Tech News

Curing Cell Lines

In a January 2011 article describing how estrogen metabolites influence the release of inflammatory molecules from human amnion-derived cells, Italian researchers took advantage of a cell line called WISH, which they describe as “constituting a model for in vitro studies of amnion functions” (1).

There was just one problem: WISH cells have been known for more than 40 years to be highly proliferative HeLa cervical cancer cells. The authors did acknowledge this fact in their conclusions, albeit obliquely and only briefly: “... considering WISH cells as a suitable model for human amnion cells, our results suggest that active estrogen metabolites may finely modulate the onset of human labor. Yet, looking on recent reports, by which WISH cells should be more meaningfully be likened to neoplastic cells, our results could support estrogen metabolite relevance in neoplastic competence via inflammation pathway activation.” (1)

This caveat makes the study’s scientific relevance open for debate. According to coauthor Alessandro Dalpiaz, “WISH cells have the advantage of a much longer survival than primary amnion cells in culture. Moreover, they behave mostly as the primary amnion cells. Therefore WISH cells can be considered as a suitable model for human amnion cells to study the effects of inflammation on the modulation of human labor.”

But the fact is, they are not. ATCC, the U.S.-based cell bank from which the authors obtained their cells, lists their origin as “HeLa contaminant.” The line is listed in CellBank Australia’s Database of Cross-Contaminated or Misidentified Cell Lines, an index of 360-odd lines that are known to be operating under assumed identities. And it was one of 19 contaminated cell lines described in a 1968 letter to *Nature* by University of Washington geneticist Stanley Gartler, (2) who, as Rebecca Skloot reported in *The Immortal Life of Henrietta Lacks*, had two years earlier rocked the cell biology community by announcing at a conference that 18 supposedly unique cell lines were all, in fact, HeLa contaminants.

“What really frustrates me is that Stan Gartler showed that a whole series of lines were cross-contaminated with HeLa cells, and yet those bloody cells are still being used now in their false guise,” exclaims John Masters, professor of experimental

Stanley Gartler first raised awareness of the cell line authentication issue decades ago. He rocked the cell biology community in the late 1960s by announcing that 18 purportedly unique human cell lines were, in fact, HeLa contaminants. Photo courtesy of Stanley Gartler.

pathology at University College London. “For nearly 50 years, people have been using falsely identified cells totally unnecessarily because they haven’t checked.”

The monetary value of all those wasted experiments—not to mention the resulting patents, drug development, and even clinical trials—is incalculable. In 2007, the BBC alleged that “thousands of studies have been invalidated” and “millions of pounds of charity donations and taxpayers’ money have been wasted on worthless cancer studies” as a result of cell line authentication issues (3). In an open letter that same year to Michael Leavitt, then-secretary of the U.S. Department of Health and Human Services (HHS), professor emeritus Roland Nardone

of Catholic University of America attempted to quantitate: “Estimates vary, but as many as 20% of scientific publications using cultured cells may be involved.” (4)

If Nardone, Masters, and others in the cell biology community have their way, such waste will be significantly diminished in the future. Masters and Nardone are members of the ATCC Standards Development Organization Workgroup, which is in the process of formulating standards that can be used in cell line authentication. A final version of the standard (ASN-0002) is anticipated this fall; the main message, says Masters, implores to test early, and often. “It says everyone should use STR [short-tandem repeat] profiling for every cell line they use for every publication

and every grant.” At the same time, those stakeholders with the most policy influence—the journals and funding agencies—are slowly enacting rules requiring authors and grant applicants to do just that [see “A Broader Resolution”]. As Nardone’s letter succinctly put it, “no authentication, no grant.”

The Source Of Contamination

There are two fundamental types of cell line contamination: infection by microbial agents, and cross-contamination by either inter- or intraspecies cell lines. Rates of all such events are surprisingly high; Hans Drexler, head of the Department of Human and Animal Cell Lines at the German Collection of Microorganisms and Cell Cultures (DSMZ), estimates that 1 cell line for every 6–7 submitted to his cell banking facility is not what it says it is. At CellBank Australia, 11% of samples submitted for testing are contaminated with mycoplasma or other cell lines, according to founding manager Amanda Capes-Davis.

In one 2010 study, researchers at the DSMZ reported that of 598 leukemia and lymphoma cell lines in their bank, “187 (31%) were contaminated with mycoplasma and/or a second cell line and 38 (6%) of cell lines [were] contaminated with both.” (5) Thirteen percent of the cells in this analysis were listed as contaminated.

Though HeLa is the primary offender in human lines, there are others. MDA-MB-435, thought to be derived from breast cancer, was shown in a series of papers published in

Hans Drexler of the German cell bank, DSMZ, estimates that one in every six or so lines submitted to the facility are not what they are believed to be. Photo courtesy of DSMZ.

2000–2007 to actually be M14 melanoma cells (6); the presumptive endothelial cell line ECV304 was shown in 1999 to be T24 bladder carcinoma (7); and in a 2009 retraction, a research team at the University of Birmingham, UK, acknowledged that what they thought were human thyroid epithelial cells (TEC61) were in fact JEG3 human choriocarcinoma cells, likely introduced into the cultures via JEG3-conditioned media used to support the cells’ growth. (8) That paper has been cited 12 times, according to the ISI Web of Knowledge.

Cell Line Authentication

Given the magnitude of the problem, it’s no surprise to learn that cell banks subject their lines to some fairly rigorous testing. At CellBank Australia, for instance, the authentication process occupies at least one full-time employee (of a total of four), according to Capes-Davis.

Incoming lines are checked for mycoplasma contamination using three separate test methods and cross-contamination using two. Species of origin is established by sequencing a PCR-amplified segment of the mitochondrial cytochrome c oxidase I gene. Human lines are tested via short tandem repeat (STR) profiling, which is the same PCR-based method the FBI uses to populate its CODIS (Combined DNA Index) database of forensic DNA samples.

Based on variation in the length and number of repeats of certain loci scattered across the human genome, STR profiles are acquired via multiplex PCR and compared against international databases such as the one maintained at DSMZ (www.dsmz.de/human_and_animal_cell_lines/main.php). That database currently lists nearly 2300 profiles from DSMZ, ATCC, and two cell banks in Japan. A new global database to be housed at NCBI and conforming to ASN-0002 recommendations is in development and should be available this fall, according to Liz Kerrigan, Director of Standards and Certification at ATCC.

Such databases help banks determine whether lines really are unique. But the fingerprint really is just a fingerprint, explains Brian

A Broader Resolution

Gartler’s announcement in the pages of *Nature* was just the first pebble in what has become a landslide of evidence of cellular subterfuge. Over the ensuing four decades, many researchers have strongly advocated for better authentication standards throughout the entire scientific community.

Finally, in 2007 the cell biology community came together to draft its open letter to HHS Secretary Leavitt calling on the NIH (and the research publishing community) to require authentication of cell lines in order to publish or receive federal funding. “This group of thought leaders essentially said that the issue of cell line misidentification is significant and plagues scientific research,” says Douglass. “It’s also a waste of tax dollars, because these are federally funded grants.”

The NIH responded (9), agreeing that cell line misidentification is “a serious problem,” but one they could not practically be expected to handle. “Because authentication methods can be quite specific and are continuously evolving, it would be impractical for the NIH to require application of particular methods in all grant applications.” Instead, the agency called on journal peer-reviewers to help ensure cell line validity.

Some small funding agencies have already implemented authentication requirements. The Project Grant Application Handbook for the UK-based Association for International Cancer Research, for instance, states: “The authentication of cell lines is considered an important part of the preliminary data described in any grant application. Applicants should also ensure that all cell lines to be used during the proposed research are adequately authenticated.”

Likewise, journals have started to incorporate cell line validation requirements. Author instructions for the American Association for Cancer Research publications stipulate that work involving human cell lines must indicate where and when the cells were obtained, whether the cell lines have been tested and authenticated, how the cells were tested, and when. Similar statements appear in the *International Journal of Cancer*, *In Vitro Cellular and Developmental Biology - Animal*, and others.

Nature has called for a global searchable database of DNA fingerprints that researchers could use to meet new, firmer guidelines that it suggests funding agencies and journals adopt. “Once this research framework is sufficiently established, major funders will be able to require the validation of all immortal cell lines in order for investigators to retain funding, and journals should (and *Nature* will) require that all lines used in a paper were verified before publication.” (10)

If the experience of the *International Journal of Cancer* is any guide, authentication requirements should pose minimal problems for journals. The *IJC*, which receives some 3,000 submissions per year, has had to “unsubmit” — or hold for further documentation — some 170 manuscripts since it initiated its rule change, says editor-in-chief Peter Lichter. Of those, 86 subsequently resubmitted with the required information, while only 28 have actively withdrawn their manuscripts. Complaints, he says, are rare.

Douglass, the market development manager for Cell Biology and Standards at ATCC. Just as a fingerprint identifies an individual yet says nothing about that person's height or eye color, so too can an STR fingerprint confirm a match or lack thereof between two lines, but nothing at all about phenotype or origin.

Generally speaking, authentication services use 8 or 15 STR loci to establish cell line identity (plus an additional marker to define gender). According to Gabriela Saldanha, Global Strategic Marketing Manager at Promega Corp., which offers kits for STR testing, the odds of a random match using eight STR loci is about one in 100 million; for 15 loci, the odds are on the order of 1×10^{-17} . But unlike crime lab forensics, cell line authentication has to allow for a little wiggle room.

Genetically speaking, cancer cells are highly stable, but STR profiles can "drift" over time due to both loss of heterozygosity and deficiencies in DNA repair mechanisms, explains Wilhelm Dirks, who leads the molecular biology group at DSMZ. Primary tumors, in contrast, are relatively heterogeneous and subject to "bottlenecking selection," which can lead to genetically distinct subclones, says Roderick MacLeod, DSMZ's head of cytogenetics. As a result, an 80% match is generally considered sufficient to prove identity.

The European Collection of Cell Cultures (ECACC) in the UK, with some 1500 mycoplasma-screened and authenticated cell lines, has been employing STR analysis of human cell lines for a decade now, says Jim Cooper, General Collection Project Manager. While some banks, including DSMZ and ATCC use 8-locus panels, ECACC currently uses Applied Biosystems' 16-locus Identifiler primer set, a service it also offers to customers, as well as a cytochrome oxidase I assay for cell line speciation.

Previously, ECACC authenticated lines with a combination of classical DNA fingerprinting (a restriction enzyme/Southern blotting-based test) and the assay Gartler used in 1966: isoenzyme analysis (a colorimetric assay of protein mobility). The change to the new methods, says Cooper, reflects not only cost, but quality. "The cost of the fingerprinting and isoenzyme analysis services seemed too much to bear for our external customers," says Cooper. "Because of concerns over the sourcing of reagents and our drive to consolidate STR profiling of all cell lines, whether human or non-human, we replaced both techniques."

Perhaps no cell bank goes to greater authentication lengths than the DSMZ, which involves six different working groups in the process. Cells submitted to that bank are tested for contamination by a collection of

A cell line early in the cross-contamination process. At this stage, there are two populations in the culture flask. The STR profile in A reveals the mixture, with multiple peaks at many loci—for example, the locus D2S11 has four peaks rather than the expected one or two (see box). The person at the microscope may also become suspicious, as shown by photos B and C; the arrows point to cells from the contaminating population. Over time, in most cases the contaminating population will take over the culture flask. Credit: CellBank Australia.

viruses and mycoplasma. They then are tested for species origin, karyotyped, and immuno-profiled for surface and/or internal markers. Human lines are DNA fingerprinted. According to Drexler, this process takes up to

two weeks to complete, and costs €500–5000; in one exceptional case, says Dirks, his team needed six weeks to confirm the authenticity of a particular line with karyotyping and cell surface marker analysis.

research +
VALIDATION
= publication

DDC has generated over 1,000,000 DNA profiles – and offers fast, accurate cell line authentication.

DNAcenter.com/cell-line

1-888-362-2669

DDC
DNA Diagnostics Center

Any gene. Any locus. Any cell line.

Endogenous gene targeting using rAAV vectors

Vector Features

- ▶ Recombinant adeno-associated virus (rAAV) vector
- ▶ Single-stranded genome, stimulates endogenous HR
- ▶ No double-strand DNA breaks, zero off-target effects

Applications

- ▶ Target any mammalian cell type
- ▶ Gene knock-in: Mutations, SNPs, Protein tag insertions
- ▶ Gene knock-out: Mutations, Optimized bioproducer lines

Available Now

Cell lines

Over 300 ready-to-ship X-MAN (Mutant And Normal) human isogenic cell line pairs

Services

Custom mammalian cell line modification, compound screening, standard & bespoke assays

Reagents

Gold-standard genetically-defined genomic DNA reagents

X-MAN 2011 Catalogue new

To receive your free copy email brochure@horizondiscovery.com

For more information on rAAV genome editing and an extensive reference library, visit www.horizondiscovery.com

horizon
precision genome editing

Best Practices

As Gartler remembers it, the reaction to his 1966 announcement was essentially denial. "People ascribed all sorts of unusual properties to the HeLa cell," he recalls: for instance, that it could survive in the open without culture media, or that it could fly through the air. "That's almost nonsense," he says.

In reality, though, cross-contamination can be just as beguiling. Mistakes can happen: double-dipping with a pipet, working with multiple lines at once, pouring liquids (which creates droplets), and so on. Fortunately, researchers can minimize the damage using some common-sense procedures. Check your lines against the Database of Cross-Contaminated or Misidentified Cell Lines. Don't passage cells too long (Masters advises 3 months or 10 passages, whichever is shorter). And of course, test the cells.

Douglass advises testing new lines when they enter the lab (if obtained from somewhere other than a cell bank), after freezing down a batch, and before publication. These days, validation is relatively inexpensive. ATCC charges \$195 for its Basic STR Profiling Service and \$295 for its Verified Profiling Service, while ECACC charges £180 for species identification and £190.55 for STR-based genotyping.

For the moment, STR testing is available only for human lines. Because mouse and rat lines are so inbred, "variation is almost undetectable," says MacLeod. That means researchers can determine whether their animal cells represent the correct species, but not if they are the correct cells. But ECACC plans to launch a murine cell line STR profiling service in July, following later with rat and primate panels as well, says Cooper. Already, the bank's murine database houses 42 unique profiles, which they hope to extend to 70 in the near future.

Perhaps most importantly, says MacLeod, get your cells from a trusted source: A cell bank. "If you get cells from colleagues and collaborators, then there's always a question mark over the identity of the cells."

References

1. Pavan, B., et al. 2011. Estrogen metabolites in the release of inflammatory mediators from human amnion-derived cells. *Life Sci.* 88:551-558.
2. Gartler, S.M. 1968. Apparent HeLa cell contamination of human heteroploid cell lines," *Nature*, 217:750-751.
3. "Cancer studies 'wasted millions,'" *BBC*, November 20, 2007. http://news.bbc.co.uk/2/hi/programmes/file_on_4/7098882.stm
4. Nardone, R.M. "An Open Letter Regarding the Misidentification and Cross-Contamination of Cell Lines: Significance and Recommendations for Correction," July 11, 2007. <http://cellbank.nibio.go.jp/cellbank/qualitycontrol/OL7-11-07.pdf>
5. Capes-Davis, A., et al. 2010. Check your cultures! A list of cross-contaminated or misidentified cell lines. *Int. J. Cancer* 127:1-8.
6. Rac, J.M., et al. 2007. MDA-MB-435 cells are derived from M14 melanoma cells—a loss for breast cancer, but a boon for melanoma research. *Breast Cancer Res Treat*, 104:13–19.
7. Dirks, W.G., R.A. MacLeod, and H.G. Drexler. 1999. ECV304 (endothelial) is really T24 (bladder carcinoma): Cell line cross-contamination at source. *In Vitro Cell Dev Biol Anim*, 35: 558-589.
8. Patel, V.A., et al. 2009. Retraction. *Am. J. Physiol. Endocrinol. Metab.* 298:E562.
9. Notice Regarding Authentication of Cultured Cell Lines. November 28, 2007. <http://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-017.html>
10. Editorial. 2009. Identity crisis. *Nature* 457:935-936.

Written by Jeffrey M. Perkel, Ph.D.

BioTechniques 51: 85-90 (August 2011) doi: 10.2144/000113714

To purchase reprints of this article, contact: biotechniques@fosterprinting.com

www.BioTechniques.com