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EDITORIAL

Editorial

The Imperative to Authenticate Cell Lines

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Contamination plagues cell culture research and requires our immediate attention. Cell culture is a powerful tool to investigate the mechanisms of disease, test new drug therapies, and potentially create and engineer tissues. In vitro culture facilitates cancer and stem cell research, in particular. These areas are of great interest to orthopaedic researchers and the subject of many publications in our translational and clinical research journal, Clinical Orthopaedics and Related Research. The integrity of our research and publication effort requires that results are accurate and reproducible. This means that contamination of cell lines must be recognized and eliminated.

Investigators often overlook the limitations and pitfalls of their cell culture technique. One of the most widely recognized is bacterial contamination [2]. The addition of increasing doses of antibiotics is the usual response, but prevention would be preferable. Even more serious is cellular contamination of the culture. As reported by Skloot, at the Second Decennial Review Conference on Cell Tissue and Organ Culture Stanley Gertler identified the phenomenon in 1966 [6]. HeLa cervical carcinoma cells took over many other cell lines, compromising many experiments. Relatively little has been done since then to address the problem. The origin of many cell lines remain misidentified [1, 2, 5, 7], and neoplastic cells contaminate other lines [1, 2, 5, 6]. This has led to many erroneous publications and spawned false lines of research [1, 2, 5, 7]. Recent controversies regarding the spontaneous transformation of mesenchymal stem cells in culture, and the misidentification of the origin of various cell lines highlight the problem [5, 7]. For example, the American Type Culture Collection (ATCC) recently found that the U251 and U373 cell lines had a common origin [3, 7]. This may have affected therapeutic research. Another example was the sarcomatous transformation of mesenchymal stem cells that in reality was attributable to contamination by H1080 cells [7]. Such problems highlight the importance of identifying the source of cell lines in use, and that it is essential to prove their identity.

The "Methods and Materials" of scientific investigations often receive too little attention by investigators, reviewers, and readers, even though we all recognize the experimental conditions will determine the results. Clinical Orthopaedics and Related Research is joining an international attempt, spearheaded by the Standard Development Organization at the ATCC [2] to address the problems affecting cell culture accuracy. The journal will require much detail regarding the provenance of cell lines. Investigators will have to prove the nature of their cells, in a manner appropriate for the investigation. The requirements will vary.

Authors are encouraged to take a two-step approach to the problem [2]. First, use existing resources to identify contaminated cell lines. Published lists are available from many sources—see the list of Cell Line Guides below. Known cross-contaminated lines include 360 cell lines, drawn from 68 references. Most contaminants arise in the same species, with HeLa still the most frequent (29%; 106/360) among human cell lines. Interspecies contaminants are less frequent (9%; 33/360), but are very important when dealing with rodent cell lines. Second, the cell line must be proven authentic, even if cross contamination has not been reported. Many investigators are not doing this: in a survey

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of 483 respondents regarding cell line usage, 35% used cell lines procured from another laboratory, and only approximately ½ were tested for cross contamination [1]. Unless a cell line has come directly from a repository or other laboratory performing identification testing, it should be tested on arrival in your laboratory, and periodically while in use. The International Reference Standard for human cell lines is to profile short tandem repeat (STR) DNA fingerprinting [2, 4]. Other approaches, such as single-nucleotide polymorphism (SNP) analysis, are accurate and acceptable. The guidelines will be flexible in this rapidly evolving field.

Clinical Orthopaedics and Related Research is committed to promoting these guidelines to encourage and publish the highest quality research.

Cell Line Guides to Identify Contamination

1. ATCC: http://www.atcc.org/
2. CellBank Australia http://www.cellbankaustralia.com/ DSMZ: http://www.dsmz.de/
3. Database of Cross-Contaminated or Misidentified Cell Lines—Amanda Capes-Davis and R. Ian Freshney: http://www.hpacultures.org.uk/media/E50/3B/Cell_Line_Cross_Contaminations_v6_0.pdf
4. ECACC: http://www.hpacultures.org.uk/collections/ecacc.jsp
5. JCRB: http://cellbank.nibio.go.jp/
6. RIKEN Bioresource Center Cell Bank: http://www.brc.riken.go.jp/lab/cell/english/guide.shtml
7. Wikipedia list of contaminated cell lines: http://en.wikipedia.org/wiki/List_of_contaminated_cell_lines

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