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Source: Radiation Research, 189(6) : 551-552

Published By: Radiation Research Society

URL: https://doi.org/10.1667/RR15086.1

RADIATION RESEARCH **189**, 551–552 (2018)
0033-7587/18 $15.00

DOI: 10.1667/RR15086.1

COMMENTARY

Identity Crisis – Rigor and Reproducibility in Human Cell Lines

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Cell line identity (more precisely, misidentification) has become a major source of concern for funding agencies, conscientious investigators, publishers and pharmaceutical companies. Worldwide, up to one-half of commonly used cell lines are believed to be misidentified (*1–4*). Incorrect or misidentified cell lines are a concern for both cancer and non-cancer research and should be a concern for the readers of *Radiation Research* due to concerns about data replicability, and the not-insignificant effects on the pyramidal nature of scientific research. Cross-contamination appears to occur shortly after cell line establishment, leading to hundreds of lines with no known authentic stock (*5, 6*). It has been estimated that studies using contaminated or misidentified cells affects up to 10% of the literature (*7*), and could cost the U.S. as much as $20 billion per year through publication of irreproducible results (*8*).

Mistaken identities of cell lines can arise as a result of cross-contamination during routine culture, accidental mistakes in labeling of cell lines or through malicious intent. Over the last 10 years, improved genomic techniques have been established that enable high-confidence identification of cell lines for relatively low cost. Short-tandem repeat (STR) testing (*9*) has become the *de facto* and recommended standard used for human cell line identification (*10, 11*). At least eight core STR loci plus amelogenin should be used, although many assay kits now assess more loci (*12*). Large cell line repositories (e.g., German Collection of Microorganisms and Cell Cultures, and American Type Culture Collection) provide STR profiles on all cell lines they distribute, which can be used to manually cross reference test results with published standards. In addition, online resources such as Cellosaurus (https://web.expasy.org/cellosaurus/) enable users to match their cell line's STR profiles to published databases. A minimum of 80% match has been recommended to discriminate between related and unrelated samples (*12, 13*). An increasing number of journals now require cell line identity confirmation, although their requirements and the rigor with which they are imposed vary greatly (*14–17*).

For our laboratory, the effort to ensure cell line identity begins prior to obtaining the cells. Whenever possible, all cell lines are obtained from verified commercial sources with published STR profiles. When this is impossible, we request shipment of cell lines directly from the originating laboratory. Cells are handled by a single technician in a dedicated incubator and undergo initial STR testing at the time of large-scale cryopreservation to confirm the identity of the cells. This initial preservation forms the reference stock for our laboratory that will be used in our ongoing and future work. Each individual laboratory member who wishes to work with a given cell line can obtain a single vial of this cell line and is responsible for establishing their own cryopreserved repository to avoid depleting the central resource. Details regarding STR testing are available on a local network and researchers are encouraged to routinely confirm the STR profile of their cells. All cell lines are cultured for less than three months before returning to an identity-confirmed early-passage frozen stock or having STR profile performed. For projects that require longer duration passaging of a given cell line, such as developing drug-resistant lines, we confirm the identity by STR at the end of the project and at regular intervals during culture. While the cost of these steps is not insignificant in a laboratory that routinely has 10–12 cell lines in use, it is a fraction of the potential cost associated with use of incorrect or misidentified cell lines and is critical for the comparison of results among research groups and the translation of research findings to clinical practice.

We would encourage the use of the following best practices:

1. Perform STR profiles of all cell lines upon receipt from both commercial and academic laboratories.
2. Perform (and publish) STR profiles of any newly established cell lines. Share STR profiles simultaneously with the sharing of cell lines and publish to a publicly accessible database such as Cellosaurus.

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3. Provide (in a supplementary table) STR profiles of cell lines used in manuscripts. Include the loci tested, reads, date of testing and where the test was performed.
4. Provide the source of the cells (commercial, collaborator, author generated) culture conditions and date (year) of receipt in all manuscripts. We suggest that this data be included in a supplementary table so that it does not require printed space.
5. Encourage journals to include supplementary data in the online pdf that most readers download.
6. As a reviewer, ask for details of STR testing on cell lines to be included as described above.

ACKNOWLEDGMENT

RJK was supported by the NIH/NCI P30 CA014520-UW Comprehensive Cancer Center Grant.

Received: March 14, 2018; accepted: March 19, 2018; published online: April 13, 2018

REFERENCES

1. Huang Y, Liu Y, Zheng C, Shen C. Investigation of cross-contamination and misidentification of 278 widely used tumor cell lines. PloS One 2017; 12:e0170384.
2. Lacroix M. Persistent use of ''false'' cell lines. Int J Cancer 2008; 122:1–4.
3. Kniss DA, Summerfield TL. Discovery of HeLa cell contamination in HES cells: Call for cell line authentication in reproductive biology research. Reprod Sci 2014; 21:1015–9.
4. Bian X, Yang Z, Feng H, Sun H, Liu Y. A combination of species identification and STR profiling identifies cross-contaminated cells from 482 human tumor cell lines. Sci Rep 2017; 7:9774.
5. MacLeod RA, Dirks WG, Matsuo Y, Kaufmann M, Milch H, Drexler HG. Widespread intraspecies cross-contamination of human tumor cell lines arising at source. Int J Cancer 1999; 83:555–63.
6. Capes-Davis A, Theodosopoulos G, Atkin I, Drexler HG, Kohara A, MacLeod RA, et al. Check your cultures! A list of cross-contaminated or misidentified cell lines. Int J Cancer 2010; 127:1–8.
7. Horbach S, Halffman W. The ghosts of HeLa: How cell line misidentification contaminates the scientific literature. PloS One 2017; 12:e0186281.
8. Freedman LP, Cockburn IM, Simcoe TS. The economics of reproducibility in preclinical research. PLoS Biol 2015; 13:e1002165.
9. Masters JR, Thomson JA, Daly-Burns B, Reid YA, Dirks WG, Packer P, et al. Short tandem repeat profiling provides an international reference standard for human cell lines. Proc Natl Acad Sci U S A 2001; 98:8012–7.
10. Barallon R, Bauer SR, Butler J, Capes-Davis A, Dirks WG, Elmore E, et al. Recommendation of short tandem repeat profiling for authenticating human cell lines, stem cells, and tissues. In Vitro Cell Dev Biol Anim 2010; 46:727–32.
11. American Type Culture Collection Standards Development Organization Workgroup ASN. Cell line misidentification: the beginning of the end. Nat Rev Cancer 2010; 10:441–8.
12. Capes-Davis A, Reid YA, Kline MC, Storts DR, Strauss E, Dirks WG, et al. Match criteria for human cell line authentication: where do we draw the line? Int J Cancer 2013; 132:2510–9.
13. Bady P, Diserens AC, Castella V, Kalt S, Heinimann K, Hamou MF, et al. DNA fingerprinting of glioma cell lines and considerations on similarity measurements. Neuro Oncol 2012; 14:701–11.
14. Blow NS. Right cell, wrong cell. Biotechniques 2011; 51:75.
15. Potash J, Anderson KC. What's Your Line? Clin Cancer Res 2009; 15:4251.
16. Editorial: Identity crisis. Nature 2009; 457:935–6.
17. Lichter P, Allgayer H, Bartsch H, Fusenig N, Hemminki K, von Knebel Doeberitz M, et al. Obligation for cell line authentication: appeal for concerted action. Int J Cancer 2010; 126:1.