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Isolation and propagation of Müller glia from human iPSC and ESC derived retinal organoids

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Footnotes

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Abstract

Purpose : Human Müller glia have been previously shown to have therapeutic benefit in rodent models of glaucoma and retinitis pigmentosa, suggesting that these cells can be used in cell based therapies. Since progress in the regulation for the use of hESCs and hiPSCs in the clinic has been made, we hypothesise that this platform may provide an attractive source of retinal cells such as Müller glia for retinal transplantation. We have therefore studied Müller glia formation in 3D retinal organoid development and have isolated these cells for characterisation *in vitro*

Methods : The human iPSC BJ and the ESC line Shef-6 were differentiated into 3D retinal organoids using a slight modification of published methods. After initiation of differentiation, retinal organoids were grown until day 280. Müller glia were isolated at various time points of retinal maturation using papain for cell dissociation. Isolated cells were grown in culture as confluent monolayers, and examined for the gene and protein expression of markers of Müller glia, including CRALBP, vimentin, nestin, glutamine synthetase, CD29 and CD44, using RT-PCR, western blotting, immunocytochemistry. FACS analysis to assess co-expression of surface markers was also performed in these cells

Results : The results showed that protein expression the Müller glia markers vimentin, CRALBP and CD29 can be detected by immunohistochemistry in retinal organoids from day 70 onwards. Isolated Müller glia in culture showed characteristic bi-polar morphology which can be expanded over 25 passages. Gene and protein expression data showed positive expression of the Müller glia markers CRALBP, vimentin, glutamine synthetase, Sox9 and Pax6. Immunofluorescence staining of isolated cells showed positive expression of CD29, CD44, CRALBP, Sox2, Sox9, nestin and vimentin. FACS analysis of the isolated cells indicated that >98% of the isolated and expanded population express Muller glia markers

Conclusions : In this study we have shown that Muller glia start to differentiate in retinal organoids in a comparable manner to *in vivo* development. In addition, these cells can be isolated *in vitro* and express Muller glia and progenitor markers. Investigation into their biological activity in transplantation studies may aid in the development of cell therapies to treat retina degenerative diseases such as glaucoma and retinitis pigmentosa

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