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Detection of a contaminant cell culture line by restriction endonuclease cleavage patterns of mitochondrial DNA*

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ABSTRACT

A putative HeLa cell culture line was discovered to be contaminated with mouse cells by examination of agarose gel profiles of restriction endonuclease digests of mitochondrial DNA. The contamination was confirmed by karyotypic analysis, and by observation of the mouse satellite band in an analytical buoyant density centrifugation of total cellular DNA. Restriction endonuclease analysis of mitochondrial DNA is suggested as a useful method for monitoring the species of cells in culture.

INTRODUCTION

Numerous reports have documented the accidental contamination of long-term cell cultures with unrelated cell lines (1, 2). Various methods have been applied for monitoring cell lines for clonal purity. Among methods in common use are biochemical procedures, karyotypic analysis and surface antigen identification (3). These are reviewed by Stulberg (4).

We suggest here the use of restriction endonuclease cleavage patterns of mitochondrial DNA (mtDNA) for species identification of cells in culture. A contamination of a HeLa cell line by mouse cells was detected in our laboratories using this method. This contamination was subsequently confirmed by two other procedures.

MATERIALS AND METHODS

Growth of Cells. Cells were grown in suspension culture with Dulbecco's modification of Eagle's Phosphate Medium (Grand Island Biological Co.) and 5% calf serum.

Isolation of Mitochondrial DNA, *Restriction Endonuclease Digestions* and *Gel Electrophoresis* were carried out as described (5, 6).

Analytical Buoyant Density Centrifugation. A 1 ml cell pellet was mixed with 8 ml of buffer (10 mM Tris-HCl [pH 7.5], 50 mM NaCl, 1 mM EDTA) and lysed by the addition of 1 ml 10% sodium dodecyl sulphate. After vigorous mixing the viscosity was reduced by several passages through a 25 gauge needle. An aliquot was mixed with a CsCl solution and centrifuged at 44,770 rev./min, 25° C for 24 hr in a Beckman model E ultracentrifuge equipped with a photoelectric scanner.

Karyotype Analysis. Preparations for karyotype analysis were carried out by a modification of a standard method (7).

RESULTS

A cell line putatively identified as HeLa was in use in one of our laboratories for the construction of restriction endonuclease cleavage maps. This cell line was sent to the second laboratory for similar studies. Both laboratories isolated mtDNA from these cells, digested it with several restriction enzymes, and analyzed the digestion products by agarose gel electrophoresis.

The results of the analysis in the first laboratory are shown in Fig. 1. Slots 3 and 6 show the cleavage products generated by the enzymes *Hin*III and *Hae*II, respectively. The molecular weights of the fragments in each of these slots total approximately twice those of mouse and human mtDNAs. Dimer mtDNA is known to consist of a head-to-tail arrangement of monomer molecules (8).

The *Hae*II digest of mtDNA from the cell line in question in slot 6 is bordered by a *Hae*II digest of LA9 mtDNA (slot 5) and HeLa mtDNA (slot 7). The largest species in slot 6 migrates indistinguishably from the products of *Hae*II on LA9 mtDNA. The latter DNA contains a single *Hae*II site (5). The remaining species in slot 6 co-migrate with the products of *Hae*II on HeLa mtDNA (slot 7).

The *Hin*III digest of the cell line in question in slot 3 is similarly compared to digests by this enzyme of mouse LA9 (slot 2) and HeLa (slot 4) mtDNAs. It is clear that the bands in slot 3 are composed of the *Hin*III products of each DNA alone.

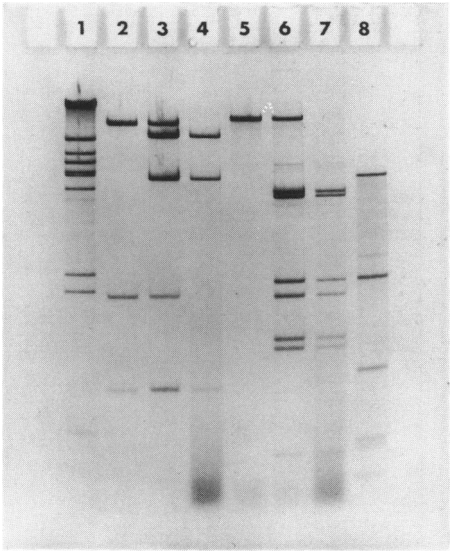

Fig. 1. Gel electrophoresis of restriction endonuclease digests of mtDNA. Electrophoresis was carried out at 50 volts, 6 hr in 1% agarose.
(1) λ x *Eco*RI + λ x *Hin*III.
(2) LA9 mtDNA x *Hin*III.
(3) "HeLa" mtDNA x *Hin*III.
(4) HeLa mtDNA x *Hin*III.
(5) LA9 mtDNA x *Hae*II.
(6) "HeLa" mtDNA x *Hae*II.
(7) HeLa mtDNA x *Hae*II.
(8) PM2 DNA x *Hin*III.

The HeLa mtDNA used for comparison with the mtDNA from the contaminated cell line was not extensively purified. The streak near the bottom of slots 4 and 7 is nuclear DNA. Slots 1 and 8 contain molecular weight standards. The *Eco*RI and *Hin*III products of bacteriophage λ DNA are shown in slot 1 (9, 10); the *Hin*III products of bacteriophage PM2 DNA are shown in slot 8 (10).

Approximately equal masses of mouse and human mtDNA are present in the isolate from the contaminated cell line, as judged by the relative intensities of appropriate DNA species in slots 3 and 6. The mtDNA content of a HeLa cell has been shown to be at least eight times that of a mouse cell (11). Assuming equal efficiencies of extraction of mtDNA from the mixed cell population, we estimate that the "HeLa" cell line contained 90% mouse cells at the time of DNA isolation.

The cleavage patterns in the recipient laboratory were generated with *Eco*RI and *Hin*III. The digests consisted exclusively of species generated by these enzymes on mouse mtDNA. We attribute the loss of the human cells to inadvertent selection during initial growth in culture dishes.

Two additional procedures were used to confirm the restriction endonuclease analysis. First, total cell DNA was extracted from the contaminated cell line and analyzed by

buoyant centrifugation in a CsCl solution, as described in Materials and Methods. The satellite DNA found in mouse cells was observed.

In addition, metaphase chromosome preparations were examined. Most spreads showed the same characteristics as those of the abnormal mouse cells (LA9) being carried in our laboratories. These cells are distinguishable from HeLa cells on the basis of several karyotypic features, including fewer total chromosomes and, most strikingly, a much higher proportion of acrocentric forms --approximately 60% *versus* 20% in HeLa cells (unpublished results).

DISCUSSION

The necessity for periodic monitoring of long-term cell cultures for contamination by other cell lines is now well established. The choice of an available method is often determined by the expertise in a given laboratory. Increasing use of restriction endonucleases and their commercial availability, as well as the widespread use of gel electrophoresis, makes the application of mtDNA cleavage patterns practical as a routine procedure, particularly in laboratories growing cells for studies with nucleic acids. The possibility of using several restriction endonucleases to more closely define a cleavage pattern enhances the appeal of this method, which has also been suggested by others (12, 13). The growing availability of restriction endonuclease cleavage maps of various mtDNAs may allow positive identification of contaminant cell species.

Variations in the cleavage patterns of mtDNAs within the same species have been documented (12). Thus, the production of reference cleavage patterns with several enzymes for cell lines in use in a given laboratory is recommended. It may be possible in this way to monitor contamination between cells of the same species.

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