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Intraluminal acidity plays a key role in the function of lysosomes in RPE cells

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Abstract

Purpose : Impaired lysosomal function in retinal pigment epithelial (RPE) cells is linked with incomplete photoreceptor outer segment (POS) degradation and their accumulation as lipofuscin; a well-defined pathway of RPE atrophy in Age-Related Macular Degeneration (AMD). Here, we tested the hypothesis that intraluminal lysosomal acidity (pH) is a key determinant in POS degradation that becomes impaired in AMD.

Methods : The molecular probe Lyso-pHluorin, which increases in fluorescence with diminishing acidification, was expressed in RPE (ARPE-19) cells and exposed to oxidative stress (10mM H_2O_2, 24Hrs) or Aβ (1μM human oligomeric $Aβ_{1-42}$, 3Hrs) which is elevated in aged/AMD retinas. Cells were then synchronously fed POS (4μg/cm^2). Parallel cultures were fed OxPOS, which were produced by UV cross-linked POS that becomes sequestered in lysosomes. RPE cells without insults acted as controls. Bafilomycin A^1 was used to obtain maximal Lyso-pHluorin response (positive control). Whilst these studies were carried out after fixation, the use of CypHer5E conjugated POS provided insights into dynamic changes to lysosomal pH in living RPE. Co-labelling with LysoTracker Green DND-26 provided readouts of lysosomal size.

Results : POS (p=0.0077) as well as OxPOS (p=0.0025) co-localisation to lysosomes significantly diminished their intraluminal acidity compared to lysosomes without cargos. Interestingly, there was no appreciable difference in the fluorescence intensity of lysosomes with POS vs. OxPOS cargos. Lysosomal acidity also diminished after exposure to oxidative stress and Aβ, whilst CypHer5E showed dynamic alterations to lysosomal acidity.

Conclusions : Our studies revealed that intraluminal lysosomal acidity becomes significantly diminished following POS and OxPOS accumulation. However, POS is rapidly degraded in healthy RPE, whilst OxPOS is known to be sequestered in RPE lysosomes for prolonged periods (akin to lipofuscin). Exposure to AMD-linked disease pathways also impaired lysosomal acidity. Our findings support a key role for intraluminal lysosomal pH in the ability to effectively degrade POS cargos, revealing novel mechanistic insights into the pathogenesis of AMD.

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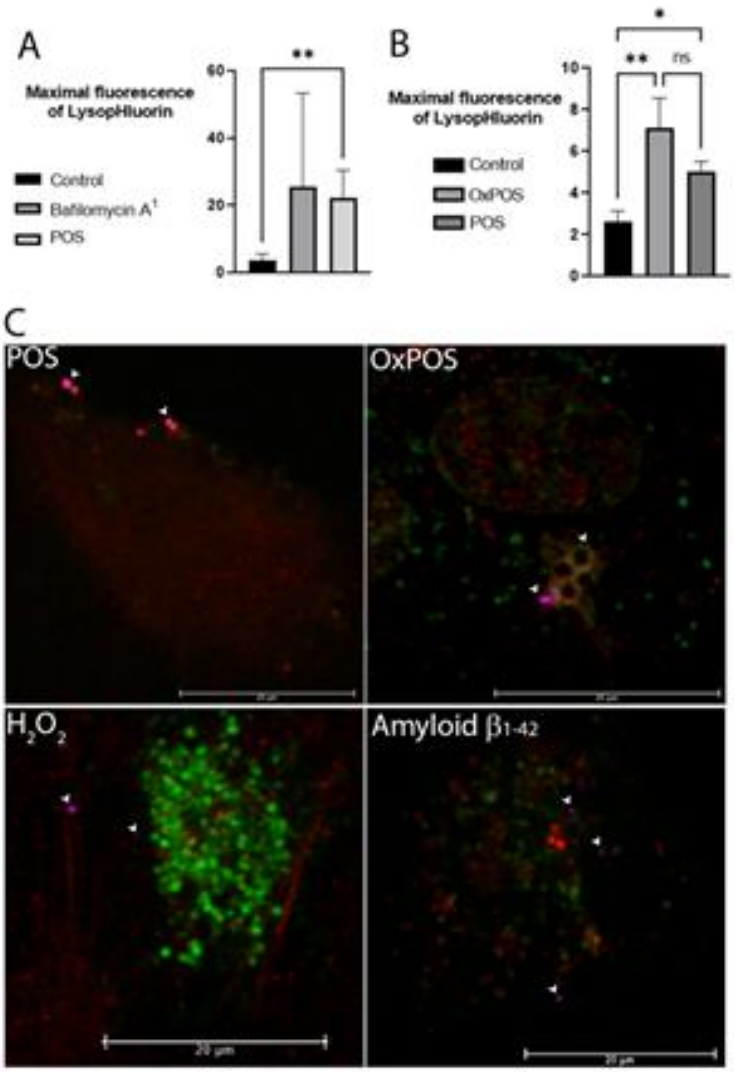

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Lyso-pHluorin response in RPE cells fed with: A: POS (n=3, p=0.0077) B: OxPOS (n=3, p=0.0025). C: Confocal images of Lyso-pHluorin (green) transfected RPE cells in each experimental group. Arrows indicate co-localisation between cargo (pink) and lysosome

(red).