Lysosomal impairment in the Retinal Pigment Epithelium (RPE)- a pathway of damage in the ageing retina

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Purpose:

A key function of the RPE is the phagocytosis/degradation of photoreceptor outer segments (POS). In age and disease, this pathway becomes impaired and lipofuscin accumulates within RPE lysosomes. Here, we used cultured RPE cells to identify the molecular pathways through which damage to the endosomal-lysosomal system occurs.

Methods:

We developed an ex-vivo model of the RPE monolayer in which cells structurally and functionally resemble native RPE[1]. ARPE-19 cells were fed 4μg/cm^2 POS-FITC. To optimise POS binding whilst synchronising internalisation[2], cultures were incubated with POS for 30min at 17^{o}C after which cells were returned to a 37^{o}C incubator before fixation with 4% PFA at 2, 4, 6, 12, 24 and 48hrs. In order to mimic oxidative stress, cultures were pre-treated with 200μM Hydrogen Peroxide (H_2O_2) for 24hrs. Parallel cultures were pre-treated with 10nM Bafilomycin (Baf) for 48Hrs, which blocks vATPase mimicking effects of the lipofuscin-derivative A2E[3]. DMSO was fed to sister cultures as a control. Cells on transwell inserts were stained with endosomal (Rab5, Rab7), lysosomal (Lamp1, Lamp2), and autophagy marker (LC3B) for each time point. Data from n>6 cells/time point/treatment. Images collected on a Leica SP8 confocal microscope and analysed using Volocity (PerkinElmer).

Results:

Our results show that trafficking of POS-FITC cargo occurs through endosomes, lysosomes and autophagosomes in a 2-48hr timescale. Pre-treatment with Baf increased accumulation in Rab5 and Rab7 ($p<0.05$) compartments at late time points (6hr-24hr), indicative of impaired maturation in late endosomes. Exposure to elevated oxidative stress (H_2O_2) increased cargo trafficking to lamp1/2 positive compartments between 2-4hrs ($p<0.01$). Exposure to Baf or H_2O_2 also increased the formation of autophagy bodies between 2-24hrs ($p<0.05$).

Conclusions:

Exposure to an A2E mimic impaired maturation of late endosomes, whilst oxidative stress caused rapid trafficking of cargos to late compartments; revealing divergent mechanisms through which the RPE endosomal-lysosomal pathway becomes disrupted in old age, and how these perturbations increase autophagy. Such insights may be informative when designing future treatments.

1. 10.1016/j.tice.2017.06.003 2. 10.1016/S0014-4835(87)80105-7 3. 10.1096/fj.03-0289fje