Carcinogenesis vol.6 no.2 pp.165–172, 1985

Commentary

Gamma-glutamyl transpeptidase – its role in hepatocarcinogenesis

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Introduction

The enzyme gamma-glutamyl transpeptidase (GGT)* is widely used as a marker in preneoplastic lesions in the liver during chemical carcinogenesis (1). The discovery of a link between GGT and liver carcinogenesis first occurred in 1972 when Fiala and co-workers reported elevated levels of the enzyme in livers of rats fed the hepatocarcinogen 3′-methyl-4-dimethylaminoazobenzene and also in transplantable, chemically induced rat hepatomas (2). Using a histochemical stain for GGT, Kalengayi and co-workers found that the increased levels of the enzyme during carcinogen feeding was due to increased GGT activity in focal areas of hepatocytes (3). Further investigation by a number of laboratories revealed elevated levels of GGT in the livers of rats fed a large variety of structurally different hepatocarcinogens, including 2-acetylaminofluorene (4), diethylnitrosamine (4), N-nitrosomorpholine (5) and aflatoxin B_1 (3). In livers from carcinogen-treated animals, focal areas of hepatocytes show a number of different enzymatic and pathologic alterations (1). In livers from rats initiated with a variety of hepatocarcinogens and promoted with phenobarbital, staining of serial liver sections shows that 85% of all the foci stain positively for GGT. GGT identifies more foci than any other strain used in these studies (6,7).

Elevated levels of GGT during hepatocarcinogenesis are found not only in the liver of rats but in the livers of other species as well. Mouse liver tumors induced by safrole (8) or o-aminoazotoluene (9) are GGT-positive. 'Spontaneous' carcinomas in the mouse do not show elevated levels of GGT (9). However, phenobarbital feeding induces GGT preferentially in the spontaneous mouse tumors compared with the surrounding liver tissue (10,11). Human hepatocellular carcinomas also show increased levels of GGT (12,13). Gerber and Thung (12) reported eight of the ten human hepatocellular carcinomas which they examined were GGT-positive. GGT which has been released from the cell membrane can be detected in the serum (14) and measurement of the level of GGT in the serum has become a common clinical test. Elevated levels of serum GGT are most often associated with liver or pancreatic disease (15). The presence of specific GGT isoenzymes in the serum has been suggested as a means of detecting hepatocellular carcinomas at an early stage (16).

There have been several proposals as to the physiological role of GGT. Meister (17) has proposed that it plays a role in amino acid transport. Recent work has led several investigators to conclude that GGT acts primarily as a glutathionase (18). This commentary is a brief review of the biochemistry, proposed functions of GGT in adult tissues and a potential pathophysiologic role of the enzyme in hepatocarcinogenesis.

*Abbreviations: GGT, gamma-glutamyl transpeptidase.

Biochemistry

Enzymatic activity of gamma-glutamyl transpeptidase

GGT was first reported as an enzyme that catalyzed the transfer of the gamma-glutamyl group from glutathione to acceptor amino acids (19). GGT remains unique as the only protease known that can cleave intact glutathione (18). The enzyme has since been found to utilize a wide variety of gamma-glutamyl compounds as substrates. GGT can catalyze the transfer of the gamma-glutamyl group to numerous peptide and amino acid acceptors. The enzyme may use H_2O as an acceptor, with resultant hydrolysis of the substrate. A general formula for the reaction catalyzed by GGT is given in Figure 1. The catalytic properties of GGT have been the subject of several reviews (18,20–22). The diversity of physiologic compounds that are potential substrates for GGT has led to a number of different theories as to the physiologic role of the enzyme, as will be discussed below. The enzyme is relatively stable and can be localized with histochemical stains (23,24) on both frozen and acetone-fixed tissues (25). For the quantitative determination of enzyme activity, assays using chromogenic or fluorogenic substrates have been developed (26–28).

Localization of GGT

GGT is present throughout the plant (29) and animal (22) kingdoms; however, this discussion will be limited to its presence in mammalian tissues. The enzyme is membrane-bound, and its active site is oriented on the outer surface of the cell membrane (30). In normal tissues, enzymatic activity is present primarily in cells that have a secretory or absorptive function. The kidney has the highest GGT activity, followed by the pancreas which in the rat has ~20% of the level found in the kidney, and then by the seminal vesicles, which have ~2% of the level in the kidney (26). All other tissues have <1% of the activity found in kidney; however, the GGT activity is usually localized to one cell type within the organ and one area of the membrane, where it is highly concentrated. Histochemical studies have shown the enzyme to be present in bile ducts and the bile canalicular regions of hepatocytes (23), glandular epithelium of the breast, the primary follicle in the ovary, epididymis, prostate (31), jejunal epithelium, choroid plexus of the brain, capillary endothelium (24), and in the lower epithelium of growing hair follicles (32). The enzyme is also present in fetal liver during the last third of gestation (33). The level of activity in the fetal mouse liver reaches ~1% of the level in the adult kidney (33). GGT is also present transiently during the development of other tissues (34–37).

Abnormally high levels of GGT are often observed in tumors of a variety of tissues, including hepatocellular carcinomas (10,38), malignant squamous carcinomas of the skin (32), squamous cell carcinomas of the buccal pouch

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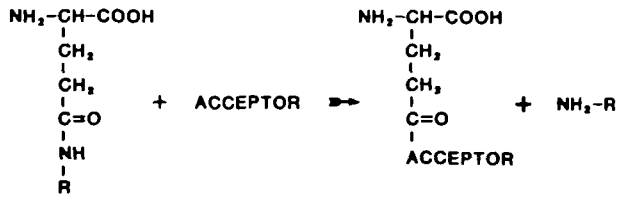

Fig. 1. The reaction catalyzed by GGT. R represents any chemical group and the acceptor can be any number of amino acids, peptides or H_2O.

epithelium (39), adenocarcinomas of the lungs (40), and in some mammary tumors (41).

Physical characteristics of GGT

GGT is a membrane-bound glycoprotein located on the outer surface of the cell membrane (30,42). The enzyme has two subunits. The enzyme from rat kidney is the most extensively characterized. Its heavy subunit (51 000 daltons) has a 6000 dalton segment at the amino terminus that is rich in hydrophobic amino acids and anchors the enzyme in the membrane (43). The light subunit (22 000 daltons), which does not interact directly with the membrane, is non-covalently bound to the heavy subunit (44). Both subunits are necessary for enzymatic activity (45). The enzyme is synthesized as a single polypeptide and is then cleaved into two subunits before it is inserted into the cell membrane (46). The complete enzyme can be purified with detergents to extract it from the membrane, or the hydrophilic regions of the enzyme can be isolated as a unit by treating membrane preparations with papain or bromelain (44,47). Protease treatment cleaves the hydrophobic segment of the heavy subunit, thereby releasing the enzyme from the membrane. The hydrophilic form retains full enzymatic activity (44).

The complete structure of the carbohydrate side chains has been reported for rat kidney GGT (48). The carbohydrate chains are complex and enriched in non-reducing terminal β-N-acetylglucosamine residues. The forms of GGT purified from various tissues within the same species are immunologically cross-reactive (49), although several of the forms differ in portions of the carbohydrate structure as well as in their amount of sialic acid residues (50). The amount of sialic acid bound to GGT also varies during the development of a single tissue. Fetal and regenerating rat liver have a sialic acid-rich form of GGT, whereas the GGT located in the bile canaliculi of adult rat hepatocytes is poor in sialic acid (51,52). Similarly, the GGT in fetal rat intestine is sialic acid-rich, while the GGT in adult intestinal cells is sialic acid-poor (51). A sialic acid-rich form of GGT is also seen in rat hepatocytes and mammary cells that have undergone malignant transformation (41,53). The half-life of rat kidney GGT *in vivo* has been estimated to be 4.3 days (53).

Inhibitors of GGT activity

GGT is irreversibly inhibited by alpha-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid (AT-125) and by the gamma-glutamyl analogues, 6-diazo-5-oxonorleucine and azaserine, all of which bind covalently to the active site of the enzyme (55,56). Sulfophthalein derivatives and serine in the presence of borate are competitive inhibitors of enzymatic activity (57,58). Studies using inhibitors have led to insights into the conformation of the active site of the enzyme (59) and the physiologic role of the enzyme as discussed below.

The presence of malate or hippurate analogues stimulates the hydrolytic activity of the enzyme while inhibiting transpeptidation, possibly by blocking the binding site for the acceptor molecule (60,61). There has been speculation that the concentrations of hippurate in the serum and urine may regulate the hydrolytic activity of GGT (60).

Function

Function of GGT in the kidney

Although GGT was discovered more than 30 years ago, there is still uncertainty as to its physiologic role. In 1973, Meister proposed the existence of a gamma-glutamyl cycle which was responsible for the transport of amino acids across cell membranes and in which GGT played a central role (17,21). In the proposed cycle, GGT transfers the gamma-glutamyl moiety of glutathione to an amino acid acceptor and transports the gamma-glutamyl dipeptide across the cell membrane. Despite the frequent citation of this theory as the physiologic function of GGT, there is now evidence that casts doubt on its validity. Curthoys and Hughey (18) have analyzed the data pertinent to the physiologic function of GGT in the kidney. They conclude that GGT does not participate in amino acid transport, but rather the enzyme catalyzes the hydrolysis of glutathione and its S-derivatives. Among the evidence cited by Curthoys and Hughey is the following. Elce and Broxmeyer (62) obtained ^{14}C glutamic acid-labeled glutathione and using physiologic amino acid concentrations measured the transfer and hydrolytic activities of GGT isolated from rat kidney. They found that hydrolysis was the principal reaction. McIntyre and Curthoys (63) reported that at pH 7.4 the K_m's for hydrolysis of reduced and oxidized glutathione were 5.7 and 8.1 μM, which is approximately equal to the levels of glutathione ($1-5$ μM) found in plasma. Curthoys and Hughey (18) have reviewed the kinetic data and show that the K_m values for various amino acids as substrates for the transpeptidation reaction are ~20-fold higher than the serum concentrations of the amino acids. They conclude that hydrolysis of glutathione and its thiol derivatives is the physiologically significant reaction catalyzed by GGT. Furthermore, Curthoys and Hughey (18) and Curthoys (64) argue that, since the active site of the enzyme is located within the heavily glucosylated hydrophilic portion of the protein, it is most likely that the catalytic activity of the protein acts exclusively in the aqueous fluids (i.e., serum or urine) that are adjacent to the cell. They dispute the idea that GGT serves as a means of transporting molecules across the cell membrane. The enzyme is present on the luminal side of the brush border membrane of the proximal tubules (30). Therefore, Curthoys and Hughey conclude that, since glutathione cannot be taken up by cells, GGT cleaves the gamma-glutamyl group, thereby releasing glutamate and leaving the remaining cysteinyl-glycine peptide susceptible to cleavage by aminopeptidase (see Figure 2). By hydrolyzing glutathione into its constituent amino acids, the kidney cells are able to reabsorb these amino acids. Hahn and coworkers (65) administered ^{14}C-labeled glutathione intravenously to adult rats and found that as the glutathione passed through the kidney it was rapidly broken down into its constituent amino acids. When ^{14}C-labeled glutathione was circulated through isolated livers, which did not express GGT activity on the surface of the hepatocytes, the tripeptide remained intact. GGT is the only enzyme known that can catalyze the cleavage of glutathione (18).

Direct evidence for Curthoy's hypothesis has been provided experimentally with inhibitors of GGT *in vivo*. Griffith and Meister (66) found that the level of glutathione in the urine of mice increased 3000-fold within 1 h after injection of the GGT inhibitor L-gamma-glutamyl-(O-carboxy)phenylhydrazine. Anderson and coworkers (67) demonstrated in the

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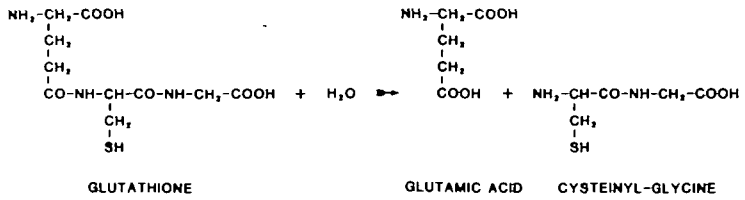

Fig. 2. The hydrolysis of glutathione by gamma-glutamyl transpeptidase.

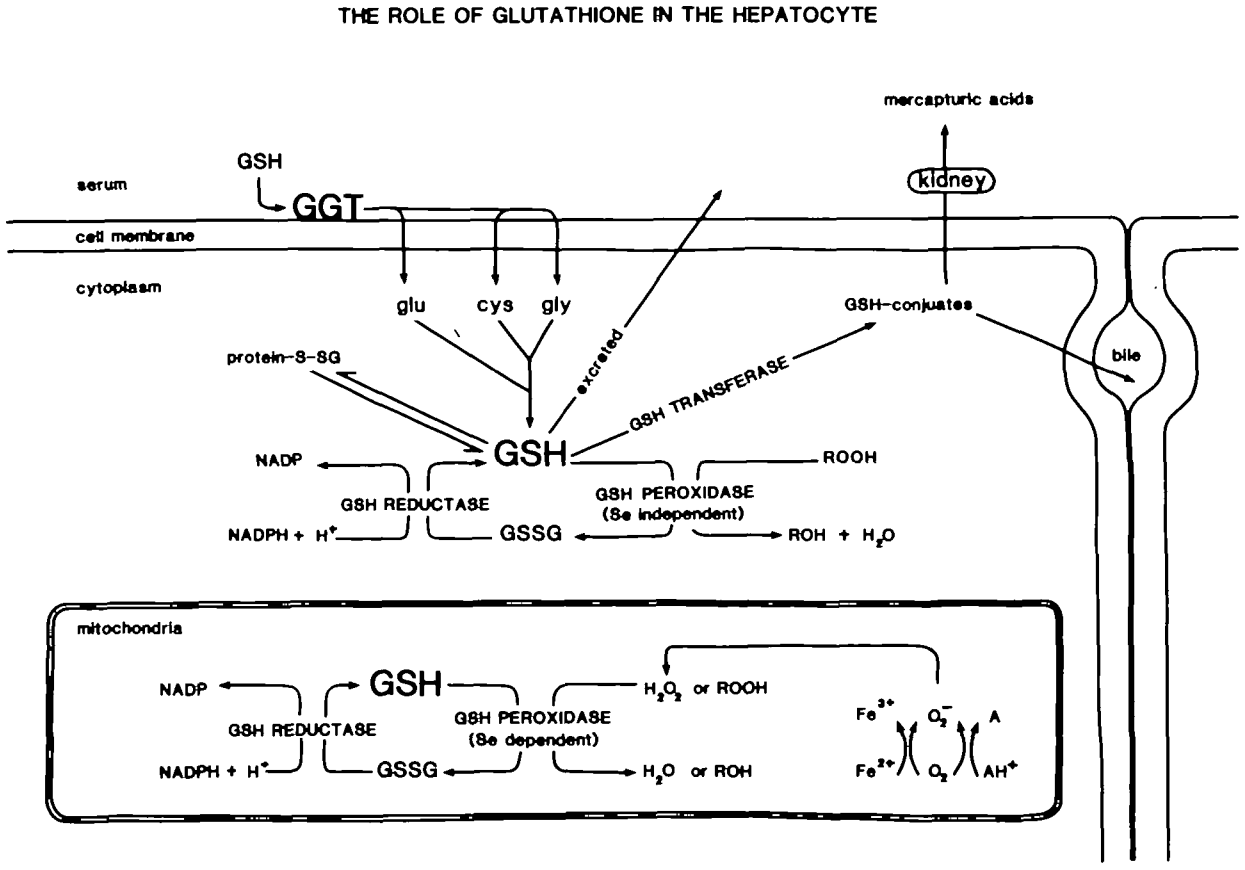

Fig. 3. Some of the functions of glutathione in the adult hepatocyte. This figure is based on figures from several of the references (18,88,89,104).

rat that the increased level of glutathione in the urine during adminstration of GGT inhibitors was the result of non-reabsorption of glutathione by the kidney. High levels of glutathione have also been reported in the urine of a patient apparently lacking GGT (68). In addition to reclaiming the amino acids from glutathione, GGT can hydrolyze oxidized glutathione (63), thereby providing a mechanism for the removal of oxidized glutathione from the serum.

It should also be emphasized that removal of the gamma-glutamyl group is the first step in the conversion of thiol-glutathione derivatives to mercapturic acids. Many foreign compounds including toxins are conjugated with glutathione in the liver. Intact glutathione conjugates are excreted via the bile. In addition, many glutathione conjugates pass through the kidneys or bile ducts (both of which have high levels of GGT activity) and are excreted as mercapturic acids. Curthoys and Hughey (18) have summarized evidence that participation in mercapturic acid formation is also a physiologic role of GGT. [For a review of mercapturic acid formation see Tate (22).] In this light, it is interesting to note that GGT is present on the luminal surface of the proximal tubules in the kidney, bile ducts of the liver, acinar cells of the pancreas, and seminal vesicle, thereby allowing reabsorption of the amino acids of glutathione from fluids that are being excreted. In addition, the presence of GGT on the jejunal epithelium would allow for the uptake of the constituent amino acids from ingested glutathione and the further metabolism of biliary glutathione conjugates (69).

Cell division and GGT levels in the liver

It has been suggested by several authors (70–72) that the presence of GGT in rat hepatocelular carcinomas and presumptive preneoplastic hepatocytes is simply a marker of cellular dedifferentiation, signaling a reversion of the hepatocyte to a fetal phenotype. This proposal is based on the observation that in most hepatocellular carcinomas there is often expression of one or more proteins that are produced by the fetal liver. Among the proteins of the fetal phenotype expressed in hepatocellular carcinomas are GGT, alpha fetoprotein, the fetal isozyme of pyruvate kinase (K-type III), and aldolase B (73; for review see 74). The basis of this theory is that all of these enzymes are present in the fetal liver late in

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gestation at a time when the hepatocytes are proliferating; therefore, GGT identifies a 'fetal-like' proliferating hepatocyte. There are several points upon which this theory can be challenged, and we would like to propose that GGT is not simply a marker for reversion of hepatocytes to a fetal state, but rather that the presence of GGT on the surface of the cell gives the hepatocyte a selective advantage in the toxic environment created by hepatocarcinogenic regimens.

It should be noted that hepatocellular proliferation and expression of GGT are not linked. In the rat, the level of GGT activity in the liver decreases dramatically at birth, reaching the level in adult liver by six days after birth (75). The level of cell proliferation declines much more slowly. Three weeks after birth the liver is still dividing at a rate more than ten times the adult level (76). Tateishi and coworkers (33) have also demonstrated a lack of correlation between GGT levels and the rate of cell proliferation in fetal and newborn mice. In the mouse the liver serves predominantly as a hematopoietic organ until the 16th day of gestation (77). GGT levels rise several hundred-fold from the 17th day of gestation until birth. At birth the GGT level begins to drop off sharply (33). Perhaps it is more relevant to assess the role of the fetal hepatocyte and how that role changes at birth. In the fetus the kidneys do not exhibit GGT activity and exhibit their excretory function only at a minimal level, while the fetal liver serves many of the functions of detoxification and removal of waste that are later performed by the adult kidney. If indeed one of the functions of GGT in the adult kidney is to hydrolyze glutathione into its amino acids so that they can be reutilized, then in the fetus some organ other than the kidney would have to serve this role; otherwise, there would be no way to break down oxidized glutathione in the serum. The rapid decline of GGT in the liver at birth concurrent with the rapid induction of GGT in the kidney (75) is consistent with the theory that the liver is responsible for hydrolysis of serum glutathione in the fetus. In the adult hepatocyte, induction of GGT may also serve to hydrolyze serum glutathione. Since cells are unable to take up intact glutathione from the serum (65), hydrolysis of the tripeptide by GGT would provide the hepatocyte with a higher level of the amino acids necessary for the intracellular synthesis of glutathione.

GGT induction as an adaptive response in the liver

GGT can be induced in the adult rat liver. After four days of fasting, rats exhibit a two-fold increase in the specific activity of GGT in the liver (78). Decreased uptake of amino acids into the cell resulting from decreased levels of serum amino acids may trigger increased GGT activity. Phenobarbital (100 μg/day for 5 days) also causes a two-fold elevation of GGT in rat liver (79), in agreement with other studies that show phenobarbital increase the level of hepatic GGT activity in many species (80 – 82). Feeding ethanol to female Sprague-Dawley rats (36% of total calories) for six weeks caused a two-fold increase in GGT activity in the liver (83). Therefore, the activity of GGT can be increased in the liver. However, under nontoxic conditions, GGT levels can apparently be increased only several-fold.

GGT induction in preneoplastic foci and tumors

The level of GGT activity in fetal rat liver is ~50-fold higher than in the adult liver (38). Hepatomas express GGT at levels equal to or higher than the level found in fetal liver (38). This extremely high level of GGT is also found in preneoplastic foci that have been isolated from livers of rats during hepatocarcinogenesis (Hanigan and Pitot, unpublished results). One possible explanation for this phenomenon is that initiation of a relatively small number of hepatocytes during hepatocarcinogenesis results in an alteration of the regulation of GGT by a unknown mechanism, which may or may not involve a mutational event. These single initiated cells remain undetected until they undergo several rounds of division and form a small focus as a result of a promoting stimulus applied to the liver.

Role of GGT in hepatocarcinogenesis

In most hepatocarcinogenic regimens, during the promotion phase of treatment the liver is subjected to a variety of stresses. Some of this stress can be lead to a depletion of glutathione levels (as described below). We suggest that hepatocytes with high levels of GGT on their cell surface will have higher levels of the amino acids needed to make glutathione. These GGT-positive cells will be able to replenish glutathione and thereby have a selective advantage during promotion.

Glutathione, which accounts for 90% of the nonprotein thiol in the cell (84) serves a variety of functions in the hepatocyte, some of which are shown in Figure 3 (85). There are two intracellular glutathione pools, one in the mitochondria and one in the cytoplasm. In hepatocytes these pools are regulated independently; the cytoplasmic pool can become depleted while the mitochondrial pool remains intact (86). Modulation of the redox state of mitochondrial pyridine nucleotides and calcium content appear to be closely coupled to mitochondrial glutathione redox status (87). The cytoplasmic pool contains 85% of the total cellular glutathione (86). Reduced glutathione is utilized to reduce organic peroxides, to bind to electrophiles directly or via glutathione transferase, to supply reduced glutathione to the serum, and to form mixed protein disulfides (88). Oxidized glutathione is excreted from the cell or reduced via the NADP system by glutathione reductase (89).

During the promotion phase of many hepatocarcinogenic treatment protocols, the promoting compounds interact with systems that utilize glutathione and stress the levels of glutathione as well as the hepatic glutathione redox state. A large number of complete hepatocarcinogens are metabolized to reactive electrophiles (90). Reduced glutathione acts as a nucleophile that protects the DNA and other nucleophilic cell components from attack by the reactive form of the carcinogen (91,92). Glutathione conjugates have been identified as major metabolites for several hepatocarcinogens (91,93). Phenobarbital, a promoting agent for the liver (94), increases the level of glutathione transferase activity (95) and induces cytochrome P450-metabolizing enzymes. These changes result in an increase in oxidative drug metabolism and lead to an increased efflux of oxidized glutathione from the liver (96). Increased amounts of oxidized glutathione are produced when there is increased glutathione peroxidase activity in the cell or when depleted levels of NADPH block the reduction of oxidized glutathione (97). Oxidized glutathione is excreted from hepatocytes when intracellular levels rise (89). Szent-Gyorgyi (98) has presented evidence that SH groups such as those of reduced glutathione play an important role in cell division. Therefore, in order for a cell to divide during promotion, it must have adequate levels of glutathione (for review see 99). Maintaining glutathione levels can be achieved either by decreased utilzation of glutathione or increased synthesis. Among the enzymatic alterations seen in preneoplastic liver lesions are decreased levels of cytochrome P450 (100,101) and monoamine oxidase (102), both of which spare

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glutathione levels by reducing the number of metabolites detoxified by glutathione (103). Also epoxide hydrolase activity is increased in many lesions (103). Increased levels of epoxide hydrolase decrease the number of epoxides that would otherwise react with glutathione (91,93). Increased synthesis of glutathione will also aid in maintaining glutathione levels. However, availability of its constituent amino acids can be rate-limiting during glutathione synthesis (84). Increased levels of GGT, which are seen in many preneoplastic lesions, lead to local hydrolysis of glutathione from the serum and provide the hepatocyte whith a higher level of the amino acids necessary for glutathione synthesis. Of the three amino acids that make up glutathione, cysteine has the lowest intracellular concentration and cysteine is generally the amino acid that becomes rate limiting in glutathione synthesis (104).

Evidence in support of this hypothesis on the role of GGT in preneoplastic liver lesions is provided from several types of experiments. Ahluwalia and Farber (105) reported that glutathione and gamma-glutamylcysteine synthetase, which is the rate-limting enzyme of glutathione synthesis, were increased in hyperplastic nodules in the rat. Ogawa and coworkers (106) induced preneoplastic lesions by the same procedure used by Ahluwalia and Farber and found almost all the lesions had high levels of GGT activity. Deml and Oesterle (107) treated rats with diethylnitrosamine, followed by promotion with polychlorinated biphenyls or with N-nitrosomorpholine. Two weeks after the carcinogen treatment had been completed, the animals were sacrificed, and serial liver sections were stained for GGT and glutathione. Areas of the liver that stained positively for GGT also showed much higher levels of intracellular glutathione than did the surrounding liver.

Promoting agents stress the liver and impair its function; therefore, a proliferative stimulus is induced in the animal. If the stress caused by the promoter depletes GSH levels within the cell, then the hepatocytes that have increased levels of GGT will be able to replenish GSH levels most rapidly. GGT-positive cells will be better able to respond to the proliferative stimulus than the GGT-negative cells.

The role of oxidation in the natural history of carcinogenesis has received increased attention recently. Administration of antioxidants can inhibit tumor formation (108). High intracellular levels of glutathione would also prevent oxidative damage. Induction of GGT on the outer surface of the cell would help maintain glutathione levels, providing the GGT-positive cells with a selective advantage.

Peroxisome proliferators are the only known class of hepatocarcinogens that do not induce GGT in preneoplastic hepatocytes nor in the hepatocellular carcinomas that result from this treatment (109). An explanation for this exception can be offered in light of this discussion. Hydrogen peroxide produced within the peroxisome is normally converted to H_2O and O_2 by the peroxisomal enzyme, catalase (110). Although peroxisomes induced by the peroxisome proliferation appear to have lower levels of catalase relative to uninduced peroxisomes, the amount of catalase per cell is higher in livers from treated rats than from untreated rats (111). Even if the level of H_2O_2 increases within the peroxisome and H_2O_2 leaks out into the cytoplasm, hydrogen peroxide is a very poor substrate for the selenium-independent glutathione peroxidase that is present in the cytoplasm (87,112). Therefore, it is likely that glutathione levels are not depleted in these cells. When the glutathione levels are not depleted in the cell, GGT induction would offer no advantage to the cell. Rao and co-workers (109) have reported that the altered foci, neoplastic nodules, and hepatocellular carcinomas induced in the rat by the hypolipidemic peroxisome proliferator Wy-14,643, show less oxidative damage (as measured by autofluorescent deposits of lipofuscin pigment) than does the adjacent uninvolved liver. Therefore, oxidative stress may play a role in promotion by peroxisome proliferators, but this does not appear to involve glutathione.

A major question in the field of carcinogenesis is whether specific alterations within a cell are both necessary and sufficient for transformation. We propose that elevated levels of GGT enable the cell to maintain a high rate of glutathione synthesis, but that this change alone is not sufficient for transformation. In many hepatocarcinogenic regimens the focal areas of GGT-positive hepatocytes regress upon cessation of the treatment. This regression or remodeling, as Farber has referred to the process (113), may involve death and removal of the GGT-positive cells by apoptosis (114). However, elevated levels of GGT reduce the stress on the glutathione level in the cell and enable the cells to respond to proliferative and other stimuli. The enhanced survival and proliferation of GGT-positive hepatocytes may facilitate transformation and tumor progression.

In summary, Curthoys and Hughey (18) have suggested that the physiologic role of GGT in the adult kidney is to cleave the gamma-glutamyl group from glutathione as it passes through the proximal tubules in the urine. This allows the reabsorption of glutathione via its constituent amino acids. It is proposed here that GGT on the surface of the hepatocytes hydrolyzes the gamma-glutamyl group from glutathione in the serum and thereby provides the cell with the amino acids necessary for glutathione synthesis. Hepatocytes with elevated levels of GGT will have an advantage over GGT-negative hepatocytes when the animal is given promoting compounds that deplete glutathione levels. On the basis of this hypothesis, GGT is not simply a marker of a fetal phenotype in preneoplastic cells, but rather an enzyme that provides a selective advantage to a cell undergoing stress, resulting in depletion of intracellular glutathione.

Implications for future study

In the past the search for a 'unique tumor enzyme phenotype' has been consistently frustrating. Although GGT by no means conforms to such a unique neoplastic phenotype, the proposal outlined in this paper suggests that neoplasms with a phenotype including high functional levels of GGT may have a distinct advantage over their normal cellular counterparts. If such a neoplastic cell is equipped with the synthetic machinery for the synthesis of glutathione and a sufficient supply of its constituent amino acids, it will be at a distinct advantage over cells not possessing this phenotype. The earlier studies suggesting the relative resistance of neoplastic cells to chemical toxic agents may be part of this same picture (115). During hepatocarcinogenesis the GGT-positive phenotype is, we believe, a distinct advantage for cells subjected to chemical adversity. This theory predicts that those cells with such a phenotype will possess higher levels of intracellular glutathione than cells that are GGT-negative. This thesis may be tested both *in vivo* and *in vitro* by relatively simple methodologies. We may have only just begun to uncover our knowledge of the role of this important enzyme in the cellular response to injury, not only in hepatocytes but also in a variety of other tissues. We hope that this discussion will stimulate further research in this area in order to extend our understan-

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ding of the role of GGT in multi-stage carcinogenesis and in environmental cellular pathology.

Acknowledgements

We would like to acknowledge Dr. Timothy Fennell, Dr. Norman Drinkwater, Dr. Jerry Hjelle and many of our other colleagues for their helpful discussions during the preparations of this manuscript. We would also like to thank Dr. Ilse Riegel for her editorial comments and Mary Jo Gilden for typing the manuscript. M.H.H. is supported by grant CA 07175 22485 from the National Cancer Institute.

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Received on 13 September 1984; accepted on 27 October 1984

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