Enzyme Polymorphisms as Genetic Signatures in Human Cell Cultures

Abstract. *The electrophoretic resolution of seven relatively polymorphic human gene-enzyme systems expressed in tissue culture cells can be used as a sensitive genetic monitor for intraspecific cell contamination. An identical genotype at each of the same allozyme loci provides a 95 percent (or greater) confidence estimate of the identity of two cultured lines, on the basis of the allelic frequencies of the seven enzyme loci in natural populations and in populations of independently derived cultured cells. Of 27 commonly used human cell lines examined, only one of 351 pairwise comparisons proved genetically indistinguishable.*

The relatively high incidence of cell-to-cell contamination within cell culture laboratories over the last 20 years has been emphasized by the publication of a list of more than 40 established human cell lines that were reported to be contaminated by the HeLa cell cervical carcinoma line (*1*). The biological detection of these discomfiting revelations has been largely karyological (absence of fluorescent Y chromosome and presence of several HeLa "marker" chromosomes), and in some cases biochemical (isoenzymes), and immunological (HL-A surface antigens) (*2–6*). The identification of cultured cell lines is especially important to a number of investigations of spontaneous or "induced" cellular transformations as well as to immunological cross-reactivity studies of cultured cells and infecting animal viruses. Since it is reasonable to suspect that human cells other than HeLa can cross-contaminate cell cultures, reliable procedures for establishing the genetic uniqueness of identity of a studied cell line with respect to possible contamination from any source (known or unknown) are required. For this purpose, we propose here the electrophoretic typing of seven gene-enzyme systems which are expressed in cultured cells and are polymorphic in natural human populations.

The detection of interspecific cell contamination is relatively easy with karyological or isoenzyme analysis (*7, 8*). For related mammalian species, the evolutionary divergence and fixation of electrophoretically distinct enzymes is a general phenomenon (*8, 9*). Thus by sampling two to three enzyme systems, the species identification of a cell line is possible. Within a species, however, the majority of electrophoretically resolvable enzymes is monomorphic, and thereby indistinguishable. Harris and others, however, have studied the character and extent of allelic variation in more than 80 gene-enzyme systems in European, Asian, and African populations (*10*). Approximately 28 percent of all the loci sampled exhibited some degree of polymorphism. The number of alleles and their frequencies vary between the gene systems, but generally the most common allele at a given locus has a characteristic frequency between 0.5 and 1.0 (*10, 11*). Approximately 85 percent of the variant genes were polymorphic within local populations, while 15 percent reflected differences between races or distinct populations within a race (*12*).

We surmised that by the resolution of the allozyme (allelic isozyme) phenotype of a cell line at several polymorphic gene-enzyme loci, a stable and statistically rigorous genetic signature could be derived which could be used to identify a specific cell line and its contaminants. Seven gene-enzyme systems were selected to test this hypothesis on the basis of the following criteria. (i) The enzyme is highly polymorphic in human populations; (ii) the enzyme is present in sufficient amounts in cultured cells to permit analysis; and (iii) the enzyme is polymorphic in the cell lines tested here.

Thirty human cell lines collected from four laboratories were harvested and tested by gel electrophoresis for ten gene-enzyme systems. The results of the seven most polymorphic allozyme systems are presented in Table 1 and the allele and genotype frequencies are tabulated in Table 2. The seven enzymes systems used were adenosine deaminase (ADA); glucose-6-phosphate dehydrogenase (G6PD); esterase-D (ESD); peptidase-D (PEP-D); phosphoglucomutase-1 and -3 (PGM_1, PGM_3); and 6-phosphogluconate dehydrogenase (PGD). The three systems that were ultimately dropped, adenylate kinase (*13*), esterase-A (*14*), and peptidase-A (*15*) were relatively monomorphic within the cell lines tested.

Of the 210 tests (seven enzymes times 30 lines) listed in Table 1, only one (ADA in cell line HEL-299) was not scorable, and the cause is not known. PGM_3 was absent in lines CCRF-CEM, EB-3, and RAJI, but these lines are the lymphoblast suspension cell cultures that normally do not express this enzyme. This property is itself a marker which may be used as a contamination monitor. Seven of the cultures were tested in duplicate from extracts prepared at different times and in different laboratories. In no case was a difference between duplicate extracts observed.

Three of the lines, KB, HEp-2, and J111, were suspected HeLa contaminants prior to our analysis since they lack a fluorescent Y chromosome, express G6PD type A, and have HeLa chromosome markers (*1, 3*). The other 26 lines were selected as representative in many cases because of existing evidence that they were not HeLa contaminants. The allozyme phenotype of HeLa was identical to that of the three suspected contaminants mentioned above and unique with respect to the other lines tested.

Excluding the three HeLa contaminants, the 27 remaining cell lines were compared in 351 individual pairwise comparisons (for example, Bewo to CCRF; Bewo to CHP 3; and CCRF to CHP 3). Of these, three pairs (A101D and A375; RAJI and CCRF-CEM; HeLa and EB-3) were identical in the initial screen. Cell lines A101D and A375 are human melanoma lines (*16*) and were obtained from HEM Research Laboratory. Both lines were obtained at the same time, were maintained at Meloy Laboratories on contract for several months on a different project, and were transferred to the laboratory of one of us (S.J.O.) where they were carried, harvested, and extracted together. The possibility that these lines were contaminants of each other seemed very likely. Subcultures from original stocks of the two lines were again obtained from HEM Laboratories and tested. In this second analysis, extracts from A375 differed from A101D extracts in two systems, PGM_1 and ESD (see Table 1), and the original identical extracts were the same as the A101D obtained later. Apparently the original A375 culture had been contaminated by A101D at some point prior to our original tests.

EB-3, RAJI, and CCRF-CEM are lymphoid lines that cannot be scored at PGM_3. The EB-3 and Hela cells were identical at the remaining allozyme loci, but lymphoid cell-specific isozyme differences in the patterns of PGM_3, ADA, and of adenylate kinase make the possibility of cell contamination unlikely, although not impossible. The match of RAJI and CCRF-CEM is probably a chance event since these lines differed with respect to the presence of the Y chromosome in a way predicted by the

sex of the donors. The remaining 350 pairwise comparisons of the 27 cell lines were distinguishable in one or more of the studied systems.

The allelic frequencies of the most common allozyme alleles in Negro and Caucasian populations, and in this survey of tissue culture lines, are presented in Table 2. The gene frequency distribution of the cell lines conforms to the population frequencies at four of the loci—those for PGM_1, PGM_3, G6PD, and ESD. There is somewhat more variability within the cell lines at the loci for ADA, PEP-D, and PGD, as compared to that reported in human populations, although each of these genes is polymorphic in man (*10, 11*). In addition, several cell lines express a slower migrating enzyme of ADA (termed ADA-*d*), (Fig. 1). The variation of ADA and PEP-D is not an artifact of sulfhydryl oxidation or reduction, since incubation of crude extracts with 25 m*M* β-mercaptoethanol or 20 m*M* oxidized glutathione alters the mobility of enzymes coordinately and not preferentially (*17, 18*). The ADA-*d* isozyme apparently results from the differential expression of a tissue-specific modifying gene, distinct from the ADA structural locus (*19, 20*). This modifying locus specifies a catalytic function that converts ADA-1, ADA-2, and ADA-1–2 to a slower migrating aggregate of ADA which is in itself difficult to type with respect to the allozyme phenotype (*19, 20*). Since the functioning of this modifier is variable and characteristic from cell line to cell line, it may be useful in diagnostic cell identification and is therefore included as a distinctive phenotype in Table 1. Diagnostic conclusions based on the ADA-*d* isozyme pattern should, however, only be used cautiously as supportive evidence along with an allozyme test of another system, since different tissue culture derivatives from the same patient have been shown to vary in their ADA-*d* isozyme patterns (*19*). Furthermore, ADA isozyme expression in fibroblasts is subject to variation under different culture conditions (*19*) and may change with passage in tissue culture. Despite these reservations, we have never observed an alteration of the ADA isozyme phenotype in any of the lines tested in different passages or from different extracts.

The genotypic frequencies derived from the survey of cultured human cells provide a statistical estimator of the efficacy of using the seven loci for establishing cell identity. The probability that any individual cell line would express the most common possible genotype at each of the seven loci is equal to the product of the individual genotypic frequencies at each locus (see column 9 in Table 2). This value $0.50 \times 0.63 \times 0.62 \times 0.78 \times 0.74 \times 0.63 \times 0.78 = 0.055$. Hence the probability that any individual will possess this genotype (column 10 in Table 2) is .05. In fact, of all the lines listed in Table 1, only VA-2 actually expresses this "most probable" genotype. Conversely, the probability that another cell line would express this same genotype by chance is also .05.

Table 1. Genotypes of established human cell lines at seven gene-enzyme loci. PGM_1, PGM_3, ADA, and ESD were resolved on starch gels, prepared and run in a tris-citrate (*p*H 7.0) buffer system (*31*). G6PD, PGD, and PEP-D were run in a tris-EDTA-borate system (*p*H 8.6) (*32*). Straining procedures have been described (*14, 17, 21, 25, 32, 33*) under these conditions, the fastest migrating (most anodal) allozyme for each enzyme system was as follows: ADA-1-1, PGM_1-2-2, PGM_3-1-1, $G6PD^A$, ESD-2-2, PEP-D-3-3, and PGD-A-A. Sources of established cell lines were as follows. American Type Culture Collection (A); Flow Laboratories (F); HEM Research Laboratories (H); VA-2 was obtained from Dr. Hayden Coon (C). The numbers in parentheses are CCC numbers of the lines from ATCC.

Designation	Source	Race	Sex	Allozyme phenotype						
				ADA	PGM_1	PGM_3	G6PD	ESD	PEP-D	PGD
Bewo	A (98)		F	2	1	1-2	B	1	1	A
CCRF-CEM*	A (119)	C	F	1	1	0	B	1	1	C
CHP 3	A (132)	N	M	d	1-2	1	A	1-2	1	A
CHP 4	A (133)	N	M	d	1-2	1	B	1-2	1	A
Cri du chat	A (90)	C	F	d	2	1-2	B	1	1	A
Detroit 525	A (65)	C	F	d	1	1-2	B	1-2	1	A
Detroit 532	A (54)	C	M	d	1	1	B	1	1	A
Detroit 562	A (138)	C	F	1	1-2	1-2	B	1	1	A
EB-3†	A (85)	N	M	1	1	0	A§	1	1	A
Flow-2000‡	F	N	F	d	1	1-2	AB	1	3	A
Flow-5000‡	F	N	M	d	1	2	A	1	3	A
Flow-6000	F	N	M	1d	1-2	1	B	1-2	3	A
Flow-7000	F		M	1d	1	2	B	1	3	A
HEL-299	A (137)	N	M	0	1	1-2	A	1	1	C
A101D‡	H		M	1	1-2	1	B	1	1	A
A375‡	H		F	1	1	1	B	1-2	1	A
A875	H		F	1	1	1	B	2	3	F
HT-1080	A (121)	C	M	1-2	1	1	B	1	1-3	A
IMR-32	A (127)	C	M	1	2	1	B	1	1	C
MCR-5	A (171)	C	M	d	1-2	1	B	1	1-3	AP
RAJI†	A (86)	N	M	1	1	0	B	1	1	C§
RD	A (136)	C	F	1	1-2	1	B	1	1-3	A
RPMI-2650	A (30)	C	M	1	1	2	B	1	1	A
SW13	A (105)	C	F	1	1	1	B	2	1-3	A
VA-2 (WI-18)	C		M	1	1	1	B	1	1	A
WI-38‡	A,F	C	F	d	1-2	1	B	1	1	A
HeLa‡	A,F (2)	N	F	1	1	1	A	1	1	A
KB*‡	A,F (17)	C	M	1	1	1	A	1	1	A
HEp-2*	F	C	M	1	1	1	A	1	1	A
J111*	F		F	1	1	1	A	1	1	A

*Cell lines previously suspected to be HeLa contaminant due to the presence of $G6PD^A$ (in lines derived from Caucasian patients), absence of fluorescent Y chromosome (in lines derived from males) or the presence of one or more distinctive HeLa chromosome markers (*1–3*). †Lymphoid suspension cell lines. These less express characteristic isozymes of ADA, but fail to express PGM_3 and an isozyme of adenylate kinase. †Cell lines which were grown, harvested, and extracted two or more times in different laboratories on different occasions. §H. Harris (personal communication) has scored RAJI as PGD-A and EB-3 as $G6PD^B$, in conflict with our observations. The reasons for these discrepancies are unclear.

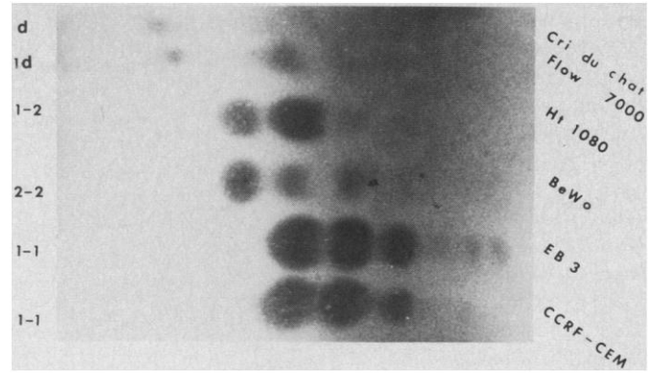

Fig. 1. Starch gel electropherogram of extracts of selected cell lines to illustrate indicated phenotype for adenosine deaminase. Cells (5×10^7) were suspended in 0.05*M* tris, 0.001*M* EDTA, *p*H 7.1 (1 ml); they were disrupted by sonication for 60 seconds and centrifuged at 6000 rev/min for 2 minutes. Conditions for electrophoresis and staining have been described (*21*, *36*). Forms ADA-1, ADA-1-2, and ADA-2 are the red cell allozyme forms. Forms ADA-1d and ADA-*d* represent those cell lines which express the tissue-specific isozymes (see text). The allozyme (1-1, 1-2, or 2-2) cannot be read easily with fully aggregated ADA-d forms, although association of these has been reported by Hirschhorn (*19*). The allozyme phenotype ADA-1d was determined from the red cell form.

In actual cell contamination tests, this value is a maximum since the probability of a cell line being identical to a second cell line by chance alone is computed according to the genotypic frequencies of the cell line in question, which only is as high as the most common genotype in less than 5 percent of all cell lines. For example, the chance that two of the lines that matched (A101D and the A101D masquerading as A375) were identical was $p(\text{ADA-1-1}) \times p(\text{PGM}_1\text{-1-2}) \times p(\text{PGM}_3\text{-1-1}) \times p(\text{G6PD}^B) \times p(\text{ESD-1-1}) \times p(\text{PEP-D-1-1}) \times p(\text{PGD-A-A}) = .026$. The probability of an identical genotype to HeLa by chance alone is .013. The estimate for cell line WI-38 is .018.

An important consideration in this proposition is the ability of cultured cells to faithfully express the phenotype of the donor and to persist in this expression after continuous passage in tissue culture. Allozyme variation is generally interpreted as involving a base substitution in an enzyme structural gene (*21*, *22*) which causes the placement of an alternative amino acid in the primary structure of the polypeptide. If the new amino acid has a different charge, the protein should exhibit an alteration in electrophoretic mobility that may not affect the enzyme's activity. Although exceptions to this interpretation have been reported (*23*), the general rule is upheld, for example, in more than 100 variants of human hemaglobin (*24*). The fidelity of expression of donor phenotype in cultured cells has been high for PGD (*25*), G6PD, and PGM_1 (*26*). The extinction of alternative allozymes in allozyme heterozygotes for autosomal genes is extremely rare in fibroblasts and has occurred in a few lymphoid cells upon establishment in culture (*27*).

Auersperg and Gartler (*26*) and Gartler (*4*) have studied the stability of G6PD, PGM_1, and PGM_3 in a human cell line (C12R), over a 3-year period or 36 passages. Although there was a dynamic variation in the karyotype during this time, the allozyme genotype remained the same despite several exposures to x-rays. One of the loci (PGM_1) was heterozygous and continued to express both allozymes over the 3-year period. Gartler (*4*) also reported the stability of enzyme phenotypes over time even when the cells were infected with mycoplasma or SV40 virus, or when exposed to bromodeoxyuridine or corticosteroid hormones. The frequency of enzyme variation after neoplastic transformation has also been studied by Gartler (*4*) and O'Brien *et al.* (*28*). Most of the transformation-associated enzyme-gene dysfunction involved generation or loss of isozymes and a very low ($\leq$ 1 percent) degree of shift in electrophoretic mobility (*28*). Thus, the expression of allozyme genotypes is both faithful and stable even after a variety of exogenous insults including viral, chemical, or spontaneous cellular transformation (*26*). Karyological monitors suffer to some extent in this respect (*5*, *29*).

It therefore appears that the allozyme phenotype can provide a confident estimation of cellular contamination or identity. The technical implementation of these procedures in a cell culture laboratory is neither expensive nor technically

Table 2. Allelic and genotypic frequencies of the seven polymorphic gene-enzyme systems in natural human populations and in the population of cultured cells described in the report. In the majority of these systems, the genotypic frequencies are in Hardy-Weinberg equilibrium. Thus with an autosomal gene-enzyme system with two alleles (1 and 2) the genotype frequencies distribute in a binomial distribution, $p^2 + 2pq + q^2$ for the frequencies of 1-1, 1-2, and 2-2, respectively; where p is the allelic frequency of 1, and q is the frequency of 2. The estimates are taken from published data for natural populations. The gene frequencies for the cell lines were calculated from the equation $p(1) = [2 \times (1\text{-}1) + (1\text{-}2)]/2 \times n$. The A375(C) contaminants and the suspected HeLa contaminants, KB, J111, and HEp-2, were not included in these calculations. The HeLa line was only counted once, so that the total population of cell lines examined number 27 ($= n$). Since G6PD is X-linked in man, the gene frequency estimates are complicated by X inactivation. Cell lines with a homozygous pattern (A-A or B-B) may result from one of three possibilities: homozygous female, hemizygous male, or heterozygous female with a single active X due to clonal derivation after X inactivation. The allelic frequency of G6PD^A was estimated by dividing the number of type A cell lines by the total. This number may be in error, depending on the frequency of the three possible situations.

Gene-enzyme system	Human chromosome	Number of alleles	Frequency of most common allele				Frequency of most common genotype				References
			N*	C*	Cell lines	Common allele	N*	C*	Cell lines	Genotype	
ADA	20	3	0.89–0.96	0.94	0.56	1	0.78–0.93	0.88	0.50	1-1	(*18*)
PGM_1	1	2	0.78	0.76	0.78	1	0.60	0.58	0.63	1-1	(*33*)
PGM_3	6	2	0.34	0.74	0.75	1	—	0.54	0.62	1-1	(*34*)
G6PD	X	2	0.7	1.0	0.80	B	0.7	1.0	0.78	B	(*35*)
ESD	13	2	0.90	0.90	0.83	1	0.80	0.81	0.74	1-1	(*36*)
PEP-D	19	2	0.93	0.99	0.70	1	0.91	0.98	0.63	1-1	(*17*)
PGD	1	4	0.95	0.95	0.80	A	0.93	0.92–.97	0.78	AA	(*25*)

*Gene and genotype frequencies in Negro (N) and Caucasian (C) populations.

difficult, and gel interpretation is straightforward. A single technician with homogenates of as few as 10^7 cells can comfortably analyze 10 to 20 cell lines for the seven allozymes discussed in a single workday (*30*).

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Regional Specialization of Reptilian Scale Surfaces: Relation of Texture and Biologic Role

Abstract. *The iridescent body scales of the fossorial uropeltid snakes produce these interference colors by keratinous ridges spaced at 2500 Å. The pattern inhibits wetting of the surface and adhesion of soil particles and thus reduces friction between the snake's trunk and walls of its tunnel. The epidermal scales of the blunt tail show a sharply defined pattern of spines and ridges with convergent flutings. Dirt caught here forms a plug that protects the snake's caudal end. The sharp transition of surface textures suggests (i) that selection for each of the two roles is great, and (ii) that the interference colors of many fossorial snakes indicate that friction as well as dirt adhesion are being reduced.*

Recent studies (*1*) have resurrected the hypothesis (*2*) that the keratinized surface ornamentation of reptilian skin reduces wear and friction. This hypothesis may explain why certain reptiles have regular microstructure that produces interference colors of unknown function (*3*). Although this explanation may be plausible, it lacks proof and does not explain the diversity of structural patterns seen (*4*). We here describe a system in which the biological role of these microstructures changes along a well-defined line so that the associated structural differences may be analyzed.

The rough-tailed (or shield-tailed) snakes (family Uropeltidae) comprise some 35 relict species of specialized (*5*) fossorial animals restricted to the southwest Indian hill country and Sri Lanka. The cylindrical trunk of these elongate cone-headed animals is always extremely smooth and shows iridescence along bands parallel to the long axis (Fig. 1). The name of the group refers to a spinose patch of variable size on the truncate caudal tip, the rough area of which is always restricted to part or all of the distally visible zone. The transition between the two zones is sharp, and structural colors extend to the immediate edge of the rough tip.

Scanning electron micrographs (*6*) show the surface texture of the two regions. The widely overlapping scales along the trunk (Fig. 2E) of all species examined show an ornamentation of regular ridges on a spacing of 2500 Å (Fig. 2H). The grooves between the ridges contain round to oval pits at an apparently random spacing. The pits are sometimes filled with irregular matrix of uncertain origin (Fig. 2, G and I). The ridges are aligned parallel to the long axis of the body, even on scales, such as the lateroventral ones, that are asymmetrical. In general, the exposures of the topmost squamous cells are transversely subelliptical, and each ellipse has a pointed lateral tip. The sutures cross the ridges at near right angles, interdigitating for 2000 to 5000 Å on a 4000 to 5000 Å spacing, so that the ridge ends of successive scales do not align. When the dorsal surface of these snakes is examined in bright light (sunlight) with the visual axis just parallel to the path of the incident light, one sees a band of interference colors on each side of the midline with the colors ranging from blue (medially) to orange-yellow (laterally). The transition of colors corresponds to the changing spacing of ridges normal to the incident light around the curvature of the body.

The scales of the tail immediately fringing the sudden blunt termination show the same kinds of ridges and pores, except that the ridges are not aligned and often appear to be more shallow. More posteriorly, the surface undulates on a spacing greater than and independent of the surface exposures of the squamous cells of the keratinized Oberhäutchen. The actual architecture of the system is species specific (*7*) (in Fig. 2, compare A–C with F). The undulant surface may show one or more ridges per scale, and the terminal scale covering the distal tip tends to become enlarged and modified into a keratinized shield (possibly underlain by an enlarged terminal ossification of the vertebral column) (*8*), that bears sharp ridges and spines of different sizes in a regular pattern. Such spines and