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MINI REVIEW

Persistent use of "false" cell lines

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From HeLa and its multiple identities, to MDA-MB-435, erroneously and widely used as breast cancer cells, the history of cancer cell lines is rich in misidentification and cross-contamination events. Despite the fact that these problems were regularly signaled during the last decades, many actors of research still seem to ignore them. A never-ending story? Solutions exist, notably based on recent technical advances in cell line authentication (short tandem repeat analysis). However, a collaborative action involving users of cell lines, cell banks, journals and funding agencies is needed to achieve success.

Key words: cell lines; misidentification; cross-contamination; authentication; short tandem repeat; HeLa; MDA-MB-435; misuse

Review

Examination of the current scientific literature indicates that a large percentage of papers reporting on experimental cancer research use human cell lines. Indeed, cell lines are expected to provide an unlimited source of specific self-replicating material, free of contaminating cells and often easily cultured in simple standard media. Alas, since the establishment of the first cancer cell lines, problems with misidentification and cross-contamination have occurred and seriously compromised research. These problems were regularly brought to light during the past decades,[1–14] but have received few audience until cell banks (American Tissue Culture Collection, ATCC; Deutsche Sammlung von Mikroorganismen und Zellkulturen, DSMZ; European Collection of Cell cultures, ECACC; Japanese Collection of Research Bioresources, JCRB) decided to act by informing their clients or even by withdrawing the false cell lines from their catalogue. It must be noted that the DSMZ has been a pioneer and is still a major actor in that process.

Various recent studies have shown that between 18 and 36% of cell lines were incorrectly designated.[5,6,14] It is likely that new false cell lines continue to be established without the knowledge of their originators. At the same time, detection of false cell lines is rendered increasingly difficult as numbers and varieties of circulating cell lines increase. Even more worrisome is the fact that many cell lines that have been proven false, sometimes since years, are still used by researchers who seem to ignore their true identity or who act as if they were ignoring it.

This is notably illustrated by Table I, which presents a nonexhaustive list of misidentified or cross-contaminated cell lines that have been cited during the first semester of 2007 by scientists apparently not aware of their exact identity. The search was performed using the HighWire database (http://highwire.stanford.edu) including PubMed journals.

A significant part of these cell lines have been contaminated with HeLa cells,[31] which, indeed, are frequently used in the laboratories, are robust, and multiply rapidly.[32] In a recent (2004) survey of 483 mammalian cell culturists, it was shown that 32% of respondents used HeLa cells and 9% well-known HeLa contaminants (including Hep-2, KB, WISH, Chang Liver, INT407). Only about a third of respondents were testing their lines for cell identity.[33] Thus, it is not surprising that many researchers are still using HeLa contaminants without apparent awareness of their true identity.

Some of the cell lines mentioned in the Table I are intensively used under their false identity. This is notably observed for Chang Liver, ECV304, KB, SK-N-MC, MCF-7/ADR, MDA-MB-435 cells… In some cases, the incriminated articles are from researchers not always familiar with the world of tumor cell lines, for instance toxicologists or chemists who wanted to test natural or modified compounds on a well-known cell line, which they therefore considered as highly representative.

For 6 of the cell lines listed in Table I, a more detailed HighWire database search was performed to identify the number of articles mentioning them under their false identity during the last years (Table II). From the Table, it appears that: (*i*) WISH and Hep-2/Hep2 cell lines are still used under their false identity by several researchers, despite the fact that their misidentification was shown in 1976[15] or 1988,[16] respectively; (*ii*) the misuse of DAMI (identified as HEL erythroleukemia cells in 1997[27]) and ECV-304/ECV304 (identified as T24 bladder carcinoma in 1999[22]) cell lines does not appear to rapidly decrease over years, and the incertitude on the exact origin of HBL-100 cells (presence of Y chromosome mentioned before 2003) is apparently not a problem for dozens of research teams.

The misuse of several cell lines appears to be relatively more frequent in works originating from various emerging countries (South Corea, India,…), and particularly from China. For instance, 45 on 102 (44%) papers published in 2006 and presented ECV-304/ECV304 cells under their false identity were from China, as there were 21 on 66 (32%) articles describing Hep-2/Hep2 cells as laryngeal cells and 5 on 22 (23%) articles in which WISH cells were used as amnion-derived cells. China was culturally isolated a long time, what could explain why so many chinese researchers seem not to be aware of cell line cross-contamination.

Paradoxically, it can arrive that false cell lines are exactly appreciated because they have a characteristic that distinguish them from other cell lines of the same supposed (and actually erroneous) origin. For instance, one of the most recently unmasked cell lines, the putative "breast cancer" cell line MDA-MB-435 had gained a great popularity due to its unrivaled metastatic efficiency in nude mice.[34,35] Contrasting with most breast cancer cell lines, which have an epithelial-like aspect, MDA-MB-435 cells express a mesenchymal-like portrait.[35] This feature has favoured the use of MDA-MB-435 cells, since it was previously widely believed that most breast cancer cells should undergo an epithelial-to-mesenchymal phenotype transition (EMT) to be able to metastasize.[36] MDA-MB-435 cells were for that reason considered as very advanced in the process of metastasization. It is now established that EMT is in fact rarely seen in breast cancer progression.[37] The melanocytic nature of MDA-MB-435 cells was first suspected following micro-array studies, where these cells were found to cluster with melanoma cells, rather than with other breast cancer cell lines.[38] Afterward, MDA-MB-435 cells were found to express several genes commonly transcribed in melanocytes, such as RXRG, TYR, ACP5 and DCP, but which are not found in vari-

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TABLE I – NONEXHAUSTIVE LIST OF MISIDENTIFIED OR CROSS-CONTAMINATED CELL LINES THAT HAVE BEEN CITED DURING THE FIRST SEMESTER OF 2007 BY SCIENTISTS APPARENTLY NOT AWARE OF THEIR EXACT IDENTITY

Cell line	Putative origin	True identity	Reference(s) identifying cross-contamination or misidentification
Chang liver	Liver cells	HeLa cells (glandular cancer of the cervix)	15
Girardi heart	Atrial myoblast cells	HeLa cells	15
Hep-2 (or Hep2)	Larynx carcinoma cells	HeLa cells	16
INT407 (or INT-407, or Intestine 407)	Embryonic intestine cells	HeLa cells	15
J111	Monocytic leukemia cells	HeLa cells	15
KB	Oral epidermoid carcinoma cells	HeLa cells	2,3,17,18
L132	Embryonic lung epithelium cells	HeLa cells	15
MT-1 (or MT1)	Breast cancer cells	HeLa cells	6
NCTC2544	Skin epithelium cells (keratinocytes)	HeLa cells	15
WISH	Amnion cells	HeLa cells	15
Wong-Kilbourne	Conjunctiva-derived cells	HeLa cells	15
RPMI-8402 (or RPMI8402)	T cell leukemia	Unknown	19
IM-9 (or IM9)	Multiple myeloma cells	Epstein-Barr virus-transfected B cell lymphoblastoid line	20
HBL-100 (or HBL100)	Breast transformed but non-tumorigenic cells	Unknown, and not female (found to contain Y chromosome)	ATCC website (http://www.lgcpromochem-atcc.com/common/cultures/probline.cfm)
TSU-Pr1 (or TSUPr1)	Prostate cancer cells	T24 cells (bladder cancer)	21
ECV-304 (or ECV304)	"Spontaneously transformed" umbilical cord endothelial cells	T24 cells	22–24
EJ138	Bladder cancer cells	T24 cells	14 and ECACC website
EJ-1 (or EJ1)	Bladder cancer cells	T24 cells	14 and ECACC website
PPC-1 (or PPC1)	Prostate cancer cells	PC-3 cells (prostate cancer)	25
ALVA-31 (or ALVA31)	Prostate cancer cells	PC-3 cells	25
ALVA-41 (or ALVA41)	Prostate cancer cells	PC-3 cells	25
SK-N-MC	Neuroblastoma cells	Ewing family tumor cells	26
DAMI	Megakaryocyte	HEL cells (erythroleukemia)	27
HS-Sultan	Plasma cell line (multiple myeloma)	Jijoye cells (Burkitt's lymphoma)	20
ARH-77 (or ARH77)	Plasma cells from a multiple myeloma patient	Epstein-Barr virus-transfected B cell lymphoblastoid line	19
WiDr	Colon cancer cells	HT-29 cells (colon carcinoma)	28
SNB-19 (or SNB19)	Glioblastoma cells	U-373MG cells (glioblastoma)	14 and ATCC website
U251	Glioblastoma cells	U-373MG cells	14 and ATCC website
$MCF\text{-}7_{ADR}$ (re-designated NCI/ADR-RES)	Breast cancer cells	OVCAR-8 cells (ovarian cancer)	29
MDA-MB-435 (or MDA-MB-435S, or MDA-MB435, or MDA-435)	Breast cancer cells	M14 cells (melanoma)	30

ATCC, American tissue culture collection; ECACC, European collection of cell cultures.

TABLE II – NUMBER OF ARTICLES CITING SEVERAL CELL LINES UNDER THEIR FALSE IDENTITY

Cell line	Year									
	1990	1995	2000	2001	2002	2003	2004	2005	2006	2007[1]
ECV-304 or ECV304	1	15	101	124	132	111	120	109	102	>53
DAMI	2	28	19	16	20	15	15	9	7	>7
HBL-100 or HBL100	22	19	57	59	51	48	47	31	40	>16
Hep-2 or Hep2	18	25	52	58	48	65	58	87	66	>53
MDA-MB-435 or MDA-MB-435S or MDA-MB435 or MDA-435	5	33	101	141	164	173	276	276	272	>140
WISH	6	7	22	19	31	30	23	23	22	>11

[1]Search performed in August 2007.

ous commonly used breast cancer cell lines.[39] Expression of melanocyte proteins tyrosinase and melan-A by MDA-MB-435 cells was also shown.[40] However, these published observations were not followed by a decrease in the use of MDA-MB-435 as breast cancer cells (see Table II). MDA-MB-435 cells are in fact derived from the melanoma cell line M14. The misidentification is likely to have occurred prior to 1982 and therefore, nearly all of the existing literature using the MDA-MB-435 cell line describes the M14 melanoma cell line, which has been far less studied under its true name.[30]

Of note, another cell line, LCC15-MB, which has not been mentioned in 2007, was recently identified as being MDA-MB-435,[41] thus in reality M14 melanoma cells. LCC15-MB had drawn attention due to its invasive and metastatic phenotype. Moreover, as these cells were believed to originate from a bone metastase in a breast cancer patient, they seemed to constitute a useful model for studying molecular mechanisms important for breast cancer metastasis to bone.[42]

In a recent white paper,[43] Dr. Roland Nardone proposed cell line authentication as a condition for the award of research grants

and for the publication of research findings. Clearly, resolution of the problem of misidentification and cross-contamination requires the conscientization and the collaboration of all involved actors: users (including originators) of cell lines, cell banks, journals and funding agencies.

Users normally do not wish to use false cell lines that are the basis of misleading publications, which can potentially have a very high cost in terms of invalid hypotheses and paradigms, misspent effort and protracted development of patient treatments. Indeed, only in a very few investigations is the exact origin of a cell line devoid of any importance. However, most (new) cell lines are freely exchanged between laboratories, rarely having their identities checked. To avoid cross-contamination of these lines, periodic reauthentication of cell lines is advisable. In addition, working from validated freeze-downs, where cells are maintained in culture, and ideally separated from other cell lines, should minimize the risk of cross-contamination.[12,44]

All reputable cell banks now employ methods to confirm the identity and origin of the cell lines they distribute. This is notably because distribution of misidentified or cross-contaminated cell lines, even when supplied in good faith, may later be the subject of costly and embarassing recall actions. Moreover, cell banks may facilitate de novo detection of cross-contamination by identifying untoward matches between new and existing cell lines. Most cell banks may also test, to a low cost, cell lines provided by their users or originators. While various techniques, not described here, have been used in the past, recent technical advances have led to the development of short tandem repeat (STR) analysis. STRs are repetitive sequences characterized by a variable number of repeated short sequence elements of 2–7 bp in length as a unit (*e.g.*, di-, tri-, tetra-nucleotide sequences), also known as microsatellites or simple sequence repeats. They are highly polymorphic, the repeat sizes are small and can be easily amplified by the polymerase chain reaction method. Furthermore, when the sizes of the products (accurate to 1 base pair) are determined, a series of numbers are generated, which can be used as a bar code for that DNA source. A registry of bar codes would make it easy to compare DNA samples and thus allow efficient cell line authentication, as notably shown by an international consortium.[45] The STR method, although not perfect,[46] is easy, reliable, inexpensive and can be done "in house" or analyzed by a commercial laboratory.[14,45,47–50]

The peer review process carried out by many (but not all) journals and funding agencies still fails to consider the authenticity of the cell lines used. Editors of journals and heads of agencies should be encouraged to examine such issues and, *in fine*, to reject papers from authors unable to substantiate the authenticity of the cell lines they have used. Along the same line, publication of new cell lines by originators, or the funding of their production should be conditional upon these lines being made freely available to other investigators, for instance by reposition in cell banks.

It is now time for a concerted action. Otherwise, days and costly resources will continue to be wasted, as a result of spurious experimental results, and some scientific reputations will continue to face the risk of being compromised.

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References

1. Gartler SM. Apparent HeLa cell contamination of human heteroploid cell lines. Nature 1968;217:750–1.
2. Lavappa KS, Macy ML, Shannon JE. Examination of ATCC stocks for HeLa marker chromosomes in human cell lines. Nature 1976;259:211–13.
3. Nelson-Rees WA, Daniels DW, Flandermeyer RR. Cross-contamination of cells in culture. Science 1981;212:446–52.
4. Satoh M, Takeuchi M. Cross-contamination of cell lines as revealed by DNA fingerprinting in the IFO animal cell bank. Res Commun Inst Ferment 1993;16:18–23.
5. Markovic O, Markovic N. Cell cross-contamination in cell cultures: the silent and neglected danger. In Vitro Cell Dev Biol Anim 1998;34:1–8.
6. 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.
7. Masters JR. Human cancer cell lines: fact and fantasy. Nat Rev Mol Cell Biol 2000;1:233–6.
8. Arlett CF. The use of dubious cell lines in research: is trust enough? Lancet Oncol 2001;2:467.
9. MacLeod RA, Drexler HG. Cell banks detect false cell lines: journals must act too. Lancet Oncol 2001;2:467–8.
10. Nelson-Rees WA. Responsibility for truth in research. Phil Trans R Soc Lond B Biol Sci 2001;356:849–51.
11. Stacey GN. Cell contamination leads to inaccurate data: we must take action now. Nature 2000;403:356.
12. Masters J. False cell lines. Int J Cancer 2002;99:154.
13. Drexler HG, Dirks WG, Matsuo Y, MacLeod RA. False leukemia-lymphoma cell lines: an update on over 500 cell lines. Leukemia 2003;17:416–26.
14. Azari S, Ahmadi N, Tehrani MJ, Shokri F. Profiling and authentication of human cell lines using short tandem repeat (STR) loci: Report from the National Cell Bank of Iran. Biologicals 2007;35:195–202.
15. Nelson-Rees WA, Flandermeyer RA. HeLa cultures defined. Science 1976;191:96–8.
16. Chen TR. Re-evaluation of HeLa, HeLa S3, and HEp-2 karyotypes. Cytogenet Cell Genet 1988;48:19–24.
17. Gartler SM. Genetic markers as tracers in cell culture. Natl Cancer Inst Monogr 1967;26:167–95.
18. Ogura H, Yoshinouchi M, Kudo T, Imura M, Fujiwara T, Yabe Y. Human papillomavirus type-18 DNA in so-called HEP-2-cells. KB-cells and F1-cells—further evidence that these cells are. HeLa-cell derivatives. Cell Mol Biol 1993;39:463–7.
19. Drexler HG, Dirks WG, MacLeod RAF. False human hematopoietic cell lines: cross-contaminations and misinterpretations. Leukemia 1999;13:1601–7.
20. Drexler HG, Dirks WG, MacLeod RAF, Quentmeier H, Steube KG, Uphoff CC, eds. DSMZ catalogue of human and animal cell lines, 8th edn. Germany: Braunschweig, 2001.
21. van Bokhoven A, Varella-Garcia M, Korch C, Miller GJ. TSU-Pr1 and JCA-1 cells are derivatives of T24 bladder carcinoma cells and are not of prostatic origin. Cancer Res 2001;61:6340–4.
22. Dirks WG, MacLeod RA, Drexler HG. ECV304 (endothelial) is really T24 (bladder carcinoma): cell line cross-contamination at source. In Vitro Cell Dev Biol 1999;35:558–9.
23. Brown J, Reading SJ, Jones S, Fitchett CJ, Howl J, Martin A, Longland CL, Michelangeli F, Dubrova YE, Brown CA. Critical evaluation of ECV304 as a human endothelial cell model defined by genetic analysis and functional responses: a comparison with the human bladder cancer derived epithelial cell line T24/83. Lab Investig 2000;80:37–45.
24. Drexler HG, Quentmeier H, Dirks WG, MacLeod RA. Bladder carcinoma cell line ECV304 is not a model system for endothelial cells. In Vitro Cell Dev Biol Anim 2002;38:185–6.
25. Varella-Garcia M, Boomer T, Miller GJ. Karyotypic similarity identified by multiplex-FISH relates four prostate adenocarcinoma cell lines: PC-3, PPC-1, ALVA-31, and ALVA-41. Genes Chromosomes Cancer 2001;31:303–15.
26. Staege MS, Hutter C, Neumann I, Foja S, Hattenhorst UE, Hansen G, Afar D, Burdach SE. DNA microarrays reveal relationship of Ewing family tumors to both endothelial and fetal neural crest-derived cells and define novel targets. Cancer Res 2004;64:8213–21.
27. MacLeod RA, Dirks WG, Reid YA, Hay RJ, Drexler HG. Identity of original and late passage Dami megakaryocytes with HEL erythroleukemia cells shown by combined cytogenetics and DNA fingerprinting. Leukemia 1997;11:2032–8.
28. Chen TR, Drabkowski D, Hay RJ, Macy M, Peterson W, Jr. WiDr is a derivative of another colon adenocarcinoma cell line. HT-29. Cancer Genet Cytogenet 1987;27:125–34.
29. Liscovitch M, Ravid D. A case study in misidentification of cancer cell lines: MCF-7/AdrR cells (re-designated NCI/ADR-RES) are derived from OVCAR-8 human ovarian carcinoma cells. Cancer Lett 2007;245:350–2.
30. Rae JM, Creighton CJ, Meck JM, Haddad BR, Johnson MD. 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 2007;104:13–19.

31. Jones HW, McKusick VA, Harper PS, Wuu KD. George Otto Gey (1899–1970). The HeLa cell and a reappraisal of its origin. Obstet Gynecol 1971;38:945–9.
32. Masters JR. HeLa cells 50 years on: the good, the bad and the ugly. Nat Rev Cancer 2002;2:315–19.
33. Buehring GC, Eby EA, Eby MJ. Cell line cross-contamination: how aware are mammalian cell culturists of the problem and how to monitor it? In Vitro Cell Dev Biol Anim 2004;40:211–15.
34. Price JE, Zhang RD. Studies of human breast cancer metastasis using nude mice. Cancer Metastasis Rev 1990;8:285–97.
35. Lacroix M, Leclercq G. Relevance of breast cancer cell lines as models for breast tumours: an update. Breast Cancer Res Treat 2004;83: 249–89.
36. Thiery JP. Epithelial to mesenchymal transitions in tumour progression. Nat Cancer 2002;2:442–54.
37. Lacroix M, Toillon RA, Leclercq G. Stable "portrait" of breast tumors during progression: data from biology, pathology and genetics. Endocr Relat Cancer 2004;11:497–522.
38. Ross DT, Scherf U, Eisen MB, Perou CM, Rees C, Spellman P, Iyer V, Jeffrey SS, Van de Rijn M, Waltham M, Pergamenschikov A, Lee JC, et al. Systematic variation in gene expression patterns in human cancer cell lines. Nat Genet 2000;24:227–35.
39. Ellison G, Klinowska T, Westwood RF, Docter E, French T, Fox JC. Further evidence to support the melanocytic origin of MDA-MB-435. Mol Pathol 2002;55:294–9.
40. Sellappan S, Grijalva R, Zhou X, Yang W, Eli MB, Mills GB, Yu D. Lineage infidelity of MDA-MB-435 cells: expression of melanocyte proteins in a breast cancer cell line. Cancer Res 2004;64:3479–85.
41. Thompson EW, Waltham M, Ramus SJ, Hutchins AM, Armes JE, Campbell IG, Williams ED, Thompson PR, Rae JM, Johnson MD, Clarke R. LCC15-MB cells are MDAMB-435: a review of misidentified breast and prostate cell lines. Clin Exp Metastasis 2004;21:535–41.
42. Sung V, Gilles C, Murray A, Clarke R, Aaron AD, Azumi N, Thompson EW. The LCC15-MB human breast cancer cell line expresses osteopontin and exhibits an invasive and metastatic phenotype. Exp Cell Res 1998;241:273–84.
43. Nardone RM. Eradication of cross-contaminated cell lines: a call for action. Cell Biol Toxicol 2007; [Epub ahead of print].
44. Kaplan J, Hukku B. Cell line characterization and authentication. Methods Cell Biol 1998;57:203–16.
45. Masters JR, Thomson JA, Daly-Burns B, Reid YA, Dirks WG, Packer P, Toji LH, Ohno T, Tanabe H, Arlett CF, Kelland LR, Harrison M, et al. Short tandem repeat (STR) profiling provides an international reference standard for human cell lines. Proc Natl Acad Sci USA 2001;98:8012–17.
46. Parson W, Kirchebner R, Mühlmann R, Renner K, Kofler A, Schmidt S, Kofler R. Cancer cell line identification by short tandem repeat profiling: power and limitations. FASEB J 2005;19:434–6.
47. O'Brien SJ. Cell culture forensics. Proc Natl Acad Sci USA 2001;98: 7656–8.
48. Ruitberg CM, Reeder DJ, Butler JM. STRBase: a short tandem repeat DNA database for the human identity testing community. Nucleic Acids Res 2001;29:320–2.
49. Dirks WG, Faehnrich S, Estella IA, Drexler HG. Short tandem repeat DNA typing provides an international reference standard for authentication of human cell lines. ALTEX 2005;22:103–9.
50. Yoshino K, Iimura E, Saijo K, Iwase S, Fukami K, Ohno T, Obata Y, Nakamura Y. Essential role for gene profiling analysis in the authentication of human cell lines. Human Cell 2006;19:43–8.