Engineering Fibroblasts into Functional Retinal Ganglion Cells

[1,2]**Biraj Mahato**, [1]Avishek Ganguly, [1]Yan Fan, [2]Subrata Batabyal, [1]Zhang Wei, [2]Samarendra Mohanty and [1,2]Sai Chavala.
[1]Department of Pharmacology and Neuroscience, North Texas Eye Research Institute, University of North Texas Health Science Center, Fort Worth, Texas. [2]Nanoscope Technologies, LLC, Bedford, Texas.

Purpose:

RGCs are primarily damaged in optic neuropathy such as in Glaucoma and once damaged they fail to regenerate, leading to irreversible blindness.

Retinal ganglion cell (RGC) replacement therapy using embryonic stem cells (ESC) and induced pluripotent stem cells (iPSCs) are promising strategy to restore visual function resulting from irreversible RGC loss.

However, protocols to derive candidate replacement cells from ES or iPS are cumbersome and time consuming making them challenging for clinical therapy.

We sought to investigate if fibroblasts can be induced to RGCs by small molecules only and if these RGCs are functional.

Methods:

A set of five small molecules (5C) were identified for efficient RGC reprogramming. An optimized RGC induction medium (RIM) is developed, and small molecules are treated for 9 days in this medium to obtain chemically induced RGCs (CiRGCs).

Mouse embryonic fibroblasts (MEFs) and human adult dermal fibroblasts (HADFs) were reprogrammed to CiRGCs by identified small molecules.

Brn3b-GFP expressing CiRGCs were FACS sorted and analyzed for RGC specific gene expression.

Reporter expressing CiRGCs were tested for functional analysis by patch clamp recording.

Fig 3: CiRGCs are functional and survived in host retina

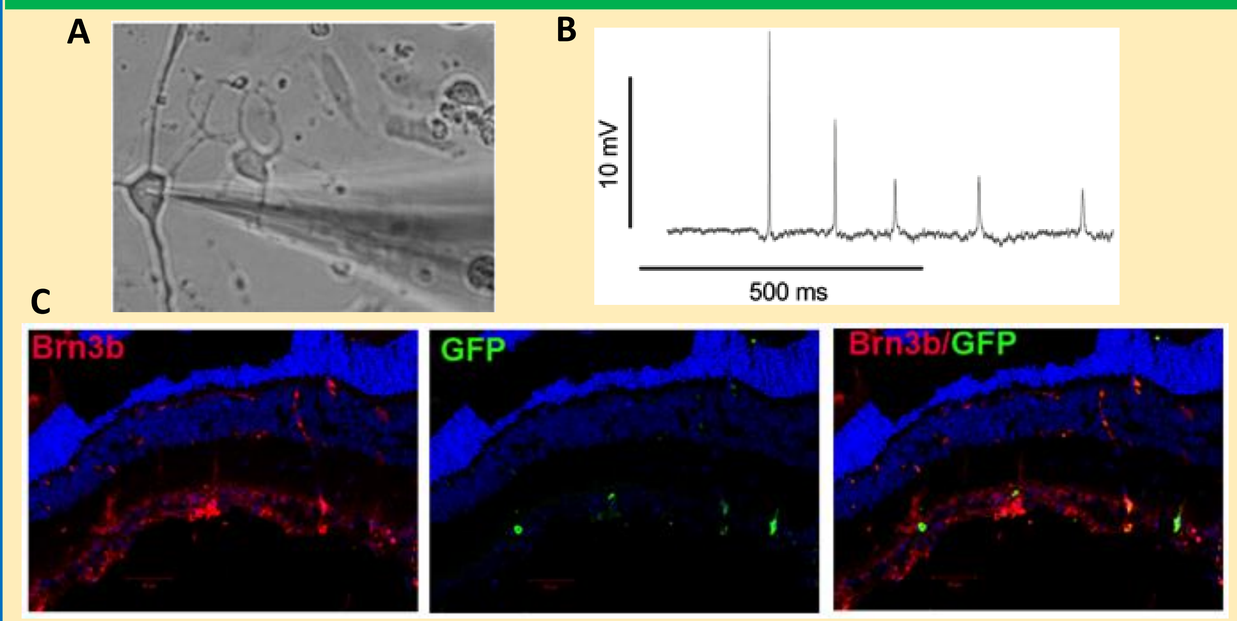

Figure 3: Functional and survival potential analysis of CiRGCs. (A) Patched CiRGC after reprograming. (B) Trace of spontaneously generated action potentials from CiRGCs (C). CiRGC survival in Sparague Dawley rat more than 2 months after transplantation.

Summary of results:

Five small molecules (5C) can reprogram mouse and human fibroblasts into retinal ganglion cells (CiRGCs).

CiRGCs express retinal ganglion cell specific transcripts such as Brn3a, Brn3b, RBPMS, ISL1, SNCG etc.

CiRGCs are functional as evidenced by generation of spontaneous action potential.

hCiRGCs were survived more than two months in rat retina after intravitreal injection.

hCiRGCs make in vivo retinal circuitry with host retina.

Fig 1: Reprogramming of MEFs to CiRGCs

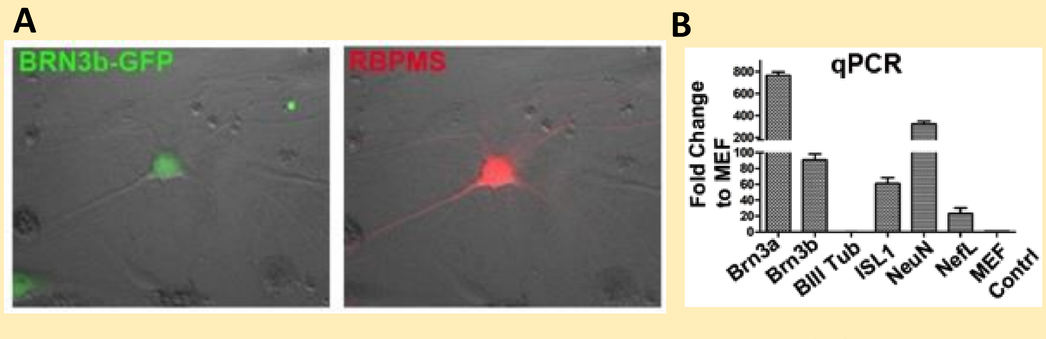

Figure 1: Reprogramming of MEFs to CiRGCs. (A) Brn3b-GFP reporter and RBPMS expressing chemically converted CiRGCs on day 7 of reprogramming. (B) qPCR analysis of Brn3b-GFP sorted cells showing expression of RGC specific markers such as Brn3a, Brn3b, ISL1,NeuN etc.

Fig 2: Reprogramming of HADF to CiRGCs

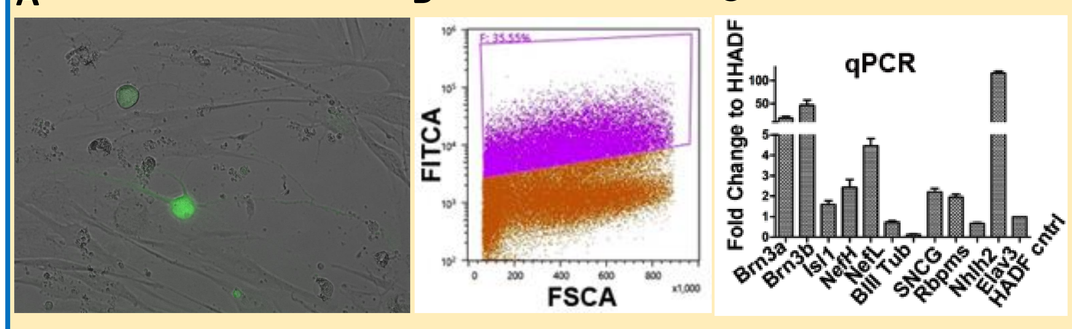

Figure 2: Reprogramming of HADF to CiRGCs. (A) Brn3b-GFP expressing CiRGCs on d 9.(B) Dot plot for FACS sorting of Brn3b-GFP Positive cells. (C) qPCR of GFP sorted cells showing RGC specific gene expression.

Conclusions and outlook:

5C can convert mouse and human fibroblasts into functional CiRGCs. Human CiRGCs survived in host retina and make synaptic connections with host retinal circuitry and improve retinal function. This method has potential implication in regenerative therapy and disease modeling for optic neuropathies such as glaucoma.

References:

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