Interplay Between TNFα-Induced Inflammation and Metabolic Dysfunction in Retinal Pigment Epithelial Cells

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Purpose

Dysfunction of the retinal pigment epithelial cells (RPE) is a key feature of age-related macular degeneration (AMD), the leading cause of blindness in developed countries. Inflammation is a known pathogenic mechanism in AMD through complement system activation, upregulation of inflammatory cytokines and macrophage recruitment. A critical cytokine mediating inflammatory responses is tumor necrosis factor-alpha (TNFα), which has been implicated in the pathogenesis of AMD. Growing evidence supports metabolic dysfunction as another key mechanism driving AMD. To date, little is known about the metabolic effects of TNFα on RPE. Here we bridge the gap between the interplay of inflammation and metabolic dysfunction in RPE showing that the pro-inflammatory effects of TNFα are associated with a dramatic change in mitochondrial morphology, function, and a rewiring of the metabolic pathways.

Methods

Matured ARPE-19 and primary human RPE cells (H-RPE, Lonza) were treated with TNFα (10 ng/ml). Glycolysis and oxidative phosphorylation (OXPHOS) were examined by high-resolution respirometry. Gene expression of EMT and metabolic markers were assessed using qPCR. The pro-inflammatory effect of TNFα was assessed by measuring IL-6 secretion using ELISA. ATP content was measured using a bioluminescent assay. Cells were stained with MitoTracker Orange and imaged using confocal microscopy to visualize changes in mitochondrial morphology. Ultrastructural features of mitochondria were assessed using transmission electron microscopy (TEM).

Results

TNFα significantly upregulated the expression of pro-inflammatory cytokines and IL-6 secretion and induced RPE to elongate into spindle-shaped cells, reminiscent of epithelial-mesenchymal transition (EMT). However, gene expression analysis showed that TNFα repressed numerous EMT genes (Col1A1, α-SMA) indicating that the elongated cells were not mesenchymal in nature. Metabolic profiling by high-resolution respirometry revealed an increase in basal OXPHOS levels and increased glycolytic capacity, associated with increased ATP content. TNFα significantly upregulated expression levels of the mitochondrial antioxidant SOD2 and induced defects in both the mitochondrial network morphology and ultrastructure with loss of cristae integrity.

Conclusions

Taken together, we show that TNFα-induced pro-inflammatory activation of RPE is associated with robust disruption of mitochondrial function, mitochondrial ultrastructure, and bioenergetic rewiring towards higher mitochondrial respiration and ATP production. Understanding the interplay between TNFα-induced inflammation and metabolic dysfunction opens new therapeutic avenues for druggable targets in treating AMD.