Mutually Exclusive Genetic Signatures of Human Breast Tumor Cell Lines with a Common Chromosomal Marker

Michael J. Siciliano, P. E. Barker, and Reilda Cailleau

ABSTRACT

Seventeen recently established human breast carcinoma cell lines of metastatic origin (MDA series), HeLa cells, and MCF-7 (a well-established breast carcinoma cell line) were studied by starch gel electrophoresis for allozymic differences at 17 enzyme loci. Ten loci proved to be informative with the exception of MDA-134 and -309, which had the same genetic signatures. The probability of these latter two lines of independent origin and finding their similar genetic signatures by chance is 0.07. These studies enable us to conclude that the chromosomal marker shown to be common to these breast carcinoma cell lines of metastatic origin is not present because of cross-contamination of the lines with other long-term lines or each other.

INTRODUCTION

Cailleau et al. (3) reported the successful establishment of 19 long-term human breast carcinoma cell lines of metastatic origin from 19 different patients. G-banding chromosome analysis of 7 of these lines indicated that each contained a translocation involving the q or long arm of Chromosome 1 (4). Preliminary cytogenetic studies (2) on an additional 6 of these lines revealed a consistency of chromosome 1 alterations in the cells. These cell lines are now under intensive investigation in this institution and elsewhere for presence of the 1q marker, tumorigenicity, estrogen receptors, and immunological properties.

A cytogenetic marker common to a particular neoplastic disease (e.g., Ph1 chromosome in chronic myelogenous leukemias) (7, 11, 16) can be an important handle in the diagnosis and understanding of the biology and evolution of the disease. However, when present in cell lines, it may reveal nothing less trivial than the possible contamination of the cells by already established long-term lines (9, 10) or with each other. Since the validity and significance of a spectrum of studies being conducted in several laboratories depend on the independent origin of the breast carcinoma cell lines, we have typed 17 of the 19 lines with respect to enzyme markers identifiable by starch gel electrophoresis. In the human population, there are many enzyme loci which contain alleles the products of which are codominantly expressed and are separable by electrophoresis (6). These separable products of different alleles at a single locus are specific forms of isozymes (formerly isoenzymes) called allozymes. The allozyme phenotype (equivalent to the genotype) at any locus is therefore fixed at conception for any individual and, except for somatic mutation, is shared by all the cells, as well as cell lines, derived from that individual. The allozyme phenotypes over a spectrum of loci, for which variant alleles have been identified at a reasonable frequency (>1%), have been referred to as the genetic signature of the cell lines in which they have been studied (12). Since these phenotypes have been shown to be stable irrespective of chromosome changes (1, 15) and to become altered only at frequencies consistent with mutational events (18), such analysis has been shown to be useful in establishing the integrity of cell lines (12, 14, 15).

We report that 16 of the 17 breast tumor cell lines can be unambiguously identified by this method so that the biological characteristics common to them cannot be attributed to cross-contamination. We also present the genetic signatures obtained for each line so that workers elsewhere can monitor the lines for their continued integrity.

MATERIALS AND METHODS

The 17 breast tumor cell lines studied are listed under their prefix MDA-MB in Table 1. The genetic signature of one of these (line 468) has been previously reported (14). Also listed in Table 1 are 2 other cell lines run as controls: HeLa and MCF-7 [a breast carcinoma cell line (19) believed to be free of contamination]. A pellet (0.1 to 0.2 ml) of cells, twice rinsed in 0.9% NaCl solution, was suspended in 2 volumes of homogenizing medium (0.01 M Tris-HCl, pH 7.5-7.001 M β-mercaptoethanol:0.001 M EDTA) and sonicated (eight 2.5-sec bursts with a Heath sonicator equipped with a microprobe and set at 80 watts). Homogenates were stored at −70° for <2 months, thawed, and cleared by centrifugation (10,000 × g, 45 min). Cleared homogenates were subjected (12 at a time) to starch gel electrophoresis as generally described by Siciliano and Shaw (17).

Three different buffer systems were used: System 1, electrode, 0.5 M Tris:0.016 M versene:0.65 M borate, pH 8.0; gel, dilute 60 ml of electrode buffer up to 600 ml with water; System 2, electrode, 0.13 M Tris:0.43 M citrate, pH 7.0; gel, dilute 40 ml of electrode buffer up to 600 ml with water; and System 3, electrode, 0.1 M Tris:0.1 M maleic acid:0.1 M EDTA:0.01 M MgCl2, pH 7.4; gel, 1:15 dilution of electrode buffer.

The products of the following 17 enzyme loci were resolvable. These were on Buffer System 1: glucose-6-phosphate dehydrogenase (G6PDH), glutathione reductase (GR), mitochondrial forms of glutamate oxaloacetate transaminase (GOTw) and malic enzyme (MEw), 6-phosphogluconate dehydrogenase (6PGD), peptidases A, C, and D (Pep-A, -C, and -D), and α-glucosidase (αGluc); on Buffer System 2:

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esterase D (EsD), glyoxalase I (GlyI), phosphoglucomutase I (PGM₁), adenylate kinase 1 (AK₁), the RBC form of adenine deaminase (ADAᵦᵣᵦ), and the cytosol form of aconitase (ACON₈); and on Buffer System 3: PGM₂ and PGM₃.

Following electrophoresis, gels were sliced into as many as 7 slabs, and on each slab an enzyme was visualized by histochemical staining. Recipes for most enzyme stains are described by either Siciliano and Shaw (17) or Harris and Hopkinson (6). GlyI was stained according to the method described by Parr et al. (13). After staining, gel slices were photographed (35 mm) and discarded.

RESULTS AND DISCUSSION

Ten enzyme loci were informative in the study in that at least one of the cell lines displayed a phenotype for the locus other than the most common one observed in human populations. Allozyme phenotypes for these loci to the extent that they were determined in the 17 breast cancer lines and 2 controls are tabulated in Table 1. Examples of the variant phenotypes observed in the cells are shown in Figs. 1 to 8.

Seven enzyme loci (GR; Pep-A, -C, and -D; αGluc; AK, and ACON₈) were uninformative in that they all displayed the most common phenotype for humans. The phenotypes for all loci comprise the genetic signature for each cell line. The possibility of HeLa contamination is eliminated in 15 of the 17 MDA-MB cell lines and MCF-7 by the presence of Caucasian-type G₆PDHᵦ. Line 468, derived from a black woman, has been previously reported as distinguished from HeLa even though the line is G₆PDHᵦ by a difference at PGM₁ (14). Line 415, derived from a woman from Paraguay originally considered Caucasian, has the α or HeLa form of G₆PDH. The line is, however, clearly free of HeLa contamination as indicated by the different phenotypes for PGM₂, EsD, MEᵦᵦ, GlyI, and 6PGD.

Sixteen of the 17 MDA-MB breast carcinoma cell lines, as well as the HeLa and MCF-7 line, all have unique genetic signatures with respect to each other. This indicates that they are of independent genetic origin and that they are not cross-contaminated. The validity of this finding is substantiated by the fact that the frequencies of the phenotypes at each locus over all the cell lines are not significantly different from the frequencies of the phenotypes in the loci in human populations.

Two cell lines, MDA-MB-134 and -309, have identical genetic signatures for the enzyme loci products resolvable from them. The frequency of just that genetic signature in the human population is calculated as the product of the gene frequencies in the human population of each of the phenotypes displayed in that genetic signature. That frequency for the MDA-MB-134 and -309 genetic signature is 0.024. If these were the only cell lines studied, the probability of their being independently derived and not contaminants of each other would be the square of the frequency of that genetic signature, or \(5.8 \times 10^{-4}\). Such a result would imply that these were not independently derived, but contaminants. However, the probability of encountering 2 such signatures by chance in a sample of 19 independently derived cell lines is best described by a binomial distribution and may be calculated by:

\[
\frac{n!}{i!(n-i)!} (P)^i (1-P)^{n-i}
\]

where \(n\) is the number of cell lines studied, \(i\) is the number of cell lines with identical signatures, and \(P\) is the frequency

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>No. of allozyme phenotypes at informative loci</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA-MB</td>
<td></td>
</tr>
<tr>
<td>134</td>
<td>1 1 1 1 1 1 1 b a 1</td>
</tr>
<tr>
<td>157</td>
<td>1 1 1,2 1 1 1 ND b 1,2 b a 1</td>
</tr>
<tr>
<td>175</td>
<td>2 1 1,2 1 1 1 ND 1,2 b a 1</td>
</tr>
<tr>
<td>231</td>
<td>1,2 1 1 1 1 1 ND 1 2 b a, c 1</td>
</tr>
<tr>
<td>253</td>
<td>1 1 1,2 1 1,2 1 1 2 b a 1</td>
</tr>
<tr>
<td>309</td>
<td>1 ND 1 1 1 1 1 b a 1</td>
</tr>
<tr>
<td>330</td>
<td>1 1 1 1 1 ND 1 b a 1, 2</td>
</tr>
<tr>
<td>331</td>
<td>1 1 1 1 1 1 2 2 b a 1</td>
</tr>
<tr>
<td>361</td>
<td>1 1 1,2 1 1 2 1 b a 1</td>
</tr>
<tr>
<td>390</td>
<td>1 1 1,2 1 1 1 ND 1,2 b a 1</td>
</tr>
<tr>
<td>415</td>
<td>1 1 1,2 1 1 2 1,2 b a, c 1</td>
</tr>
<tr>
<td>416</td>
<td>1,2 1 1 2 1 1 ND 1,2 b a 1</td>
</tr>
<tr>
<td>431</td>
<td>1 1 2 1 1 ND 2 b a 1</td>
</tr>
<tr>
<td>435</td>
<td>2 1 1 1 1 1 1 2 b a 1</td>
</tr>
<tr>
<td>436</td>
<td>1 1 1 1 1 1 1 2 b a 1</td>
</tr>
<tr>
<td>453</td>
<td>1 1 1,2 1 1 1 ND 1 b a 1</td>
</tr>
<tr>
<td>468</td>
<td>1 1 2 1 1 1,2 2 a a 1</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
</tr>
<tr>
<td>HeLa</td>
<td>1 1 1 1 1 1 1 2 a a 1</td>
</tr>
<tr>
<td>MCF-7</td>
<td>1,2 1 ND 1,2 1 ND 1,2 b a 1</td>
</tr>
</tbody>
</table>

\(\text{a}\) Some cell lines produce large quantities of ADA tissue conversion factor which obscures the ADAᵦᵦᵦ phenotype (8). Such samples are not read for that phenotype.

\(\text{b}\) Some samples were run before a particular enzyme-staining procedure was operational in the laboratory. For such situations, ND indicates not done.
of that signature in the human population (5). In this case, that probability is 0.07. Therefore, the identity of the genetic signatures of 134 and 309 is not too disturbing, and they may sort out as more enzymes are added to the program or perhaps when we can resolve $M_{\text{ex}}$ in 309.

We conclude that the chromosomal marker common to these cell lines, as well as any other biological similarities among them, is not present because of cross-contamination with other established long-term lines or each other.

REFERENCES


Figs. 1 to 8. Zymograms (stained starch gel slices) demonstrating informative phenotypes for enzyme loci observed after electrophoresis and histochemical staining. The anodal end is toward the top of each figure. The direction of migration was toward the anodal end with the exception of $GOT_{\alpha}$ (Fig. 8), which migrates cathodally. For each figure, the order of samples is from left to right.

Fig. 1. PGM. a and b are of the same gel slice (Tris-maleic acid, pH 7.4, buffer system), with a photographed after 10 mm of staining and b taken after 45 mm. Products of the PGM1 locus are fastest in migration, late staining, and therefore best observed in b. Of the 6 samples, one has the products of only the PGM1 allele (first sample). The other 5 all appear as PGM1 homozygotes. Products of the PGM2 locus are slowest in migration and stain so rapidly that they can be clearly viewed with this buffer system after only 10 min of staining (a). The 3 most common types are represented among the 6 samples. The first, second, and fourth samples are PGM3 homozygotes. The third and fifth samples are PGM2 homozygotes. The last sample is a 1,2 heterozygote. After further staining, secondary bands appear which make difficult the unambiguous distinction of PGM1 homozygotes versus PGM2 heterozygotes. The secondary bands do not appear when samples are run on the Tris-citrate, pH 7.0, buffer system (not shown), so that the proper phenotypes can be confirmed on that system. The products of PGM2 are faster migrating than those of PGM1. All samples shown here have the products of only the PGM1 allele.

Fig. 2. ADA$^+$. The 4 samples shown produce the RBC form of adenosine deaminase. The first sample is a 1,2 heterozygote. The remaining 3 samples are homozygotes for the most common allele, ADA$^0$.

Fig. 3. EaD. Of the 3 samples shown, the middle one is EaD$^0$. The remaining 2 samples are EaD$^+$ homozygotes.

Fig. 4. ME. Three zones of malic enzyme activity can be seen on our gels. The 2 most anodal regions represent the superantigenic form of malic enzyme (indicated by a at the left). The differential activity of these a bands does not appear to be under genetic control. The most cathodal region of activity (m) represents the products of $M_{\text{ex}}$. The first sample has the fastest migrating form, which is type 2. The fourth sample is the slowest migrating form, type 1. The second and third samples are 1,2 heterozygotes, which appear as a broad smear between the region of 1 and 2.

Fig. 5. 6PGD. The first sample is 6PGD$^+$. The remaining 3 samples are type a homozygotes.

Fig. 6. $G_{\text{PDH}}$. The first sample is $G_{\text{PDH}}^+$. The remaining 3 are $G_{\text{PDH}}^0$.

Fig. 7. Glyl. The first sample is type 1, the third is type 2, and the second is a 1,2 heterozygote.

Fig. 8. $GOT_{\alpha}$. The first sample is a 1,2 heterozygote. The remaining 2 samples are type 1 homozygotes.

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