Examination of HeLa cell contamination of human cell lines derived from primary hepatomas using glucose-6-phosphate dehydrogenase and lactate dehydrogenase isozymes.

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Abstract

Isozyme patterns of glucose-6-phosphate dehydrogenase (G6PD) and lactate dehydrogenase (LDH) in human cell lines derived from primary hepatomas were compared with those in HeLa cells. Some cell lines derived from primary hepatomas having type B G6PD showed one or two isozymes of LDH. On the other hand, HeLa cells having type A G6PD showed four LDH isozymes. These findings suggest that not only G6PD, but also LDH may be useful for the detection of HeLa cell contamination of a culture in some cases.

KEYWORDS: lactate dehydrogenase, isozyme, HeLa cell contamination, human cell lines, primary hepatomas

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Examination of HeLa Cell Contamination of Human Cell Lines Derived from Primary Hepatomas Using Glucose-6-Phosphate Dehydrogenase and Lactate Dehydrogenase Isozymes

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Keywords: lactate dehydrogenase, isozyme, HeLa cell contamination, human cell lines, primary hepatomas

The suspicion that human tissue culture cell lines may be contaminated by another cell type has often been raised (1). It is, therefore, very important to check interspecies contamination, especially HeLa cell contamination when human cells are established in culture. Isozyme patterns of glucose-6-phosphate dehydrogenase (G6PD), which is a polymorphic isozyme variant showing types A and B, have been examined for this purpose (1). However, it is often difficult to discriminate cultured human cells from HeLa cells on the basis of this isozyme, because the difference in electromobility between types A and B is very small. In the present study, we studied not only the isozyme patterns of G6PD, but also those of lactate dehydrogenase (LDH), which has routinely been used for the detection of interspecies contamination, in human cell lines derived from primary hepatomas and HeLa cells to examine whether or not LDH is useful for the detection of HeLa cell contamination.

The cell lines tested were derived from human primary hepatomas (2). They were HuH-6 (hepatoblastoma) (3), HuH-7 (hepato-cellular carcinoma, HCC) (4), JHH-1 (HCC, 5), HLF (HCC) (6), PLC/PRF/5 (HCC) (7), HuH-28 (cholangiocellular carcinoma, CCC) (8) and HuCCT1 (CCC) (9). Cells were propagated on 100 mm plastic dishes (Falcon, Oxnard, CA, USA) in RPMI-1640 medium supplemented with $3 \times 10^{-8}$M selenium, $3 \times 10^{-4}$M oleic acid, $3 \times 10^{-7}$M linoleic acid and trace elements for HuH-7 and in RPMI-1640 medium supplemented with 5% bovine serum (inactivated at 56°C.
for 30 min) for the other cell lines. Cells were cultured in a humidified atmosphere containing 5% CO₂ in air at 37°C. Electrophoresis study was carried out as follows: Cells harvested by trypsinization were suspended in extraction buffer (Corning AuthentiKit, Innovative Chemistry, Inc., Marshfield, MA, USA) for 15 min at 0-4°C and then centrifuged at 2000 × g for 10 min. The supernatant was used for electrophoretic separation of isozymes. Mouse L929 and HeLa S3 (HeLa) cell extracts in Corning AuthentiKit were used as the standard and control, respectively. Isozyme electrophoresis was performed on 1% agarose Corning universal gels using Corning AuthentiKit.

G6PD in HeLa cells was of type A, while in cell lines derived from primary hepatomas (JHH-1 was not tested) it was of type B, indicating that cell lines derived from primary hepatomas were not contaminated by HeLa cells (Fig. 1). In the present system, however, the difference in mobilities between types A and B was very small, and therefore, it was often difficult to

![Fig. 1: Electrophoretic patterns of G6PD in human cell lines derived from primary hepatomas and HeLa cells. Mouse L929 and HeLa in Corning AuthentiKit were the standard and control, respectively. Extracts of cells derived from primary hepatomas were subjected to electrophoresis. “O” indicates the origin.](image)

![Fig. 2: Electrophoretic patterns of LDH in human cell lines derived from primary hepatomas and HeLa cells. Mouse L929 and HeLa in Corning AuthentiKit were the standard and control, respectively. Extracts of cells derived from primary hepatomas were subjected to electrophoresis. “O” indicates the origin.](image)
Table 1 Distribution of lactate dehydrogenase (LDH) isozymes in cell lines derived from human primary hepatomas

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>LDH isozymes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>HeLa</td>
<td>13</td>
</tr>
<tr>
<td>HuH-6</td>
<td>63</td>
</tr>
<tr>
<td>HuH-7</td>
<td>99</td>
</tr>
<tr>
<td>JHH-1</td>
<td>100</td>
</tr>
<tr>
<td>HLF</td>
<td>41</td>
</tr>
<tr>
<td>PLC/PRF/5</td>
<td>22</td>
</tr>
<tr>
<td>HuH-28</td>
<td>74</td>
</tr>
<tr>
<td>HuCCT1</td>
<td>100</td>
</tr>
</tbody>
</table>

\(a\) : HeLa cells in Corning AutentiKit were the control. Extracts of cells derived from primary hepatomas were subjected to electrophoresis.

discriminate cell lines derived from primary hepatomas from HeLa cells by the G6PD isozyme as shown in HuH-7 or PLC/PRF/5. The isozyme patterns of LDH and the relative distribution of the various LDH isozymes are shown in Fig. 2 and Table 1, respectively. HeLa cells, in the present study, showed four isozymes of LDH, although four or five LDH isozymes have been reported in the literature (1). On the other hand, three of seven cell lines derived from primary hepatomas, namely, HuH-7, JHH-1, and HuCCT1, showed one or two isozymes, indicating that these cell lines can easily be discriminated from cell lines showing four or five LDH isozymes such as HeLa cells. HuH-28 showed three LDH isozymes, one of which had much smaller relative distribution than the other ones. From these data, it is possible to say that cellular cross contamination, especially HeLa cell contamination, should be considered if cell lines showing originally one or two LDH isozymes as shown here show four or five LDH isozymes. Thus, the present study suggests that not only G6PD, but also LDH may be useful for the detection of HeLa cell contamination in some cases.

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References


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