOP Ablations Modify the Cellular Signaling in T17M Retina

In T17M retina we found that the level of mTOR protein was significantly (over 2-fold) higher compared to wt (Figure 4). The CSP7 ablation led to down-regulation of the mTOR by 33% in these mice that was not statistically different compared to wt. In T17M CSP7 retina this alteration was accompanied by 160% increase in pAKT compared to T17M suggesting that in T17M CSP7 the mTOR/AKT pathway is modified in similar to wt manner.

In T17M retina we also found that the levels of PARP1, TNFα, TRAf2 and pcJUN proteins were significantly higher compared to wt by 180%, 235%, 217% and 300%, correspondingly. The CSP7 ablation, however, significantly reduced the level of these proteins by 51%, 72%, 31% and 54%, respectively

Ablation of the CHOP protein also altered the cellular signaling in T17M retina. For example, we found that the HDAC1 protein was over 2-fold elevated in T17M CHOP retina compared to T17M. On the contrary, the P300 transcription factor, the co-activator of the RHO gene transcription [7] was found to be decreased by 77% in T17M CHOP mice. Along with RHO mRNA the CRX and NRL gene expressions were diminished in T17M CHOP retina as well (data not shown).

58.4. Discussion

In this study, we tested the hypothesis of whether the ablation of pro-apoptotic UPR markers, CSP7 and CHOP proteins are beneficial for ADRP retina. Despite the fact that the increase of the a- and b-waves of ERG amplitudes in T17M CSP7 did not reach the level of wt, the vision loss in these animals was significantly prevented. The results of the SD-OCT and histology confirm the ERG data and indicate the significant improvement of the T17M CSP7 retinal morphology. Controversially, the CHOP ablation expedites the ADRP

progression. The ERG, SD-OCT and histological results indicate the acceleration of the vision loss and the loss of photoreceptor cells. We found that the reprogramming of the PERK pathway is a common consequence in ADRP retina deficient either in CSP7 or CHOP proteins. For example, in T17M CSP7 mice the decrease in ATF4 could negatively regulate the CHOP protein production and dampen the apoptosis. In T17M CHOP mice the increase in peIF2α at P30 suggests that these animals experience a long-term activation of the PERK UPR arm compared to previously detected in these mice at P15 [3]. Therefore, a prolonged activation of the PERK pathway may induce the inhibition of a global protein translation in ADRP retina and expedite the rate of photoreceptor degeneration. In favor of this hypothesis, we found that the sXBP1 protein (the IRE1 pathway) is significantly diminished in T17M CHOP mice, suggesting that the function of the pro-survival arm is compromised in this retina [3]. The second UPR arm, the ATF6 pathway is also modulated in T17M CSP7. Diminishing of the pro-apoptotic outcomes from the ATF6 activation could be potentially responsible for slowing down the ADRP progression.

Both ablations modify cellular signaling in T17M retina. We found first that the PARP1-TNFα-TRAF2-pcJUN pathway is elevated in T17M retina and the ablation of the CSP7 attenuates the apoptosis in these mice via the modulation of the PARP1 protein and decrease in pcJUN. Evidently, the reduction in pro-apoptotic pcJUN protein is responsible for slowing down the rate of the ADRP progression in T17M CSP7 mice suggesting that the anti-apoptotic therapeutic strategy could be applied to treat the ADRP photoreceptors. This data also reveal that the inflammatory component may be involved in the mechanism of the ADRP pathology.

In T17M CHOP mice, the elevation of HDAC1, a binding partner of the CHOP transcriptional factor [8], evidently suggests that increased histone deacetylation could repress a general transcription and accelerate retinal degeneration in these mice. The HDAC's function is an opposite of the histone acetyl-transferase P300. Therefore, it is not surprise, that the level of P300 is diminished in T17M RHO, evidently leading to decrease in the CRX, NRL and RHO mRNA. This down-regulation serves as an essential proof for the decline in P300 expression, and additionally for global translation attenuation in ADRP photoreceptors by increased peIF2α.Therefore, in this study we demonstrated that the rate of the ADRP progression could be modulated by reprogramming of the UPR and, in order to achieve a therapeutic effect based on manipulation with pro-apoptotic UPR proteins in ADRP retina, a careful examination should be taken.

Acknowledgments

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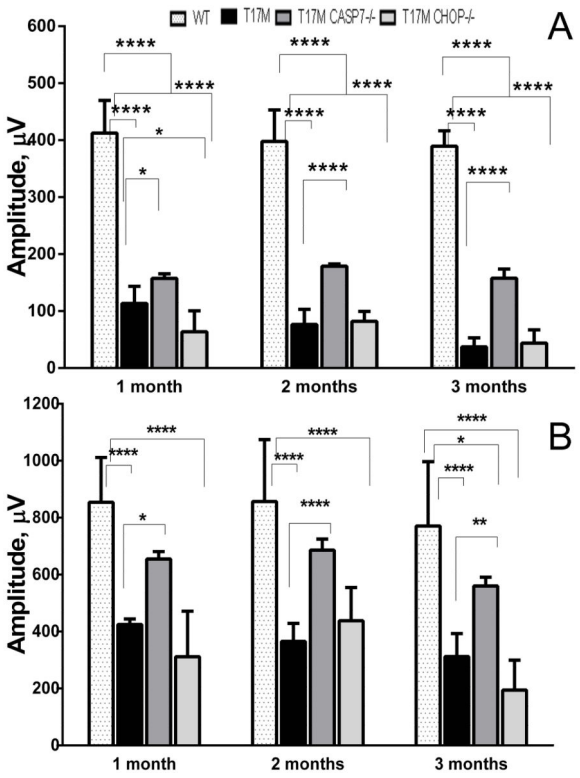

Fig. 58.1.

The lack of CSP7 and CHOP proteins modulates the vision loss in T17M photoreceptors. A: The a-wave of ERG amplitudes are modified in T17M CSP7 and T17M CHOP mice compared to control. B: The B-wave of ERG amplitudes are modified in T17M CSP7 and T17M CHOP mice compared to control. (*−P<0.05, **−P<0.01, ***−P<0.001, ****−P<0.0001).

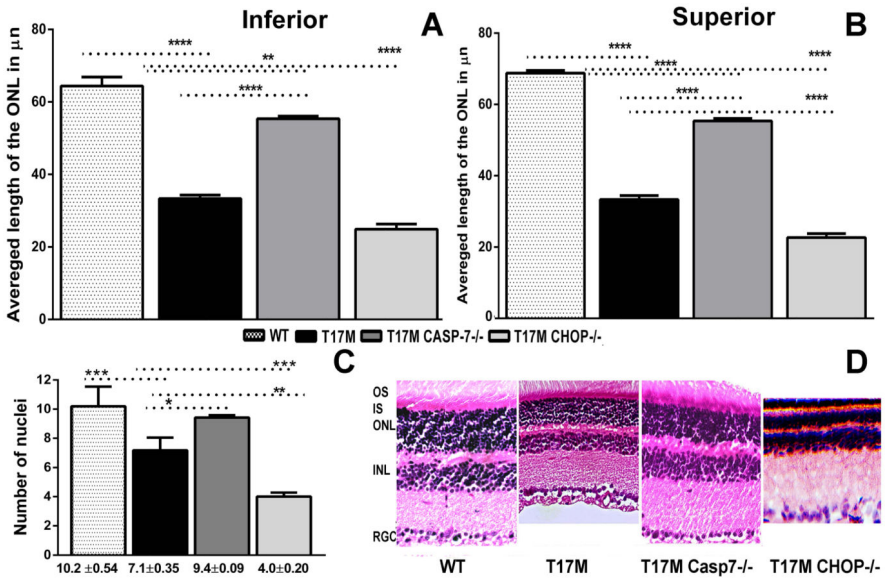

Fig. 58.2.

The lack of CSP7 and CHOP proteins modifies the retinal structure and morphology in T17M mice. A and B: the average thickness of the ONL in the superior and inferior retinas correspondingly. C: The number of the nuclei measured by H&E histological analysis in the retina. D: Images of the H&E staining in the retina. (*−*P*<0.05, **−*P*<0.01, ***−*P*<0.001, ****−*P*<0. 0001).

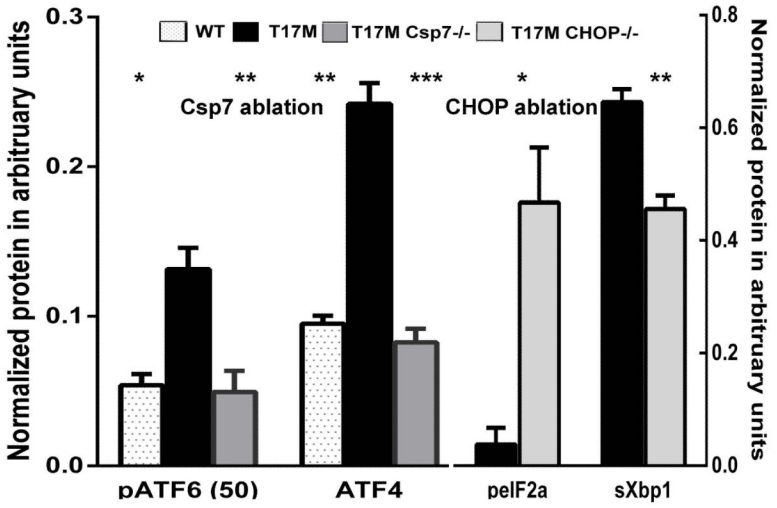

Fig. 58.3.

The UPR is modified in T17M retina deficient either in CSP7 or CHOP protein. A: The ATF4 and pATF6 protein are reduced in T17M retina deficient in CSP7. B: Increase in peIF2 and decreased in sXBP1 protein are found in T17M retina deficient in the CHOP. (* −P<0.05, ** −P<0.01, *** −P<0.001).

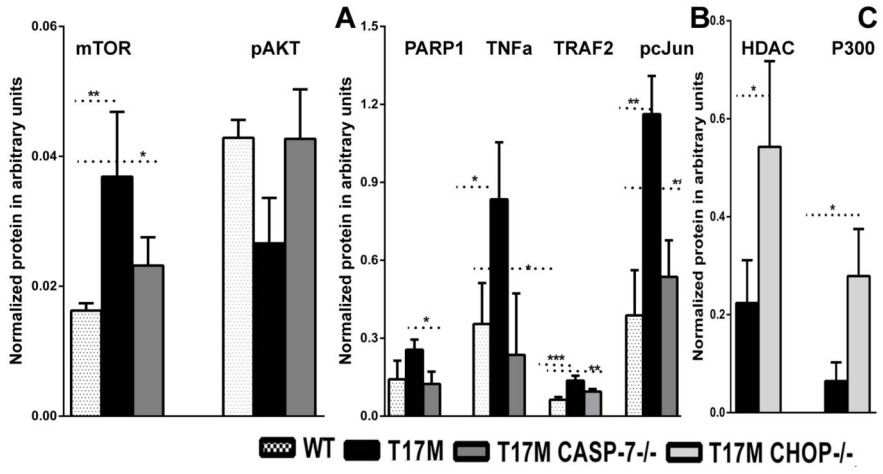

Fig. 58.4.

Modulation of the cellular signaling in T17M CSP7 and T17M CHOP mice. A: Modulation of the mTOR/pAKT signaling in T17M CSP7 retina. B: Modulation of the PAR1-TNFa-TRAF2-pcJUN pathways in T17M CSP7 retina. C: The HDAC and P300 protein expressions are modified in T17M CHOP retina. (*−P<0.05, **−P<0.01, ***−P<0.001, ****−P<0. 0001).