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Stability of Polymorphic Enzyme Phenotypes in Human Tumor Cell Lines (40711)

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Hundreds of cultured cell lines from a large variety of human tumors have now been established as experimental models of cancer (1–11). The risk of cross-contamination with cells of other lines has been demonstrated by a number of cases of contamination by the HeLa cell line (12–14), and by another detected contamination among a group of G-6-PD type B lines (15–17). By analysis of polymorphic enzymes with inherited electrophoretic mobility variants observed in human tissues, cell lines can be efficiently characterized so that contamination can be either detected or shown not to exist (1, 12, 13, 15–20).

A prerequisite for valid results is that phenotypes of polymorphic enzymes remain stable *in vitro* and *in vivo.* Ideally, to demonstrate phenotype stability, cell strains derived from normal tissues and lines from malignant tumors should be compared with each patient's normal and malignant cells. The logistics, however, have usually been too complicated to completely achieve this end. The present paper reports on a partial stepwise approach toward a satisfactory answer to this question (21) and provides some of the rationale and validation of our ongoing analysis of about 400 human tumor cell lines during the last several years which has been reported briefly (15, 16), and will be published in detail.

Materials and methods. Specimens of both malignant tumors and normal tissues corresponding to the tissue of origin of each tumor were removed at biopsy or autopsy from each of 11 patients with the following carcinomas: breast, 3; colon; renal cortex; lung, 4; stomach, 2; and from one patient with Hodgkin's lymphoma. The tissues were kept frozen until extracts for enzyme analysis could be made by homogenization with 1–3 vol of distilled water, followed by centrifugation and collection of the supernatant. Human tumors propagated in nude mice were similarly processed.

Human tumor cell lines, grown as monolayer or suspension cultures, and cultured skin fibroblast strains were harvested and washed with saline. Samples were lysed with 1–2 parts distilled water followed by freeze–thawing two to three times and centrifuged at 2000 rpm for 15 min at 4°C. References to data on the origin and other characteristics of the cell lines reported in this paper are given in Tables II and III. Human erythrocytes were collected from heparinized blood, the serum separated by centrifugation was discarded, and the erythrocytes were washed three times with saline.

The cell samples were stored at −80°C which was found to preserve enzyme activity and mobility patterns for months; in some cases, as an extra precaution, NADP and mercaptoethanol were added to stabilize enzyme activity and maintain enzyme electrophoretic mobility patterns. Electrophoresis was carried out according to methods of Harris and Hopkinson (22). The following enzymes were analyzed by the methods given in the references: glucose-6-phosphate dehydrogenase, G-6-PD (22–24); acid phosphatase, ACP_1 (22, 25); glyoxalase I, GLO (26); malic enzyme, mitochondrial, MEm (27–29); phosphoglucomutase, PGM_3 (30); α-fucosidase, FUC, by isoelectric focusing (31); phosphoglucomutase, PGM_1 (32); esterase D, ESD (33); adenosine deaminase ("red cell" isozyme), ADA (34); adenylate kinase, AK_1 (35); 6-phosphogluconate dehydrogenase, PGD (22, 36); glutamate–oxaloacetate transaminase, GOTm (22); and peptidases, PEPA (22, 37), PEPB (38), PEPC (39), and PEPD (40). The buffer system for GLO consisted in part of 0.03 *M* diethylbarbituric acid (MW 184.19) rather than barbituric acid (MW 128.09) as stated in Ref. (26). The peptidases are included as a polymorphic group of loci, although strictly speaking PEPA, B, or C may not quite qualify as polymorphic in the Caucasian or Negro populations using the methods indicated. The phenotypes for these enzymes are expressed with standard accepted

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terminology: homozygotes are 1, 2, A, B, or C, where the same allele occurs at the same locus on homologous chromosomes in an individual or cell line; heterozygotes are 2-1, BA, CB, or CA where two different alleles occur together.

Control samples for various enzyme phenotypes were kindly provided by Drs. Lynda J. Donald, Department of Pediatrics, University Hospital, London, Ontario, Canada (PGM_1, PGM_3); Phyllis J. McAlpine, Children's Centre, Health Science Centre, Winnipeg, Manitoba, Canada (ESD, ADA, AK_1, PGD); Susan M. Povey, MRC Human Biochemical Genetics Unit, the Galton Laboratory, University College London, England (controls for many of the enzymes); Bryan M. Turner and N. G. Beratis, Division of Medical Genetics, Department of Pediatrics, Mount Sinai School of Medicine, New York, (FUC); and Geoffrey O'Neill, Sloan-Kettering Institute for Cancer Research, New York (GLO). In addition, erythrocytes of W.C.W., typed at the Galton Laboratory, were used as a control for several enzymes. Phenotype frequency products were obtained by multiplying the frequencies of all observed phenotypes for a cell line using frequencies published for normal populations of the same race as the patient from which the particular cell line originated (22).

Results. During the stages from normal human tissue to malignant tumor to established tumor line *in vitro*, and to tumor propagated from cells of this line in nude mice, the following comparisons demonstrate that phenotypes of the polymorphic enzymes used in our characterization studies of cultured human tumor cell lines remain stable.

1. When the 12 malignant human tumors were compared with 12 corresponding normal tissues for isozyme patterns of G-6-PD, PGM_3, FUC, PGM_1, ESD, ADA, AK_1, and PGD, concordant phenotypes (57 total) were observed in all sets, showing that the conversion of normal to malignant cells in the patients had not changed the phenotypes (Table I). The data presented here are not complete for all 24 tissues, since some enzymes in certain specimens were not sufficiently active to be phenotyped. FUC 2-1 and 2 phenotypes could not be distinguished from each other in these specimens, but usually were distinguished in cultured cells.

2. A comparison of a skin fibroblast culture with red blood cells (Table II) from the same normal donor showed five concordant phenotypes out of five comparisons (G-6-PD, PGM_1, ESD, AK_1, PGD). Thus, cultivation *in vitro* had not changed these phenotypes.

3. Two cultured human tumor cell lines were compared with normal skin fibroblasts cultured from the same two patients (Table II). The kidney tumor line, Caki-1, and the related skin fibroblast strain had nine out of nine phenotypic comparisons identical for G-6-PD, PGM_3, FUC, PGM_1, ESD, AK_1, and PEPA, B, and C. The melanoma line, Malme-3M, and the fibroblast strain from the same patient had 13 identical phenotypes out of 13 enzymes including the above 9 enzymes, and including ACP_1, GLO, MEm, and PEPD.

TABLE I. IDENTICAL PHENOTYPES OF EIGHT ENZYMES FOR SETS OF MALIGNANT AND CORRESPONDING NORMAL TISSUE FROM 12 PATIENTS.

Tissue set	G-6-PD	PGM_3	FUC	PGM_1	ESD	ADA	AK_1	PGD	Freq. Product
Breast				1	1		1		0.4318
Breast		2-1		1	2-1	1	1		0.0333
Breast		1	1	2-1	1	1	1	A	0.0674
Colon	B	2-1	[a]	2-1	2-1				0.0253
Kidney		2-1	[a]	2-1	1		1		0.1052
Lung		1	[a]	1	1		1		0.2333
Lung		1	[a]	1	1		1		0.2333
Lung	B	1	1	2-1	1				0.0880
Lung	B	1		1	1		1		0.2333
Hodgkin's dis.		2	1	2-1	1		1		0.0089
Gastric		1	1	1	2-1	1		A	0.0266
Gastric		2-1	1	1	1		1		0.0970

[a] Two cathodal bands of equal intensity: probably phenotype 2-1 (frequency 0.3814), less likely phenotype 2 (frequency 0.0567).

TABLE II. IDENTICAL PHENOTYPES OF 16 ENZYMES IN ERYTHROCYTES/CULTURED SKIN FIBROBLASTS, CULTURED KIDNEY CARCINOMA LINE/CULTURED FIBROBLASTS, AND CULTURED MELANOMA LINE/CULTURED FIBROBLASTS, EACH SET DERIVED FROM SAME DONOR.

Donor diagnosis	Cell type	G-6-PD	ACP_1	GLO	MEm	PGM_3	FUC	PGM_1	ESD	ADA RBC	Tiss.	AK_1	PGD	GOTm	PEPA	PEPB	PEPC	PEPD	Freq. product	Passages tested	Reference
Normal	RBC	B			No act.	No act.		2-1	2-1			1	A						0.0548		
	Cultured skin	B			2-1	1		2-1	2-1			1	A						0.0150	7, 10, 11	
Ca. kidney	Caki-1	B	B	2-1	2	2-1	2-1	1	2-1	1	d	1	A	1	1	1	1		0.0001	14, 19, 26, 43	4
	Caki-1 skin	B				2-1	2-1	1	2-1		d	1			1	1	1	1	0.0139	14, 20	
Melanoma	Malme-3M	B	BA	2-1	1	1	1	1	1	1		1	CA	1	1	1	1	1	0.0004	10, 43, 48, 50, 51	4
	Malme-3 skin	B	BA	2-1	1	1	1	1	1		d	1			1	1	1	1	0.0112	3	

These phenotypes, therefore, were stable during tumorigenesis, as well as cultivation during many passages of both malignant and normal cells.

4. Constancy of phenotypes shown in Table III of G-6-PD, ACP_1, GLO, MEm, PGM_3, FUC, PGM_1, ESD, ADA, AK_1, PGD, GOTm, and peptidases A, B, and C was also found between lines derived from the same patient. Included were two series of tumor lines, with each series derived from a different patient: the MDA-MB-175 series of lines I, II, III, IV, VI, and VII and the MDA-MB-331 series, II and IV. Each line was derived from a pleural effusion, taken at intervals of a number of weeks. Also included in the analysis were the following eight pairs or sets of cell lines where lines in a pair were either derived from the same tumor or one line was later cultured as a subline: SK-LU-1 and SK-LU-1b, lung carcinoma; 734B and MCF-7, breast carcinoma; C-33A and C-33B, C-4 I and C-4 II, and two HeLa lines ($HeLa_1$ and $HeLa_2$) cultured separately for many years, carcinomas of the cervix; SK-UT-1 and SK-UT-1b, primary mixed mesodermal tumor of the uterus; HEC-1A and HEC-1B, adenocarcinoma of the endometrium; SK-MEL-26 monolayer and SK-MEL-26 suspension, malignant melanoma. All 95 comparisons of phenotypes within sets were concordant. The sum of the numbers of passages of different lines within sets (for example, HEC-1A and HEC-1B, 117 + 122 = 239 passages) attests to the high genetic stability of these phenotypes during the course of separate culture.

5. A comparison of two previously unpublished lines established from the same carcinoma specimen (probably lung) in this laboratory, Calu-6 as a cultured cell line and PR 164 as a tumor line in nude mice, showed identical phenotypes for MEm 1 (frequency = 0.44) and PGM_3 2 (frequency = 0.06). These human isozymes, therefore, were stable in human tumor cells grown in parallel *in vitro* and in the nude mouse.

Discussion. We are not aware of previous extensive comparisons of polymorphic enzyme phenotypes between human tumor cell lines and the normal or malignant tissues of the patients of origin. In the present study, many enzymes were shown to have stable electrophoretic phenotypes in all the *in vivo*

TABLE III. IDENTICAL PHENOTYPES OF 15 ENZYMES IN 10 SETS OF CULTURED HUMAN TUMOR CELL LINES, EACH SET DERIVED FROM ONE PATIENT.

Tissue	Patient no.	Cell line	G-6-PD	ACP_1	GLO	MEm	PGM_3	FUC	PGM_1	ESD	ADA	AK_1	PGD	GOTm	PEPA	PEPB	PEPC	Freq. product	Passages tested	References
Lung	1	SK-LU-1	B		2	1	1	2	2	2		1						2.0×10^{-6}	35, 48	4
		SK-LU-1b	B				1	2	2	2	1	1	A		1	1	1	1.2×10^{-5}	19	
Breast	2	734 B	B	CB	2-1		1	1	2-1	2-1	1				1	1	1	0.0005	6 (ours)	41
		MCF-7	B	CB	2-1		1	1	2-1	2-1	1	1	A	1	1	1	1	0.0004	22, 31	41
	3	MDA-MB-175 I	B				2-1		2	1		1						0.0049	10, 25	4
		MDA-MB-175 II	B				2-1		2	1								0.0051	45, 50	4
		MDA-MB-175 III	B		2						1				1	1	1	0.1322	42	4
		MDA-MB-175 IV	B				2-1		2	1								0.0050	16, 40	4
		MDA-MB-175 VI	B				2-1		2	1								0.0050	5, 18	4
		MDA-MB-175 VII	B				2-1		2	1								0.0050	20, 38, 41, 45	4
	4	MDA-MB-331 II	B			2	1		1	1		1						0.0124	5	
		MDA-MB-331 IV	B				1		1	1		1						0.2333	5	
Cervix	5	C-33A	B	BA	2	2	1	1	1	1	1	1	A	1	1	1	1	0.0007	89, 93	42, 43
		C-33B	B	BA		2	1		1	1	1	1	A	1	1	1	1	0.0041	86, 92	42, 43
	6	C-4 I	B	B	1	1	1	2	1	1	1	1	A	2-1				8.6×10^{-6}	86	42, 43
		C-4 II	B		1	1	1	2	1	1	1	1	A	2-1				2.2×10^{-5}	91	42
	7	$HeLa_1$	A	BA	2	2-1	1	1	1	1	1	1	A		1	1	1	0.0002		44
		$HeLa_2$	A	BA	2	2-1	1	1	1	1	1	1	A		1	1	1	0.0002		45
Uterus	8	SK-UT-1	B	B	2-1	2-1	1	2-1	1	1	1	1	A					0.0073	3, 30	4
		SK-UT-1b	B	B	2-1		1	2-1	1	1	1	1	A	1				0.0144	29	
	9	HEC-1A	B	BA	2	1	2-1	1	1	1	1	1	A	1					117	46, 47
		HEC-1B	B	BA	2		2-1	1	1	1	1	1	A	1					122	46
Melanoma	10	SK-MEL-26[a]	B	BA	2-1	1	1	1	2-1	2-1	1	1	A	1				0.0013	8, 9, 21, 69	9
		SK-MEL-26[b]	B	BA		1	1	1	2-1	2-1	1			1				0.0029	70	9

[a] Monolayer.
[b] Suspension.

and *in vitro* situations compared. Paired samples of tumor and normal tissues from 100 patients have previously been reported to have concordant G-6-PD and PGM_1 phenotypes (13). However, our study also includes PGM_3, FUC, ESD, ADA, AK_1, and PGD in paired samples from 12 patients. Several fibroblast strains derived from normal persons who were typed directly for G-6-PD and PGM_1 were found to maintain the enzyme phenotypes in culture (13). Our study confirms this comparison and extends it to include ESD, AK_1, and PGD. Stability has also been reported for phenotypes of G-6-PD and PGM_1 in cell strains after cultivation for 3 years, after infection with mycoplasma and SV40, after treatment with drugs (13), and after X irradiation (13, 48). In our analysis of two tumor lines and normal fibroblast strains from the same patients, all phenotypes within a pair were concordant. Stability of isozyme mobilities has been observed between normal and transformed cells. For example, established cell lines of the Chinese and Syrian hamsters and the mouse maintained the same species-specific isozymes as seen in fresh tissues (49). Furthermore, when fresh erythrocytes of human donors were compared with lymphoblastoid lines derived from their leukocytes stability of phenotypes was observed (18).

Long-term cultivation of human tumor lines, separated by as many as 239 passages, did not change the phenotypes, thus confirming previously observed stability over 3 years of *in vitro* cultivation for two human tumor lines (13). Likewise, phenotype stability after long-term culture was reported for 26 enzymes in 66 human lymphoblastoid lines with only two exceptional changes, from heterozygosity to homozygosity, observed in two lines grown for many years in several laboratories (18). However, in the present study 35 heterozygous phenotypes in tissue samples or cell lines were observed with complete concordance with the heterozygous phenotypes observed in other material or cell lines from the same patient.

The observation that MEm and PGM_3 phenotypes were the same in a cultured line and a nude mouse-grown tumor line derived from the same human tumor agrees with previous reports that growth of human tumor cells in the nude mouse does not affect the electrophoretic mobilities of several other enzymes (50, 51). Similar results have been observed for tumors in nude mice originating from cell lines of the Chinese and Syrian hamsters, mouse, rat, rabbit, and man, and with human–rodent cell hybrids (52–56).

An interesting example of phenotype stability, which might have been considered as a case of instability, was reported for a group of black female patients who were G-6-PD type BA (57–60). Benign or malignant tumors and tumor cell lines from these patients were either A or B, not BA. Taking into account that G-6-PD is X linked and subject to the Lyon effect, the explanation given was that these tumors arose as single cell clones. This problem would not occur for other enzymes studied presently since they are not X linked.

The stability of polymorphic enzyme phenotypes contrasts with the relative instability of chromosomes in tumors (61) and cultured cell lines (62). Because of variation of karyotypes within a cell line karyotypic variation occurs between sublines or clones established from a line. Similarly, since cases of cross-contamination are likely due to the transfer of only a few or even single cells, a cross-contaminated line may have a karyotype different from that of the contaminating lines. This interpretation can be made from the karyotypic analysis of a number of human tumor cell lines determined by polymorphic enzyme analysis to be a contamination group (17).

Whether the demonstration of a few identical marker chromosomes in two or more cell lines carries sufficient weight to declare them as cross-contaminated has become more problematical as a consequence of recent karyotypic studies of human tumors and tumor cell lines with chromosome banding techniques. These studies have shown that specific translocations and other karyotypic modifications involving a nonrandom selection of chromosomes occur frequently and can be correlated with specific types of cancer (63–70). Therefore, tumor cell lines derived from tumors of different patients with certain types of cancer could be expected, in some cases, to have certain similar marker chromosomes. Also, marker chromosomes resembling the HeLa markers were detected in a pleural effusion of a black female patient with breast cancer and in a cell line derived from her pleural effusions (71). Such a cell line might have been considered as HeLa cell contaminated if the patient's own effusions had not been examined directly. Furthermore, the results of analysis of 15 polymorphic enzymes showed that two enzymes could distinguish the patient and the cell line from HeLa cells. Therefore, because of (a) the lack of frequency data on the occurrence of the independent origin of identical marker chromosomes in particular types of tumors and derived cell lines and (b) the inconsistent presence of markers in all cells, clones, or sublines of a line, one cannot give a statistical test of significance to evaluate karyotypic data as can be done with polymorphic enzyme data.

The overwhelming concordance of phenotypes in the various comparisons made in this study of many polymorphic enzymes on human tumor cells confirms and extends other reports on stability. This signifies that these enzyme phenotypes are stable in spite of cellular changes to malignant or transformed status and on long-term tissue culture. Therefore, these enzymes can be effectively used to individually distinguish human tumor cell lines, and the phenotype frequency data obtained from normal human populations are applicable for the calculation of the frequency of the combination of phenotypes of a cell lines.

Summary. Concordance of polymorphic enzyme phenotypes in various comparisons of human normal and malignant cells *in vitro* and *in vivo* establishes isozyme stability after change to the malignant state and after long-term tissue culture. Such enzymes, therefore, can be used with considerable confidence to individually distinguish human tumor cell lines. The stability validates the use of phenotype frequency data obtained from surveys of normal human populations in the analysis of data on human tumor cell lines.

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