

Cultured Human Retinal Ganglion Cells

New approaches in laboratory research could help identify targets for glaucoma intervention.

BY ABBOT F. CLARK, PhD

For anyone who wants to better understand glaucoma, studying the retinal ganglion cells (RGCs) is very compelling. It is known that vision loss in glaucoma results from damage to the RGCs and their axons, but there is much more to learn about these important cells. For example, if scientists better understood molecular mechanisms that initiate the loss of connections in the RGC dendritic arbor, neuroprotective agents to keep RGCs healthy could be developed.

There are several hurdles to studying RGCs in the laboratory. For one, RGCs make up less than 2% of all retinal cells, and this tiny portion of the retina is not homogeneous. There are more than 20 different types of RGCs, each with slightly different structures and functions as well as possibly differing susceptibility to damage.

Most of what is known about RGCs comes from scientific research on rodents, but even animal research has been hampered by the difficulties associated with harvesting a sufficient number of cells from complex retinal tissue. To get around this problem, scientists have cultured the cells in the laboratory. Primary RGCs, however, do not proliferate in culture. In addition, one transformed proliferating rat RGC line that was used extensively was recently discovered to be faulty (the cells were actually mouse photoreceptor cells), calling into question the more than 200 articles based on this cell line as surrogates of RGCs. To date, no one has successfully cultured human RGCs.

CONDITIONALLY IMMORTALIZED RGCs

In my laboratory, my colleagues and I are developing a mouse RGC line that will indefinitely proliferate. Using a special research mouse (Immortomouse; Charles River Laboratories), our goal is to take RGCs that are normally terminally differentiated (meaning they do not divide) and “immortalize” them so that the cells will constantly propagate in culture medium, mak-

ing it easy to generate large numbers of identical cells for research. Importantly, these cells will only be conditionally immortalized. Their proliferation is temperature dependent. At low temperatures, large numbers of cells can be grown; at higher temperatures, the cells stop replicating and behave more like normal RGCs.

Access to such a cell line would be a major asset for research into the pathophysiology of glaucoma and potential targets for glaucoma therapy. We intend to use the cell line as a stepping stone to developing a conditionally immortalized human RGC line. If this process works in the Immortomouse, our next step will be to attempt to culture RGCs from human donor tissue. We believe it may be possible to clone the same conditionally immortalizing vector that has been used in the mouse cells into a virus that could “infect” the human RGCs and turn them into conditionally immortalized cells. We have already successfully cultured cells from human trabecular meshwork and optic nerve head tissue in both normal and glaucomatous eyes. We have also harvested ribonucleic acid from the cultured cells and donor eye tissue to compare gene expression in normal versus glaucomatous eyes.

Fresh tissue will be needed to culture RGCs. The retina deteriorates very rapidly after death, with autolytic changes in the tissue making the RGCs less usable with every passing hour. For this reason, we are working closely with the Lions Eye Institute for Transplant & Research (LEITR). Not only can LEITR obtain tissue quickly and efficiently, but the institute also offers sleeping quarters and laboratory facilities on site. At LEITR, researchers can obtain enough tissue over a 1- to 2-week period to begin the development of the cell line. The initial cell dissociation and purification would be performed at LEITR, while the more advanced work, such as transforming the cells, would continue at my laboratory.

(Courtesy of Andrew Huberman, PhD.)

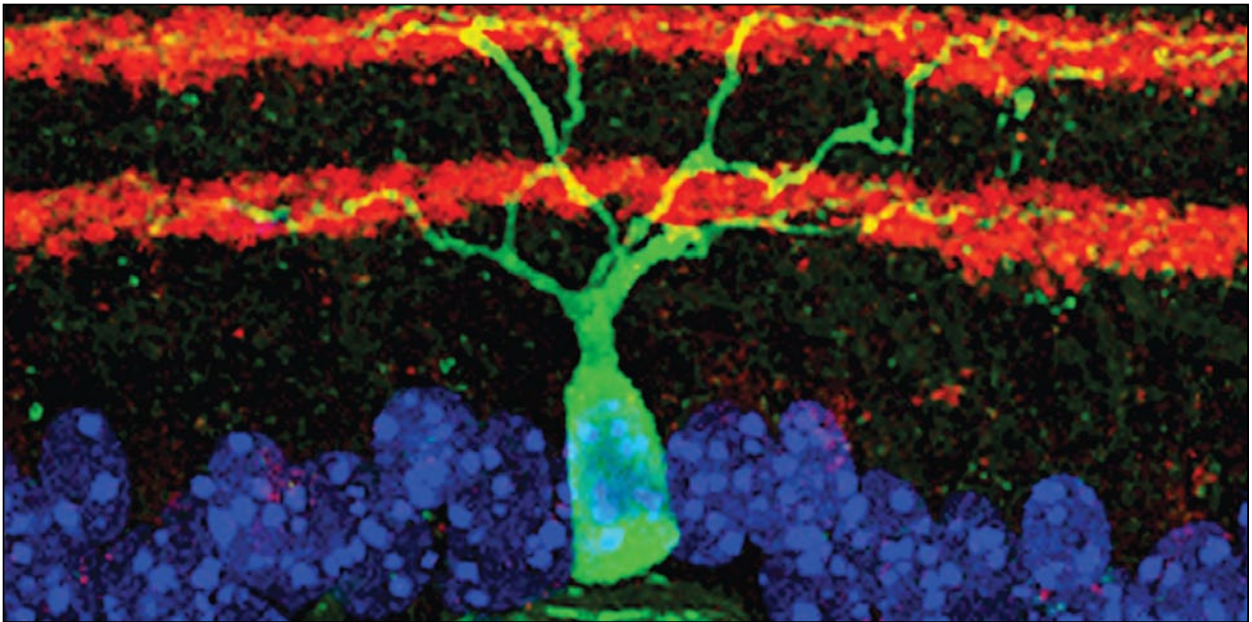


Figure. An RGC that has been genetically labeled with green fluorescent protein. The red portions represent other retinal cells that connect to the RGC, and blue labels the cell bodies of all cells in the vicinity.

THE PROMISE OF RGCs

Many hypotheses about glaucoma could be tested with conditionally immortalized human RGCs. For example, we could expose normal, healthy RGCs to hypoxia, add excitotoxic amino acids, activate oxidative stress, or withdraw growth factors. It is hard to tease out the impact of each of these insults in eyes with later-stage glaucoma, but they could be studied individually or in combination in cultured cells. In all of these experiments, gene expression could be closely examined to help us understand the molecular pathways that are activated as glaucoma damages healthy cells.

In earlier work with cultured trabecular meshwork and optic nerve head cells, my colleagues and I identified several pathogenic signaling pathways that not only seem to be common across different sites of damage in glaucoma but also to be interrelated (see “Why Human Tissue Is Essential to Glaucoma Research” in the November/December 2012 issue of *GT*).¹⁻⁴ It will be very interesting to see whether these same pathways are implicated in RGC damage.

In addition to testing the effects of potential sources of damage, we might also be able to test whether certain compounds protect the RGCs from damage or alter the patterns of gene expression.

There are exciting possibilities for comparisons between mouse- and human-derived cell lines. Potentially, mice with particular subsets of RGCs labeled with green fluorescent protein (Figure) could

be compared with the cells cultured from human tissue to evaluate them for similar characteristics. Ultimately, we want to validate any findings in cultured mouse or human cells in human glaucomatous eyes. If a pathway is turned on following a particular insult in cultured cells, we can then look at histopathology of human donor eyes with glaucoma to further validate those findings.

The future of RGC research is bright. Although there is much work yet to be done, I am optimistic that soon we will be able to culture human RGCs, providing a great resource for better understanding glaucoma. ■

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