Acta Medica Okayama

Volume 43, *Issue* 4 1989 *Article* 7

AUGUST 1989

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

Takayoshi Tokiwa[*] Yasunori Kusaka[†]

Atsushi Muraoka[‡] Jiro Sato[**]

[*]Okayama University,

[†]Okayama University,

[‡]Okayama University,

[**]Okayama University,

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

Takayoshi Tokiwa, Yasunori Kusaka, Atsushi Muraoka, and Jiro Sato

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

---

*PMID: 2552753 [PubMed - indexed for MEDLINE]

Acta Med Okayama 43 (4) 245-247 (1989)

—Brief Note—

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

Takayoshi Tokiwa*, Yasunori Kusaka, Atsushi Muraoka and Jiro Sato

Division of Pathology, Cancer Institute, Okayama University Medical School, Okayama 700, Japan

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.

Key words : 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 intraspecies 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 (hepatocellular 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 3×10^{-8}M selenium, 3×10^{-8}M oleic acid, 3×10^{-7}M linoleic acid and trace elements for HuH-7 and in RPMI-1640 medium supplemented with 5 % bovine serum (inactivated at 56°C

* To whom correspondence should be addressed.

for 30 min) for the other cell lines. Cells were cultured in a humidified atmosphere containing 5% CO_2 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 $2000 \times g$ 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

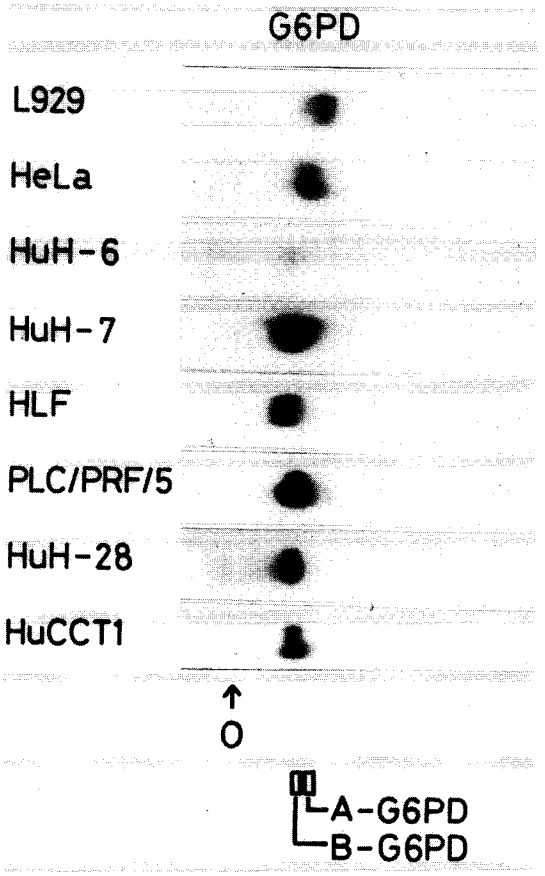

Fig. 1 Electrophoretic of patterns G6PD in human cell lines derived from primary hepatomas and HeLa cells. Mouse L929 and HeLa in Corning AuthentiKit were the standard and control, respectively. Extracts of cells derived from primary hepatomas were subjected to electrophoresis. "O" indicates the origin.

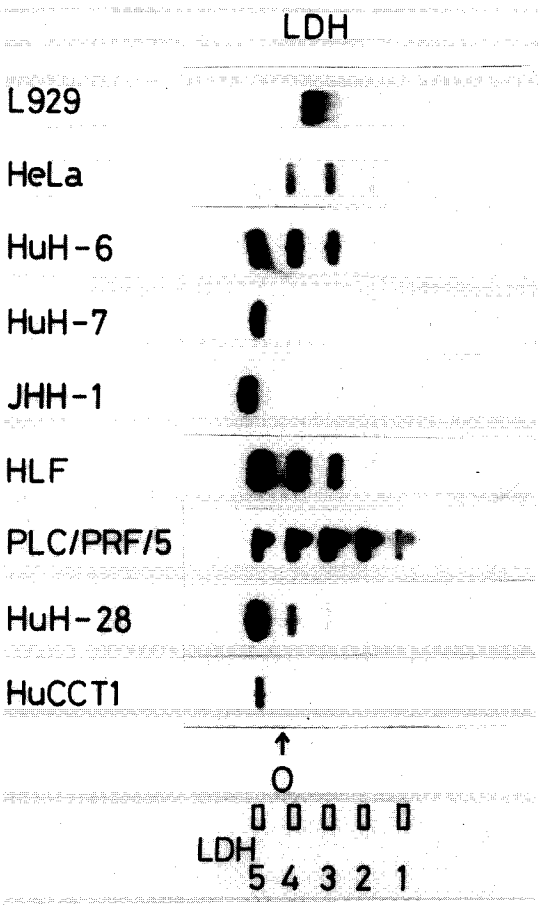

Fig. 2 Electrophoretic patterns of LDH in human cell lines derived from primary hepatomas and HeLa cells. Mouse L929 and HeLa in Corning AuthentiKit were the standard and control, respectively. Extracts of cells derived from primary hepatomas were subjected to electrophoresis. "O" indicates the origin.

Table 1 Distribution of lactate dehydrogenase (LDH) isozymes in cell lines derived from human primary hepatomas

Cell lines[a]	LDH isozymes (%)				
	5	4	3	2	1
HeLa	13	33	42	12	—
HuH-6	63	22	14	1	—
HuH-7	99	1	—	—	—
JHH-1	100	—	—	—	—
HLF	41	39	19	1	—
PLC/PRF/5	22	19	24	23	12
HuH-28	74	22	4	—	—
HuCCT1	100	—	—	—	—

a : HeLa cells in Corning AuthentiKit 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.

Acknowledgments. JHH-1 cells were kindly supplied by Dr. Nagamori, Dept. of Internal Medicine, Jikei Medical School, Tokyo, Japan. The authors wish to thank Ms. K. Miyano and Ms. T. Henmi for their technical assistance.

References

1. Stulberg CS : Extrinsic cell contamination of tissue cultures ; in Contamination in Tissue Culture, Fogh ed, Academic Press, New York (1973) pp 1-27.
2. Tokiwa T and Sato J : Human cancer cell lines (I). Protein Nucleic Acid Enzyme (1988) **33**, 2603-2605 (in Japanese).
3. Doi I : Establishment of a cell line and its clonal sublines from a patient with hepatoblastoma. Gann (1977) **67**, 1-10.
4. Nakabayashi H, Taketa K, Miyano K, Yamane T and Sato J : Growth of human hepatoma cell lines with high differentiated functions in chemically defined medium. Cancer Res (1982) **42**, 3858-3863.
5. Homma S : Studies on the establishment and some biological characteristics of cultured human liver cancer cell lines—Their growth, functional and morphological characteristics and temperature sensitivities—. Jikei Med J (1985) **32**, 289-315.
6. Doi I, Namba M and Sato J : Establishment and biological characteristics of human hepatoma cell lines. Gann (1975) **66**, 385-395.
7. Alexander JJ, Bey EM, Geddes EW and Lactsas G : Establishment of a continuously growing cell line from primary carcinoma. S Afr Med J (1976) **50**, 2124-2128.
8. Kusaka Y, Tokiwa T and Sato J : Establilshment and characterization of a cell line from a human cholangiocellular carcinoma. Res Exp Med (1988) **188**, 367-375.
9. Miyagiwa M, Ichida T, Sasaki H, Tokiwa T and Sato J : A new cholangiocellular carcinoma cell line (HuCCT1) producing carbohydrate antigen 19/9 in serum free medium. *In Vitro* Cell Dev Biol (1989) (in press).

Received January 24, 1989 ; accepted March 7, 1989