Acta Pharmacol Sin 2008 Jul; 29 (7): 877–880

Letter to Editor

Cell line cross-contamination in biomedical research: a call to prevent unawareness

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Key words

cross-contamination; false cell lines; pharmacological screening

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Received 2008-03-04
Accepted 2008-04-10

doi: 10.1111/j.1745-7254.2008.00809.x

Abstract

During the 1950s, cross-contamination of cell lines emerged as a problem with serious consequences on the quality of biomedical research. Unfortunately, this situation has worsened over years. In this context, some actions should be urgently undertaken to avoid the generation of misleading data due to the increasingly and sometimes neglected use of cross-contaminated cell lines. Unawareness about this problem may then turn many scientists into victims or even perpetrators of this unwanted situation. Collaborative actions involving researchers, cell banks, journals, and funding agencies are needed to save the scientific reputation as well as many public or private resources that are used to produce misleading data.

False cell lines: ghosts in biomedical research

Cell lines are widely used in several aspects of biomedical research, particularly in pharmacological sciences as valuable tools to test pharmacological activities and dissecting mechanisms of drug actions and to study putative pharmacological targets among others experimental approaches, in addition to the classical advantages, such as easy handling, an unlimited self-replicating source, a high degree of homogeneity, and easily replacement of frozen stocks[1]. However, the use of cell lines in biomedical research has intrinsic limitations, such as genotypic and phenotypic drifts. A serious extrinsic problem, referred to the concept of false cell lines, has largely been ignored over years. The problem arises from cross-contamination and the continued use of cross-contaminated cell lines under false descriptions.

Cross-contamination of cell lines has thus emerged as a real problem which seriously compromises the quality of research, and unfortunately, a large portion of the scientific community is still apparently unaware of or indifferent to this problem [2,3].

This problem was first reported in the 1950s, largely due to interspecies contamination[4]. In the late 1960s, a shocking report demonstrated that most (if not all) of the putative unique human cell lines available at that time were actually derivatives of the HeLa cell line[5]. In the next 2 decades, the situation remained without major improvements[6,7].

However Pandora's box was really opened in the 1990s, mainly due to the availability of new techniques for checking cell line identities, showing that the problem is still expanding and affects many cell lines used as classical *in vitro* models for many years. At present, the incidence of research papers flawed by the use of misidentified and cross-contaminated cell culture is estimated to be between 15% and 20%[8].

In 1999, a survey performed by the German Collection of Microorganisms and Cell Cultures, identified that 18% of cell lines analyzed (45/252) were cross-contaminated by the originators[9].

However, the magnitude of this situation is probably higher because many cell lines currently in use in individual laboratories throughout the world are obtained indirectly and not from recognized cell repositories.

One of the most affected areas is cancer research, and particularly cancer pharmacology, considering that *in vitro* models using cancer cell lines are widely used in many laboratories. Obviously, this situation also reaches tumor implantation models.

In this area, HeLa cells, the first established human cancer cell line, are reported to be the origin of many cell lines now in use by researchers, apparently derived from different

tissues as demonstrated for heart (Girardi heart), epidermoid cancer (KB), liver (Chang liver), eye, and amnion (WISH) cell lines[10] The real magnitude of this problem was highlighted by Masters in 2005[11]; a search in Medline (2000–2004) showed that many cell lines, known as HeLa cell cross-contaminants, were claimed to be from different origins by authors, appearing in 9 citations for Int-407 ("intestinal" cells), 45 citations for WISH ("amnion" cells), 59 citations for Chang liver ("liver" cells), 470 citations for Hep-2 ("human nasal carcinoma cells"), and 556 citations for KB ("oral carcinoma" cells). In total, there were 1149 papers in which false cell lines were used.

A similar situation has also occurred with some prostate carcinoma cell lines[12,13]. TSU-Pr1 was originally described in 1987 as having been derived from a prostate carcinoma lymph node metastasis[14]. Later, JCA-1 was described in 1990 as having been derived from a primary prostate carcinoma[15]. Both cell lines, originally believed to be of prostatic origin, and therefore widely used for many years as prostatic carcinoma models, are actually derivatives of the bladder carcinoma cell line T24[16].

Multidrug resistance is an intensive research area in cancer pharmacology in which the use of cell lines as experimental models is widely accepted. MCF-7/AdrR cells, expressing P-glycoprotein (ABCB1), were among the first multidrug-resistant cell lines derived by continuous *in vitro* exposure to increasing drug concentrations[17,18]. Both MCF-7/AdrR cells and the presumed parental cell line MCF-7 were part of the famous NCI 60 panel, a group of 60 selected human cancer cell lines that has been utilized since 1990 in various pharmacological[19,20], pharmacogenomic[21], and proteomic screening programs[22]. In the late 1990s, the MCF-7 and MCF-7/AdrR cell lines were reported as most likely derived from 2 different donors[23]. Very recently, MCF-7/AdrR, which was then redesignated as NCI/Adr-RES, was found to be derived from OVCAR-8 ovarian adenocarcinoma cells[24]. Furthermore, human leukemia–lymphoma cell lines are extremely important resources for research in many disciplines, not only those dealing with cancer. Recently, a large study carried out in 550 leukemia–lymphoma cell lines demonstrated alarming results. The overall incidence of cross-contaminated cell lines reached almost 15%, either among cell lines obtained directly from original investigators (59/395) or when cell lines were obtained from secondary sources (23/155). It is noteworthy that classic leukemia cell lines, such as CCRF-CEM, HL-60, JURKAT, K-562, and U-937 were found to account for the majority of cross-contaminations[25].

Vascular biology is another important area where cross-contamination is a real menace to research quality. Today the role of endothelium in cardiovascular and immune systems, as well as in processes, such as inflammation, tumor angiogenesis and metastasis, is well-documented[26,27].

In this sense, many endothelial cell lines are widely used as alternative models to the time-consuming and low-yielding approach of human umbilical cord endothelial cell primary culture.

For many years, one of these cell lines (ECV304) was considered to be a spontaneously transformed line derived from a Japanese human umbilical vein endothelial cells (HUVEC) culture[28,29]. However, differences between ECV304 and HUVEC were increasingly reported, leading to the conclusion that ECV304 is a derivative of the human urinary bladder carcinoma T24 cell line because of cross-contamination[30].

At present, and after almost 1 decade of the original report that alerted the scientific community, many papers are still published using ECV304 as a human endothelial cell line[31].

In regards to the cross-contamination of ECV304, great effort has been made to publicize the fact that this cell line is a derivative of T24, particularly those carried out by important cell and tissue repositories, including the German Culture Collection of Microorganisms and Cell Cultures, who were the first to report the cross-contamination, the American Type Culture Collection (ATCC), and the Japanese Collection of Research Bioresources. This information is explicitly stated in their catalogues and websites, and the ATCC has even contacted all customers who have ever purchased this cell line, alerting them about it. In spite of this, a simple search in Pubmed showed more than 35 papers published during 2007. Some authors still argued its usefulness considering the endothelial cell-like features displayed by this cell line[32]. However, all scientists should seriously take into consideration that ECV304 is not of HUVEC origin and is therefore an inappropriate cell line to study endothelial cell biology.

Calling for ghostbusters

It is important that scientists, the main actors in this expanding situation, assume that the authentication of all cell lines should be an essential part of any cell culture operation in either research or academic laboratories. It is not a theoretical problem anymore; the consequences of working with a cell line that is misidentified or cross-contaminated with cells from different origins has became a real problem with serious consequences, such as invalidating results, loss of scientific credibility, and even devaluing products or drugs[33].

It is therefore important to perform identity-checking procedures in all cell cultures. At present, many methods are

available from different complexities and costs, covering enzyme polymorphisms determination[34], cytogenetic analysis[35], HLA typing[36], immunophenotypic and immunocytochemical analyses[37], and finally, DNA fingerprinting[38]. More recently, short tandem repeat profiling of DNA has been shown to provide an international reference standard that could be applied to human cell lines[39].

Different authors have discussed different approaches to stop the problem of using false or cross-contaminated cell lines[3-8]. However, one important point is that many researchers, reviewers, and even journals appear to be unaware of which cell lines are known or suspected to be contaminated, even after they has been reported. Therefore, important actions have been taken by cell banks by increasingly checking submissions, stopping the delivery of contaminated cell lines, and informing all users of the corresponding information in their websites. However, it should be mentioned that many cell lines are shared between researchers before being submitted.

It has become increasingly important that researchers demonstrate that research is conducted to the highest standards. In this context, laboratory directors must ensure the implementation of quality assurance programs as part of the quality control system established for any standard operating procedures, and they should ensure that all staff members are made aware of the problem of cross-contamination.

Researchers should also know that if the performance of any cell lines is not consistent or if results are unexpected, identity checking is highly recommended. If identity-checking procedures are not available in their laboratories, there are many institutions worldwide that offer speedy cell line identity-checking services at reasonable prices.

Journal Club has become a well-known and important activity in any research group, offering a scenario for analysis and discussion of scientific papers, experimental data, techniques, as well as many other activities. The inclusion of topics dealing with research quality particularly cell line cross-contamination, undoubtedly help to disseminate this information. It is worth mentioning that graduate and doctoral students are always very motivated to attend these activities, and therefore the message will be also directed to a new generation of scientists.

Another important role should be assumed during the peer-review process, particularly by reviewers. Reviewers should check that the papers do not use cell lines already declared as false or cross-contaminated by cell repositories, taking in mind that the work the researchers carried out is essential in sustaining the reputation of the journal they are contributing to, in addition to all efforts made by editorial boards and editors. Unfortunately, it is apparent that many referees and even some editors are unaware of the problems associated with false cell lines. Perhaps the availability of this information inside the journal's webpage, particularly in the instructions to authors section, explicitly or through links to a list of cell lines known to be cross-contaminated, will make many researchers aware that their cell lines may be from sources other than cell banks. This action may have positive effects considering that many researchers have become victims or even perpetrators of this unwanted situation, mainly because they were unaware of the situation.

In summary, this situation is of major concern. It must be faced with seriousness to stop the apparent unawareness of some researchers about cell line identity. Collaborative actions involving researchers, cell banks, journals, and funding agencies are needed to save scientific reputations, as well as many public or private resources that are used to produce misleading data.

Author contribution

Armando ROJAS, Ileana GONZALEZ, Héctor FIGUEROA jointly developed the plan and outline of the article, and worked together on performing the literature review, write and edit the manuscript

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