Development of long-term in vitro culture method for human induced pluripotent stem cells-derived retinal ganglion cells

Rong Kung Tsai [1,2], Yi Ling Tsang [2], Yao Tseng Wen [2]

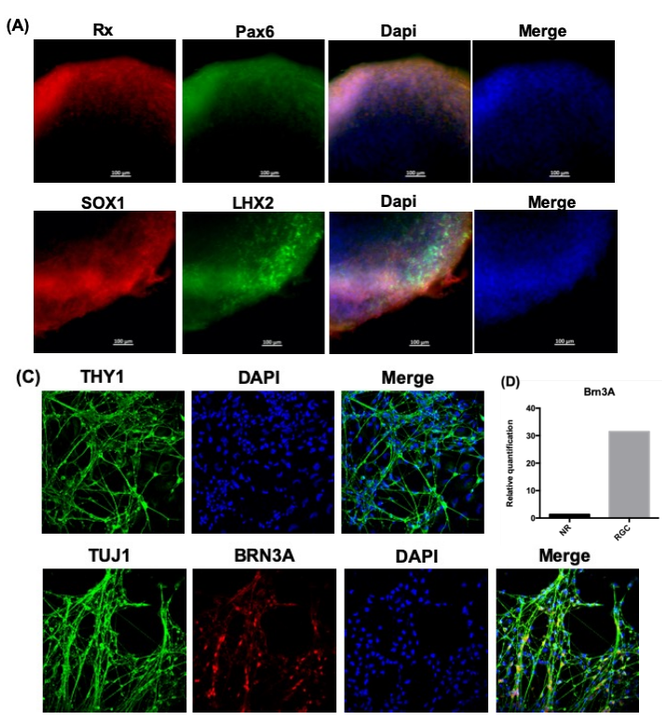
1. Institute of Medical Sciences, Tzu Chi University, Hualien, Taiwan
2. Institute of Eye Research, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan

Purpose

Retinal ganglion cells (RGCs) play an important role in visual transmission from eye to brain through the axons. Optic nerve injuries lead RGCs damages and irreversible RGC loss with permanent visual deficits. However, RGCs fail to regenerate after optic nerve injury and to survive during long-term *in vitro* expansion. Thus, our goal is to establish a long-term RGCs expansion method for culturing human induced pluripotent stem cells (hiPSCs)-derived RGCs.

Methods

Normal human epidermal keratinocytes (NHEK) were reprogramed by Yamanaka factors to generate hiPSCs. For RGC differentiation, the retinal neuron conditioned medium (CM) was gradually replaced by neuron induction medium (NIM) in the retinal neuron culture every day. For RGC expansion, NIM was changed every day. The neuron retinal precursor cells were characterized by immunofluorescence staining of Rx, Pax6, SOX1, and LHX2. The RGCs were characterized by immunofluorescence staining of Tuj1, Brn3a, and Thy1. To trace the transplanted hi-RGCs, hi-RGCs were labeled with GFP before transplantation, and optical coherence tomography was used to obtain cells location *in vivo*.



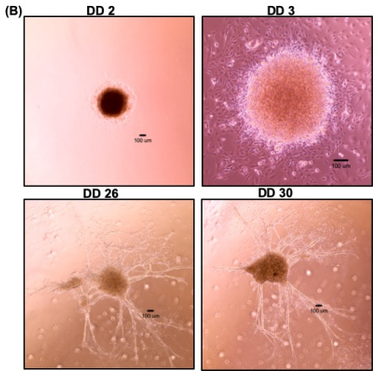


Fig. 1. Differentiation of retinal ganglion cells from iPS-derived neuron retinal precursor cells

(A) Neuron retinal precursor cells express neuron specific marker. (B) Treatment with conditioned medium induced iPSCs-derived RGCs proliferation and kept cell survival for 50 days. (C) Neuron retinal precursor cells started to generate axon and express RGC specific markers TUJ1, BRN3A, and THY1. (D) qPCR results revealed that *Brn3a* level was higher in RCG differentiation compare to retinal neuron. DD: Differentiation day

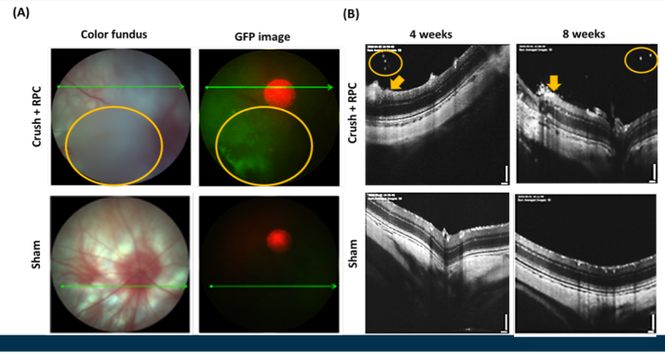
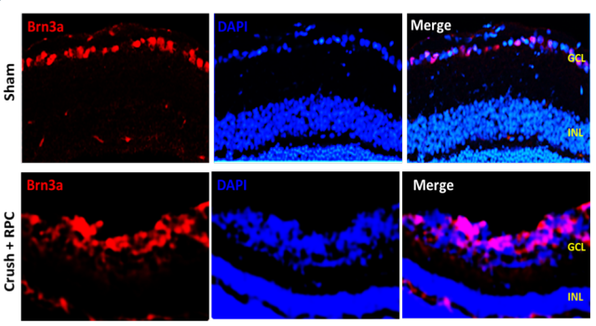


Fig. 2. Transplantation of GFP-labeled hi-RGCs in the optic nerve crush model

(A) The yellow circle indicates the GFP-labeled hi-RGCs 4 weeks after transplantation. (B) OCT imaging shows the position of the transplanted hi-RGCs at 4 weeks and 8 weeks after transplantation. The yellow circle and arrow indicate the transplanted cells.



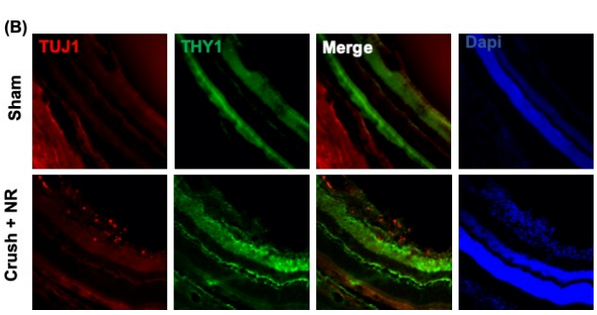


Fig. 3. Immunofluorescence staining of NR transplants at 8 weeks

(A) Cells in the vitreous space were highly expressed BRN3A at 8 weeks(B) Co-expression of TUJ1 and THY1 were also seen in the vitreous space at 8 weeks

Conclusion

The conditioned medium of retinal neuron contains many key factors involved in RGC growth and survival. This long-term RGC expansion method might help to establish a large scale of RGC production for clinical use.