Histochemistry 53, 135–142 (1977)

Histochemistry

The Activity and Distribution of Gamma-Glutamyl Transpeptidase (y-GT) in Human Lung Cancers Serially Transplanted in Nude Mice*

Peter Groscurth, Norman Fleming**, and Gonzague S. Kistler
Division of Cell Biology, Institute of Anatomy, University of Zurich, Gloriastrasse 19, CH-8006 Zurich, Switzerland

Summary. Gamma-glutamyl transpeptidase (y-GT) activity and distribution were investigated in different types of human lung cancers (three epidermoid carcinomas, one large cell carcinoma) which were maintained by serial transplantation in nude mice. All transplanted tumour fragments were positive for the enzyme. In the epidermoid carcinomas, y-GT levels were related to the degree of tumour differentiation. Enzyme activity in tumour fragments was always higher than that found in normal adult human lung tissue, and was, in general, maintained throughout the transplantation series.

Introduction

Gamma-glutamyl transpeptidase (y-GT, E.C. 2.3.2.1.), a membrane bound enzyme which facilitates extra-intracellular amino acid transport, can be detected in a range of human organs (Glenner et al., 1962; Albert et al., 1964; Albert et al., 1970). In some of these, the enzyme shows a progressive increase or decrease in activity with pre- and postnatal development. For example, y-GT levels are higher in adult kidney than in the foetal organ, while in adult liver, brain and lung, activity is lower than in the corresponding foetal tissue (Albert et al., 1970; Fleming et al., 1977). In adult organs, enzyme activity may also vary under pathological conditions. Thus, in tumour bearing livers, y-GT levels are elevated, both in the tumour cells, and in the surrounding hepatic tissue (Aronsen et al., 1969; Goldberg and Martin, 1975), whereas, in renal adenocarcinomas, the enzyme content is lower than in the normal kidney (Albert, 1965). In both these cases, the change in activity may represent a reaquisition of a foetal characteristic by malignant tissue (Fleming et al., 1977).

* This work was supported by a grant from A.S.F.C., Switzerland, and the Jubiläumsspende 1970 der Zürcher Kantonalbank
** N.F. was supported during part of the work by a Royal Society European Science Exchange Programme Fellowship

Table 1. Histological types, background information and transplantation details of the human lung cancers examined. The tumours were classified according to the World Health Organization recommendation (Kreyberg, 1967)

Histological type of tumour	Age of donor (years)	Sex of donor	Number of passages in nude mice	Average interval between transplantations (days)
Epidermoid carcinoma (well differentiated)	64	M	19	26
Epidermoid carcinoma (moderately differentiated)	70	M	17	26
Epidermoid carcinoma (poorly differentiated)	70	M	4	55
Large cell carcinoma	44	M	18	26

Whether such a reversion is also a property of human lung neoplasms is not known. Thus in the present study, the activity and distribution of y-GT were examined in different types of human lung cancers which were maintained by serial transplantation in nude mice.

Materials and Methods

Animals. Mice, homozygous for the mutation "nude" (nu/nu) were bred under SPF conditions by mating nu/+ females with nu/nu males, both with the genetic background Balb/c. Animals of both sexes, aged 5 to 8 weeks, were transfered to conventional conditions for the transplantation experiments.

Tumours and Transplantation. Human lung cancers were obtained immediately after surgery. The age and sex of the donors, and the histological classification of the tumours are recorded in Table 1. Tumour tissue was washed in sterile, phosphate buffered saline containing 0.5% Minocycline® and chopped into fragments approximately $1 \times 1 \times 5$ mm, three or four of which were implanted subcutaneously by trocar in the scapulary region. Samples of each carcinoma were inoculated into three to five animals. When the implanted tumours had grown to a diameter of 15–20 mm, they were removed from the hosts and fragments were passaged into new recipients.

The total number of passages and the mean passage duration for each tumour are shown in Table 1.

Histochemistry. Fragments of transplanted tumour tissue, 1–2 mm^3, were fixed in freshly prepared 4% paraformaldehyde in 0.1 M potassium phosphate, pH 7.4, at 4°C. The tissues were rinsed in the same buffer, dehydrated through an ethanol series and infiltrated with polyethylene glycol (M.W. 1000, M.P. 37°C). Sections were cut at 5 μ and stained for y-GT by the method of Rutenburg et al. (1969), with y-glutamyl-4-methoxy-2-naphthylamide (Vega-Fox Biochemicals, Tucson, Arizona) as substrate.

Enzyme Assay. Tumour tissue was chopped into fragments approximately 3 mm^3, blotted and weighed. The fragments were homogenized in 0.1% aqueous sodium cholate (w:v 1:10 or 1:50) and the homogenate centrifuged at 25,000 g for 30 min at 4°C. The clear supernatant fluid was assayed for y-GT by the Boehringer (Mannheim) y-GT monotest system, based on the method of Szasz (1969). Protein content was determined by the Biuret method (Weichselbaum, 1946). The serum of tumour bearing nude mice was also regularly assayed for y-GT.

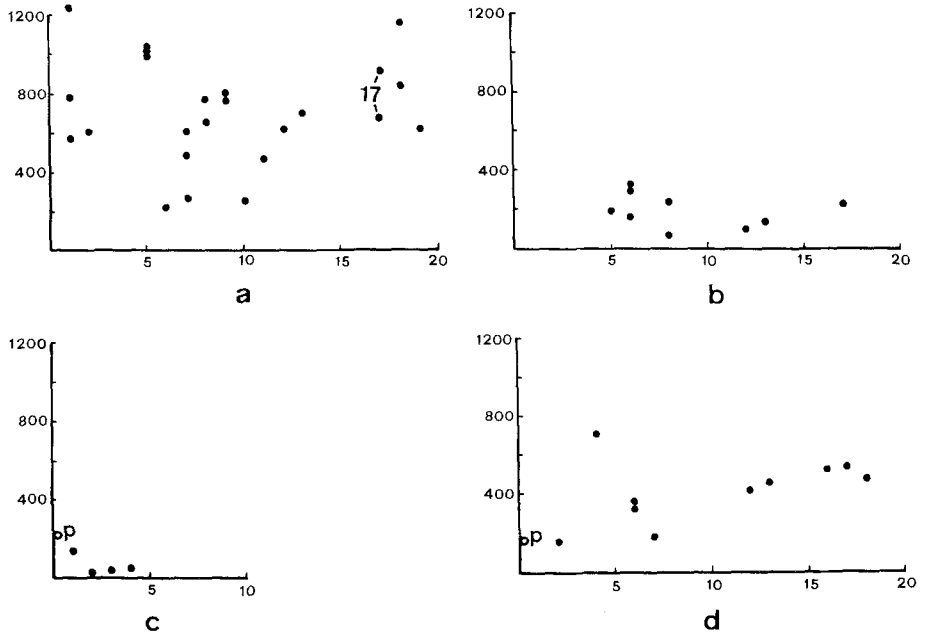

Fig. 1a–d. y-GT activity in four types of lung carcinomas serially transplanted in nude mice. **a** Well differentiated epidermoid carcinoma. **b** Moderately differentiated epidermoid carcinoma. **c** Poorly differentiated epidermoid carcinoma. **d** Large cell carcinoma. Abscissa: passage number. Ordinate: y-GT activity, milli U per g tissue wet weight. *p*, primary tumour. A unit (U) of enzyme activity transforms 1 μ Mol of substrate in 1 min at 25°C

Results

Biochemistry

Only two of the primary tumours were assayed for y-GT (Fig. 1) and samples from subsequent passages of all four cancers were not always available for investigation. The enzyme was detected in all tumours transplanted in nude mice.

The highest activity was found in the well differentiated epidermoid carcinoma (Table 2). Fragments of this tumour from different animals showed a large variation in enzyme levels. In addition, activity was not always consistent in different fragments of the same tumour from a single animal (Fig. 1a, passage 17). Nevertheless, the results indicate that a relatively constant level of y-GT activity was maintained throughout the series of transplants.

The moderately differentiated epidermoid carcinoma was not examined until the fifth passage in the nude mouse. The enzyme activity measured in the tumour at this stage was also fairly consistent with that assayed in subsequent passages (Fig. 1b). Levels were, in general, lower that those found in the well differentiated epidermoid carcinoma (Table 2). The lowest content of y-GT in the three epidermoid carcinomas investigated was observed in the poorly differ-

Table 2. Average y-GT activity of tumour fragments serially transplanted in nude mice

Tumour	Average y-GT activity ± S.E.M.	
	Milli units per g wet weight	Units per g protein
Epidermoid carcinoma (well differentiated)	701 ± 55	14.99 ± 1.51
Epidermoid carcinoma (moderately differentiated)	185 ± 29.3	3.58 ± 0.54
Epidermoid carcinoma (poorly differentiated)	90.4 ± 37.6	2.07 ± 0.72
Large cell carcinoma	381 ± 52.2	7.06 ± 1.66

entiated tumour (Table 2). Enzyme activity seemed to be reduced over the first two passages to a level which was maintained to passage four (Fig. 1c).

The large cell carcinoma also showed some variation in its y-GT levels during the first seven passages (Fig. 1d), but thereafter tumour fragments displayed a constant activity up to passage 18.

The sera of tumour bearing nude mice were always negative for y-GT.

Histochemistry

Because of the small size of the primary tumour fragments available they were not examined histochemically.

Well Differentiated Epidermoid Carcinoma. In all the passages examined, y-GT was detected over large areas of tumour tissue, only a few strands being negative for the enzyme (Fig. 2). The carcinoma showed varying degrees of keratinization, both in different mice and from passage to passage. The keratinized regions were confined to the central areas of tumour nodules and strands, and exhibited an intense y-GT reaction (Fig. 2). In the basal cells, the enzyme was localized as a fine particulate deposit at the cell surface. A similar distribution was observed in the "prickle" cells, some of which also demonstrated a positive reaction in the cytoplasm (Fig. 2).

The connective tissue supporting the transplanted tumour was negative for y-GT, as in all the other lung carcinomas examined.

Moderately Differentiated Epidermoid Carcinoma. y-GT was visualized at the surface of most of the tumour cells (Fig. 3). Many tumour strands had a central necrotic region in which a diffuse enzyme reaction was also observed. Small keratinized areas, which displayed intense y-GT activity, were occasionally noted.

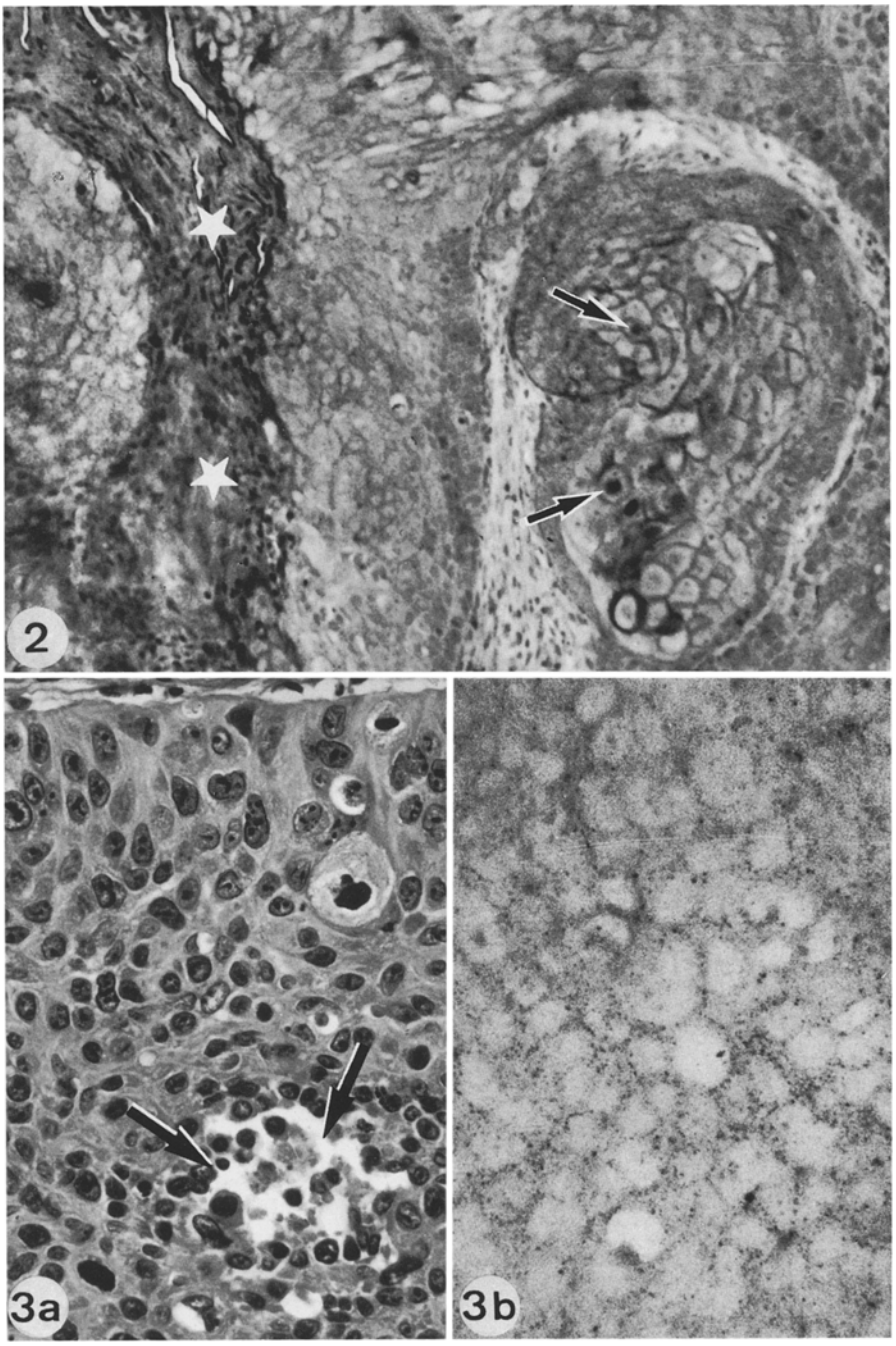

Fig. 2. Well differentiated epidermoid carcinoma, 5th passage in the nude mouse. The keratinized regions (asterisks) show intense y-GT activity. Some of the prickle cells display a cytoplasmic reaction (arrows) in addition to that observed at the cell surface. ×150

Fig. 3a and b. Moderately differentiated epidermoid carcinoma, 8th passage in the nude mouse. **a** Haematoxylin and eosin. Arrows indicate a necrotic area. ×390. **b** y-GT activity, localized at the surface of most of the tumour cells. ×430

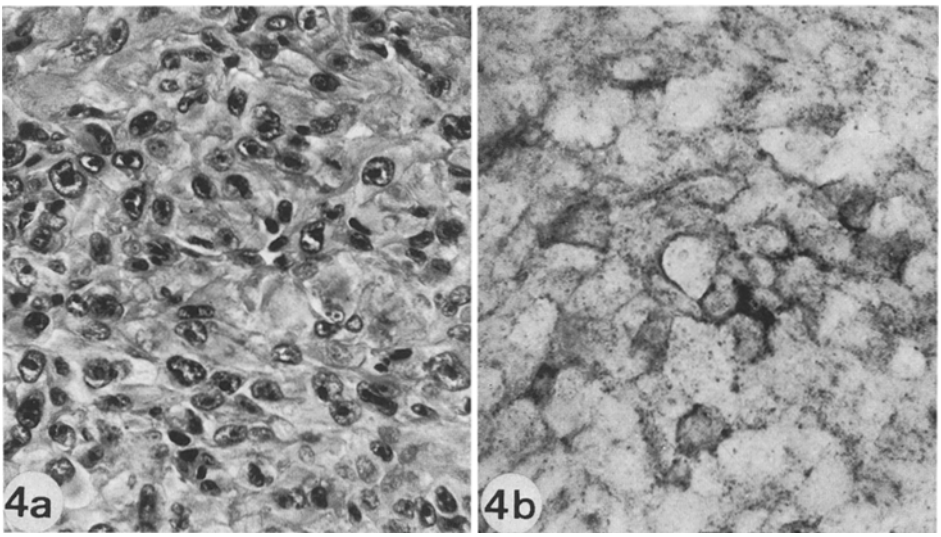

Fig. 4a and b. Large cell carcinoma, 7th passage in the nude mouse. **a** Haematoxylin and eosin. ×400. **b** y-GT is distributed at the surface of the tumour cells. ×350

Poorly Differentiated Epidermoid Carcinoma. The enzyme could be detected histochemically only in the first passage, when weak activity was observed in isolated, randomly distributed cells close to the basal lamina. There was no keratinization of the carcinoma in any of the passages examined.

Large Cell Carcinoma. This tumour was characterized by polymorphism of cell size and nuclear shape (Fig. 4). In all fragments examined, y-GT was demonstrated by a fine granular deposit at the surface of most of the tumour cells. A diffuse reaction was noted in the large, frequently occurring necrotic areas.

Discussion

The thymus-dysgenetic nude mouse is a suitable host for the long-term maintenance of morphology and function of different types of transplanted human tumours (Povlsen et al., 1973; Sordat et al., 1974; Povlsen et al., 1975; Shimoto et al., 1976). In the present study, samples of primary carcinomas or of transplanted tumours were not always available for examination. However, the results indicate that human lung cancer fragments of the types examined here can retain consistent levels of y-GT activity for several passages in nude mice. The poorly differentiated epidermoid carcinoma showed an initial drop in activity, which then remained fairly constant over the next few passages. Because of the limited information available on this tumour, no obvious trend could be established.

In the transplanted tumours, y-GT was assayed at levels which varied with histological type and degree of differentiation. Of the three epidermoid carci-

nomas, the highest activity was found in the well differentiated tumour. Fragments of this tumour in the same passage had a wide range of y-GT values in different mice. This variation was probably related to the proportion of enzyme-positive keratinized regions in the samples examined. Lower levels of the enzyme were found in the moderately and poorly differentiated epidermoid carcinomas, which were only slightly, and not keratinized, respectively.

The other type of lung cancer investigated, the large cell carcinoma, was also y-GT positive. Whether all types of lung neoplasms contain the enzyme is not known. To our knowledge, only Kokot et al. (1965) have measured y-GT in a lung tumour. They reported "low values" of the enzyme in a carcinoma, the histological type of which was not recorded. In normal adult human lung, Albert et al. (1970), found an average y-GT activity of 0.7 units per g protein. The enzyme was localized in the bronchial epithelium, but y-GT-positive cell types were not specified by these authors. In foetal lungs, the enzyme is also found in the undifferentiated bronchial epithelium (Fleming et al., 1977). It has been measured at levels of 4.0 U/g protein in embryos and young foetuses (Albert et al., 1970); and of 1.7 U/g protein in older foetuses (22–24 weeks post menstruationem, own observations). These findings suggest a progressive reduction in y-GT activity in the lung with pre- and postnatal development. The carcinomas examined in our study had levels of y-GT which were clearly higher than those in normal adult lung tissue (Table 2). This increase in activity may represent a reacquisition by malignant tissue of a foetal property (Fleming et al., 1977).

Some types of human tumours, e.g. nephroblastoma, transplanted in the nude mouse may produce detectable levels of y-GT in the host's serum (Wise and Müller, 1976). In our study, the enzyme could not be measured in the serum of nude mice implanted with human lung tumours. This is consistent with the fact that in man, lung carcinomas do not generally lead to an increase in serum y-GT (Rutenburg et al., 1964; Kokot et al., 1965). Serum levels of y-GT are, however, significantly elevated in 90% of patients with either primary liver tumours, or with liver metastases from malignancies of different origin (Rutenburg et al., 1964; Kokot et al., 1965; Aronson et al., 1969). Thus, increased y-GT activity in the serum of patients with lung cancers indicates metastatic involvement of the liver.

Acknowledgements. We thank Miss M. Balzer, Mrs. K. Fenner and Mr. W. Scherle for excellent technical assistance, Misses M. Erni and M. Tunesi for the animal husbandry and Miss. E. Meier for typing the manuscript.

References

Albert, Z.: Gamma-glutamyl transpeptidase in cancers of different human organs. Nature **205**, 407 (1965)

Albert, Z., Orlowska, J., Orlowski, M., Szewczuk, A.: Histochemical and biochemical investigations of gamma-glutamyl transpeptidase in the tissues of man and laboratory rodents. Acta Histochem. (Jena) **18**, 78–89 (1964)

Albert, Z., Rzucidlo, Z., Starzyk, H.: Biochemical and histochemical investigations of the gamma-

glutamyl transpeptidase in embryonal and adult organs of man. Acta Histochem. (Jena) **37**, 74–79 (1970)

Aronsen, K.F., Hägerstrand, I., Nordén, J.G., Phil, B.: On the cause of the increased activity of alkaline phosphatase and gamma-glutamyl transpeptidase in serum of patients with liver metastases. Acta Chir. Scand. **135**, 619–624 (1969)

Fleming, N., Groscurth, P., Kistler, G.S.: The activity and distribution of gamma-glutamyl transpeptidase (γ-GT) in human foetal organs. Histochemistry **51**, 209–218 (1977)

Glenner, G.G., Folk, J.E., McMillan, P.J.: Histochemical demonstration of a gamma-glutamyl transpeptidase-like activity. J. Histochem. Cytochem. **10**, 481–489 (1962)

Goldberg, D.M., Martin, J.V.: Role of γ-glutamyl transpeptidase activity in the diagnosis of hepatobiliary disease. Digestion **12**, 232–246 (1975)

Kokot, F., Kuska, J., Grzybeck, H., Cekanski, A.: γ-glutamyl transpeptidase in tumour diseases. Arch. Immunol. et Ther. Exper. **13**, 586–592 (1965)

Kreyberg, L.: Histological Typing of Lung Tumours. Geneva: World Health Organization 1967

Povlsen, C.O., Fialkow, P.J., Klein, E., Klein, G., Rygaard, J., Wiener, F.: Growth and antigenic properties of a biopsy-derived Burkitt's lymphoma in thymus-less (nude) mice. Int. J. Cancer **11**, 30–39 (1973)

Povlsen, C.O., Visfeldt, J., Rygaard, J., Jensen, G.: Growth patterns and chromosome constitutions of human malignant tumours after long-term serial transplantation in nude mice. Acta path microbiol. scand. Sect. A. **83**, 709–716 (1975)

Rutenburg, A.M., Pineda, E.P., Fischbein, J., Goldbarg, J.A.: A study of serum peptidases in anicteric patients with cancer. Cancer **17**, 781–784 (1964)

Rutenburg, A.M., Kim, H., Fischbein, J.W., Hanker, J.S., Wasserkrug, H.L., Seligman, A.M.: Histochemical and ultrastructural demonstration of γ-glutamyl transpeptidase activity. J. Histochem. Cytochem. **17**, 517–526 (1969)

Shimosato, Y., Kameya, T., Nagai, K., Hirohashi, S., Koide, T., Hayashi, H., Nomura, T.: Transplantation of human tumours in nude mice. J. Nat. Cancer. Inst. **56**, 1251–1260 (1976)

Sordat, B., Fritsche, R., Mach, J.-P., Carrel, S., Ozzello, L., Cerottini, J.-C.: Morphological and functional evaluation of human solid tumours serially transplanted in nude mice. Proceedings of the First International Workshop on Nude Mice (ed. Rygaard, J., Povlsen, C.O.), Stuttgart: Gustav Fischer Verlag 1974

Szasz, G.: A kinetic photometric method for serum γ-glutamyl transpeptidase. Clin. Chem. **15**, 124–136 (1969)

Weichselbaum, T.E.: Determination of proteins in blood serum and plasma. Amer. J. Clin. Path. **16**, 40–49 (1946)

Wise, K.S., Müller, E.: γ-glutamyl transpeptidase in human nephroblastoma grown in nude mice. Experientia (Basel) **32**, 294–296 (1976)

Received March 9, 1977