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

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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 regulatory framework for the use of hESC and iPSC 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 Ishiel-6 were differentiated into 3D retinal organoids using a slight modification of published methods. After induction of differentiation, retinal organoids were grown until day 280. Müller glia were isolated at various time points of retinal maturation using papanicolaou for cell dissociation. Isolated cells were grown in culture as confluent monolayers, and examined for the presence of marker expression on markers of Müller glia, including CRALBP, vimentin, nestin, glutamine synthetase, CDO29 and CDO44, using confocal microscopy, immunocytochemistry, FACS analysis to assess co-expression of surface markers was also performed in these cells.

Results: The results showed that protein expression of the Müller glia markers CRALBP, CD29 and CD44 was detected by immunocytochemistry in retinal organoids from day 70 onwards. Isolated Müller glia in culture showed characteristic bipolar morphology which could be expanded over 25 passages. Gene and protein expression data showed positive expression of the Müller glia markers CRALBP, vimentin, glutamine synthetase, Sox3 and Pax6. Immunofluorescence staining of isolated cells showed positive expression of CD29, CD44, CRALBP, Sox2, Sox5, nestin and vimentin. FACS analysis of the isolated cells indicated that >98% of the isolated and expanded population express Müller glia markers.

Conclusions: In this study we have shown that Müller 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 Müller glia and progenitor markers. Investigation into their biological activity in transplantation studies may aid in the development of cell therapies to treat retinal degenerative diseases such as glaucoma and retinitis pigmentosa.

This is an abstract that was submitted for the 2018 ARVO Annual Meeting, held in Honolulu, Hawaii, April 29 - May 3, 2018.

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