Autophagosome catabolism of visual transduction proteins prevents retinal degeneration

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BACKGROUND

• Autophagy plays important roles in maintaining cellular homeostasis, preventing the accumulation of toxic proteins and regulating the response to environmental stresses[2]

• Light exposure results in the translocation of phototransduction proteins, arrestin and α-transducin (Tα), between the IS and OS of the rods[3]

• The bimodal pattern of autophagy activation in the photoreceptor is partially driven by the light-induced translocation of (Tα) and arrestin[4]

METHODS

1. Generation of gnat1−/− Atg5fl/fl mice (gnat1−/−; Atg5fl/fl; Rho)-Cre whose rod photoreceptors cells are deficient in both the gnat1 and Atg5 gene products

2. Knockout of Gnat1 delays retinal degeneration in the Atg5−/− mice

3. Isolation of autophagosomes in vitro – Assay development

4. Immunization of retinal autophagosomes from the LC3-GFP mouse retinas

RESULTS

1. Detection of the ATG5 from the rod photoreceptors (Atg5fl/fl) results in an accumulation of Tα and the degeneration of the photoreceptors[6]

2. The generation of gnat1−/− Atg5fl/fl mice for ex vivo and in vivo

3. Generation of gnat1−/− Atg5fl/fl mouse retina whose rod photoreceptors are deficient in both gnat1 and Atg5 gene products

4. Isolation of autophagosomes from GFP-LC3 transfected cells (HEK and 661W), and from retinas of the GFP-LC3 transgenic mice using an immunopurification-based autophagosome enrichment technique[5]

• Immunoprecipitation of GFP-tagged LC3 by using 6×His magnetic beads coated with anti-GFP (MACS Milteny Biotec)

• Western blot analysis

• Mass spectrometry analysis

• Transmission electron microscopy and fluorescence microscopy

CONCLUSIONS

• Targeted deletion of both gnat1 and Atg5 in the rod photoreceptors resulted in significantly decreased rate of retinal degeneration as compared to the Atg5−/−-mouse retina

• Immunolabeled autophagosomes from GFP-LC3 mouse retinas confirmed that the visual transduction proteins transducin and arrestin are associated with autophagosome-specific proteins

• This study shows that degradation of phototransduction proteins by autophagy is necessary to prevent retinal degeneration

• We demonstrate a simple and easily reproducible immunoprecipitation technique for enrichment of autophagosomes from GFP-LC3 mouse retinas, providing a novel application to the study of autophagosome contents across different organs and specific cell types in vivo

REFERENCES


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