Identity tests: determination of cell line cross-contamination

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Abstract Cell line cross-contamination is a phenomenon that arises as a result of the continuous cell line culture. It has been estimated that around 20% of the cell lines are misidentified, therefore it is necessary to carry out quality control tests for the detection of this issue. Since cell line cross-contamination discovery, different methods have been applied, such as isoenzyme analysis for inter-species cross-contamination; HLA typing, and DNA fingerprinting using short tandem repeat and a variable number of tandem repeat for intra-species cross-contamination. The cell banks in this sense represent the organizations responsible for guaranteeing the authenticity of cell lines for future research and clinical uses.

Keywords Cell lines · Cross-contamination · Fingerprinting · Isoenzyme analysis · Short tandem repeat · Variable number tandem repeat

Abbreviations

AFLP Amplified fragment length
polymorphism
MCB Master cell bank

STR Short tandem repeat
SBT Sequence based typing
SSO Sequence specific oligonucleotide
SSOP Sequence specific oligonucleotide probe
VNTR Variable number tandem repeat
WCB Working cell bank

Introduction

Fifty years ago, there was intense competition between cancer researchers to develop the first human cancer cell line. The first human cell line, HeLa, was established in 1952 and was derived from a carcinoma of the uterine cervix in an African-American woman (Gey et al. 1952). It was not established in a tissue-culture laboratory as we know it today. There were no laminar-flow cabinets or bottles of sterile culture media and sera. Tissue culture was done on the open bench, with the help of a Bunsen burner to give a small window of relatively sterile air, using materials collected and prepared on an almost daily basis (Masters 2002). During the next 15 years, many human cell lines from different origin tissues were established (Buehring et al. 2004). In 1968, Gartler noted that many cell lines originated from Caucasian donors have enzyme forms rare in Caucasian individuals but with 30% of frequency

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in people of African origin, specifically the A form of glucose-6-phosphate dehydrogenase (G6PD), found in the HeLa cells, which migrates faster in gel electrophoresis than the B form typically found in Caucasian individuals (Gartler 1968). As a result of this finding Stan Gartler suggested that perhaps all of these cell lines were HeLa cells (Gartler 1968).

With the development of karyotyping and isoenzyme techniques, Nelson-Ress et al. (1975, 1981) and Nelson-Ress and Flandermeyer (1976) found that many cell lines had been switched or cross-contaminated with HeLa cells. The term of cross-contamination is used to indicate misidentification of one cell line by another, rather than contamination by a microbiological organism. This terminology follows the internationally agreed nomenclature (Schaeffer 1990), and is used by the United Kingdom Coordinating Committee on Cancer research guidelines (United Kingdom Coordinating Committee 2000).

Today, it is estimated that around 20% of the cell lines are cross-contaminated with HeLa cells (Stacey 2000). In a recent publication by the German Collection of Microorganisms and Cell Cultures (DSMZ), it is estimated that the 18% of the 252 human tumour cell lines studied are misidentified (MacLeod et al. 1999). Several methods have been used in the past to identify the presence of cross-contamination, such as isoenzyme analysis, karyotyping, HLA typing, and determination of amplified fragment length polymorphisms (AFLP); however with the use of the short tandem repeat (STR) technology it is possible to make the determination of cell line cross-contamination of the more sensitive, and rapid form (ATCC 2000; Masters et al. 2001).

Cell banks must assure the quality, traceability and safety of cell products for research and future clinical uses. This should be achieved with close attention to the standardization of processes and the implementation of quality control programmes and methods, which reflect current best practices. The frequent monitoring of the cell lines is vital for any research centre that uses cell cultures, and would be mandatory if these cultures are intended for clinical use in cell therapy and regenerative medicine. Thus, within the

quality assurance programmes in these establishments, one of them is the adequate authentication of cell lines.

This review intends to give an overview of this methodology that should be used in cell banks to carry out the authentication of the cell lines in order to assure their quality and to avoid the cross-contamination.

Methods to detect cross-contamination

Isoenzyme analysis

The isoenzyme technique is used on a routine basis for the determination of inter-species cross-contamination (Stacey et al. 1997). This methodology is based on the existence of enzymes with similar or identical specificity, but different molecular structures (isoenzymes). Using isoenzyme analysis of a set of enzymes such as: glucose-6-phosphate dehydrogenase (G6PD), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and malate dehydrogenase (MDH), a characteristic “mobility pattern” can be established for each species (Authentikit, Innovative Chemistry, Marshfield MA, USA). If isoenzymes from different species result in distinct electrophoretic mobilities, these samples can individually be distinguished from each other (Stacey et al. 1997). This technique is able to determine inter-species cross-contamination if the contaminating cells represent at least 10% of the total cell population (Nims et al. 1998).

Identity testing by HLA typing

Genetic identity testing refers to the determination of one or more unique characteristics of an individual's genetic material that makes it distinguishable from all other humans. Although the majority of the human genomic sequences are identical between individuals, the relatively small number of sequence differences (about 1 every 300–1,000 nucleotides) is enough to distinguish all individuals. The human lymphocyte antigen (HLA) system is one of the most polymorphic genetic systems known. More than 1,250 alleles are currently identified and distributed between

the classical HLA class I loci (HLA-A, B, and C), and the classical HLA-II loci (HLA-DR, DP, and DQ) (Nomenclature for factors of the HLA system 2000). HLA typing was primarily used for forensic analysis based on detection of sequence polymorphisms at HLA-DQA1 (or HLA-DQ α) with sequence-specific oligonucleotide (SSO) or allele-specific oligonucleotide (ASO) probes (Page-Bright 1982), and equally has been used in the identification of cell lines (Defendi et al. 1960).

The HLA system contributes to the immune response, it is made up of a set of molecules expressed at the surface of almost all the cells that are responsible for lymphocyte recognition of “self” and “non-self”. They belong to a group of molecules known as the Immunoglobulin Superfamily. The main function of the HLA molecules is to present the antigen determinants to the T lymphocytes and initiate the specific immune response (Stevanovic 2002).

HLA typing can be performed at several levels of discrimination between alleles. The serological methods used antigen-specific sera. The sera are human-derived preparations that react to specific HLA antigens expressed on cell surfaces (Gerlach 2001). The molecular methods used synthetic probes (Sequence Specific Oligonucleotide Probes, SSOP) (Cao et al. 1999) (Fig. 1), and primers (Sequence Specific Primers, SSP) (Welsh

and Bunce 1999) (Table 1). The major HLA typing resolution is obtained by the Sequence-Based Typing (SBT) method, with direct DNA sequencing (Rajalingam et al. 2004) (Table 1). At present, the HLA typing is a complementary test in the determination of intra-species cell line cross-contamination (Defendi et al. 1960).

DNA fingerprinting

The term “DNA fingerprinting” was firstly defined and used by Jeffreys et al. (1985a, b) to refer to restriction fragment length polymorphism (RFLP) analysis of hypervariable “minisatellite” regions of DNA. The RFLP polymorphisms are DNA fragments of different lengths between individuals that can be obtained when genomic DNA is cleaved with a particular restriction endonuclease. The resulting different-sized DNA fragments are separated by electrophoresis, followed by capillary transfer to a membrane in a technique known as Southern blot. The fragments can be visualized on the blot by hybridization with labelled DNA probe that recognizes the locus of interest. The resulting band pattern can be compared between individuals.

The RFLP technique is applied to hypervariable regions of DNA, which are abundant and dispersed throughout the genome, and composed of tandem repeats of a core nucleotide sequence. Two types of tandem repeats exist and are classified based on the numbers of nucleotides in each core repeat (Wong et al. 1987; Koreth et al. 1996). Minisatellites, also known as variable number of tandem repeat (VNTR) loci, have core repeats of 8 to more than 80 base pairs (bp). Each VNTR locus has between two and several hundred core repeats on each allele (Wong et al. 1987). Microsatellites, also known as STR loci, have core repeats of 2–7 bp. STR usually have between 3 and 20 tandem core repeats in each allele but in some cases may have 100 repeats or more (Koreth et al. 1996). Both VNTRs and STRs are useful for DNA fingerprinting testing because they are inherited in a mendelian fashion, that is, an individual receives one allele from each parent. The two alleles can have the same number of tandem repeats, making the individual homozygous at that locus, or a different number of

Fig. 1 SSOP technique. The HLA loci are amplified by PCR with specific biotin-labelled primers for each locus. These PCR products are hybridized to a membrane strips containing SSO probes specific for each of the known alleles bound to the membrane in an array of “dots”. The reaction is revealed with streptavidin conjugated to peroxidase

Table 1 Methods commonly used for identity tests

Polymorphisms	Method of analysis	Commercial reagent example
Single nucleotide polymorphism	PCR-SSO	Dynal RELI™ SSO Typing Kit (HLA-A, B, Cw, DRB1, and DQB1) (Dynal) (http://www.dynal.com) INNO-LiPa (Innogenetics) (http://www.innogenetics.com)
	PCR-SSP	Dynal AllSet + SSP (Dynal) (http://www.dynal.com)
	DNA sequencing	SBT-HLA kits (Applied Biosystems) (http://www.appliedbiosystems.com)
STR	Multiplex PCR	AmpF/STR PCR amplification kits (Applied Biosystems) (http://www.appliedbiosystems.com) Gene Print fluorescent STR kits (Promega) (http://www.promega.com)

SSO, sequence specific oligonucleotide; SSP, sequence specific primer; SBT, sequence based typing; STR, short tandem repeat

repeats, making the individual heterozygous at that locus.

Today, the VNTRs and STRs are mainly amplified by polymerase chain reaction (PCR) technique using primers that flank target locus (Koreth et al. 1996; Tamaki and Jeffreys 2005) (Fig. 2). The different length alleles are distinguished by comparison of the size of the amplified

fragments. The PCR products are separated by gel electrophoresis, and the sizes of the bands are compared to an allelic ladder composed of the most common alleles in the human population. The PCR products can be detected by radioactivity with incorporation of a radioactive nucleotide in the PCR, by silver stain of the gel, or by fluorescence with incorporation of either a labelled primer or a labelled nucleotide in the PCR. The detection of the fluorescent products by capillary electrophoresis is also possible. At present there are different commercial methods available for the determination of cell line cross-contamination by STRs (Table 1).

Quality control in cell-banking

The large scale culture of animal cells is now recognized as an approach for the manufacture of biological materials. It was in 1987 when the World Health Organization (WHO) introduced safety testing guidelines for the use of continuous cell lines used as substrates for the production of inactivated viral vaccines (World Health Organization 1987). Since then, progress in the biomedical sciences and the exploitation of biotechnology has led to the development of new biological medicinal products at an unprecedented rate. To ensure the maximum safety level of biopharmaceuticals there are several guidelines

Fig. 2 Schematic of STR PCR. The number of tandem repeats at an STR locus can be determined by PCR amplification across the repeat locus with flanking primers (arrows, primer 1 and 2)

Table 2 Quality control tests applied to MCBs and WCBs

- Viability (trypan blue, propidium iodide PI, diacetyl fluorescein, etc).
- Karyotyping
- Microbiological controls (viruses, bacteria, fungi, and mycoplasma)
- DNA fingerprinting (intra-species cross-contamination)
- Isoenzyme analysis (inter-species cross-contamination)

MCB, master cell bank; WCB, working cell bank

that define international standards (International Conference on Harmonisation 1997; MCA 2002). In this sense, the creation of cell banks for the production of biopharmaceuticals offers the advantage that the process can be performed with an already well characterized, and homogeneous starting lot of cells (AATB 2002). The first step in generating a cell bank is the production of the Master Cell Bank (MCB) by expanding the cell substrate. The Working Cell Bank (WCB) that is used to start the manufacturing process is derived from a single or a few vials of cells of the MCB. Thus, using this cell bank system (MCB and WCB) a continuous supply of cells is provided.

In both banks (MCB and WCB) are applied different types of quality control tests that appear listed in the Table 2. The viability tests measure the proportion of viable cells following freezing or thawing cells in the banking process (Freshney 1994). The microbiological controls guarantee the absence of any adventitious agents like viruses, mycoplasma, bacteria, and fungi (Cobo et al. 2005). Karyotyping assess the chromosomal stability of the cells in the continuous culture process. Finally, the DNA fingerprinting and isoenzyme analysis detect respectively the presence of intra-species and inter-species cross-contamination (Masters et al. 2001; Stacey et al. 1997). Therefore, both techniques (DNA fingerprinting, and isoenzyme analysis) are an essential tool in the quality control of cell-banking.

Conclusions

In the earliest days of tissue culture (1950s), there was little danger of cross-contamination of cell

lines, however the increasing number of new cell lines produced and the high rate of their use, has made the problem of cross-contamination relevant. The widespread use of cell cultures in different fields, either for research purposes or for large-scale production of biological compounds, requires that quality control tests be systematically performed to evaluate the authenticity of the cell lines used. So the cell banks are the major organisms responsible for guaranteeing the authenticity of the cell lines, because they have the material and logistic tools to make it possible (AATB 2002).

The intra-species and inter-species cross-contamination represent an important problem not only due to the research articles which use misidentified cell lines with the corresponding false results (Buehring et al. 2004), as well in the production of cell lines destined to clinical uses such as the stem cell lines (Borge and Evers 2003; Hyslop et al. 2005). The stem cell lines are subjected to rigorous characterization and quality because in the future they will be applied to human transplant therapies (Hyslop et al. 2005).

Both the isoenzyme analysis and the DNA fingerprinting are the methods chosen for the detection of inter-species and intra-species cross-contamination respectively, and their application of the routine manner will prevent the derived problems of the misidentification in cell lines. Today, the American Type Culture Collection (ATCC) carried out the identification of all their cell lines by STR analysis amplified eight STR loci and the amelogenin gene in a multiplex PCR (<http://www.atcc.org>). Equally, in cell and stem cell banks (Healy et al. 2005) the aforementioned tests (isoenzyme analysis and DNA fingerprinting) are essential to guarantee the identity of the cell lines.

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