OPEN ACCESS
ARVO Annual Meeting Abstract | July 2018

Development of a human reporter stem cell line for mature cones in retinal organoids

Marta Zuzic; Stephanie Wieneke; Anka Kempe; Volker Busskamp; Mike Karl

+ Author Affiliations & Notes

Investigative Ophthalmology & Visual Science July 2018, Vol.59, 3103. doi:

Abstract

Purpose : Much of our knowledge about photoreceptor development comes from the mouse retina that is rich in rods but has significantly lower amount of cones. The derivation of three-dimensional (3D) retinal organoids from human stem cells allowed us to study differentiation of human photoreceptors. Therefore, our aim was to develop a fluorescent reporter human induced pluripotent stem cell (hiPSC) line that would allow identification and isolation of postmitotic cones from 3D retinas and further transcriptomic analysis.

Methods : For the reporter production, the PGP1 hiPSC line was nucleofected with piggyBAC plasmid coding for green fluorescent protein (GFP) under the mouse cone arrestin promoter (mARR3) and Blasticidin resistance under the human Ubiquitin C promoter (pUBC). Retinal organoids from the monoclonal PGP1-pmARR3-GFP were produced using a modified variant of a previously published protocol. Expression of retinal markers was analysed by immunohistochemistry.

Results : After 100 days, we observed stratified retinal tissue with an outer nuclear layer consisting of photoreceptors confirmed by recoverin immunostaining. Some of the recoverin-positive cells have also shown GFP-expression. Co-expression of GFP and endogenous ARR3 indicated the specific transgenic fluorescent labelling of cone photoreceptors. Further immunohistochemical analysis showed some GFP-positive cells expressing S- and M-opsin. Other retinal cell types analysed by staining were negative for GFP.

Conclusions : We produced a fluorescent hiPSC reporter line and tested its specificity in human 3D retinas. We could show that the cone photoreceptors were successfully expressing GFP indicating that the reporter indeed functions specifically. Furthermore,

cones derived from the reporter 3D retinas can be sorted based on fluorescence and used for transcriptome analysis to elucidate gene regulatory networks imperative for cone differentiation. We emphasize the potential use of our reporter cell line for disease modelling and studying underlying mechanisms of disease as well as development.

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