Modulation of the PTEN/mTOR pathway to enhance survival of cone photoreceptors in retinal degeneration disorders

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KEY MESSAGES

1. Phosphatase and tensin homologue (PTEN) overexpression down-regulated mTOR and S6K1 activity and induced 661W cone cell apoptosis.
2. S6K1 knockdown prevented 661W cone cell survival to a similar degree as PTEN.
3. S6K1 expression improved 661W cone survival in the presence of PTEN.
4. PTEN deletion activated S6K1 and improved the survival and function of cones and visual performance in the rd10 mouse model of retinitis pigmentosa.
5. S6K1 treatment rescued cones from degeneration in the rd10 retina.

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Introduction

Retinitis pigmentosa refers to a type of inherited retinal photoreceptor degeneration. Mutations in rod-specific genes cause photoreceptor death, and cones die subsequent to the rod cell loss in this disease.[1] The molecular mechanisms that lead to the secondary cone death are not fully understood. Down-regulation of the insulin/mTOR metabolic pathway has been reported to be a major cause of cone death in several mouse models of retinitis pigmentosa.[2] Down-regulation of the PI3K/mTOR pathway can be modulated at several levels. For instance, phosphatase and tensin homologue (PTEN) opposes PI3K function, leading to inactivation of the PI3K/mTOR pathway. However, it remains unclear whether inactivation of the PI3K/mTOR pathway is directly modulated by PTEN in retinitis pigmentosa. To determine whether PTEN is involved in down-regulating the PI3K/mTOR pathway, we used the Cre-loxp system to generate a cone-specific deletion of PTEN to assess the effect of PTEN deletion on the PI3K/mTOR pathway and on the structural integrity and function of cones in the rd10 mouse model of retinitis pigmentosa.[3]

Methods

Wild-type (C57BL/6) mice, rd10 mice, and PTEN$^{loxP/loxP}$ mice were obtained from Jackson Laboratory (Bar Harbor [ME], US). To investigate whether PTEN deletion slowed down cone degeneration in rd10 retinas, rd10 mice were backcrossed with PTEN$^{loxP/loxP}$ mice, and the littermates from rd10/PTEN$^{loxP/loxP}$ mice and rd10/PTEN$^{+/loxP}$ mice were used for experiments.

The plasmid CAG-Cre was purchased from Addgene (#13775). The plasmid human red opsin promoter–Cre was constructed by replacing CAG with a human red opsin promoter. To create a conditional PTEN deletion in cone photoreceptors, we injected an adeno-associated virus vector expressing Cre driven by a human red opsin

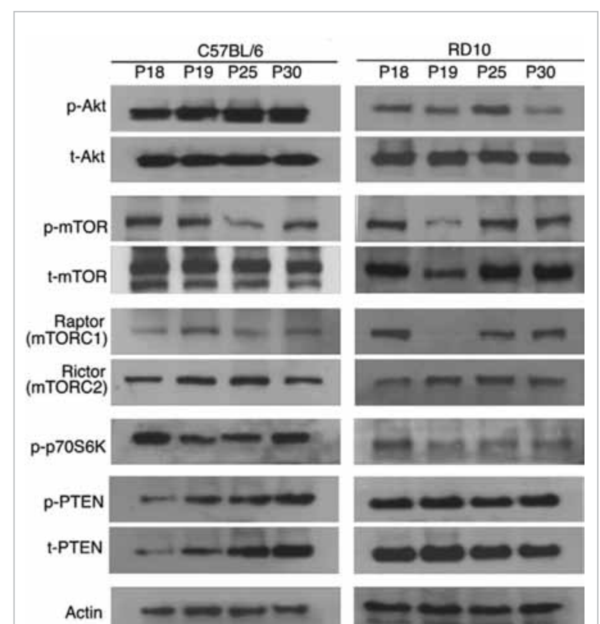

FIG 1. PI3K/mTOR pathway dysregulated in rd10 retinas. Western blots of whole retinal phosphorylated Akt (p-Akt) and total Apt (t-Akt), p-mTOR and t-mTOR, Raptor (mTORC1), Rictor (mTORC2), p-p70S6K, p-PTEN, and t-PTEN from rd10 mice and C57BL/6 mice. β-actin levels were used as a loading control. The PI3K/mTOR pathway was downregulated, and PTEN was upregulated in rd10 retinas.

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promoter. DNA (0.5 μl of 5 μg/μl) was injected into the subretinal space of right eyes of newborn mouse pups. After DNA injection, 80V pulses were applied using a square pulse electroporator ECM830.

We then assessed Cre expression and cellular localisation in retinas by immunofluorescence staining using an anti-Cre antibody. To ensure that cones were selectively targeted, we stained Cre-treated $PTEN^{loxP/loxP}$/rd10 retinas using both anti-Cre and anti-red/green opsin antibodies. Primary antibodies used were rabbit anti-red/green opsin, rabbit anti-blue opsin, and rabbit anti-Cre recombinase. A secondary antibody conjugated to either Alexa 488 or Alexa 594 was applied for 2 hours at room temperature. Confocal micrographs of fluorescent specimens were captured using a Zeiss LSM 700 Meta Axioplan 2 laser scanning confocal microscope (Carl Zeiss, Oberkochen, Germany) equipped with argon and helium-neon laser.

We assessed the effect of PTEN deletion on photoreceptor function using scotopic and photopic electroretinography (with an Espion ERG Diagnosys machine). Scotopic, rod-mediated responses were obtained from dark-adapted animals. Photopic, cone-mediated responses were performed following 10-minute light adaptation.

Vertical sine wave gratings were projected on computer monitors. Images of head movements were monitored using an infrared-sensitive camera.

661W cells were transfected with a mixture composed of lipofectamine 2000 (Invitrogen) and pCMV/Flag/WT-PTEN, with over-expression of pCMV/Flag/dPDZ-PTEN as the negative control. p70S6K1 overexpression was realised by transfecting

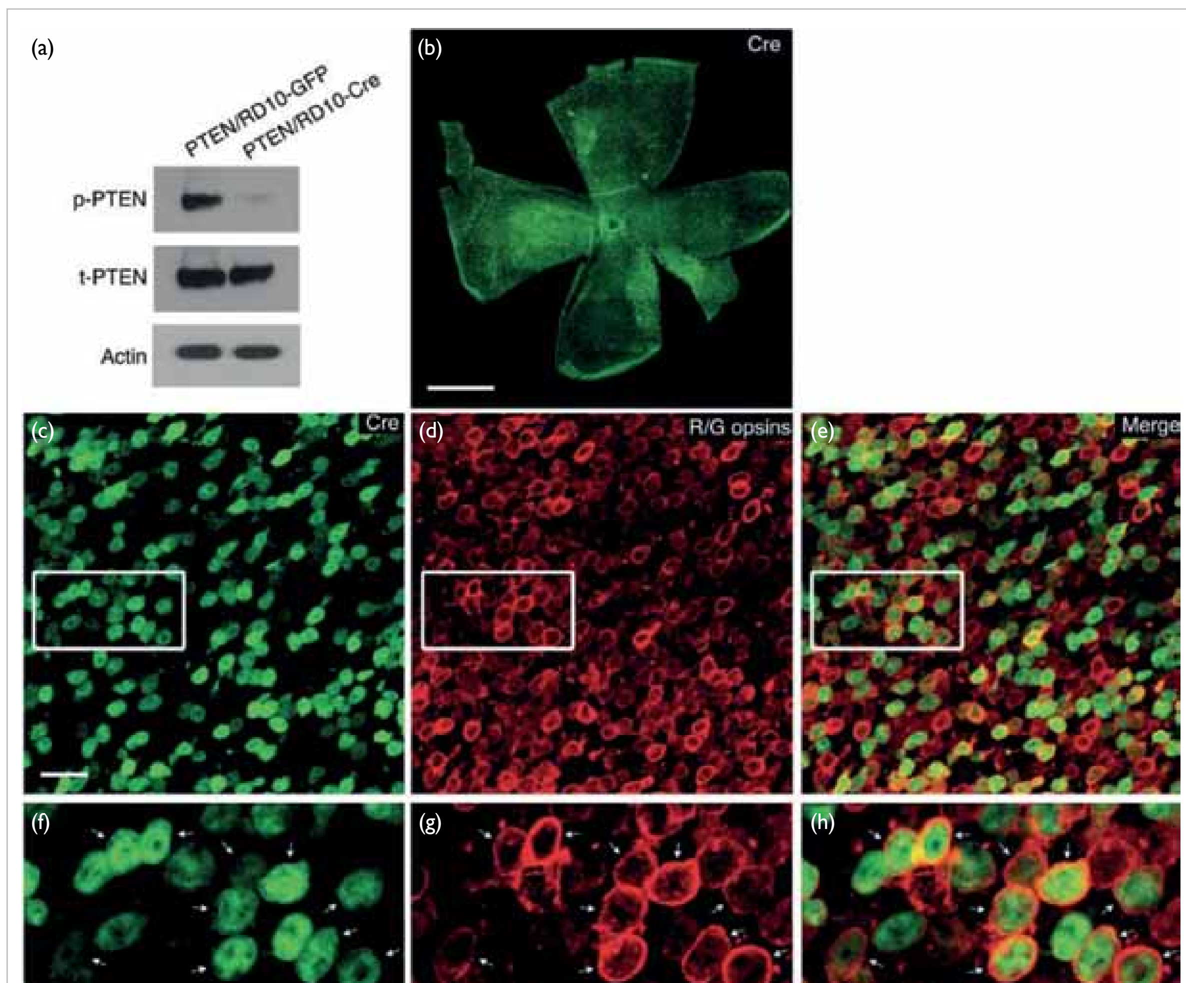

FIG 2. Conditional deletion of PTEN in cone photoreceptors of rd10 mice. (a) Western blots of PTEN protein levels in Cre-treated $PTEN^{loxP/loxP}$/rd10 mice and GFP-treated $PTEN^{loxP/loxP}$/rd10 mouse retinas. β-actin levels were used as a loading control. (b) Flat mounted preparation of rd10/$PTEN^{loxP/loxP}$ mice treated with adeno-associated virus vector expressing Cre and harvested at P25. Representative retinal flat mount showed extensive Cre expression stained by an anti-Cre antibody (green) throughout the whole retina. Adeno-associated virus–human red opsin promoter–Cre–treated $PTEN^{loxP/loxP}$/rd10 retinas were co-labelled with antibodies against (c) Cre (green) and (d) red/green opsins (red). (e) Most cre-positive cells were also positive for red/green opsins. (f to h) Highly magnified images of the boxed regions above. Red/green cones colocalised with Cre (arrows).

pRK7-HA-S6K1-WT, and pRK7-HA-S6K1-KR was used as the negative control.

The total and phosphorylated forms of PTEN, Akt, Raptor, Rictor, mTOR, and p70S6K proteins were measured using western blot analysis.

Results

Phosphorylated PTEN was upregulated, whereas phosphorylated Akt, phosphorylated mTOR, mTORC1 and p70S6K were downregulated in the rd10 retina relative to control retinas (Fig 1). These suggested the possible correlation between down-regulation of PI3K/Akt survival pathway and up-regulation of PTEN in rd10 retinas.

PTEN overexpression increased the proportion of TUNEL positive 661W cells (data not shown). p70S6K1 knockdown induced 661W cone cell apoptosis. S6K1 increased cell survival in PTEN-overexpressed 661W cone cells. These data confirmed that PTEN functions through p70S6K1 to regulate cone cell apoptosis.

Most Cre-positive cells were co-labelled with red/green opsins, indicating specific Cre transduction in cones (Fig 2).

Scotopic b-wave amplitudes at two different light intensities were significantly higher in Cre-treated PTEN$^{loxP/loxP}$/rd10 mice than in GFP-treated PTEN$^{loxP/loxP}$/rd10 mice (Fig 3). Photopic electroretinographic a- and b-wave amplitudes in Cre-treated PTEN$^{loxP/loxP}$/rd10 mice were similarly larger compared to GFP-treated PTEN$^{loxP/loxP}$/rd10 controls (Fig 3). Additionally, we measured the optomotor response of mice to moving gratings. Photopic visual acuity was approximately 2.5-fold higher in P35 Cre-treated PTEN$^{loxP/loxP}$/rd10 mice than that in age-matched GFP-treated PTEN$^{loxP/loxP}$/rd10 (Fig 3g). Taken together, PTEN deletion improved retinal function and visual performance in rd10 mice.

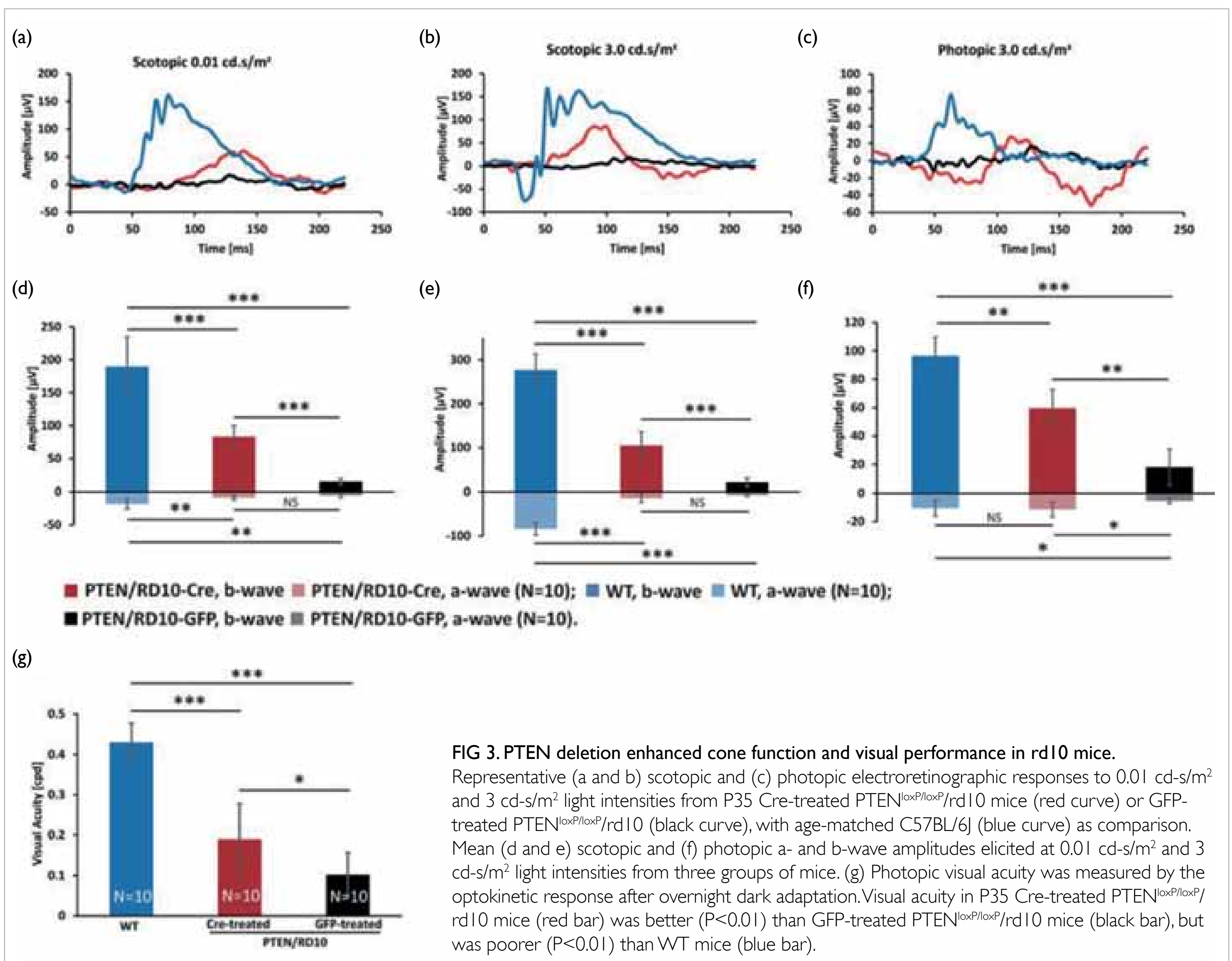

FIG 3. PTEN deletion enhanced cone function and visual performance in rd10 mice. Representative (a and b) scotopic and (c) photopic electroretinographic responses to 0.01 cd-s/m^2 and 3 cd-s/m^2 light intensities from P35 Cre-treated PTEN$^{loxP/loxP}$/rd10 mice (red curve) or GFP-treated PTEN$^{loxP/loxP}$/rd10 (black curve), with age-matched C57BL/6J (blue curve) as comparison. Mean (d and e) scotopic and (f) photopic a- and b-wave amplitudes elicited at 0.01 cd-s/m^2 and 3 cd-s/m^2 light intensities from three groups of mice. (g) Photopic visual acuity was measured by the optokinetic response after overnight dark adaptation. Visual acuity in P35 Cre-treated PTEN$^{loxP/loxP}$/rd10 mice (red bar) was better (P<0.01) than GFP-treated PTEN$^{loxP/loxP}$/rd10 mice (black bar), but was poorer (P<0.01) than WT mice (blue bar).

Discussion

PTEN activity played a major role in down-regulating the PI3K/mTOR survival pathway and contributed to photoreceptor apoptosis in the rd10 mouse model of retinitis pigmentosa. Conversely, PTEN deletion in cones activated the PI3K/mTOR pathway and subsequently facilitated cone photoreceptor survival. PTEN overexpression induced 661W cone cell apoptosis by direct inhibition of the PI3K/mTOR survival pathway. Furthermore, S6K1 was identified as one of the downstream effectors of PTEN neurotoxicity. The effect of PTEN demonstrated *in vitro* was further confirmed *in vivo* by conditional deletion of PTEN in cone photoreceptors of rd10 mouse retinas. PTEN deficiency activated the PI3K/mTOR pathway and its downstream target S6K1, enhanced cone survival and function, and improved visual performance in rd10 mice, confirming that PTEN activation contributed to the induction of cone death in rd10 retinas.

Acknowledgement

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