Cross-Contamination of Human Esophageal Squamous Carcinoma Cell Lines Detected by DNA Fingerprint Analysis

Paul D. van Helden, Ian J. F. Wiid, Carl F. Albrecht, Elize Theron, Alan L. Thornley, and Eileen G. Hoal-van Helden

MRC Centre for Molecular and Cellular Biology, Department of Medical Biochemistry [P. D. v. H., I. J. F. W., E. G. H.-v. H.] and Department of Pharmacology [C. F., E. T.], University of Stellenbosch Medical School, P.O. Box 63, Tygerberg, 7505, South Africa and Department of Zoology, University of the Witwatersrand, Johannesburg, 2050, South Africa [A. L. T.]

ABSTRACT

DNA "fingerprint" analysis has recently become known as a valuable technique for positive identification of any given individual. The chances for mistaken identity have been estimated to be 10⁻⁴ for close siblings or as little as 10⁻²⁵ for randomly selected individuals. This methodology thus represents a significant improvement over previously established identification tests using protein or enzyme analysis techniques and has already found application in forensic medicine.

One of the chief problems in tissue culture studies is the question of the unequivocal identity of the cultured cells used and the very real possibility of their being contaminated by cells of a similar morphological appearance. We report here the application of the DNA "fingerprint" technique to the genotypic analysis of cultured human squamous carcinoma cells. The results show that a number of lines, designation HCu, have become cross-contaminated. Lines SNO, HCu 10, and HCU 13 are genetically distinct, however lines HCU 10, 18, 33, 37, and 39 are genetically identical and are in fact subcultures of the same cell. In addition, a myocardial line known as Girardi is shown to be identical to HeLa cells. The introduction of this technique to tissue culture laboratories could therefore prevent contaminated cultures from being disseminated or used in research studies.

INTRODUCTION

Contamination of cell lines by other cells is a phenomenon not unknown to scientists and its has been estimated that such events occur quite frequently (1, 2). HeLa cells in particular are known to be aggressive invaders of other cultured cells and are thought to have invaded more than 90 individual (separate) lines (1). As far as could be ascertained from the literature, no concerted effort has been made since 1981 to investigate this problem. The reasons may simply be that the techniques required to type the cells in question were not sufficient to unequivocally identify any cell line (1, 2) and that the application of these techniques requires a great deal of effort for little reward.

A similar situation has existed until recently in forensic medicine, where various techniques could be used to eliminate certain suspects, but not identify one individual unequivocally (3, 4). However, the recent discovery that satellite DNA may be used for effective unequivocal genotyping of any individual will certainly revolutionize forensic medicine (3–7). These satellite DNA probes allow one to calculate the probability of relationship of two DNA samples based on the hybridization pattern obtained from genomic DNA probed with the minisatellite probes (Southern blot procedure).

Earlier work in our laboratories had shown that many apparent differences in a number of human esophageal squamous carcinoma (HCu) cell lines could be as a result of Mycoplasma contamination and that differences in morphology and growth rate became insignificant if Mycoplasma species were removed from culture. In addition, similar cytokeratins had been observed from lines Hcu 18, 33, and 39 (8) and polyacrylamide gel electrophoresis of cytoplasmic and nuclear proteins from some of these cell lines failed to detect any differences between the lines. We therefore suspected that the cell lines may not have maintained individuality in spite of a number of reports where authors claim differences in their behavior, for example, in prostaglandin release (9) or epidermal growth factor receptor content (10).

In order to address this problem, we have applied a minisatellite (Jeffreys) DNA probe to the study of a commercially available myocardial line and the genotype of 10 different subcultures of esophageal cell lines (representing eight purported lines; two lines of which were checked at high and low passage number). HeLa cell DNA was obtained from cells grown in a laboratory geographically distant from locations used for culturing other cells.

MATERIALS AND METHODS

Cell Cultures. Cell lines purported to be human esophageal SCC lines (Hcu 18, 33, and 39) were obtained directly from the originator, Dr. K. Robinson (11, 12). Other lines originating from the same source plus alternative cultures of the three mentioned above were obtained from a liquid nitrogen storage facility at the University of Cape Town (South Africa). These samples were the closest to the primary cultures available, having been stored in liquid nitrogen from some months after the primary cultures were established until March 1987. These cells were cultured in our laboratories in separate departments and separate buildings by different personnel to avoid any possibility of cross-contamination. The cells were maintained in culture as described elsewhere (11, 12). The esophageal line SNO (13), and a derivative SN-T4, as well as a sample of D98 HeLa cells were cultured by one of us in a facility situated geographically distant (Johannesburg) from that used for HCU line cultures (Cape Town). The derivative SN-T4 is derived from a 4th passage nude mouse tumor. The myocardial cell line, Girardi, is available from Flow Laboratories.

DNA Isolation, Electrophoresis, and Hybridization. Approximately 10⁶ cells of each line were harvested and DNA extracted as described elsewhere (14). Incubation of the DNA with restriction enzyme Hinf I, followed by horizontal agarose gel electrophoresis, Southern blotting, and hybridization to the λ33.6 Jeffreys human satellite DNA probe was done following the procedures described (4–7).

RESULTS AND DISCUSSION

The pattern of hybridization to genomic DNA of the Jeffreys minisatellite DNA probe (33.6) is shown in the accompanying figures. Fig. 1 shows the results of hybridization to DNA from human esophageal cell lines Hcu 18, 33, and 39. These cells were established between 1975 and 1979 and have therefore
DETECTION OF CARCINOMA CELL-CONTAMINATION BY DNA FINGERPRINT

been maintained in culture for at least 8 years (12).

In addition, Fig. 1 shows the hybridization pattern for DNA from the cell line SNO (13) and the nude-mouse passage derivative, SN-T4. It is evident that there are no detectable genetic differences between the three HCu lines, although these are different from the SN lines. There are also no detectable differences between SNO and SN-T4, illustrating the genetic stability of cells which have been passaged through nude mice.

Since the three continuously grown HCu lines appeared identical, low passage number (less than 15 passages) samples of these lines plus additional human esophageal (SCC) lines established in the same laboratory were obtained (designation HCu). All of these samples had been stored in a separate facility in liquid nitrogen since 1979. The DNA from these lines was hybridized as before and apart from one line, HCu 13, all the others appear to be genetically identical, whether of high (greater than 150 passages) or low passage number (Fig. 2). None of these lines proved to be HeLa cells, as is clearly evident (Fig. 2).

The same cannot be said for Girardi cell DNA. This myocardial line (available from Flow Laboratories) was a suspect line, first listed as a possible HeLa line in 1981 (1) and advertised as carrying an HeLa-like isozyme marker (Flow). We have been unable to detect any genetic differences between HeLa and Girardi cells, although they are clearly different from the esophageal lines HCu 10 and SNO (Fig. 3).

Visual inspection of these results shows that of the 10 different human esophageal (SCC) cultures tested, only three genetically distinct cell lines are evident, namely (a) SN variety (SNO and SN-T4), (b) HCu 13, and (c) all other HCu lines, which are identical.

It has been estimated that the mean probability that a fragment in the DNA fingerprints of one human is present in a second, randomly chosen, individual is approximately 0.26 (5, 15). The chance therefore that one cell line is not related to one other in our case, is approximately 0.26 33 (since 33 distinguishable bands can be seen on the autoradiograph of lines HCu 18 and family) or 10^-20. The probability that all the Hcu lines sharing identical DNA fingerprints are not related or not genetically identical is therefore considerably less than 10^-20. We therefore conclude that lines Hcu 10, 18, 33, 37, and 39 are the same cell lines. Karyotypic analysis of lines HCu 18, 33, and 39 showed a large degree of commonality, but differences were observed in marker chromosomes. Analysis of karyotypes has been helpful in defining the identity of cell lines, but in long-term cultures, the possible evolution of new marker chromosomes from ongoing chromosome rearrangements can make karyotypic resolution of a suspected contamination problem almost impossible. It has been observed in HeLa cells, for example, that marker chromosomes can differ according to the strain studied. In our work therefore, the results of karyotypic analysis were inconclusive, and DNA fingerprint analysis
DETECTION OF CARCINOMA CELL-CONTAMINATION BY DNA FINGERPRINT

Fig. 3. DNA fingerprint analysis of high relative molecular mass DNA from cultured human cells. Lane 1, Girardi cells; lane 2, HeLa D98 cells; lane 3, HCu 13; lane 4, SNO.

The probe 33.6 is the subject of a patent and enquiries should be directed to ICI Diagnostics, Gadbrook Park, Rudheath, Northwick, Cheshire CW9 7RA, England. We thank ICI for permission to use this probe.

REFERENCES


proven to be of greater value in defining cell line genotype.

We are unable to ascertain at what stage in the cultivation of these cells the problem of a common genetic origin could have arisen. However, it is clear that within months of the establishment of these cultures the cells had the genotype reported in this manuscript. There are two possibilities to be considered as the source of error: (a) an aggressive line was derived from an early tumor sample which invaded other cultures and gradually replaced the cells during a number of passages (compare behavior of HeLa cells) or (b) an aggressive line invaded dishes in which dormant tumor cells were being maintained and that the cells were mistakenly grown as new lines (3). HCu 13 appears to have escaped contamination (Fig. 2). It is not possible to establish which of these scenarios is correct, nor can we establish the origin of the common cell.

The results of this study emphasize the need for careful monitoring and screening of cell lines for specificity and purity, as has been stated (1, 16). As far as we are aware, this work describes a new application for the Jeffreys probe and illustrates their value for the verification of cell line identity. Since contaminated cultures are still available commercially, the exact extent of the dissemination of cells masquerading as others represents a serious source of error to researchers. We propose that this technique be used to genotype cells in the future and that the genotype of cells which are made available for dissemination from culture banks be carefully controlled and classified by genotype comparison with reference cells, such as HeLa. Clearly the use of incorrectly identified cells will invalidate any findings and at best can lead to the retraction of published work (17).

ACKNOWLEDGMENTS

The probe 33.6 is the subject of a patent and enquiries should be directed to ICI Diagnostics, Gadbrook Park, Rudheath, Northwick, Cheshire CW9 7RA, England. We thank ICI for permission to use this probe.
Cross-Contamination of Human Esophageal Squamous Carcinoma Cell Lines Detected by DNA Fingerprint Analysis


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/48/20/5660

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/48/20/5660. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.