In Vivo Human-Hamster Somatic Cell Fusion Indicated by Glucose 6-Phosphate Dehydrogenase and Lactate Dehydrogenase Profiles

David M. Goldenberg, Raj D. Bhan, and Rose A. Pavia

Department of Pathology, Temple University School of Medicine, Philadelphia, Pennsylvania 19140 [D. M. G., R. A. P.], and Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213 [D. M. G., R. D. B., R. A. P.]

SUMMARY

Transplantation of a human lymphoma to the cheek pouches of unconditioned, adult golden hamsters resulted in permanently transplantable tumors which grow progressively and kill their hosts. This tumor system, GW-478, has a hamster-specific lactate dehydrogenase isoenzyme mobility pattern and both human and hamster glucose 6-phosphate dehydrogenase enzyme profiles when propagated in the hamster or in vitro, thus suggesting that GW-478 is a human-hamster hybrid.

INTRODUCTION

The fusion of somatic cells in vitro has been described for allogeneic, xenogeneic, normal, and neoplastic cell combinations (2, 3, 6, 16, 26, 30). We here present evidence, based upon the isoenzyme profiles of LDH and G6PD, that an interspecific in vivo fusion of human lymphomatous and presumably normal hamster cells resulted in a highly malignant and anaplastic tumor cell population in untreated, adult hamsters.

MATERIALS AND METHODS

An aliquot of the gastric stem cell lymphoma of a 62-year-old white man was injected as a tumor mince into both cheek pouches of 6 unconditioned, adult golden hamsters (Mesocricetus auratus) of both sexes (60 to 70 g) which were randomly bred by our supplier (Hilltop Lab Animals, Scottdale, Pa.), our transplantation technique has been described elsewhere (10, 15). As early as 7 days after grafting, one-fourth of the pouches showed microscopic and macroscopic evidence of viable and expansively growing tumors. Morphologically, the transplants resemble immature neoplastic cells somewhat similar to the cells of the patient's lymphoma but with more basophilia and mitotic figures (Fig. 1). Tumor cell spread from the cheek pouch to the regional submental lymph nodes and also to all major organs of the hamster was witnessed in the 2nd animal passage, but, our findings with other tumor heterografts suggest that it probably already occurred early in the 1st transplant generation. This tumor line, designated GW-478 (14), has now been continuously propagated for over 100 cheek pouch passages in a period of about 2 years and has consistently shown invasive and metastatic properties in this host regardless of transplantation site. Also, an ascites tumor cell line (GW-478A) has been carried in hamsters for over 50 successive passages. The GW-478 tumor can easily be grown in monolayer cultures, where approximately an 8-fold increase in cell number occurs in 7 days. A detailed account of the transplantation history, growth behavior, species-specific antigen, and karyology of this tumor is in preparation; a brief communication of some of these data has already appeared (14).

GW-478 tumor cells were taken from various in vivo and in vitro sources for enzyme separations (Table 1). Monolayer cultures of GW-478 grew on glass in Eagle's minimal essential medium supplemented with 10% fetal calf serum, penicillin, and streptomycin. The detached tumor cells were harvested, washed several times in 0.9% NaCl solution, and disrupted in 0.25 M sucrose by a motor-driven Potter-Elvehjem grinder. After the homogenate was centrifuged for 30 min at 31,000 X g in a refrigerated Sorvall centrifuge, 0.1 ml of the supernatant fluid was used for enzyme separation on 5% polyacrylamide gel with a Shandon disc electrophoresis apparatus. Bromphenol blue was used to visualize the mobility front. After electrophoresis at 4° for 1.5 hr in Tris-glycine buffer (pH 8.2) at a constant current of 3 ma/column, the gels were stained in the dark at 37° for 30 min. The G6PD stain contained 0.02 M glucose 6-phosphate, 5 mg NADP, 0.1 M MgCl₂, 0.25 mg phenazine methosulfate, 3 mg nitro blue tetrazolium salt, and 13 ml 0.05 M Tris-HCl buffer (pH 7.6). LDH was stained in 6 ml 1 M lithium lactate, 40 mg NAD, 4 mg phenazine methosulfate, 10 mg nitro blue tetrazolium salt, and 90 ml 0.05 M Tris-phosphate buffer (pH 8.6). The enzyme test for each cell line was run in duplicate gels and frequently repeated on the following day. As control cell lines also maintained in monolayer cultures, we chose to use HeLa-S3 as our human representative and...
Fig. 1. Microscopic morphology of GW-478 tumor in 2nd cheek pouch passage resembling relatively uniform, immature neoplastic cells with an abundance of mitotic figures. H & E, X 400.

Table 1
Transfer history of GW-478 cell populations demonstrating G6PD profiles typical for both human and hamster cells

<table>
<thead>
<tr>
<th>No.</th>
<th>No. of cheek pouch generations</th>
<th>No. of ascites i.p. passages</th>
<th>Days in cell culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31</td>
<td>None</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>15</td>
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</tr>
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<td>4</td>
<td>40</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>18</td>
<td>5*</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>30</td>
<td>None</td>
</tr>
<tr>
<td>7</td>
<td>86</td>
<td>None</td>
<td>86</td>
</tr>
<tr>
<td>8</td>
<td>86</td>
<td>None</td>
<td>123</td>
</tr>
</tbody>
</table>

* Cells of same source as those of No. 3 but frozen at \(-64^\circ\) for an additional 41 days prior to preparation of enzyme extract.

Fortner’s amelanotic melanoma, A.Mel. 3 (8) as our hamster reference line. These were grown and processed for enzyme electrophoresis in the same manner as was GW-478.

RESULTS

The isoenzyme mobility pattern of LDH is specific for hamster and human cells. As is demonstrated in Fig. 2, 5 distinct isoenzyme bands are present in the hamster A.Mel. 3 cell line obtained from tissue culture passage, in which 2 heavy, slower cathodic bands (LDH4-5) and 3 weaker, more anodic, bands (LDH1-3) can be seen. In comparison, the human HeLa cell line shows, as is the case for all human LDH separated by this method of disc electrophoresis, only 4 bands (LDH1-4). LDH5 is more cathodic and thus beyond the origin of this system (7). In addition to the difference in isoenzyme number, the spacing between the individual bands and their relative distances from the bromphenol band (i.e., their relative mobility) is distinctly different from that of the LDH of the HeLa cells. The LDH isoenzyme profile shown for GW-478, taken here from the 86th hamster cheek pouch transplant maintained in culture for 4 months, is consistent with that of the hamster. This LDH isoenzyme profile has been found in all of the 9 runs made from different cell culture passages and cheek pouch transplants of the GW-478 tumor.

Acrylamide gel disc electrophoresis of G6PD from HeLa cells usually reveals the presence of 2 distinct, closely related bands, probably representing subbands (Fig. 3), which is consistent with the results of Bakay and Nyhan (1) and of Nabholz et al. (21). Hamster G6PD subbands, however, usually number 3 to 5 and are more rapid in their mobility than those of human cells (Fig. 3, A.Mel.3). GW-478 tumor cells derived from culture, as well as directly from the hamster once, have on 8 different occasions (Table 1) been found to contain G6PD bands consistent in subband number and mobility with both those of human and of hamster cells (Figs. 3 and 4). The presence of G6PD enzyme profiles of both species is further attested to by the almost identical picture resulting from mixing G6PD enzymes from human (HeLa) and hamster (A.Mel. 3) cells prior to electrophoresis in the same gel column (Fig. 4, Mixture). The presence of both human and hamster G6PD in GW-478 cells was only noted in 8 of 24 enzyme runs from different GW-478 cell populations. At no
HeLa
GW-478
A.Mel.3

Fig. 2. Acrylamide gel disc electrophoretic separation of LDH from human (HeLa), hamster (A.Mel.3), and GW-478 tumor cells. Isoenzyme profiles compared with bromphenol blue bands as the common reference point (extreme right of each gel).

HeLa
GW-478
A.Mel.3

Fig. 3. Comparison of human (HeLa) and hamster (A.Mel.3) G6PD patterns (top) and relationship to G6PD enzyme profile of GW-478 (bottom). Enzyme run corresponds to that of No. 7, Table 1.

HeLa
GW-478
A.Mel.3

Fig. 4. G6PD isoenzyme mobility pattern of human (HeLa), hamster (A.Mel.3), and GW-478 tumor cells (No. 5, Table 1) and an in vitro mixture of enzyme extracts from HeLa and A.Mel.3.

The continuous propagation in a normal, presumably immunologically mature, xenogeneic animal of human tumor cells which subsequently exhibit invasive and metastatic properties could, at first glance, suggest that spontaneous or induced oncogenesis had occurred. Such an explanation would then be further supported by our finding that the GW-478 tumor karyologically resembles its hamster host (14), although the similarity between the karyotypes of the hamster and of man obviously prevents our recognizing the possible presence of 1 or more human chromosomes by mere chromosome morphology, particularly for an aneuploid tumor. Our repetition of this event in a number of human tumors heterografted to hamsters (12) would, by itself, seem to argue against the possibilities of spontaneous or viral oncogenesis, especially with latent periods of about 1 week between heterotransplantation and tumor growth.

The presence of human G6PD enzyme in the GW-478 tumor growing in hamsters, at least in 8 cell populations derived from it, supports the hypothesis of retention of a part of the human genome and thus suggests that these tumor cells in the hamster are genetically related to those of the patient's lymphoma. In addition, we have found that GW-478A tumor cells do not have a typically hamster-like stathmokinetic response to Colcemid but have a response compatible for human cells or intermediate between both extremes (13). [Hamster cells are known to be 50 to 100 times more resistant to Colcemid than are human cells (23, 29).] In view of the universal presence of

* D. M. Goldenberg, R. A. Pavia, and M. Chen, manuscript in preparation.
hamster LDH isoenzymes and the many cell populations demonstrating only hamster G6PD enzyme, one is apt to interpret these results as indicating either that the GW-478 tumor is a mosaic of human and hamster cells or that it is composed of human-hamster heterokaryons, at least in part. In either case, it appears that the hamster contribution to the genome of the GW-478 cells is the more dominant of the 2 species. Our karyological data, however, showing that the GW-478 tumor has about 60% of its cells within its hyperdiploid modal chromosome range, 4 with no cells in the population showing a typically human or diploid hamster karyotype, leads us to favor the hybridization explanation at this time.

Our failure to demonstrate human-hamster heteropolymer “hybrid” G6PD enzyme formation in GW-478 does not in itself militate against considering these results indicative of human-hamster somatic cell fusion, since such enzyme heteropolymers, at least in Ruddle’s experience (25), are found in only a few of the hybrid clones examined for G6PD. We are of course aware that a final resolution of some of these questions must await the completion of our cloning experiments with GW-478.

The similarity in mobility between the human G6PD bands of our presumptive GW-478 hybrids and that of HeLa would seem to indicate that GW-478, although derived from a Caucasian, has the Negro “A” type variant of G6PD. This is compatible with the reports that almost all spontaneous human cell lines studied in vitro have the common A electrophoretic variant although not necessarily derived from Negroes. Since the ubiquitous HeLa cell line contains the A type of G6PD, Gartler (9) and Petersen et al. (24) suggested that most of these lines were HeLa cell contaminants. The presence of the A type variant of G6PD in GW-478 cultured in vitro from hamster cheek pouch or ascites tumor transplants on several occasions but with a karyotype other than that of HeLa cells as well as a hamster-specific LDH isoenzyme pattern does, however, constitute evidence contradicting this view. This contention is further supported by the studies of Steele (27) which suggest that the A type electrophoretic variant of G6PD in many spontaneous human cell lines could arise as the result of point mutation and is not necessarily a reflection of HeLa cell contamination.

The evidence currently available for GW-478 and 2 of our other tumor systems in the hamster suggests that human-hamster hybrids, predominantly hamster-like in character and behavior, have been produced in vivo. In this model, it seems that the presumptive fusion of tumor and nontumor cells of 2 different species involves the concomitant loss of chromosomes, since no predominantly tetraploid tumors have resulted. Because of the dominance of the contribution of the animal host, it appears likely that such chromosomal loss is principally from the malignant human complement, which might indeed be a basic phenomenon of this in vivo xenogeneic situation. This, as well as the expression of a portion of the human genome (G6PD) in only a few cell populations of GW-478, is in agreement with the reported loss of human chromosomes in human-mouse hybrids in vitro (19, 20, 31). Its presence at these times, nevertheless, suggests the retention of at least a part of a human X chromosome, since G6PD in man and some animals is considered to be X-linked (17, 18, 22, 28, 32). If the hamster and human G6PD forms present in our presumptive hybrid tumor cells are indeed X-linked enzymes, this would support the intriguing conclusion that 2 X chromosomes from different species can remain active when coexisting in the same cell. It is thus of interest to determine whether other X-linked human and hamster enzymes are functional in the GW-478 tumor.

Since nonhybrid human tumor xenografts have hitherto rarely shown the highly malignant properties characteristic of our presumptive human-hamster hybrid tumors when propagated in unconditioned, adult animals, the hypothesis is entertained (11) that in vivo somatic cell fusion might be an important mechanism in the evolution and progression of tumors to their ultimate stages of malignancy.

ACKNOWLEDGMENTS

We extend our appreciation to Dr. Emmanuel Farber, Dr. Renato Baserga, and Dr. Wallace H. Clark, Jr., for helpful suggestions and criticism. We also thank Miss V. F. Ward and Miss P. Poznik for their skillful technical assistance. The HeLa-S3 cell line was provided courtesy of Dr. Bruce Philips, Department of Microbiology, University of Pittsburgh.

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Cancer Res 1971;31:1148-1152.

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