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Communication

γ-Glutamyl Transpeptidase and Malignant Transformation of Cultured Liver Cells[1]

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ABSTRACT

The relationship between γ-glutamyl transpeptidase (GGT)and malignant cell transformation was analyzed in malignant and nonmalignant culture epithelial cell lines derived from rat livers and fibroblastic cell types derived from hamsters and mice. GGT activity was prominent (25 to 90% of cells) in 3 of 5 malignant epithelial liver cell lines. None of the 9 fibroblastic or 4 nonmalignant epithelial cell lines exhibited GGT activity. Our results suggest that by use of GGT activity we can detect in cultured liver cells a significant fraction of the spontaneously or chemically induced malignant cells. Thus, in conjunction with other markers, this marker may help in identifying tumorigenic cells in liver epithelial cultures.

INTRODUCTION

In vitro mammalian cell culture systems are useful for studying the mechanism of chemical carcinogenesis as well as for identifying potential carcinogens in our environment (1, 3, 5, 6, 10–15, 20, 27). In such experiments, the detection of malignant cell transformation is studied frequently in fibroblasts, even though the most prevalent human tumors are derived from epithelial cell origin. The fibroblasts are used mainly because of their ease of cultivation and because only a few reproducible and objective criteria are available for predicting tumorigenicity in cultured epithelial cells (21).

GGT,[3] an enzyme presumably involved in the transport of neutral amino acids across cell membranes (25), was found to be present in hepatomas and preneoplastic liver nodules but absent in adult liver (4, 7–9, 16).[4] It was, therefore, of interest to determine whether this enzyme might also be related to malignant transformation in cultured liver cells. In recent years, a series of methods has been developed for the isolation and long-term maintenance of epithelial cells derived from liver (22, 27, 30). Once established, such cells can be transformed *in vitro* both spontaneously and by different chemical carcinogens (17, 21–24, 27–30). GGT activity was analyzed in 9 such cultured epithelial cell lines derived from rat liver in order to determine a possible relationship between this enzyme activity and malignant transformation of liver cells in culture. For comparison, 8 different normal and transformed hamster and mouse fibroblastic cell lines which are commonly used in chemical carcinogenesis studies were also included. The presence or absence of GGT activity was determined cytochemically (16, 26).

MATERIALS AND METHODS

Cell Lines. The various epithelial cells used in this study were established by Montesano *et al.* (18, 22, 23) from normal rat livers. IAR-6 and IAR-27 cells were derived from 8- to 10-week-old BD-IV rats, IAR6-1 cells were derived from IAR-6 cells after treatment with the liver carcinogen dimethylnitrosamine, and IAR-6-1-RT7 cells were derived from an adenocarcinoma obtained after inoculation of IAR-6-1 cells into newborn BD-IV rats. IAR-2-19 and IAR-20 cells originated from livers of 10-day-old normal BD-VI rats, and IAR-20-PC1-3 cells were derived from a cloned population of IAR-20 cells that were treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. The other cell lines used in this study included secondary golden hamster embryo cultures (Ham normal) and hamster embryonic cells transformed by 3-methylcholanthrene (Ham MCA1, Ham MCA2) and 4-nitroquinoline 1-oxide (Ham NQO1) as well as cells obtained from tumors induced in golden hamsters after inoculation with Ham MCA1 and MCA2 cells (15). Control and SV40-transformed BALB/3T3 cells, kindly supplied by Dr. R. Tennant (Oak Ridge National Laboratory), were also included. The tested cells were cultured at 37° in Dulbecco's modified Eagle's medium supplemented with 2 mM glutamine and antibiotics with 10% fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.) in humidified incubators supplied with 10% CO_2 in air.

Assays for Tumorigenicity. IAR cells were inoculated into newborn syngeneic rats at 1 to 2 × 10^6 cells/animal (22, 23). The normal and transformed hamster cells were inoculated s.c. into 4- to 6-week-old hamsters. Tumors from the transformed cells appeared after a latency period of 7 to 10 weeks, and those from the tumor cells appeared after 1 to 3 weeks.

Histochemical Assay for GGT. In the *in vitro* studies, 1 to 5 × 10^6 cells were seeded onto glass slides which had been immersed previously in 10 ml of growth medium in 100-mm plastic Petri dishes. After the cells grew for 3 to 8 days, the slides were rinsed 3 times with phosphate-buffered saline, 8 g NaCl, 0.2 g KCl, 0.2 g KH_2PO_4, 0.1 g $MgCl_26H_2O$, 2.16

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[3] The abbreviation used is: GGT, γ-glutamyl transpeptidase.

[4] D. T. Pugh and S. Goldfarb. Quantitative Histochemical and Autoradiograph Studies of 2-Acetylaminofluorene Hepatocarcinogenesis, submitted for publication.

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$Na_2HPO_47H_2O$ dissolved in 1 L water (Grand Island Biological Co.), and then stored at −80° (storage at this temperature up to 4 weeks did not alter staining for GGT activity). After slides were collected from a series of cell lines, they were thawed, fixed in acetone (16), stained for GGT activity (26), and lightly counterstained with hematoxylin. The percentage of cells that were stained and their intensity were determined on duplicate sets of slides. For each cell type, at least 1.5×10^4 cells were scanned; results were considered negative when no GGT-positive cells were found. No significant differences in GGT staining were obtained in sparse or confluent cultures.

RESULTS

A series of epithelial cell lines derived from rat livers was tested cytochemically for GGT activity. Staining for GGT activity, when positive, was localized only to the cytoplasm of cells and, in general, the intensity of staining was proportionate to the percentage of positive cells (Fig. 1). The results in Table 1 indicate that the epithelial cells from the cell lines IAR-6 at 26 weeks, IAR-20 at 32 and 40 weeks, and IAR-20-PC1-3 at 21 weeks were not tumorigenic and did not exhibit GGT-positive cells. Two of the 5 tumorigenic cell lines, IAR-6-1 and IAR-27 at their 65th and 73rd passages, respectively, contained 80 to 90% GGT-positive cells. IAR-6-1-RT7 cells, which were derived from an adenocarcinoma induced after inoculation of IAR-6-1 cells, contained 25% GGT-positive cells. The IAR-20-PC1-3 cell line at 54 weeks in culture also contained rare, positively stained clusters of cells. The cell line IAR-2-19, which was tumorigenic, did not exhibit GGT-positive cells. The highest GGT activity was observed in the cells (IAR-6-1, IAR-27, and IAR-6-1-RT7) that gave rise to epithelial tumors, most being adenocarcinomas, upon their inoculation into syngeneic animals (Table 1).

Six different golden hamster fibroblastic cell types were also studied, including a secondary embryo culture, 2 independent 3-methylcholanthrene-transformed cell lines, a 4-nitroquinoline 1-oxide-transformed cell line, and 2 cell lines derived from tumors induced by the 3-methylcholanthrene-transformed cells. With the exception of the normal embryo cells, all of these cell lines induced tumors in golden hamsters upon s.c. injection. In addition, we tested the BALB/3T3 cells and an SV40-transformed-derived cell line. None of these 8 fibroblastic cell types exhibited GGT activity as determined by cytochemical staining (Table 1). On the other hand, GGT activity was prominent (more than 90% of the cells) in 8 different epithelial rat tracheal cells, including 5 primary cultures and 3 malignant epithelial cell lines obtained after *in vivo* treatment with 7,12-dimethylbenz(a)anthracene (E. Huberman, M. Terzaghi, T. D. Pugh, and S. Goldfarb, unpublished observations).

DISCUSSION

The present studies indicate that transformation of cell lines derived from rat liver is associated with acquisition of cytoplasmic GGT activity in a considerable proportion of cell cultures. In contrast, nontransformed and transformed fibroblasts derived from hamsters and mice were uniformly negative for GGT activity, whereas nonmalignant and malignant epithelial rat tracheal cells were uniformly positive for GGT activity. The liver cell findings are especially notewor-

Table 1
GGT in malignantly transformed cells in vitro

Cell line	Time in culture when tested for GGT (wk)	GGT-positive cells (%)	Intensity of GGT in positive cells	Time in culture when tested for tumorigenicity (wk)	Tumors/ inoculated animals	Tumor type[a]
Derived from rat liver						
IAR-6	26	0	−	23	0/4	
IAR-6-1	65	90	+++	35	6/6	6 EP[b]
IAR-6-1-RT7	12	25	++	12	15/15	15 EP
IAR-20A	40	0	−	35	0/5	
IAR-20B	32	0	−	31, 37	0/11	
IAR-20-PC1-3	21	0	−	21	0/7	
IAR-20-PC1-3	54	1	+	30	6/6	4 EP, 1 MX, 1 AT
IAR-27	73	80	+++	63	3/3	3 EP
IAR-2-19	74	0	−	51	2/3	1 EP, 1 MX
Derived from golden hamster embryos						
Ham normal	1	0	−	1	0/5	
Ham MCA1	35	0	−	35	5/5	
Ham MCA1 Tu	8	0	−	8	5/5	
Ham MCA2	38	0	−	38	5/5	
Ham MCA2 Tu	12	0	−	12	5/5	
Ham NQO1	42	0	−	42	5/5	
Derived from BALB mouse embryos						
3T3	?	0	−	NT	−	
3T3-SV40	?	0	−	NT	−	

[a] The tumors observed with hamster embryo cells are all mesenchymal tumors.
[b] EP, epithelial tumors; MX, mixed tumors (epithelial and anaplastic); AT, anaplastic tumor; NT, not tested.

thy, in view of observations (4, 7-9, 16, 19)[4] that, *in vivo*, most rat hepatocellular carcinomas and putative preneoplastic hepatocellular nodules are positive for GGT activity.

Primary and transplantable hepatomas contain GGT activity that is localized to canalicular membranes, closely mimicking the pattern found in fetal and neonatal liver (16). In the case of the Morris hepatoma 7777, we found that the carcinoma has maintained its canalicular distribution despite serial transplantation for many years. However, the same hepatoma cells, adapted to culture more than 3 years ago and which produce α-fetoprotein (2), now show mainly intracytoplasmic distribution of enzyme activity when reinoculated into appropriate Buffalo rats. The cultured cells which formed the tumors still showed intracytoplasmic GGT activity (T. D. Pugh and S. Goldfarb, unpublished observations). These findings and the results of the present study support the conclusion that, regardless of its localization, the acquisition of GGT activity by cultured hepatocytes probably reflects the tumorigenicity of the cell.

We, therefore, suggest that this simple stain for enzymatic activity, in conjunction with other cellular markers (21), may help in identifying tumorigenic cells in epithelial liver cultures.

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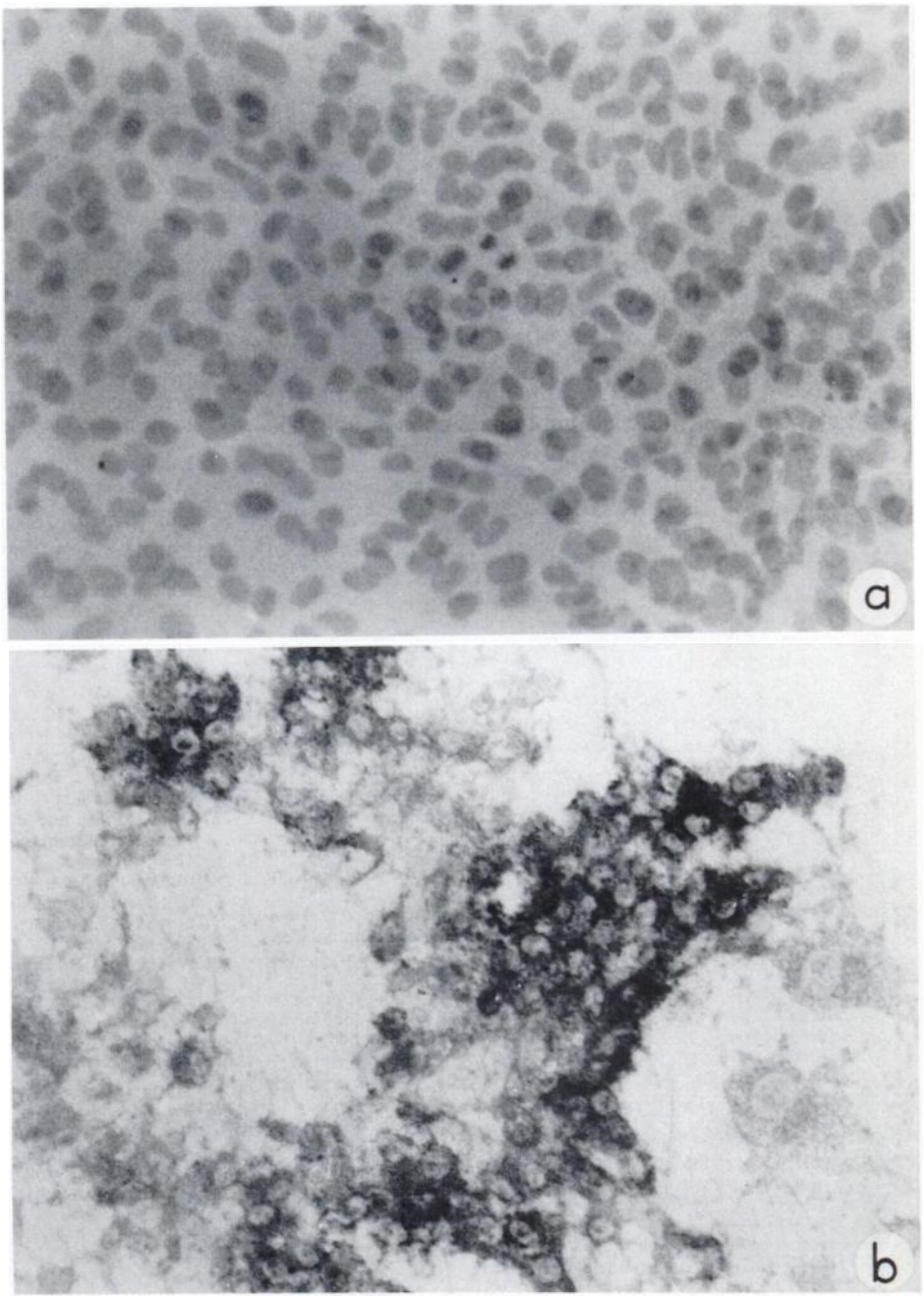

Fig. 1. Staining for GGT activity in cells derived from (*a*) a nonmalignant 26-week-old IAR-6 culture and (*b*) the malignant IAR-6-1-RT7 cells which originated from an adenocarcinoma obtained after inoculation of IAR-6 cells which were treated previously with the liver carcinogen dimethylnitrosamine. The cells were counterstained with hematoxylin. × 370. Cytoplasmic enzyme activity in the IAR-6-IRT7 cells varied from + to ++++ with an average of ++, whereas in the IAR-6 cells it was entirely absent.