OPINION

Human cancer cell lines: fact and fantasy

John R. W. Masters

Cancer cell lines are used in many biomedical research laboratories. Why, then, are they often described as unrepresentative of the cells from which they were derived? Here, I argue that they have been unjustly accused. Under the right conditions, and with appropriate controls, properly authenticated cancer cell lines retain the properties of the cancers of origin.

Cell lines provide an almost unlimited supply of cells with similar genotypes and phenotypes. Their use avoids variation between individuals and bypasses ethical issues associated with animal and human experiments. However, many scientists question whether they retain the characteristics of the cells from which they were derived. How have cancer cell lines attained this dubious reputation, and what can cancer researchers do to ensure their appropriate use?

Culture of human cancer cells

There is a misconception that because cancers seem to have unlimited growth potential in patients, the cells are easy to culture and have limitless growth potential in the laboratory (BOX 1). Nothing could be further from the truth — for many types of cancer, it is far easier to grow the normal cells than the cancer cells[1]. Even for cancers that are relatively easy to grow, such as melanomas, only the metastatic cancers can be established as continuous cell lines in most cases[2].

So are the human cancer cell lines that have been produced representative of the cancers from which they were derived? There are two aspects to this question and, as discussed below, two opposite answers.

Representing the cancer of origin

Individual cancer cell lines "provide a snapshot of the tumor at the time the biopsy was taken"[3]. Evidence to support this statement includes the following:

Histopathology. When human cancer cell lines are transplanted subcutaneously into immunodeficient mice (such as the nude strain), most can form tumours. Of 127 human cancer cell lines that produced tumours in nude mice after subcutaneous injection, the histopathology correlated with the tumour of origin in every case[4].

Molecular genetics and receptor expression. Few direct comparisons have been made of the genotypic and phenotypic characteristics of cancer cell lines with those of the tumours from which they were derived. Two exceptional studies compared a series of breast cancer cell lines[5] and lung cancer cell lines[6] with the cancers from which they were derived, and showed that the cultures retain many of the phenotypic (such as oestrogen receptor expression) and genotypic properties of their corresponding tumours for long periods of time. Similarly, mutations in cyclin-dependent kinase genes and p53 were almost always identical in cell lines and the lymphomas or leukaemias from which they were derived[7,8].

Gene expression. Complementary DNA microarray studies of over 8,000 genes in 60 human cancer cell lines revealed consistent similarities between cell lines from the same

Box 1 | Cell line models

There are a multitude of definitions for each tissue culture term. This perspective follows the definitions of the terminology committee of the Society for *In Vitro* Biology (formerly the American Tissue Culture Association)[33].

Primary culture
Produced by growing cells from tissue taken directly from an individual.

Cell line
A primary culture becomes a cell line when it is transferred into the next culture vessel. For adherent cultures, the cells are detached using a protease, such as trypsin, and/or a chelating agent, such as EDTA, and subdivided — this process is known as passaging. For cells that grow in suspension, the culture is split into new culture vessels. Unless specialized culture conditions are used, within a few passages a relatively uniform population of proliferative cells is selected. This population is probably representative of the cells that divide when the tissue of origin is wounded, and will carry on growing until the end of the natural proliferative lifespan is reached and senescence occurs. As long as the cells proliferate, they show little or no evidence of tissue-specific differentiation. However, given the appropriate signals, they can regenerate a functional tissue.

Immortal cell line
Normal human cells have a limited lifespan in culture and almost never spontaneously immortalize (in contrast to rodent cells). Consequently cell lines can only be used over a limited period until they senesce. Most human cancers express telomerase, but either cannot be cultured or undergo senescence. To delay senescence, the lifespan can be extended by transfection with viral genes. The products of the viral genes sequester proteins such as p53 and Rb, allowing the cells to continue dividing for more passages. The cultures still senesce (this period is described as 'crisis'), but if one is patient, in some cultures the occasional cell will acquire the mutation(s) that make it immortal and sometimes tumorigenic. Cell immortalization and carcinogenesis have much in common.

Conditionally immortalized cell lines
The advantages of immortal cell lines (a constant supply of almost identical cells) can be achieved, without the disadvantage of transforming the cells into the equivalent of cancer cells, by using conditional immortalization with a temperature-sensitive mutant of the viral gene. For example, one mutant of the SV40 T-antigen is functional at 33 °C, but conformationally inactive at 39 °C (REF. 34). Cells conditionally immortalized with this construct grow exponentially at the permissive temperature (33 °C), but stop dividing and can express tissue-specific features at the non-permissive temperature (39 °C)[35,36]. However, there is often a degree of 'leakiness', where dividing cells escape and grow at the non-permissive temperature.

Continuous cancer cell lines
Generally, it is only highly aggressive cancers that have accumulated the genetic changes necessary for unlimited growth *in vitro* that spontaneously become continuous cell lines. Cancer cell lines tend to be grown in commercial tissue culture medium that contains fetal calf serum, under which the main selection pressure is for proliferative cells.

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tissue of origin, and consistent differences between cell lines of different origins[9].

Drug sensitivity. In contrast to most other solid cancers in adult humans, testicular germ cell tumours are cured in over 80% of cases using cisplatin-based combination chemotherapy. Cell lines derived from testis tumours retain their hypersensitivity to cisplatin and other DNA-damaging agents[10].

Not representing cancer type

Are cancer cell lines derived from a particular type of cancer representative of the clinical spectrum of cancers at that site? This is a completely different question, and the answer this time is a resounding no. In the clinic, cancer is classified by stage and grade (BOX 2). The cancers that yield continuous cell lines tend to be fast growing, high stage and poorly differentiated tumours that have accumulated the mutations required for indefinite growth *in vitro*. There are few cell lines derived from primary well-differentiated cancers, which form the majority of some types of cancer, such as bladder tumours[11].

The genetic changes required to immortalize cells (BOX 1) are mostly late events in cancer progression, and therefore it is not surprising that most primary cancers are not immortal. Also, few scientists have had the interest, the patience or the funding to develop cell lines from slow-growing cancers. Consequently, the population doubling times of cancer cell lines are mostly short. This deficit reflects the greater difficulty of establishing slow-growing tumour cell lines and the preference of the scientific community for fast-growing cells that are easy to handle.

Quality control

Careful, regular quality control is a vital, but sadly often neglected, part of cell culture. This neglect has played an important part in tarnishing the reputation of *in vitro* cancer models. What are the pitfalls, and how can we avoid them?

Genomic instability

The problem. Why do phenotypic and genotypic differences arise between sublines of the same cancer cell line? The first reason is that cancer cell lines constantly generate variants with phenotypic and/or genotypic differences from the predominant population. If the cells are grown continuously over many generations, faster growing and less representative clones may be selected. Second, if the cells are sent to other laboratories and exposed to different environments (such as media, sera, trypsin, carbon dioxide levels, humidity and temperature), variants that are better adapted to the new conditions are likely to be selected. The uncontrolled passing of cells between laboratories is the cause of much unreliable data[12].

The solution. As long as cells are not grown indefinitely and passed between laboratories, they retain most of the features of the cancers from which they were derived. Adequate frozen stocks of each cell line should be produced, and users should return to frozen stocks at regular intervals (for example, for adherent cell lines, every 10 passages or at 3-month intervals, whichever is shorter; and for cell lines growing in suspension, such as those derived from leukaemias and lymphomas, every 4–6 weeks). Treated like this, if the cells are kept under identical culture conditions, they are relatively stable phenotypically and genotypically[5–8].

Cross-contamination

Cross-contamination of cell lines happens in two ways. It can result from poor culture technique when, for various reasons, two cell lines accidentally get into the same culture. After a few passages, there is no trace of the slower-growing cell line, and it has been completely displaced by the faster-growing cell line. The second reason is clerical error — mislabelling of growing cells or frozen stocks. Such accidents can and do occur frequently in any laboratory, and have devastating consequences unless simple quality control measures are adopted.

The two most notorious examples of human cell line cross-contamination are 'KB' cells and 'ECV304' cells. 'KB' cells are widely used as a model for keratinocytes, despite being HeLa cells, derived from a glandular cancer of the cervix. 'ECV304' cells are widely used as a model of endothelium, despite being T24 cells, derived from a bladder cancer[13]. It was first shown over 30 years ago that 'KB' cells are in fact HeLa cells[14], but this false cell line continues to appear in hundreds of publications every year.

A common misconception is that cross-contamination leads to hybrids of the cross-contaminating cell line and the original cell line. But consider what happens during cross-

Box 2 | Cancer stage and grade

Cancer is classified in three ways — the site of origin (such as breast or prostate cancer), the stage of the cancer (how far it has spread) and the grade of the cancer (how similar to the normal cells it appears under the microscope).

Stage

Specific staging classifications are available for each type of cancer and there is a choice of classifications. The stage of the cancer is important for the patient, because the treatment options are mainly dictated by the stage. One of the most widely used staging systems is the tumour, node, metastasis (TNM) system. The details differ for each type of cancer, but the following definitions provide a rough guide.

T1: Small localized cancer.
T2: Larger cancer, but confined within the organ.
T3: Cancer at the limits of the organ.
T4: Cancer that has spread locally into other organs.
N0: No cancer cells detected in those regional nodes that have been examined.
N1: Cancer cells detected in one or more of the regional lymph nodes examined.
M0: No metastases.
M1: Metastases present.

The classification can be refined for cancers where there are reliable serum markers (such as prostate and testis cancer). A more accurate stage is obtained once the surgical specimen has been examined under the microscope, giving the pathological stage (pT).

In general, T1 and T2 cancers can be treated by local means (surgery or radiotherapy), but most T3 cancers and virtually all T4, N1 and M1 cancers need systemic treatment. Most cell lines are derived from high-stage cancers that are beyond local treatment.

Tumour grading

The histopathologist examines sections of cancers under the microscope to determine the pT stage (as described above) and to decide how aggressive the tumour is (grade). Various features are used to determine the grade, including the similarity of the morphology of the cancer to that of the normal tissue from which it is derived, the extent of morphological changes in the nucleus and the frequency of mitotic figures (dividing cells). The grade is also important for the patient, because it gives an idea of the prognosis (likelihood of the cancer progressing). There are many grading systems for each type of cancer, and the following definitions provide only a rough guide.

G1: Well-differentiated cancer with good prognosis.
G2: Moderately differentiated cancer with intermediate prognosis.
G3: Poorly differentiated cancer with bad prognosis.

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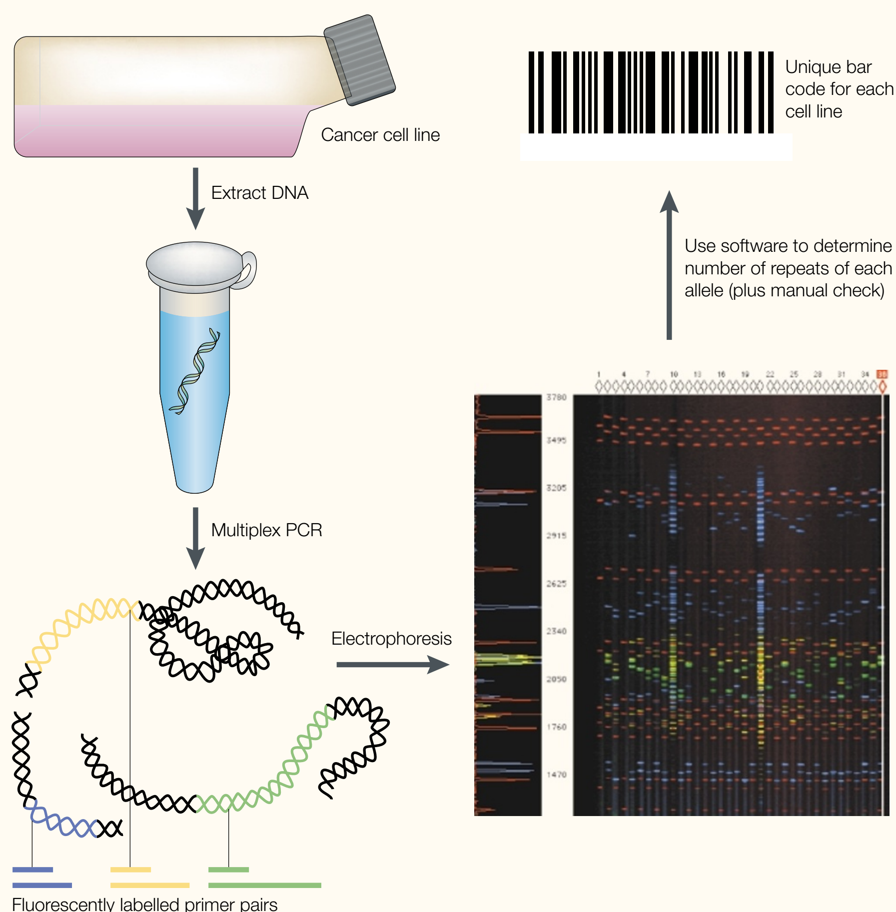

Figure 1 | **DNA profiling of short tandem repeats.** Every genome, whether it belongs to a crime suspect or a cancer cell line, has a characteristic pattern of repetitive sequences, including short tandem repeats (STRs). These are loci (specific sites in the DNA) at which highly variable numbers of a short repeated sequence (2–6 nucleotides in length) are found. For STR profiling, the number of repeats at 5–10 different loci is calculated by extracting the DNA, adding different primers (fluorescently tagged with coloured dyes — blue, green or yellow) for each locus and amplifying the DNA using the polymerase chain reaction (PCR). The PCR products are then separated by gel electrophoresis and compared with standard size markers (fluorescently tagged with a red dye)[37]. Cells derived from the same clone will have an identical pattern of markers that can be converted into a series of numbers corresponding to the number of repeats in each allele at each locus. This provides a 'bar code' or international reference standard for that cell line. Although small differences can occur between cells that originated from the same culture but have subsequently been cultured separately for long periods of time, they can still be accurately matched to a consensus bar code using appropriate cut-off values

contamination: within a few passages, the faster-growing cell line outgrows the slower-growing cell line, and all trace of the slower-growing cell line is gone. There is no evidence that HeLa(KB) or T24(ECV-304) are somatic cell hybrids or have acquired any genetic information from the cells they contaminated. Claims of mixed parentage have little, if any, foundation. Any genotypic or phenotypic differences between the various sublines of HeLa probably reflect the different selection pressures under which they have been placed in different laboratories.

The problem. In the 1970s, Walter Nelson-Rees fought a bitter campaign to expose the use of cross-contaminated cell lines[15] — a thankless task for which he became notorious. He showed that a large proportion of the cell lines being used worldwide and being distributed by cell banks were HeLa cells[16,17] — the first human cancer cell line developed[18]. The campaign was so successful that by the time he retired in 1981, some people thought that all human cancer cell lines were HeLa cells.

Sadly, Nelson-Rees's campaign was rapidly forgotten after his retirement, and the use of cross-contaminated cell lines is now greater than ever. Of the cancer cell lines submitted for cell banking, 17–36% are either from a different individual or from a different species to that claimed[19,20]. The implication of these figures — that a similar proportion of the publications describing work with human cell lines contain misleading data — is impossible to avoid. It is bizarre that this problem continues to be ignored, particularly as it so easy to detect.

The solution. Cross-contamination can be detected by karyotyping[21], isozyme analysis[22], HLA (human leukocyte antigen)typing[23] or DNA fingerprinting[24], but none of these methods provides a simple numerical output that can be compared between laboratories. However, DNA profiling (FIG. 1), the technique used by forensic services for the identification of individuals, is now available for cell lines. DNA profiling provides a simple, cheap and universal solution applicable to all human cell lines. If it is adopted routinely and incorporated into standard operating protocols, it will provide an international reference standard for every cell line and prevent all but the most deliberate fraud.

Problems such as those outlined above could be tackled by the careful training of all users of cancer cell lines. Information on how best to use cancer cell lines is readily available[12,25]. Journal editors and referees could also play a part, by requesting evidence that all the cell lines discussed in a research paper have been authenticated.

Microbial contamination

The problem. Just as serious is the widespread contamination of cell lines with microorganisms, especially Mycoplasma. On the basis of submissions to cell banks, it is estimated that 15–35% of cell lines are contaminated with Mycoplasma[26,27]. Mycoplasma infection can have marked effects on gene expression and cell behaviour[26] and work done with Mycoplasma-infected cells cannot be regarded as valid. Infection is often at a low level that is undetectable with microscopic techniques, and Mycoplasma is highly infectious and can rapidly spread through all cell stocks.

The Mycoplasma problem, like the HeLa problem, is probably only the tip of the iceberg. There are many other insidious microorganisms lurking in cell cultures. For example, *Mycobacterium avium* has been found in cell lines in London and Berkeley, California (J.R.W.M. and G. Buehring, unpublished observations), and is probably far more widespread. Even more worrying, screening for viruses is completely non-existent in almost all research laboratories. Expression of viral products could influence experiments and biotechnology products, as

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well as transcriptome and proteome analyses.

Another bad — but extremely common — practice is the inclusion of antibiotics in tissue culture medium. For routine management of cell lines, antibiotics are unnecessary and only provide a cover for inadequate technique[12]. The antibiotic could reduce an infection to a level where it is not noticed, despite the fact that microorganisms are present. Their products will contribute to DNA, RNA and protein analyses and could alter the behaviour of the cells. Also, antibiotics might influence the behaviour and characteristics of the cells.

The solution. Sensitive tests for Mycoplasma contamination (including polymerase chain reaction (PCR)-based assays, indicator cells, and broth and agar culture) are available[12,26]. All laboratories that use cell lines should test their cell stocks for Mycoplasma. Laboratories that do not test can reasonably make the assumption that all their cells are contaminated with Mycoplasma.

Screening for viruses, in addition to Mycoplasma, could be carried out by cell-line suppliers. For example, the German Collection of Microorganisms and Cell Cultures screens all human cell lines for some of the more frequently occurring viruses.

In common with cross-contamination, there is a lack of awareness of the magnitude and seriousness of the problem. Scientists using cell lines must be thoroughly trained and educated. Tests for Mycoplasma infection are readily available, and their use should be built into routine laboratory practice.

Future needs

Most cancer cell lines have already acquired all the changes needed for the cells to grow as metastatic deposits in distant sites. Consequently, they are of questionable value for studying the changes associated with cancer progression, except perhaps as models with which to reverse these changes. Cell lines are needed from early stage and well-differentiated cancers, with matching cell lines from the corresponding normal tissues from the same patients. We also need cell lines from inherited cancers. Cell lines are essential gene discovery tools in human cancer and more are needed because most molecular genetic changes are much easier to detect using these pure populations of cancer cells. For example, it is difficult to detect homozygous deletions in tumour tissue because of the presence of contaminating non-malignant cells. Identification of such mutations in cell lines is relatively easy[28] and can result in the detection of new tumour suppressor genes. Methylation and loss of heterozygosity (often the first evidence of a new tumour suppressor gene) are usually first detected in cell lines[29,30].

We need to know why, in many types of tissue, the normal cells grow much more readily than the cancer cells — have the cancer cells lost some of the properties needed for them to grow in isolation? One of the main goals for cancer research is to routinely culture every human cancer from every patient. For the patient, this advance will facilitate individualized drug therapy, autologous immunotherapy, and more accurate molecular staging and prognosis using transcriptome and proteome analyses[31,32].

Most of the criticisms concerning phenotypic and genotypic drift in cell lines are due to lack of quality control. All cell lines need to be authenticated by DNA profiling, and contamination by Mycoplasma and other microorganisms excluded. Once these simple quality control measures are taken by every laboratory working with cancer cell lines, we will be able to rebuff most of the criticisms levelled against these cells.

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Links

FURTHER INFORMATION

Laboratory of the Government Chemist | German Collection of Microorganisms and Cell Cultures | Society for *In Vitro* Biology | Time-lapse movies of five cancer cell lines

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Acknowledgements

I thank Alan Entwistle, Ludwig Institute for Cancer Research, University College London for help preparing the time-lapse movies and Jim Thomson, Laboratory of the Government Chemist, London, UK for providing images for Figure 1.

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