

## **Introduction**

Inflammation and oxidative stress are key drivers of retinal pigment epithelium (RPE) dysfunction in the pathogenesis of age-related macular degeneration (AMD), a leading cause of irreversible blindness globally. Tumor necrosis factor-alpha (TNF $\alpha$ ), a pro-inflammatory cytokine involved in AMD, induces defects in mitochondrial health and function in the RPE. Sirtuins, a family of enzymes involved in maintaining metabolic homeostasis, mitochondrial function and longevity, are downregulated in aged RPE cells and AMD retinal specimens. Here we investigated the efficacy of resveratrol, a potent activator of sirtuin1 (SIRT1), in suppressing TNF $\alpha$ -induced inflammation, metabolic dysfunction and oxidative stress in RPE.

## **Methods**

Matured primary human RPE (H-RPE) cells were treated with TNF $\alpha$  (10 ng/ml) and/or resveratrol (50  $\mu$ M) or DMSO at equal volume as the vehicle control. Oxidative phosphorylation (OXPHOS) and glycolytic metabolic profiles were determined by the Seahorse Xfe96 Mito Stress Test and Glycolytic Stress Test, respectively. Gene expression of metabolic and inflammatory markers was assessed using qPCR. Interleukin-6 (IL-6) secretion was quantified by enzyme-linked immunosorbent assay (ELISA). Intracellular levels of reactive oxygen species (ROS) were measured using CellROX green reagent.

## **Results**

TNF $\alpha$  induced elongation and loss of the regular cuboidal cobblestone morphology of H-RPE cells. Concurrent treatment with resveratrol blocked TNF $\alpha$ -induced H-RPE morphological changes. TNF $\alpha$  robustly upregulated IL-6 levels in H-RPE conditioned media with a 19-fold increase while concurrent treatment with resveratrol significantly suppressed TNF $\alpha$ -induced IL-6 secretion by almost 50% to a 10-fold decrease. Moreover, resveratrol suppressed TNF $\alpha$ -induced transcriptional upregulation of proinflammatory genes (IL-6, IL-8, TLR2, and MCP-1) and metabolic genes (ENO1, PFKFB3, HK2). Bioenergetic profiling using the Seahorse Xfe96 showed enhanced maximal mitochondrial respiration, spare respiratory capacity, and basal glycolysis levels in H-RPE treated with resveratrol. While co-treatment with resveratrol and TNF $\alpha$  further increased oxygen consumption rate, resveratrol suppressed TNF $\alpha$ -dependent accumulation induction of the ROS-producing NADPH oxidase NOX4. The protective effect of resveratrol on TNF $\alpha$  was further validated by evidenced reduction of TNF $\alpha$ -dependent accumulation of cytoplasmic ROS.

## **Conclusions**

RPE cells are profoundly affected by the pro-inflammatory cytokine TNF $\alpha$ . TNF $\alpha$  not only disrupts the structural epithelial morphology of H-RPE cells, it also causes dysfunction of mitochondria and metabolism. Treatment with the natural organic compound, resveratrol, efficiently blocks TNF $\alpha$ -induced proinflammatory activation and bioenergetic reprogramming of RPE. These results reveal a critical interplay between inflammation and metabolic dysfunction in RPE, identifying resveratrol as a potential drug against AMD progression.