Letters

RGC-5 Cells

Our laboratory previously reported the generation of a retinal ganglion cell 5 (RGC-5) cell line with phenotypic expression of many retinal ganglion cell markers.¹ The RGC-5 cell line has been widely used since then, reproducing several of the original findings and their retinal ganglion cell phenotype.^{2–8} ISI Web of knowledge gives 260 references by using "RGC-5" as a search word under topic.

However, last month the first author of the original study¹ published a report⁹ that, along with previous studies,^{10,11} stated that the RGC-5 line is the same as 661W, a cell line believed to be of cone photoreceptor origin.¹² As the two cell types are very different by molecular phenotyping, it is difficult to reconcile this discrepancy. In fact, the same group who published this recent report⁹ previously used RGC-5 cells for other studies, confirming their retinal ganglion cell origin (Dauphin et al. *IOVS* 2003;44:ARVO E-Abstract 2249; Krishnamoorthy et al. *IOVS* 2005;46:ARVO E-Astract 2215; and Refs. 13 and 14). Furthermore, the originator of 661W cells and many others used RGC-5 cells in several published studies¹⁵⁻¹⁸ and reported different phenotypic properties between RGC-5 and 661W cell types. So, what is the true phenotype of 661W cells?

Interestingly, the two coauthors of Krishnamoorthy et al.⁹ published a paper on sigma-1 receptor (sigma-1r) in purified retinal ganglion cells,¹⁹ in which they corroborated their coimmunoprecipitation data of sigma-1r with L-type volted gated calcium channels in purified RGCs with that of RGC-5¹³ and suggested, "Our co-localization data in purified RGCs is in agreement with the above studies" done with RGC-5 cells.

Furthermore, there were two editorials on authentication of RGC-5 cells,^{20,21} suggesting RGC-5 may have never existed. These editorials have totally ignored the vast published literature on their ganglion cells' origin even by some of the coeditors' own studies. As I have been without an active laboratory for the last several years, I am in no position to validate the published reports.

In light of these revelations from the first author of the original study who could not reproduce his own work, it became imperative for me to dissociate myself from the original work and, as a corresponding author, have requested that the original manuscript be retracted from the *Molecular Brain Research* journal.¹ I sincerely regret this incidence and apologize to my colleagues in vision research—and I agree that one has to be very careful while using cultured cells in research.

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References

- 1. Krishnamoorthy RR, Agarwal P, Prasanna G, et al. Characterization of a transformed rat retinal ganglion cell line. *Mol Brain Res.* 2001;86:1–12.
- 2. Fressetto LJ, Schlieve CR, Lieven CJ, et al. Kinase dependent differentiation of a retinal ganglion cell precursor. *Invest Ophtbalmol Vis Sci.* 2006;47:427-438.
- 3. Zhang B, Rusciano D, Osborne NN. Orally administered epigallocatechin gallate attenuates retinal neuronal death in vivo and light induced apoptosis in vitro. *Brain Res.* 2008;1198:141–152.

- 4. Lieven CJ, Millet LE, Hoegger MJ, Levin LA. Induction of axon and dendrite formation during early RGC-5 cell differentiation. *Exp Eye Res.* 2007;85:678–683.
- 5. Jung SH, Kang KD, Ji D, et al. The flavonoid baicalin counteracts ischemic and oxidative insults to retinal and lipid peroxidation to brain membranes. *Neurochem Int.* 2008;53:325-337.
- Umapathy NS, Dun Y, Martin PM, et al. Expression and function of system N glutamine transporters (SN1/SN2 or SNAT3/SNAT5) in retinal ganglion cells. *Invest Ophthalmol Vis Sci.* 2008;49: 5151–5160.
- Nieto PS, Acosta-Rodriguez VA, Valdez DJ, Guido ME. Differential responses of the mammalian retinal ganglion cell line RGC-5 to physiological stimuli and trophic factors. *Neurochem Int.* 2010; 57:216–226.
- 8. Ganapathy PS, Dun Y, Ha Y, et al. Sensitivity of staurosporineinduced differentiated RGC-5 cells to homocysteine. *Informa Healthcare*. 2010;35:80–90.
- 9. Krisnamoorthy RR, Clark AF, Daudt D, Vishwanatha JK, Yorio T. A forensic path to RGC-5 cell line identification: Lessons learned. *Invest Ophthalmol Vis Sci.* 2013;54:5712–5719.
- Van Bergen NJ, Wood JP, Chidlow G, et al. Recharacterization of the RGC-5 retinal ganglion cell line. *Invest Ophthalmol Vis Sci.* 2009;50:4267-4272.
- 11. Wood JPM, Chidlow G, Tran T, Crowston JG, Casson RJ. A comparison of differentiation protocols for RGC-5 cells. *Invest Ophthalmol Vis Sci.* 2010;51:3774–3783.
- Tan E, Ding XQ, Saadi A, Agarwal N, Naash MI, Al-Ubaidi MR. Expression of cone-photoreceptor-specific antigens in a cell line derived from retinal tumors in transgenic mice. *Invest Ophthalmol Vis Sci.* 2004;45:764–768.
- 13. Tchedre KT, Huang RQ, Dibas A, Krishnamoorthy RR, Dillon GH, Yorio T. Sigma-1 receptor regulation of voltage-gated calcium channels involves a direct interaction. *Invest Ophthalmol Vis Sci.* 2008;49:4995–5002.
- 14. Yang MH, Krishnamoorthy RR, Jong SB, et al. Protein profiling of human nonpigmented cilliary epithelium cell secretome: The differentiation factors characterization for retinal ganglion cell line. *J Biomed and Biotech*. 2011;2011:1–28.
- 15. Kanan Y, Moiseyev G, Agarwal N, Ma JX, Al-Ubaidi MR. Light induces programmed cell death by activating multiple independent proteases in a cone photoreceptor cell line. *Invest Ophtbalmol Vis Sci.* 2007;48:40-51.
- Mackey AM, Sanvicens N, Groeger G, Doonan F, Wallace D, Cotter TG. Redox survival signaling in retina-derived 661W cell. *Cell Death Differ*. 2008;15:1291–1303.
- Tsuruma K, Yamauchi M, Inokuchi Y, Sugitani S, Shimazawa M, Hara H. Role of oxidative stress in retinal photoreceptor cell death in N-methyl-N-nitrosourea-treated mice. *J Pharmacol Sci.* 2012;118:351–362.
- Schnichels S, Hagemann U, Januschowski K, et al. Comparative toxicity and proliferation testing of aflibercept, bevacizumab and ranibizumab on different ocular cells. *British J Ophthalmol.* 2013;97:917–923.
- 19. Mueller IIBH, Park Y, Daudt III DR, et al. Sigma-1 receptor stimulation attenuates calcium influx through activated L-type voltage gated calcium channels in purified retinal ganglion cells. *Exp Eye Res.* 2013;107:21–31.
- 20. Boatright JH, Cammarata PR, Nickerson JM, Zhang QJ. On authentication of cell lines. *Mol Vision*. 2013;19:1848-1851.
- Clark A, Tamm ER, Al-Ubaidi MR, Hollyfield JG. On the use of immortalized ocular cell lines in vision research: The unfortunate story of RGC-5 [published online ahead of print September 7, 2013]. *Exp Eye Res.* doi:10.1016/j.exer.2013.08.002

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