A Human Trophoblastic Isozyme (Lactate Dehydrogenase-Z) Associated with Choriocarcinoma

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ABSTRACT

A unique electrophoretic form of lactate dehydrogenase (LDH-Z), formerly observed in a choriocarcinoma cell line (JEG-3) and first-trimester placenta, has been shown to be the same as that produced in hydatidiform mole and term placenta. We have also observed LDH-Z in second-trimester placenta, choriocarcinoma metastasized to the liver, and five of five additional independently derived (from different patients) choriocarcinoma cell lines. The only exception to the production of LDH-Z in a choriocarcinoma was in the cell line BEWO, which was established from the same tumor as JEG-3. Since BEWO has been in culture for over 200 passages more than any of the independently derived lines, its lack of LDH-Z is viewed as being consistent with the loss of certain expression characteristics upon such long-term culture. Analysis of JEG-3 subclones revealed the expression of LDH-Z to be independent of the products of the LDH-A genetic locus. Present data do not allow us to determine whether LDH-Z is the product of a newly discovered LDH locus or is a modified form of the product of the LDH-B locus. LDH-Z has not been observed in other human tissues, nor have we observed it in homogenates prepared from over 60 cell lines established from a wide variety of human neoplasms. We therefore conclude that LDH-Z is an isozyme associated with human choriocarcinoma and is indicative of the trophoblastic origin of the cells.

INTRODUCTION

Five common forms of LDH (EC 1.1.1.27) are identifiable in cleared homogenates from most mammalian tissues and cells after electrophoresis and histochemical staining. These 5 isozymes have been demonstrated to be the result of the random combination into tetramers of electrophoretically variant subunits specified by 2 different genetic loci (LDH-A and LDH-B). The subunit structures of the 5 isozymes from most anodal to most cathodal are B2A2, B2A2, B2A2, B2A2, and A4, known as LDH-1, -2, -3, -4, and -5, respectively. The relative activities of the isozymes are skewed in the direction of LDH-1 or -5 depending on the relative expression of the LDH-B locus as opposed to LDH-A in the cells or tissues sampled (13, 15, 22).

Edlow et al. (5) using starch gel electrophoresis observed a weak lactate-dependent band of activity slightly cathodal to LDH-2 in 3 of 4 first-trimester placentas. This band was not observed in second- or third-trimester placenta material. They noted an earlier observation (29) that an extra LDH isozyme was associated with LDH-3 in young placenta. However, careful study of the zymograms of the latter group reveals that the extra LDH band was also slightly cathodal to LDH-2. Van Bogaert et al. (29) further stated that this band was observed in a sample of a hydatidiform mole, an intrauterine hyperplastic growth of trophoblastic origin which may become invasive and is believed to be responsible for 50% of the cases of choriocarcinoma of gestational origin (8). Later, Edlow et al. (6) observed the band to be quite prominently present after polyacrylamide electrophoresis of extracts from the choriocarcinoma cell line JEG-3. They concluded that the presence of a first-trimester isozyme in JEG-3 cells indicated the possibility that the choriocarcinoma had undergone cellular dedifferentiation to produce an atypical, phase-specific protein.

Here, we confirm the presence of this additional LDH isozyme (called LDH-Z) in the first-trimester placenta, hydatidiform mole, and JEG-3 cells. In addition, we establish its association with choriocarcinoma by demonstrating it in 5 of 5 independently derived choriocarcinoma cell lines as well as in an autopsy sample of choriocarcinoma metastasized to the liver. However, our data are inconsistent with the dedifferentiation hypothesis (6) since we observed LDH-Z in second- and third-trimester placenta material. Finally, with respect to LDH-Z itself, we report that subclone analysis of JEG-3 cells reveals the expression of the isozyme to be independent of the activity of the LDH-A locus and to be generally present in JEG-3 cells (i.e., the JEG-3 cell population is not heterogeneous with respect to LDH-Z activity).

MATERIALS AND METHODS

Cell Lines Used

Choriocarcinoma Lines. BEWO (18) was established by Hertz (10) from the Woods strain of the Erwin-Turner tumor carried in hamster cheek pouch.

The JEG-1, JEG-2, JEG-3, JEG-7, and JEG-8 (12) cell lines were later cloned from the Woods strain of the Erwin-Turner tumor in its 387th passage in hamster cheek pouch.

The JAR (27), OMEGA, ELFA, and ZALB cell lines were independently derived (from 4 different patients).

Nontrophoblastic, HCG-producing Lines. CaSki (19) cervical carcinoma produces subunit of HCG. EICo (11) breast carcinoma produces subunit of HCG. ChaGo (28) lung carcinoma produces both subunits of HCG. All above cell lines, with the exception of ChaGo, and the JEG series were provided

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by R. Pattillo, Medical College of Wisconsin, Milwaukee, Wis. ChaGo was provided by S. Rosen, National Institute of Arthritis, Metabolism and Digestive Diseases, Bethesda, Md.

Other Human Tumor Cell Lines. HeLa was provided by J. Clarkson, The University of Texas System Cancer Center Science Park, Smithville, Texas.

Eighteen independently derived breast carcinoma lines of metastatic origin (4, 23) were provided by R. Cailleau, M. D. Anderson Hospital and Tumor Institute, Houston, Texas.

Over 40 cell lines (supplied by Col. A. Leibovitz, formerly of the Scott and White Clinic, Temple, Texas) derived from a wide variety of human neoplasms including: adenocarcinomas of the colon, rectum, adrenal cortex, ovary, endometrium, kidney, and thyroid; squamous cell carcinomas of the vulva, thyroid, cervix, lung, breast, bladder, and larynx; melanoma; mesothelioma; meningioma; synovial sarcoma; chondrosarcoma; chemodectoma; alveolar carcinoma; astrocytoma; spindle cell carcinoma of the bladder; Wilm’s tumor; liposarcoma; fibrosarcoma; and rhabdomyosarcoma.

Cell Culture

Cells were grown in F-12 medium with modifications to augment glucose and certain salts [F-12 Mod, available from Grand Island Biological Co. (Grand Island, N. Y.) as F-12 modified by Bordon]. Medium was supplemented with 13.5% horse serum, 2.5% fetal calf serum, penicillin (100 units/ml), and streptomycin (100 mg/ml).

Subcloning

JEG-3 cells at passage 70 were subcloned by 2 methods. Cells were diluted to 20 cells/ml, and 0.1 ml was placed into each well of a Microtiter II plate. The wells were scanned for individual cells, and these cells were followed for growth and carried as individual cell lines. The JEG-3 cells were also plated as individual cells in 100-mm Petri dishes, and independent colonies were transferred for carrying as subcloned cell lines.

Passages at Which Choriocarcinoma Cell Lines Were Studied

The JEG-3 cells have been analyzed for LDH at intervals of every 10 to 15 passages spanning the 58th to 210th passage over a 2-year period. JEG-1 was tested at passage 72; JEG-2 was tested at passage 64; JEG-7 was tested at passage 78; and JEG-8 was tested at passage 60. BEWO was tested at passage 508; ZALB was tested at passage 277; OMEGA was tested at passage 268; JAR was tested at passage 306; and ELFA was tested at passage 278.

Autopsy Material

P. G., a 21-year old Latin-American woman, was admitted to M. D. Anderson Hospital on March 17, 1976. She had delivered a full-term normal infant 10 months previously. Histological examination of a previously obtained biopsy of a lung lesion and an elevated HCG level enabled the diagnosis of choriocarcinoma to be made. Liver and brain scans were positive for metastasis. Despite intensive chemotherapy and radiotherapy to resistant metastatic areas, the patient failed to respond and died on December 2, 1977. Postmortem examination confirmed choriocarcinoma metastases to the liver, lungs, spleen, and lymph nodes. The brain was not examined. Autopsy material (~0.5 g each) of tumor metastatic to the liver, lung, and spleen and grossly normal specimens from liver, lung, heart, and uterus were prepared for isozyme analysis.

Placental Material

Specimens from voluntary terminations of early pregnancy (first trimester), spontaneous premature deliveries (second trimester), and normal term pregnancies (term placenta) were prepared for isozyme analysis.

Isozyme Analysis

A pellet (0.1 to 0.2 ml) of cells or 0.5 g of tissue was Dounce homogenized at 4°C in 2 volumes of homogenizing medium (0.01 M Tris-HCl, pH 7.5-0.001 M β-mercaptoethanol-0.001 M EDTA). Homogenates were stored at -70°C for <2 months, thawed, and cleared by centrifugation (10,000 × g for 45 min). Cleared homogenates were subjected (12 at a time) to starch gel electrophoresis as generally described in the paper of Siciliano and Shaw (25).

Three different buffer systems were used. Buffer System 1: electrode, 0.5 M Tris-0.16 M EDTA-0.65 M borate, pH 8.0; gel, dilute 60 ml electrode buffer up to 600 ml with water and add 20 mg NADP after degassing. Buffer 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. Buffer 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 enzyme loci were resolvable on: Buffer System 1, G6PD, G0Tm, PGD, and PEP-A, PEP-C, PEP-D; Buffer System 2, ESD, GLO, PGAM, AK1, and ADA; Buffer System 3, PGM2. Forms of LDH were resolvable on Buffer Systems 1 and 2.

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 in the papers of either Siciliano and Shaw (25) or Harris and Hopkinson (9). GLO was stained according to the method described in the report of Parr et al. (17). After staining, gel slices were photographed (35 mm) and discarded.

RESULTS

Following electrophoresis and histochemical staining of their homogenates for LDH, HeLa cells and mammary carcinoma cell lines displayed patterns typical of tissues with greater expression of LDH-A than LDH-B (Fig. 1). Homogenate from the choriocarcinoma cell line JEG-3 had greater expression of LDH-B as indicated by the great intensity of LDH-1 and the inability to detect the Aα homotetramer (LDH-5). Its most interesting feature, however, was the extra band of LDH activity observed in the JEG-3 cells studied by Edlow et al. (6). The same bands (including LDH-Z) appeared under UV after reverse staining (conversion of fluorescent NADH to nonfluorescent NAD in the presence of pyruvate under UV).

LDH patterns exhibited by 6 subclones of JEG-3 are shown in Fig. 1. A total of 25 subclones were produced, and all had LDH-Z at approximately the same intensity. The subclones fell...
LDH isozymes 2, 3, and 4. Class i is typified by the subclones into 2 classes with respect to the expression of LDH-A as visualized by the activity of the heterotetramers responsible for LDH isozymes 2, 3, and 4. Class i is typified by the subclones samples a and b (Fig. 1). In these, LDH-A expression was essentially nil, as indicated by the lack of heterotetramer activity (none even in LDH-2). A total of 11 Class i type subclones were produced. The remaining 14 subclones fell into Class 2 and are typified by subclone samples c to f (Fig. 1). In these, LDH-A was expressed as indicated by the activity of the LDH 2, 3, and 4 isozymes. Therefore, LDH-Z was clearly expressed in all JEG-3 subclones, independent of the expression of the products of LDH-A.

At the same time that JEG-3 was cloned from the original choriocarcinoma that had been carried through animals, 4 other clonal isolates were established (JEG-1, JEG-2, JEG-7, and JEG-8) (12). These 4 lines showed the same LDH patterns as did the subclones of JEG-3, i.e., greater expression of LDH-B than of LDH-A and clear expression of LDH-Z independent of the variable expression of LDH-A. The LDH patterns in JEG-1 and JEG-7 are shown in Fig. 2 on either side of JEG-3. BEWO, an uncloned cell line derived from the same tumor many years earlier, on the other hand, displayed none of the above unique LDH characteristics shown by the JEG clones and subclones (Fig. 2).

Four additional choriocarcinoma cell lines (JAR, ZALB, ELFA, and OMEGA) believed to be independently derived from different individuals were then assayed for their LDH patterns. All had patterns as displayed by the JEG clones and subclones (Fig. 2). OMEGA is not shown but had an LDH pattern indistinguishable from JAR and ELFA.

Because of the possibility that the cell lines showing LDH-Z might all be contaminants from the same source, these cell lines were subjected to genetic signature analysis (16, 20, 23). In this procedure, each cell line was genotyped for 12 enzyme loci known to be polymorphic in the human population. The allozyme phenotypes for these loci are tabulated for each locus on Table 1. No 2 cell lines had the same phenotype at each locus. Such phenotypes have been shown to be stable in cell culture (1, 20) and altered to produce random forms only at mutation rates that were quite different from each other from locus to locus (26). Since these phenotypes at each locus over all the cell lines did not represent random forms (all variants are common polymorphisms seen in human populations) and were present at frequencies not inconsistent with Hardy-Weinberg expectations, we conclude that the cell lines expressing LDH-Z were in fact of independent origin and not contaminants from the same source.

None of the other of over 60 independently derived human tumor cell lines tested for LDH, which included the 3 lines producing HCG as an ectopic event, produced LDH-Z.

We have resolved LDH-Z in an autopsy specimen of choriocarcinoma metastasized to the liver (Fig. 3) as well as to the lung and spleen. Unaffected liver tissue adjacent to the tumor site as well as grossly normal uterine, heart, spleen, and lung tissue failed to show LDH-Z. Allozyme analysis of polymorphic enzyme loci from the unaffected liver and adjacent tumor tissue revealed the tumor to be truly of trophoblastic origin. The PGM1 locus was informative in this regard in that the tumor material was PGM1\(^1\), and the maternal liver was PGM1\(^1\)\(^2\). This result is consistent with the fetal origin of the tumor cells located in the affected mother host. LDH-Z was not detectable in the serum of the patient in samples monitored up to a week before death.

We confirmed the findings of Edlow et al. (5) and Van Bogaert et al. (29) by resolving the extra LDH band cathodal to LDH-2 in 5 of 5 first-trimester placenta samples as well as in a sample of hydatidiform mole and showed the band to comigrate with LDH-Z. However, unlike Edlow et al. (5), we found LDH-Z well expressed in all 7 term placenta samples (Fig. 4) as well as in all 7 second trimester material (not shown but pattern was identical to that of term placenta). Our findings of LDH-Z in term placenta are consistent with the results of Prasad et al. (21).

### DISCUSSION

The earlier observation of an extra LDH isozyme, cathodal to LDH-2, in early placenta (5, 29), in hydatidiform mole (29), and in the choriocarcinoma cell line JEG-3 (5) has been confirmed and shown to be the same in electrophoretic mobility in all 3 types of material. Since the band is lactate dependent in the presence of NADH (5), and we have shown it also to be pyruvate dependent in the presence of NADH, we consider it a LDH and provisionally designate it LDH-Z. In the event that further work uncovers the genetic basis of the band to rest in a heretofore unrecognized structural LDH locus, that locus would be designated LDH-D taking its place behind the already identified LDH-A, LDH-B, and LDH-C (see review on the evolution of LDH loci by Markert et al. (14)).

Present data do not allow us to determine whether LDH-Z is the product of a new locus or represents some posttranslationally modified form of one of the established loci products. Precedent for such a modification of LDH-5 (A\(_4\) homopolymer) by a viral protein producing a new band of LDH activity anodal to LDH-5 (LDH-Sex) exists (30). LDH-Z is clearly not LDH-5Sex. Also, the migrational position of LDH-Z is quite different from the testis-specific form of LDH, LDH-X (2), which is the product of its own genetic locus, LDH-C (3).

The various choriocarcinoma cell lines, clones and subclones are informative with respect to the relationship of LDH-Z to the products of LDH-A. In none of this material is LDH-5 visualized. This indicates the possibility of a modification of LDH-5 similar to the LDH-5Sex situation. However, as seen in the cell lines and clones and as most clearly demonstrated among the JEG-3 subclones, the activity of LDH-Z appears to be independent of the expression of the LDH-A locus. Therefore LDH-Z does not appear to be a modified form of any LDH.
isoenzyme involving the participation of LDH-A subunits (LDH-2, -3, -4, or -5). Our present working hypothesis is that LDH-Z is either the result of some modification of the products of the LDH-B locus or the product of a new structural locus either in combination with B subunits or alone. Further immunological and biochemical work is in progress to resolve this question.

The greater production of LDH-B than of LDH-A subunits and production of LDH-Z in 5 of 5 independently derived choriocarcinoma cell lines establishes a relationship between those expressional characteristics and choriocarcinoma cells. Since in the characterization of human cell lines and tissues greater production of LDH-B than of LDH-A subunits is frequently observed, it is the expression of LDH-Z that is of particular interest. The lack of expression of LDH-Z in BEWO is not consistent with the results from the independently derived choriocarcinoma cell lines. (BEWO is not independently derived since both it and the JEG series were derived from the Hertz tumor.) However, it might be expected that during long-term passage certain expressional characteristics of cells may become altered. When sampled here, BEWO had been passed 500 times (over 200 passages more than any of the other lines).

Identification of LDH-Z in the metastasized choriocarcinoma autopsy specimens indicates that the expression of this isozone is not limited to choriocarcinoma cells in culture. The presence of LDH-Z in second- and third-trimester placenta allows an alternative suggestion to that of Edlow et al. (8), who stated that choriocarcinoma cells necessarily go through a dedifferentiation to produce an atypical, phase-specific protein. Our data indicate the possibility that the choriocarcinoma cells represent an enrichment of cells already producing a typical phase-specific protein. The LDH-Z isozone appears to be a marker for these cells, indicating their trophoblastic origin. LDH-Z may cease being expressed without necessarily affecting other characteristics of choriocarcinoma. This is indicated by the lack of LDH-Z expression of BEWO, whereas BEWO retains the capability of producing HCG, placental lactogen, and tumors in immunologically privileged sites (7, 18). That LDH-Z production is not necessarily related to the production of HCG or its subunits is further indicated by its absence in HCG-producing tumor cell lines (ChaGo, CaSki, and EICo) of nonplacental origin.

We conclude, therefore, that LDH-Z production in choriocarcinoma is real and that its production there is a function of the trophoblastic origin of the cells. Such a finding illustrates that unexpected isoforms in tumor material may be present for reasons other than altered regulation of tumor cells. An alternative explanation may be that such isoforms are markers for the cells of origin of tumors that develop in tissues of heterogeneous cell types (such as trophoblast). In such cases, presence of "fetal" or earlier stage proteins may be a consequence of the expansion of relatively undifferentiated cells that were a minority in the tissue of origin.

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REFERENCES


Figs. 1 to 4. Starch gel slices stained, after electrophoresis, for LDH. Anodal ends (+) and origins (o) are marked on the left when present. Positions of the 5 usual LDH isozymes (1 to 5) and LDH-Z are marked either on the right (Figs. 1 and 4) or through the middle (Figs. 2 and 3). Samples run are indicated along the bottom of each figure.

Fig. 1. Homogenates from HeLa cells, the choriocarcinoma cell line JEG-3, 2 different breast carcinoma cell lines (BC), and 6 subclones of JEG-3 (a to f) are shown.

Fig. 2. Homogenates from 7 choriocarcinoma cell lines are shown.

Fig. 3. Homogenates from choriocarcinoma metastasized to the liver, adjacent uninvolved liver, and grossly normal uterus extracted at autopsy from the same patient are shown. HeLa and JEG samples are also shown as controls.

Fig. 4. Homogenates from 2 different term placenta samples are shown along with JEG and HeLa controls. Sample from second trimester samples produced LDH patterns essentially identical to those from term placenta.
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