TK Talk ~ Demystify science, Kant not cant

Tag Archives: *Cross-contaminated cell lines*

Are all HeLa cells from a commercial source equivalent to numbers or replications? I wager they are not.

27 Sunday Sep 2015

Posted by Tirumalai Kamala in Biomedical research, Cancer, Cell lines, Human cell lines

≈ Comments Off

Tags

Cancer cell lines, *Cell line authentication*, *Cell line contamination*, *Cross-contaminated cell lines*, *HeLa*, *Henrietta Lacks*, *John R. Masters*, *Roland Nardone*, *Short Tandem Repeat (STR) profiling*, *Stanley Gartler*, *Walter Nelson-Rees*

Cell lines like HeLa are the bread and butter of biomedical research, especially human research, yet this could be one of the safest wagers.

Laypersons are largely unaware that for decades misidentified and cross-contaminated cell lines (1) were the vast, seedy underbelly of human biomedical research, a problem well-known in the scientific community with no one, not the scientists, not the funding agencies, not the professional societies nor the scientific journals taking the necessary, largely simple steps to ensure clean up. Though the problem maybe slightly better acknowledged today, it persists and will continue to do so unless and until the key stakeholders with power, namely, funding agencies and scientific journals enforce mandatory policies, rather than continue their head-in-the-sand policy stemming from the wrongheaded notion that science is apparently self-correcting, i.e. that 'blatantly poor science' will correct itself.

Scientific editorials casually opine that up to a third of all human cell lines in existence may be misidentified. No way to know for sure. Cell lines circulate freely between colleagues and collaborators. For long, the attitude was casual, along the lines of '*who has the time or money to get authenticated cell lines from a cell bank?*'. After all, the key stakeholders with the power to effect change, namely, the funding agencies and scientific journals, didn't mandate such effort. We can surmise that the problem reached epidemic proportion if a mainstream non-science news source such as the Wall Street Journal published a detailed 2012 article on the topic. Titled 'Lab Mistakes Hobble Cancer Studies But Scientists Slow to Take Remedies' (2), the article identifies the key sources of the problem:

- Inexperienced or distracted technicians use the same tool when working with different samples
- A pipette or other tool isn't sterilized
- Cultures are stored closely together
- Containers are mislabeled or labels are missed
- Cell samples from colleagues and other labs aren't thoroughly tested

The story of cell lines in general and of HeLa in particular is thus operatic for its scale of shoddiness, both unwitting and knowing, and the steadfast courage and integrity of a handful who chose to highlight the increasingly vexing problem of cell line cross-contamination and misidentification, including some like Walter Nelson-Rees, who doggedly pursued a clean up, even in the face of vilification of their scientific reputation, with finally, vindication, though only in posterity (4).

My brief, largely graphic, history starts with this 2002 list (1) by John R. Masters, one of the heroes in this story, that says

- There was never a common stock of HeLa.
- Many different HeLa sublines exist.
- HeLa sublines are quite different from each other.

HeLa cell line heterogeneity has been observed for decades. For example, different alkaline phosphatase expression between HeLa sublines (4).

Another example, differences in expression of bone morphogenic proteins (BMPs) (5).

A recent comparison (6; the only non-peer-reviewed study I refer to in this answer) shows that dramatic karyotype differences have existed between HeLa sublines for decades.

Recently the HeLa genome was sequenced, creating more headaches (7, 8, 9) because the authors publicly posted the genome without first seeking the approval of the Lacks family. Discussions between the family and the US NIH resulted in the condition that US federal grant recipients who use and publish on HeLa need to acknowledge Henrietta Lacks and her family (10).

The HeLa story doesn't end with the multiple sublines and their many documented and as yet undiscovered differences. HeLa is probably one of the most robust cell lines in existence. Over the decades, this characteristic feature of Hela combined with shoddy cell culture techniques and lax oversight ensured that many cell lines commonly used in human biomedical research became contaminated with HeLa (11, 12, 13).

Yet scientists continue using these contaminated cell lines with an unknown number of peer-reviewed scientific papers containing utterly erroneous and even useless data. In fact, such data is not just useless but costly because it results in wrong research avenues being pursued, sometimes for years, at tremendous cost to taxpayers (13, 14).

Finally, a consensus of sorts on cell line authentication led to the ATCC (American Tissue Culture

Collection) paper Standard 'ASN-0002 – Authentication of Human Cell Lines: Standardization of STR Profiling' (15). A brief history and overview of current cell line authentication.

What method does ASN-002 advocate for cell line authentication? That everyone using cell lines 'should use STR (Short Tandem Repeat) profiling for every cell line they use for every publication and for every grant' (16, 17).

Advantages of STR.

- Accessible
- Easy to perform
- Inexpensive
- Robust

STR is the same PCR (Polymerase Chain Reaction)-based method the FBI uses on its forensic DNA samples (16).

- In STR, the PCR amplifies across tetra- or pentanucleotide repeats
- Varying numbers of repeats produce different sized DNA fragments
- Fragments generated by each cell line are unique, and identified and assigned a numerical value by comparing to size standards.
- Result? A database of STR for cell lines that can be compared between labs, using appropriate controls and protocols.

Drawbacks of STR

- Cannot account for genetic drift, which is a common issue in cancer cells.
- Not useful for discriminating human cell lines derived from different tissues of the same person.
- So not useful for comparing HeLa sublines.
- Need guidelines on minimum number of STR loci needing to be tested for each cell line being authenticated.
- Human-specific, not much useful for other species.
- Complicated data interpretation stemming from two sources. One, human cell lines have numerous genomic changes such as chromosome duplications, mutations and rearrangements. Such chromosomal abnormalities yield complex STR profiles that are difficult to interpret. Two, PCR can create artifacts, requiring experience to diagnose correctly.

Finally a list of online resources for list of Misidentified cell lines, and databases of cross-contaminated or misidentified cell lines:
misidentified cell line[Filter – BioSample – NCBI]
Database of Cross-contaminated or Misidentified Cell Lines – ICLAC
Page on atcc.org
Page on atcc.org
Page on atcc.org
Home – BioSample – NCBI

Bibliography

1. Masters, John R. "HeLa cells 50 years on: the good, the bad and the ugly." Nature Reviews Cancer 2.4 (2002): 315-319.
2. Lab Mistakes Hobble Cancer Studies But Scientists Slow to Take Remedies

3. American Type Culture Collection Standards Development Organization Workgroup ASN-0002. "Cell line misidentification: the beginning of the end." Nature Reviews Cancer 10.6 (2010). Page on calis.edu.cn
4. Benham, Frances J., M. Susan Povey, and Harry Harris. "Heterogeneity of alkaline phosphatases in different HeLa lines." Somatic cell genetics 4.1 (1978): 13-25.
5. Kochanowska, I. E., et al. "Osteogenic properties of various HeLa cell lines and the BMP family genes expression." Annals of Transplantation 7.4 (2002): 58-62.
6. Rutledge, Samuel. "What HeLa Cells Are You Using?." The Winnower (2014). The Winnower | DIY Scientific Publishing
7. Landry, Jonathan JM, et al. "The genomic and transcriptomic landscape of a HeLa cell line." *G3: Genes| Genomes| Genetics* 3.8 (2013): 1213-1224. The Genomic and Transcriptomic Landscape of a HeLa Cell Line
8. Mittelman, David, and John H. Wilson. "The fractured genome of HeLa cells." *Genome biology* 14.4 (2013): 111. Page on biomedcentral.com
9. Andrews, Brenda J., and Tracey DePellegrin. "HeLa Sequencing and Genomic Privacy: The Next Chapter." *G3: Genes| Genomes| Genetics* 3.8 (2013): vii-vii. The Next Chapter
10. Greely, Henry T., and Mildred K. Cho. "The Henrietta Lacks legacy grows." *EMBO reports* 14.10 (2013): 849-849. Page on embopress.org
11. Gartler, Stanley M. "Apparent HeLa cell contamination of human heteroploid cell lines." Nature, 1968; 217 (5130): 750-751.
12. Perkel, J.M. Curing cell lines. Biotechniques 2011, 51, 85–90; Page on biotechniques.com
13. American Type Culture Collection Standards Development Organization Workgroup ASN-0002. "Cell line misidentification: the beginning of the end." Nature Reviews Cancer 10.6 (2010). Page on calis.edu.cn
14. MacLeod, Roderick AF, Wilhelm G. Dirks, and Hans G. Drexler. "Where have all the cell lines gone?." International Journal of Cancer 132.5 (2013): 1232-1234. Page on wiley.com
15. Barallon, Rita, et al. "Recommendation of short tandem repeat profiling for authenticating human cell lines, stem cells, and tissues." *In Vitro Cellular & Developmental Biology-Animal* 46.9 (2010): 727-732.
16. Perkel, J.M. Curing cell lines. Biotechniques 2011, 51, 85–90. Page on biotechniques.com
17. Reid, Yvonne, and Joe Mintzer. "The current state of cell contamination and authentication—And what it means for biobanks." *Biopreservation and biobanking* 10.3 (2012): 236-238.

https://www.quora.com/Are-all-HeLa-cells-from-a-commercial-source-equivalent-to-numbers-or-replications/answer/Tirumalai-Kamala