Keepin' it real: Authenticated cell lines in biomedical research

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The use of authenticated cell lines is recommended to ensure the integrity of research results. This mini-review provides practical tips to prevent contamination of cell lines as well as details new publication requirements which address the importance of cell line integrity

Cell lines have been important tools in biomedical research for over 50 years. The proper use of cell lines in the right hands can generate data to test a multitude of scientific hypotheses. Yet, throughout history, cell line research has been plagued by contamination and misidentification. In this mini-review, we will look at the importance of cell line authentication, steps to ensure cell line identity, as well as new publication requirements for cell line studies.

Cautionary Tales

We can learn a lot about the importance of cell line authentication by studying the history of cell line misidentification and the resulting negative consequences. The most notorious case of cell line contamination dates back to the 1960's, when Dr. Stanley Gartler performed isoenzyme comparisons that revealed 19 independently derived human cell lines were contaminated with the HeLa cervical adenocarcinoma cell line (1,2). That was just the beginning. HeLa cell line contamination turned out to be so rampant that the American Type Culture Collection (ATCC) still designates some of the cell lines in its inventory as "cross-contaminated" or "misidentified" due to the confounding presence of HeLa cells. Many of these cell lines were deposited in the collection before the advent of modern authentication techniques. The ATCC suggests that "HeLa contaminated cell lines should not be chosen for study when the specific organ or tissue of presumptive origin is of importance to the validity of the research as results can be compromised." Yet, compromised results continued over the years. It would take additional episodes of cell line misidentification accompanied by improvements in authentication methods to advance the field to present day standards.

In a much more recent case, forensic analysis of the RGC-5 rat retinal ganglion cell line demonstrated that these cells were neither rat cells nor retinal ganglion cells. Instead, they were actually 661W cells, a mouse SV-40 T antigen transformed photoreceptor cell line (3). The most likely explanation for this confusion was that the 661W cell line was also being studied in the laboratory of origin and cross-contaminated the cultures designated as RGC-5. These findings undermined the validity of dozens of publications based on RGC-5 studies (3, 4). The RGC-5 episode has brought cell line validation back into the spotlight, in terms of cell culture methodology, cell culture forensics, new verification methods, as well as updated journal requirements for publication of cell line studies.

Cell Line Authentication

There are steps that we can take to prevent the problems of contamination and misidentification of cell cultures. In your own lab, you can screen cell cultures for signs of trouble by ensuring that the cell line under investigation: a) reacts with species-specific probes (eg. human cells react with human primers or human-specific antibodies) and b) expresses markers consistent with the expected phenotype (eg. retinal markers are expressed in retinal cells). Primer or probe incompatibility is a red flag that needs to be addressed before proceeding with additional experiments.

With the development of new technologies, cell line authentication has become more routine and less expensive. The gold standard for cell line authentication is single tandem repeat (STR) DNA analysis. Each cell line has a unique DNA pattern that can be compared to an initial reference standard, preferably an early sample of the original cell line. Mammalian-specific primers can determine whether a particular cell line is contaminated with cells of non-human or non-rodent species, while sex-specific loci can determine gender. There are now several companies that provide cell line authentication services in the $100-$300 range, depending on the extent of testing needed. Once validation is complete, a report is provided to the investigator to

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keep on file. As an example, the R28 retinal precursor cell line (KeraFAST, Eur201) has undergone verification and shown to be of rat origin without contamination by other mammalian cell lines. A baseline genetic profile of these cells is available for comparison testing with other presumptive R28 cells under investigation. In this way, we can ensure the integrity and uniformity of identifiable cell cultures worldwide.

Preserving Cell Line Integrity

Once cell lines are authenticated, there are some very straightforward steps that can be taken in any cell culture laboratory to preserve cell line integrity and prevent cross-contamination. Many of these tips can be found at this website, presented by The International Cell Line Authentication Committee:

In my own lab, we follow some basic rules to prevent cross-contamination and mislabeling. For most of these rules, the underlying reason is the same—to prevent cell-laden droplets from transferring between flasks, pipets and bottles to contaminate other cell cultures:

1. We obtain cell lines from reputable vendors.
2. We assign one separate bottle of cell culture medium for each individual cell line.
3. We do not double-dip pipets into cell culture flasks and then back into bottles.
4. We work with only one cell line at a time in the laminar flow hood.
5. We wipe down the laminar flow hood surface with 70% ethanol after each cell line is used.
6. We always make sure to work with clean hands and clean gloves.

Publication Requirements

With renewed concern about cell line authenticity, coupled with new technologies available for cell line analysis, peer-reviewed journals have recently instituted stricter requirements for publication of research involving cell lines. As one example, the editors of the journal *Molecular Vision* wrote a comprehensive editorial about their expectations for all submitted manuscripts that involve the use of cell lines (5). Proof of cell line authentication is now compulsory. The editors note that manuscripts must show that cell lines are the "correct species of origin, the correct sex and genotype, and express genes and gene products that are specific to the pertinent cell type" (5). The responsibility of cell line authentication remains with the investigator prior to manuscript submission. Manuscripts that report on data derived from the misidentified RGC-5 cell line are now automatically rejected without further review. Clearly, journals are taking cell authentication very seriously, as should we all.

If we desire credible and publishable results, we need to use authenticated cell lines and take the necessary steps to preserve cell line integrity before initiating any cell line experiments. If we all remain vigilant, we can do our part in preserving the identity of cell lines so that we can continue to make conclusive and meaningful contributions to our respective fields in biomedical science.

References

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Link to the R28 cell webpage: http://tinyurl.com/l4t8v6f

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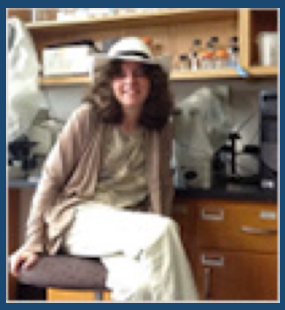

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