

Unravelling the Effects of Blue Light Exposure on Human Choroidal Melanocytes

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Introduction: Chronic exposure to environmental blue light, that can reach the retina, retinal pigment epithelium and choroid, may play key roles in both the physiological development and photooxidative decline of ocular tissue. A similar mechanism has been identified in skin melanocytes where blue light can impact melanogenesis via light-sensitive pathways involving Opsin 3 (OPN3; a non-visual opsin). This study explores the possibility that human choroidal melanocytes (HCMs) may exhibit blue-light sensitivity through the expression of non-visual opsins. **Methods:** Human donor eyes provided by the Lions NSW Eye Bank, with human research ethics approval and consent, and without obvious ocular pathology were dissected and imaged. Primary HCMs were isolated from donor eyes (n=4; 63-77 years), grown to 80% confluence, with passage (P) 2 to P7 cells used for experiments. HCMs seeded at 50,000 cells/well were exposed to 1.3mW/cm² 460nm blue light for 24 hours; HCMs not exposed to blue light served as controls. Following this, RNA was extracted and purified, and bulk RNA sequencing performed (Novogene, Singapore) for blue-light and control samples. Differentially expressed genes (Log2FC > |1|, FDR < 0.05) were identified and gene set enrichment analysis of hallmark gene sets, Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG), was performed to identify enriched signaling pathways. HCMs were also immunolabelled for melanogenesis and oxidative stress-related proteins including TYR, TRYP1, DCT, MC1R, OPN3 and HMOX1, visualized using fluorescence and confocal microscopy. **Results:** Bulk RNA sequencing of HCMs with and without blue light identified 84 differentially expressed genes (34 upregulated, 50 downregulated). Highly upregulated genes included *HMOX1*, *NQO1* and *SLC7A11*. Highly downregulated genes included *DCT*, *CXCL13*, and *ADAMTS15*. Key positively enriched pathways included 'glutathione metabolism' and 'oxidative stress'; negatively enriched pathways included 'melanogenesis' and 'ferroptosis'. Immunolabelling of HCMs confirmed the expression of melanocyte markers including TYR, TYRP1 and DCT, and OPN3. **Conclusion:** HCMs responded to low energy blue light exposure via regulation of oxidative stress, pigmentation, and ferroptosis gene transcription. HCMs expressed OPN3, a blue-light-sensitive opsin previously identified in skin melanocytes, suggesting a potential blue-light response mechanism in the choroid.

Funding: Australian Vision Research (AVR) (MCM)