Characterization of 5-Oxo-L-prolinase in Normal and Tumor Tissues of Humans and Rats: A Potential New Target for Biochemical Modulation of Glutathione

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ABSTRACT

5-Oxo-L-prolinase (5-OPase) is an enzyme of the γ-glutamyl cycle involved in the synthesis and metabolism of glutathione (GSH), which is known to protect cells from the cytotoxic effects of chemotherapy and radiation. Previous studies on rats have shown that administration of the cysteine prodrug L-2-oxothiazolidine-4-carboxylate, a 5-oxo-L-proline analogue that is metabolized by 5-OPase, preferentially increases the GSH content of normal tissues while paradoxically decreasing it in the tumor and results in an enhanced in vivo tumor response to the anticancer drug melphalan. These observations initiated the present study of 5-OPase in experimental models and clinical specimens to investigate the potential role of this enzyme in the selective modulation of GSH in normal and tumor tissues. First, 5-OPase activity was measured in tissues of tumor-bearing rats, in the peripheral mononuclear cells of normal human subjects, and in surgically resected tumor and the adjacent normal tissues from patients. We found that the activity of 5-OPase in human kidney, liver, and lung is significantly lower than that found in rats. Second, we have raised a polyclonal IgG anti-5-OPase antibody by immunizing rabbits with purified 5-OPase from rat kidney. This antibody has very high affinity (shown by immunoprecipitation) and specificity (shown by Western blot) and cross-reacts with human 5-OPase (shown by Western blot and immunohistochemistry). It was then used to examine the distribution of 5-OPase in paired normal and neoplastic human specimens using Western blot and immunohistochemistry. Examination of paired normal and neoplastic tissues of stomach and lung revealed a significantly lower level of 5-OPase in tumor tissues than in the paired normal tissues. In colon tissues, there is no significant difference in 5-OPase level between the normal and tumor tissues. These findings could have implications for both carcinogenesis and therapy.

INTRODUCTION

5-OPase is one of the five enzymes involved in the γ-glutamyl cycle, an interrelated series of reactions involved in the synthesis and metabolism of GSH. 5-OPase catalyzes the ATP-dependent hydrolysis of 5-oxo-L-proline to L-glutamate. Whereas 5-oxo-L-proline is the metabolite of the γ-glutamyl residue in GSH, L-glutamate is one of the three amino acid substrates for GSH synthesis. Therefore, 5-OPase links the reactions involved in GSH metabolism with those involved in GSH synthesis in the γ-glutamyl cycle (1–3).

Because GSH plays a critical role in protecting cells from toxic oxygen species and various xenobiotics, including many anticancer drugs and carcinogens, numerous studies have been undertaken to modulate the cellular GSH levels to improve the effect of chemotherapy on cancer or produce chemoprotection against carcinogens. Most of the enzymes involved in the γ-glutamyl cycle have been investigated thoroughly. However, neither 5-OPase itself nor its role in GSH metabolism has been as extensively studied (4–8). The main reason is the limited characterization of the enzyme and lack of probes such as antibodies for its study. Another reason may be the observation that under normal conditions, cells do not necessarily depend on S-OPase for its study. Another reason may be the observation that under normal conditions, cells do not necessarily depend on S-OPase for GSH synthesis, because 5-oxo-L-proline has no significant feedback inhibition on GSH synthesis, and glutamate from many other sources is usually available and sufficient for this purpose (9). Rare cases of an inborn deficiency of 5-OPase have been reported that are associated with a variety of clinical manifestations but do not necessarily lead to GSH deficiency (9, 10). However, several studies have shown that OTZ, an analogue of 5-oxo-L-proline, can significantly increase the cellular GSH level when cells are under oxidative stress (11–13). Because 5-OPase intracellularly converts OTZ to L-cysteine, a rate-limiting substrate for GSH synthesis, these studies suggest that in certain circumstances, the role of 5-OPase in maintaining cellular GSH content seems to be more important than under normal conditions.

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3 The abbreviations used are: 5-OPase, 5-oxo-L-prolinase; GSH, glutathione; OTZ, L-2-oxothiazolidine-4-carboxylate; ECL, enhanced chemiluminescence.
A number of studies have identified another potentially important role of 5-OPase in GSH modulation in the context of cancer chemotherapy. It was observed that tumor cell lines usually have lower 5-OPase content than normal cells in culture (14). Russo et al. (12) showed that OTZ administration can increase the GSH content in a normal lung fibroblast cell line but not in a lung cancer cell line, resulting in a selective potentiation of the cytotoxicity of several anticancer drugs in the cancer cells. We have recently shown that administration of OTZ to tumor-bearing rats increases the GSH level in normal tissue (including bone marrow) while paradoxically decreasing the GSH level in the tumor (15), and administration of OTZ together with the anticancer drug melphalan significantly enhances the in vivo tumor response to melphalan (16). These observations stimulated our investigation of 5-OPase in experimental models and clinical specimens. We have purified the enzyme from rat kidney and generated a high-affinity antibody against it. Here we present our results on the tissue distribution of 5-OPase as well as the preliminary enzymatic and immunohistochemical examination of 5-OPase in rat and clinical human specimens.

MATERIALS AND METHODS

Reagents and Tissues

5-Oxo-L-[14C]-proline was prepared by cyclization of L-[14C]-glutamate (DuPont New England Nuclear, Mississauga, Canada) as described previously (17).

The animal model used in this study was the MatB tumor-bearing female Fisher 344 rat. Five × 10^5 MatB cells (rat mammary carcinoma cells) were s.c. injected into 10–12-week-old rats, and a solid tumor was palpable in approximately 10 days. At that time, the rats were sacrificed, and tissue samples of kidney, liver, lung, and bone marrow from limb long bones and tumors were excised, assayed immediately for 5-OPase activity, and processed routinely for histology. The bone marrow cells were collected by centrifugation through Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden).

Human tissue samples were obtained immediately after surgical excision. The tumor and normal tissues were dissected by the pathologist (L. C. A.) and processed separately for 5-OPase activity and for histological analysis within 30 min. Samples of normal kidney included both cortex and medulla. Samples of normal colon and stomach consisted of mucosa and scant amounts of attached submucosa. Samples of normal lung contained only subsegmental bronchi and alveolar parenchyma.

Human peripheral blood mononuclear cells were collected from 13 normal donors in the fasting state before 10 a.m. and purified by centrifugation through Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden).

Enzymatic Assay of 5-OPase

Tissues or cells were homogenized in ice-cold buffer A [50 mm Tris (pH7.3), 0.1 mm EDTA, 2 mm DTT, and 5 mm 5-oxo-L-proline]. Purification of 5-OPase followed the liquid chromatography method described previously (18). The activity of 5-OPase was determined by measuring the conversion rate of 5-oxo-L-[14C]-proline to L-[14C]-glutamate (18). The stability of 5-OPase in tissues was tested by assaying the 5-OPase activity in rat kidney after the kidney had been kept at 4°C for different time intervals up to 30 h after sacrificing the rat, and after the dialyzed rat kidney solution was frozen at −80°C for up to 1 week. Results showed no decline of 5-OPase activity in the delayed assay samples as compared to the fresh tissue samples. The enzyme activity was therefore stable under the conditions tested. The standard curve of this assay method was tested with purified rat kidney 5-OPase and proven to be linear and sensitive even within the low range of 10–100 milliunits (data not shown).

Western Blot

An ECL Western blotting protocol (Amersham, Buckinghamshire, United Kingdom) was followed. The primary antibody at a 1:50,000 dilution was incubated for 3 h, and the secondary antibody at a 1:2000 dilution was incubated for 1 h at room temperature.

Immunohistochemistry

Histological sections of formalin-fixed paraffin-embedded tissue samples were examined for immunodetectable 5-OPase using standard techniques. The protein blocking agent, the secondary antibody solution, and the streptavidin peroxidase reagent were from Lipshaw Immunon (Pittsburgh, PA). The avidin/biotin blocking kit was from Vector Laboratories, Inc. (Burlingame, CA). The primary antibody, diluted to 1:500 and 1:1000 in 5% normal goat serum, was applied and incubated overnight at 4°C. Preimmune rabbit serum diluted to 1:750 was used as control. Incubation with the biotinylated secondary antibody and then with the streptavidin-peroxidase conjugate was 30 min each.

Antibody Production and Validation

5-OPase Purification and Immunization. Rat kidney 5-OPase was partially purified from frozen kidneys using a previously described method (18). The 5-OPase in the semipurified pool was separated by preparative SDS-PAGE. The 5-OPase band, identified by its molecular weight (140,000; Ref. 19) and by the fact that it was the most dominant band on the gel, was then cut from the gel, minced, and emulsified with an equal volume of incomplete Freund's adjuvant (IFA; Sigma Chemical Co., St. Louis, MO) and injected into multiple i.m. or s.c. sites (0.5–1 ml/site) of a New Zealand strain White rabbit. The rabbit was bled 12 days after the priming immunization. The same procedure was followed for additional boost immunizations (once in 4–8 weeks or when the antibody titer was found to be declining).

ELISA to Detect Antibody. 5-OPase was coated onto a 96-well microtiter plate (Dynatech Laboratories, Chantilly, VA) at 0.2 µg/well overnight under vacuum. ELISA followed protocols described previously (20). A goat antirabbit IgG (H+L) horseradish peroxidase conjugate (Bio-Rad, Richmond, CA) was used as secondary antibody at a 1:4,000 dilution. The primary antibody exposure was 2 h, and that of secondary antibody was 1 h. The developing solution was 0.03% tetramethylbenzidine [stock solution was 0.125% (v/v) tetramethylbenzidine in methanol] and 0.02% H₂O₂ in citrate phosphate buffer [0.1 M Na₂HPO₄ (pH 5.0) and 0.05 M citric acid monohydrate]. Development time was 30 min. The plate was read on a Dynatech ELISA reader (Dynatech; Fisher) at 405 nm wave-
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Immunoprecipitation to Determine the Affinity of Antibody. Twenty-five μg of 5-OPase (47 milliunits) and the primary antibody at various dilutions were mixed, and the mixture was rotated for 5 h at 4°C. This antigen/antibody mixture was then mixed with protein A-Sepharose CL-4B (Pharmacia LKB), rotated for 2 h at 4°C, and then centrifuged. The supernatant and the Sepharose precipitate were used for the enzymatic activity assay and Western blot experiment, respectively. All samples were in duplicates. The result showed that the preimmune serum had no specific effect on 5-OPase activity in the supernatant (the small decline in the activity observed is more likely due to the dilution of the reaction mixture), whereas the amount of 5-OPase activity left in the supernatant after precipitation by the immune serum was proportional to the dilution of the serum. A 1:11.5 dilution of the immune serum precipitated over 90% of the 5-OPase activity compared to that of the control (Fig. 1B). The Sepharose resin of the 1:11.5 antiserum dilution sample was washed with SDS sample buffer and subjected to Western blot analysis together with the supernatant and 47 milliunits of purified 5-OPase as control. The 5-OPase band was not seen in the supernatant but appeared in the elution of the Sepharose (data not shown). These results demonstrated that this polyclonal antibody recognizes and binds to 5-OPase with very high affinity.

Western Blot to Test the Specificity of the Antibody. An ECL Western blot was performed with the semipurified 5-OPase preparation. As shown in Fig. 1C, at the position of the 5-OPase protein (Mr 140,000) on an ECL Western blot film, no band was detected in the lane blotted with either the preimmune serum (Lane 1) or the primary immune serum (Lane 2), whereas there was a strong band in the lane blotted with the first booster serum (Lane 3). To test if the band density corresponds to the amount of 5-OPase activity in tissue samples, both an ECL Western blot and a 5-OPase activity assay were performed with protein extracts from rat kidney, liver, lung, and MatB tumor. The same amount of protein (20 μg) was loaded on the gel. As shown in Fig. 1D, a specific band with the correct molecular weight was detected, and its density correlated with the 5-OPase activity measured in these samples.

Immunohistochemical Method to Test Specificity of the Antibody. Immunohistological examination of 5-OPase in rat and human kidney samples confirmed that the polyclonal ant-
Table 1  5-OPase activity in the normal tissues of female Fisher rats bearing MatB tumors, and patients with the corresponding cancer

The experimental error was within 10% of the values. See “Materials and Methods” for experimental procedures.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Rat</th>
<th>Human</th>
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<tr>
<td>Kidney</td>
<td>187.6 ± 23.5 (n = 6)</td>
<td>8.3 ± 2.3 (n = 6)</td>
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<td>Liver</td>
<td>26.1 ± 2.9 (n = 5)</td>
<td>19.6 (n = 2)*</td>
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<td>Lung</td>
<td>3.4 ± 1.6 (n = 5)</td>
<td>3.3 ± 2.4 (n = 4)</td>
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<td>Colon</td>
<td>9.6 ± 2.7 (n = 12)</td>
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<td>Breast</td>
<td>11.2 ± 7.2 (n = 3)</td>
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<tr>
<td>Uterine</td>
<td>4.4 (n = 1)</td>
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<td>BM*</td>
<td>2.1 (n = 2)</td>
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<td>PBMCs†</td>
<td>11.3 ± 5.8 (n = 13)</td>
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<tr>
<td>Tumor</td>
<td>3.4 ± 2.6 (n = 5)†</td>
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* Samples from two patients were assayed, and the 5-OPase activity was 30.0 and 9.1 milliunits/mg, respectively.
† BM, bone marrow.
‡ PBMCs, peripheral blood mononuclear cells.
§ Data from MatB mammary tumors grown in female Fisher rats.

The 5-OPase activity in normal tissues from patients were: liver, 19.6 milliunits/mg (two specimens, 30 and 9.11 milliunits/mg, respectively); peripheral blood mononuclear cells, 11.3 ± 5.8 milliunits/mg; breast tissue, 11.2 ± 7.2 milliunits/mg (n = 3); colon, 9.6 ± 2.7 milliunits/mg (n = 12); kidney, 8.3 ± 2.3 milliunits/mg (n = 6); uterine smooth muscle, 4.4 milliunits/mg (n = 1); and lung, 3.3 ± 2.4 milliunits/mg (n = 4). Therefore, humans do not have a tissue distribution pattern of 5-OPase activity similar to that of rat. It is noted that: (a) whereas rat kidney had strikingly high 5-OPase activity [up to 187.6 ± 23.5 milliunits/mg (n = 6)], human kidney only had an average 5-OPase activity of 8.3 ± 2.3 milliunits/mg (n = 6), a 30-fold difference between species; and (b) 5-OPase activity in humans presented a significant individual variation; in the peripheral mononuclear cells, 5-OPase activity ranged from 5–25 milliunits/mg (5-fold interindividual variation), and in the normal colon mucosa, it ranged from 3.6–12.8 milliunits/mg (3-fold interindividual variation).

RESULTS

Enzymatic Characterization of 5-OPase in Rat and Human Tissues. The 5-OPase activity in rat tissues presented a very specific distribution pattern (Table 1). Rat kidney had the highest activity [187.6 ± 23.5 milliunits/mg (n = 6)], followed by liver [26.1 ± 2.9 milliunits/mg (n = 5)], lung [3.4 ± 1.6 milliunits/mg (n = 5)], and bone marrow [2.1 milliunits/mg (n = 2)]. 5-OPase activity in MatB tumors [3.4 ± 2.6 milliunits/mg (n = 5)] was relatively low. As a comparison, the mean values of 5-OPase activity in normal tissues from patients were: liver, 19.6 milliunits/mg (two specimens, 30 and 9.11 milliunits/mg, respectively); peripheral blood mononuclear cells, 11.3 ± 5.8 milliunits/mg; breast tissue, 11.2 ± 7.2 milliunits/mg (n = 3); colon, 9.6 ± 2.7 milliunits/mg (n = 12); kidney, 8.3 ± 2.3 milliunits/mg (n = 6); uterine smooth muscle, 4.4 milliunits/mg (n = 1); and lung, 3.3 ± 2.4 milliunits/mg (n = 4). Therefore, humans do not have a tissue distribution pattern of 5-OPase activity similar to that of rat. It is noted that: (a) whereas rat kidney had strikingly high 5-OPase activity [up to 187.6 ± 23.5 milliunits/mg (n = 6)], human kidney only had an average 5-OPase activity of 8.3 ± 2.3 milliunits/mg (n = 6), a 30-fold difference between species; and (b) 5-OPase activity in humans presented a significant individual variation; in the peripheral mononuclear cells, 5-OPase activity ranged from 5–25 milliunits/mg (5-fold interindividual variation), and in the normal colon mucosa, it ranged from 3.6–12.8 milliunits/mg (3-fold interindividual variation).
To investigate whether 5-OPase activity changes along with tumorigenesis in humans, paired specimens, i.e., tumor and the adjacent normal tissue from the same patient, were assayed for 5-OPase activity. Among the eight colonic tumor and normal tissue pairs studied, 5-OPase activity was higher in normal colonic mucosa than in the matched colon carcinoma in three pairs, lower in three pairs, and equal in two pairs. Therefore, no consistent pattern of 5-OPase activity in normal colonic mucosa versus colonic carcinoma could be documented. 5-OPase activity level in these colonic carcinomas did not seem to be related to the grade of tumor differentiation, nor to the age or gender of the patient. Similar results were found in four pairs of lung samples but were less confident due to the limited number of samples (Fig. 3).

**Immunohistocharacterization of 5-OPase.** Because the amount of tissue required for the activity assay of 5-OPase is rarely available, and the heterogeneity of cells within tissue samples may complicate interpretation of the 5-OPase activity, we characterized 5-OPase in greater detail using Western blot and immunohistochemistry methods. Paired normal and tumor tissues from patients with stomach, lung, and colon cancer were examined, because our interest in the patient. Similar results were found in four pairs of lung samples, 5-OPase activity was higher in normal tissue pairs studied, 5-OPase activity was higher in normal colonic mucosa than in the matched colon carcinoma in three pairs, lower in three pairs, and equal in two pairs. Therefore, no consistent pattern of 5-OPase activity in normal colonic mucosa versus colonic carcinoma could be documented. 5-OPase activity level in these colonic carcinomas did not seem to be related to the grade of tumor differentiation, nor to the age or gender of the patient. Similar results were found in four pairs of lung samples but were less confident due to the limited number of samples (Fig. 3).

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Four pairs of normal stomach tissue and gastric tumor from patients were examined by Western blot. 5-OPase levels in the normal stomachs were shown to be 4.1-, 1.8-, 2.0-, and 10.1-fold (cases 1-4, respectively) higher than in the corresponding tumors (Fig. 4A). By immunohistochemistry, the four normal stomach specimens showed a consistent pattern in which 5-OPase immunopositivity was localized strongly to the cytoplasm of normal parietal cells and localized weakly to glands in the normal gastric antral mucosa. In the areas where intestinal metaplasia was observed, 5-OPase was also localized to the cytoplasm of enterocytes in the superficial mucosa, being mostly found in cytoplasm of columnar absorptive cells. However, immunohistochemistry showed a rather different pattern of 5-OPase immunoreactivity among the four stomach tumor specimens, ranging from completely negative in the first case to moderately positive in some other cases. This might be due to the pathological differences among these tumors. The tumor in case 1 was classified as a mucinous adenocarcinoma, in which tumor cells were dispersed in abundant mucus, and virtually no 5-OPase immunopositivity was found by immunohistochemistry (Fig. 4B). Tumors from the three other cases (cases 2-4) were solid and histologically classified as intestinal-type gastric adenocarcinomas. In these tumors, tumor cell cytoplasm was variably but convincingly immunoreactive for 5-OPase. On average, tumors from cases 2 and 3 showed moderate immunopositivity, whereas case 4 showed weak and only focally moderate immunoreactivity (data not shown). These results from immunohistochemistry correlated with those obtained from Western blot experiments (Fig. 4A), in which we observed a greater difference in 5-OPase level between normal and tumor tissues in case 4 than in cases 2 and 3. Case 1 provided enough tissue to perform 5-OPase activity assay and Western blotting as well as immunohistochemistry. The activity assay revealed a 4.9-fold higher 5-OPase activity in the normal stomach tissue (6.4 milliunits/mg) compared to that in the tumor (1.3 milliunits/mg). Western blot showed a 4.1-fold higher 5-OPase band density in the normal stomach tissue than in the tumor (Fig. 4A).

Immunohistochemistry detected strong 5-OPase positivity in the parietal cells in the normal tissue, but the tumor was 5-OPase negative (Fig. 4B). These three approaches showed essentially the same result and serve to validate the observation as well as the utility of these different assay techniques.

In three paired human colon specimens, Western blot detected no obvious or consistent difference of 5-OPase level between the normal and neoplastic tissues, which is compatible with the enzyme activity measured in eight other pairs of colon samples. By immunohistochemistry, 5-OPase was localized to colonic enterocyte cytoplasm in the superficial mucosa in two of three cases. Tumor cell cytoplasm was immunopositive, varying from nonreactive to focally moderately reactive (data not shown).

The localization of 5-OPase in human breast, liver, and kidney was also investigated by immunohistochemistry. In the breast, 5-OPase was shown to be localized to cells of the terminal ducts and ductules in normal breasts and also to the cytoplasm of invasive ductal cancer cells (data not shown). In normal liver, 5-OPase was detected in both hepatocytes and bile duct epithelium. Hepatocyte cytoplasm was diffusely, mildly to moderately immunoreactive, and no regional pattern was ob-
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Band density ratio (N vs. T)

- Lung: 13.0:1, 47.8:1, 1:1, 4.1:1, 10.3:1, 4.0:1
- Stomach: 4.1:1, 1.8:1, 2.0:1, 10.1:1

B

Fig. 4 A. Western blot of 5-OPase in seven pairs of normal and tumor lung tissues and in four pairs of normal and tumor stomach tissues. The density of bands was measured using NIH Image 1.60 software. N, normal tissue; T, tumor tissue. B. Immunohistochemical localization of 5-OPase in normal human stomach (b, ×130) and stomach cancer (c, ×260) in the tissues of stomach case 1, as in A. Negative control (a, ×130) was reacted with preimmune serum. 5-OPase is localized to cytoplasm of parietal cells (arrow) in benign body mucosa (b) and is weakly reactive in this mucin-producing gastric adenocarcinoma (c).

DISCUSSION

This is the first report on 5-OPase activity in rat tissues by measuring the conversion rate of L-[14C]-5-oxoproline to L-[14C]-glutamate, which directly reflects the activity of 5-OPase. Our results (Table 1) reflected a pattern of 5-OPase distribution in rat tissues consistent with a previous study (2) that showed that the relative rates of oxidation of L-[14C]-5-oxoproline to 14CO₂ in rat kidney (9.7 milliunits/mg dry tissue weight), spleen (2.7 milliunits/mg dry tissue weight), liver (1.6 milliunits/mg dry tissue weight), intestine (0.26 milliunit/mg dry tissue weight), heart muscle (0.25 milliunit/mg dry tissue weight), and brain (0.14 milliunit/mg dry tissue weight) are 100:29:16:2.7:2.7:1.4.

Previous studies of 5-OPase focused on its catalytic mechanism and its activity in some animal tissues (2, 17). This is also therefore the first report on 5-OPase activity and cellular distribution in human tissues. The present study shows that (a) compared to rats, human 5-OPase activity is generally lower, and, unlike rats, whose 5-OPase is mainly localized to the kidney and liver (2), human 5-OPase shows no obvious preferential organ distribution pattern; and (b) in humans, there is significant individual variation of 5-OPase, as indicated by the
enzymatic assay of 5-OPase in the normal colon (n = 12; 3-fold individual variation) and peripheral mononuclear cells (n = 13; 5-fold individual variation). Despite the limited number of samples, this individual variation in 5-OPase activity may suggest heterogeneity of cells within the tissue samples, different exposures to inducers of 5-OPase, or a genetically based polymorphism yet to be defined.

We have raised a polyclonal antibody against rat kidney 5-OPase and proved that it has high affinity and specificity and cross-reacts with human 5-OPase in various tissues. Using this antibody, we showed a similar distribution pattern of 5-OPase in the normal kidney of both rats and humans: 5-OPase is localized to the renal tubular cells. This result is consistent with those of a previous study that measured the 5-OPase activity in microdissected renal segments and showed that rat 5-OPase activity was highest in the renal tubules and low in the glomeruli (21). Because the physiological role of renal tubules, especially the proximal tubules, is to reabsorb the glomerular filtrate including massive amount of amino acids (22), the γ-glutamyl cycle is an active inward amino acid transporter system (22), and rat kidney is the richest source for 5-OPase in rats (2), our results on 5-OPase distribution in the kidney are consistent with these physiological aspects and suggest that 5-OPase has an important function in the γ-glutamyl cycle at this site. This deduction is further substantiated by our observation of 5-OPase distribution in colon tissue: 5-OPase is strongly, if not exclusively, localized to the enterocytes in the superficial mucosa, whose function is to reabsorb several types of substances, including amino acids (23).

5-OPase was also clearly localized to cells involved in secretory functions such as the parietal cells that secrete hydrochloric acid in gastric mucosa and the cells of the ducts, ductules, and lobules that secrete milk in breasts as well as the cells of bile ducts, which also have secretory functions (23). The functional relevance of this distribution might be that the absorptive function of cells may be up-regulated when its secretion is elevated as compared to other cells.

The focus of this report is to compare the levels of 5-OPase in tumors and their adjacent normal tissues in humans. Because of the implication of chemical carcinogens in their genesis and the role of GSH in their detoxification mechanisms, we focused on lung, colon, and stomach cancers. Two of four pairs of lung specimens studied by enzymatic assay, six of seven separate pairs by Western blot, and four of six separate pairs by immunohistochemistry showed lower levels of 5-OPase in tumors as compared to the paired normal tissues. Four pairs of stomach specimens were examined by both Western blot and immunohistochemistry, all of which showed a higher 5-OPase level in the normal tissues than in the paired tumors. These results are consistent with the previous finding that in culture, tumor cells usually have lower 5-OPase levels than normal cells (14). In all cases studied, the heterogeneity of tumor cells is significant, and their 5-OPase positivity ranged from completely negative in some cases to moderately positive in some other cases. Because the stomach and lung contain several cell types, the cellular origins of gastric and lung neoplasms have yet to be conclusively elucidated. The absence of 5-OPase in foveolar cells (that typically secrete mucus) and in mucinous adenocarcinoma may thus be significant. The presence of 5-OPase in intestinal metaplastic columnar absorptive cells and in intestinal-type cancers may also reflect derivation of this histological subset from this cell type. In comparison with paired stomach and lung tissues, paired colon specimens showed a rather inconsistent relationship between the 5-OPase levels in normal and tumor tissues.

The mechanism by which 5-OPase expression is decreased in particular tumors is of interest to future studies. We have previously demonstrated decreased expression of another GSH-related enzyme, GSH S-transferase α and μ, in human breast tumor tissues compared to adjacent normal tissues (24). The significance and mechanism of this phenomenon will be more easily studied once the 5-OPase gene and its regulatory elements are isolated and characterized.

Our findings in this report also have potential implication for cancer chemotherapy. OTZ, a 5-oxo-L-proline analogue and a substrate for 5-OPase, has been shown to differentially increase the GSH level in normal tissues while decreasing it in tumor tissues, thus accentuating the relative susceptibility of tumor cells to the anticancer drug melphalan (13, 14). An ideal model in which to explore this selectivity of OTZ should have significantly lower 5-OPase levels in the tumor than in the paired normal tissue. Although the physiological significance of differences in 5-OPase level in normal versus tumor tissue is not yet certain in both stomach and lung cancers, a lower 5-OPase level was found in the tumor tissues as compared to normal tissues, suggesting that a decrease in this particular enzyme occurs at some point in the process of tumorigenesis. Because OTZ is metabolically activated by 5-OPase, these data suggest a potential use of OTZ as a biochemical modulator in the treatment of these cancers. Although we have not assessed human bone marrow 5-OPase activity, previous reports demonstrated that OTZ is activated in this tissue and may result in a protective effect (25).

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5-OPase in Cancer Chemotherapy


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