On authentication of cell lines

The Editors of Molecular Vision

THE RGC-5 CELL LINE MAY HAVE NEVER EXISTED

The RGC-5 cell line was first described in 2001 as being an immortalized cell line derived from rat retinal ganglion cells (RGCs) [1]. At least 230 articles have been published in which this cell line was used to test hypotheses. Eighteen of these were published in *Molecular Vision* [2-19].

Work recently published by several of the co-authors of the original RGC-5 paper indicates that the cell line may have never existed outside of the originating laboratory [20]. Cryopreserved RGC-5 passages, some as early as the second passage (P2), were obtained from the originating laboratory. These cells were found to be genetically identical to 661W cells, an immortalized mouse photoreceptor cell line [21] that was in use in the originating laboratory at the time that the RGC-5 cell line was being developed [20]. It is likely that from P2 on, all RGC-5 clones were instead 661W cells [20]. We commend the authors and *Investigative Ophthalmology and Vision Science* for providing this correction.

Previous concerns about the identity of the RGC-5 cell line, some published [22,23], resulted in a policy of *Molecular Vision*, which was until now:

“Cells of questionable characterization cannot be used to test hypotheses specifically concerning retinal ganglion cells. Manuscripts reporting experiments in which putative RGC-5 cells are used must include data that demonstrate that the RGC-5 cells, as used under the culture conditions of the other experiments reported in the manuscript, are of rat origin and express genes and proteins that are specific to retinal ganglion cells (i.e., they should be positive for Thy-1, Brn-3C, Neuritin, NMDA receptor, GABA-B receptor, and synaptophysin expression and negative for GFAP, HPC-1, and 8A1, as reported in Krishnamoorthy et al.)” - from *Molecular Vision Instructions to Authors*, dated January 1, 2013 to August 23, 2013.

This policy has been revised to: “New manuscripts containing data derived from RGC-5 cells will be editorially rejected without review.”

MOLECULAR VISION POLICY ON USE OF CELL LINES: COMPULSORY AUTHENTICATION

The editors of *Molecular Vision* recognize that the proper use of immortalized cell lines can result in the rapid generation of substantial data in testing many hypotheses. However, due to continual problems of misidentification of cell lines for at least 55 years [24-28], including estimates of mammalian cell misidentification of 15%-35% [27,29,30], the new *Molecular Vision* policy for reporting data based on immortalized cell lines is:

“Manuscripts reporting experiments in which immortalized cell lines are used must include data, documentation, or citations that demonstrate that the actual cells used in the experiments reported in the manuscript exhibit the correct phenotype and genotype. It must be demonstrated that the cells actually used are of the correct species of origin, the correct sex and genotype, and express genes and gene products that are specific to the pertinent cell type. Where possible, phenotype analysis should include the effects of differentiation. Cells used in experiments should be within a few passages of authentication (typically five passages). These standards hold even if the cell lines are considered “established” and were obtained from reputable sources. A statement of cell handling protocol that includes passage information and authentication data, certification documentation, and/or citation of published authentication by the co-authors must be provided in Methods sections of submitted manuscripts. These will be part of the freely-available article.” - *Molecular Vision Instructions to Authors*, August 27, 2013.

MEETING THESE CRITERIA

Authors are encouraged to study the history and consequences of misidentification of cell lines and the array of solutions available to avoid this chronic problem. Several excellent reviews and primary papers are available (e.g., [22,24-28,31]). Even a cursory reading of these sources provides insight into the historical lack of scientific rigor and expensive outcomes in terms of research monies and careers that has plagued the misuse of immortalized cell lines.

The editors of *Molecular Vision* agree with The International Cell Line Authentication Committee (ICLAC) and the National Institutes of Health (NIH) in their statements.
of the need for cell line authentication (NOT-OD-08-017) and approaches to accomplish this authentication [25]. The ICLAC provides guidelines for incorporating authentication into good tissue-culture practice (Advice to Scientists: Incorporating Authentication into Everyday Culture Practice). A similar online resource is published by the National Center for Biotechnology Information [30]. Briefly, once a cell line is authenticated, it is expanded to create a “master stock” of cell aliquots for cryopreservation. An aliquot from the master stock is expanded to create a “distribution stock” of cell aliquots. An aliquot from the distribution stock is expanded into aliquots of cells that are used in the actual experiments reported in submitted manuscripts. Thus, by best practices, the cells actually used in experiments should be within five passages of authentication (this is not five passages from the initial establishment of the cell line, which may have occurred many passages earlier). These guidelines must be followed and documented in order for data based on the use of immortalized cell lines to be published in Molecular Vision. Exemptions for incidental use of immortalized cells (e.g., simple expression confirmation assays) might occur following scrutiny by reviewers and editors. The journal’s Instructions to Authors will contain the above policy statement and supporting details, including examples of potential exceptions.

Authentication itself principally involves short-tandem-repeat (STR) profiling using standards and protocols developed by the American National Standards Institute for human cell lines [30,32] and by the National Institute of Standards and Technology for mouse cell lines [33]. The articles reporting the re-characterization of the RGC-5 cell line also provide insight into authentication approaches [20,22,23]. Cell lines also may be authenticated by replicating the experiments published for initial characterization. Authors can either provide data in their submitted manuscripts demonstrating that cell lines were authenticated by these various standards, or they can contract with external services (e.g., ATCC, Promega, Identicell, DSMZ, Genetica, and others not listed) to provide authentication. If contract services are used, documentation from the service must be supplied to Molecular Vision as part of the manuscript submission. Note that only some, but not all, cell lines sold by commercial suppliers are authenticated. It is the authors’ responsibility to confirm authentication.

VISION RESEARCHERS SHOULD LEAD IN ENDING MISIDENTIFICATION

The need for authentication of immortalized cell lines has previously been called for in our field [34]. However, the use of misidentified cell lines has harmed the vision research community. Resources were wasted and misinformation was propagated (which itself will require further expenditures for remediation). Molecular Vision has committed to halt these problems by enforcing the above-stated policy. However, a wider approach is needed to effect change. To that end, we have worked with the editors of Experimental Eye Research, the flagship journal of the International Society for Eye Research (ISER) and Investigative Ophthalmology and Visual Science, the flagship journal of the Association for Research in Vision and Ophthalmology (ARVO), in formulating our policy. The policies of these journals are presented in recent editorials and in the journals’ Instructions to Authors. We encourage other vision research journals to consider joining in this editorial effort to preempt the replication of this type of error in future work by mandating cell line authentication for all submitted manuscripts. Further, we urge scientific and professional organizations to set standards for cell line maintenance and authentication and to develop educational resources (online and at major meetings) to aid their members in meeting these standards. We urge universities to hold similar courses. Finally, we encourage funding agencies to require that cell line maintenance and authentication protocols be incorporated into project proposals.

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REFERENCES

1. Krishnamoorthy RR, Agarwal P, Prasanna G, Vopat K, Lambert W, Sheedlo HJ, Pang IH, Shade D, Wordinger RJ, Yorio T, Clark AF, Agarwal N. Characterization of a transformed rat retinal ganglion cell line. Brain Res Mol Brain Res 2001; 86:1-12. [PMID: 11165366].

2. Kim SH, Park JH, Kim YJ, Park KH. The neuroprotective effect of resveratrol on retinal ganglion cells after optic nerve transection. Mol Vis 2013; 19:1667-76. [PMID: 23901250].

3. Li GY, Li T, Fan B, Zheng YC, Ma TH. The D(1) dopamine receptor agonist, SKF83959, attenuates hydrogen peroxide-induced injury in RGC-5 cells involving the extracellular
4. Tsui L, Fong TH, Wang JJ. YC-1 targeting of hypoxia-inducible factor-lalpha reduces RGC-5 cell viability and inhibits cell proliferation. Mol Vis 2012; 18:2882-95. [PMID: 23233790].

5. Lee JJ, Hsiao CC, Yang IH, Chou MH, Wu CL, Wei YC, Chen CH, Chuang JH. High-mobility group box 1 protein is implicated in advanced glycation end products-induced vascular endothelial growth factor A production in the rat retinal ganglion cell line RGC-5. Mol Vis 2012; 18:838-50. [PMID: 22511847].

6. Li H, Ao X, Jia J, Wang Q, Zhang Z. Effects of optineurin siRNA on apoptotic genes and apoptosis in RGC-5 cells. Mol Vis 2011; 17:3314-25. [PMID: 22194658].

7. Li GY, Fan B, Ma TH. Visible light may directly induce nuclear DNA damage triggering the death pathway in RGC-5 cells. Mol Vis 2011; 17:3279-89. [PMID: 22194654].

8. Nakamura-Yanagida T, Takahashi Y, Sano K, Murata T, Hayashi T. Development of spontaneous neuropathy in NF-kappaBp50-deficient mice by calcineurin-signal involving impaired NF-kappaB activation. Mol Vis 2011; 17:2157-70. [PMID: 21850191].

9. Brar VS, Sharma RK, Murthy RK, Chalam KY. Bevacizumab neutralizes the protective effect of vascular endothelial growth factor on retinal ganglion cells. Mol Vis 2010; 16:3848-53. [PMID: 21031022].

10. Ju WK, Kim KY, Duong-Polk KX, Lindsey JD, Ellisman MH, Weinreb RN. Increased optic atrophy type 1 expression protects retinal ganglion cells in a mouse model of glaucoma. Mol Vis 2010; 16:1331-42. [PMID: 20664796].

11. Biswas SK, Zhao Y, Sandirasegarane L. Imatinib induces apoptosis by inhibiting PDGF- but not insulin-induced PI 3-kinase/Akt survival signaling in RGC-5 retinal ganglion cells. Mol Vis 2009; 15:1599-60. [PMID: 19693287].

12. Martin PM, Ananth S, Cresci G, Roon P, Smith S, Ganapathy V. Expression and localization of GPR109A (PUMA-G/HM74A) mRNA and protein in mammalian retinal pigment epithelium. Mol Vis 2009; 15:362-72. [PMID: 19223991].

13. Ju WK, Kim KY, Lindsey JD, Angert M, Patel A, Scott RT, Liu Q, Crowston JG, Ellisman MH, Perkins GA, Weinreb RN. Elevated hydrostatic pressure triggers release of OPA1 and cytochrome C, and induces apoptotic cell death in differentiated RGC-5 cells. Mol Vis 2009; 15:120-34. [PMID: 19169378].

14. Lin S, Cheng M, Dailey W, Drenser K, Chintala S. Norrin attenuates protease-mediated death of transformed retinal ganglion cells. Mol Vis 2009; 15:26-37. [PMID: 19137075].

15. Surgucheva I, Weisman AD, Goldberg JL, Shnaya A, Surguchov A. Gamma-synuclein as a marker of retinal ganglion cells. Mol Vis 2008; 14:1540-8. [PMID: 18728752].

16. Wood JP, Lascaratos G, Bron AJ, Osborne NN. The influence of visible light exposure on cultured RGC-5 cells. Mol Vis 2008; 14:334-44.

17. Agarwal N, Agarwal R, Kumar DM, Ondricek A, Clark AF, Wordinger RJ, Pang H. Comparison of expression profile of neurotrophins and their receptors in primary and transformed rat retinal ganglion cells. Mol Vis 2007; 13:1311-8. [PMID: 17679933].

18. Shimazawa M, Inokuchi Y, Ito Y, Murata H, Aihara M, Miura M, Araie M, Hara H. Involvement of ER stress in retinal cell death. Mol Vis 2007; 13:578-87. [PMID: 17438523].

19. Khalyfa A, Chlon T, Qiang H, Agarwal N, Cooper NG. Microarray reveals complement components are regulated in the serum-deprived rat retinal ganglion cell line. Mol Vis 2007; 13:293-308. [PMID: 17356516].

20. Krishnamoorthy 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; 54:5712-9. [PMID: 23975727].

21. al-Ubaidi MR, Font RL, Quiambao AB, Keener MJ, Liou GI, Overbeek PA, Baehr W. Bilateral retinal and brain tumors in transgenic mice expressing simian virus 40 large T antigen under control of the human interphotoreceptor retinoid-binding protein promoter. J Cell Biol 1992; 119:1681-7. [PMID: 1334963].

22. Van Bergen NJ, Wood JP, Chidlow G, Trounce IA, Casson RJ, Ju WK, Weinreb RN, Crowston JG. Recharacterization of the RGC-5 retinal ganglion cell line. Invest Ophthalmol Vis Sci 2009; 50:4267-72. [PMID: 19443730].

23. Wood JP, Chidlow G, Tran T, Crowston JG, Casson RJ. A comparison of differentiation protocols for RGC-5 cells. Invest Ophthalmol Vis Sci 2010; 51:3774-83. [PMID: 20181845].

24. American Type Culture Collection Standards Development Organization Workgroup ASN. Cell line misidentification: the beginning of the end. Nat Rev Cancer 2010; 10:441-8. [PMID: 20448633].

25. Masters JR. Cell-line authentication: End the scandal of false cell lines. Nature 2012; 492:186-186. [PMID: 23235867].

26. Nelson-Rees WA, Daniels DW, Flandermeyer RR. Cross-contamination of cells in culture. Science 1981; 212:446-52.

27. Nardone RM. Curbing rampant cross-contamination and misidentification of cell lines. Biotechniques 2008; 45:221-7. [PMID: 18816888].

28. Gartler SM. Genetic markers as tracers in cell culture. Natl Cancer Inst Monogr 1967; 26:167-95. [PMID: 4864103].

29. Nardone RM. Eradication of cross-contaminated cell lines: a call for action. Cell Biol Toxicol 2007; 23:367-72. [PMID: 17522957].

30. Reid Y, Storts D, Riss T, Minor L. Authentication of Human Cell Lines by STR DNA Profiling Analysis. In: Sittampalam GS, Gal-Edd N, Arkin M, Auld D, Austin C, Bejcek B, Glicksman M, Inglese J, Lemmon V, Li Z, McGee J, McManus O, Minor L, Napper A, Riss T, Trask OJ, Weidner J, editors. Assay Guidance Manual. Bethesda (MD); 2004.
31. Fialkow PJ, Gartler SM, Yoshida A. Clonal origin of chronic myelocytic leukemia in man. Proc Natl Acad Sci USA 1967; 58:1468-71. [PMID: 5237880].

32. American National Standards Institute. Authentication of Human Cell Lines: Standardization of STR Profiling. ANSI/ATCC: ASN-0002-2011; 2012.

33. Almeida JL, Hill CR, Cole KD. Mouse cell line authentication. Cytotechnology 2013; in press. [PMID: 23430347].

34. Folberg R, Kadkol SS, Frenkel S, Valyi-Nagy K, Jager MJ, Pe'er J, Maniotis AJ. Authenticating cell lines in ophthalmic research laboratories. Invest Ophthal Vis Sci 2008; 49:4697-701. [PMID: 18689700].