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View Abstract

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Commercial Relationships Disclosure: Ioan Matei: Commercial Relationship: Code N (No Commercial Relationship) | Tanachapa Jantalika: Commercial Relationship: Code N (No Commercial Relationship) | Daniel Grimes: Commercial Relationship: Code N (No Commercial Relationship) | Luminita Paraoan: Commercial Relationship: Code N (No Commercial Relationship)

Study Group: (none)

ABSTRACT

TITLE: AMD-associated cystatin C variant impacts RPE differentiation and mitochondrial activity

ABSTRACT BODY:

Purpose: The AMD-associated variant B cystatin C (var B CysC) expressed in the RPE is the result of a point mutation impairing its intracellular trafficking and secretion. While its partial mitochondrial association is suggestive of impact on mitochondrial function and dynamics, the specific mechanism underpinning AMD pathogenesis has not yet been elucidated. We aim to assess the impact of var B CysC on differentiation and mitochondrial activity.

Methods: Differentiation of wildtype (wt) CysC low passage (12-14) ARPE-19 cells and isogenic gene edited var B CysC ARPE-19 cells was achieved on ECM-coated inserts and wells, in low serum, high glucose-containing DMEM, supplemented with pyruvate and nicotinamide. Cultures were kept for up to 6 months with celll morphology, TER, gene/protein expression (qPCR/immunoblotting), and mitochondrial function (TMRE, ROS, MitoTracker and MitoSOX) assayed monthly.

Results: Wt CysC cells shifted towards a pigmented, polyhedric phenotype at 2 weeks, achieving a high degree of pigmentation at 2 months, plateauing at 6. Var B CysC RPE cells also assumed a polyhedric shape, however pigmentation was faint after 1 month in culture, comparable at 5 months with that of wt cells at 1. TER values steadily increased for both groups, with lower values at 1, 2 and 3 months in culture for var B CysC compared to wt cells. Expression analysis of functional RPE genes *CST3, BEST1* and visual cycle genes *CRALBP, RDH5* and *LRAT* showed upregulation compared with undifferentiated controls at all time points in the wt RPE, and decreased expression at 1, 2, 3 and 5 months for var B CysC cells compared to wt. No differences were found for mitochondrial dynamics/mitophagy markers *MFN1, MFN2, OPA1, DRP1, PINK1, SPPL2B. PARK2* was consistently upregulated and *LC3B* consistently downregulated in var B CysC cells. Immunoblotting confirmed downregulation of CST3, CRALBP, RDH5 and VDAC1 in var B CysC cells, with temporally-regulated expression increase in wt cells. TMRE and MitoTracker assays revealed increased mitochondrial membrane potential and mass in var B CysC cells. By contrast, ROS and SOX production were decreased in the var B CysC cells, suggesting a maladaptive/insufficient compensatory response to be at play.

Conclusions: Var B cysC RPE cells demonstrate lower propensity towards phenotypic and functional maturation, as well as impairments in polarity, visual cycle and mitochondrial function.

(No Image Selected)

DETAILS

PRESENTATION TYPE - PLEASE NOTE, IF YOU CHANGE YOUR PRESENTATION TYPE AFTER APPLYING FOR AN AWARD (BELOW), YOU MUST GO BACK AND RESELECT THE APPLY BUTTON.: Poster Only : Travel Award Applicant

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CURRENT SECTION: Biochemistry/Molecular Biology

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