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Immunohistochemical Localization of Glutathione S-Transferase α and π in Human Esophageal Squamous Epithelium, Barrett's Epithelium and Carcinoma

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High tissue levels of glutathione S-transferases (GSTs), a family of detoxification enzymes, are inversely correlated with cancer risk in the human gastrointestinal tract. Patients with Barrett's esophagus, wherein squamous epithelium is replaced by columnar epithelium, have an increased risk for developing esophageal adenocarcinoma. Biochemical analyses revealed that Barrett's epithelium contains lower levels of GST enzyme activity as well as some GST isoforms, as compared with squamous epithelium. So far, little information on the immunohistochemical distribution of the GST α and π isoforms in normal squamous epithelium, in Barrett's metaplastic epithelium or in adeno- and squamous cell carcinomas of the esophagus is available. Tissues were fixed in formalin and embedded in paraffin. Three 4 μm thick sections were used for hematoxylin and eosin staining and for immunostaining with antibodies against GST α and π. GST α and π were seen in normal squamous epithelium (0% and 75%, respectively), Barrett's epithelium (75% and 100%), adenocarcinoma (25% and 100%) and squamous cell carcinoma (27% and 91%). Staining was mainly cytoplasmic, though some nuclear staining with the GST π antibody was apparent. The varying expression of GST α and π in normal and (pre)neoplastic esophagus may have consequences for the treatment of these diseases and may contribute to an understanding of the development of these esophageal disorders.

Key words: Barrett's esophagus — Glutathione S-transferases — Esophageal cancer — Immunohistochemistry

Barrett's esophagus is a pathological condition in which stratified squamous epithelium has been replaced by columnar epithelium in the lower part of the esophagus.[1–4] Three types of epithelium have been identified: gastric-cardiac or junctional type, gastric-fundic type and intestinal-type epithelium. The latter is a distinctive type of specialized epithelium with the villous surface lined by columnar epithelium and goblet cells.[2] Barrett's esophagus is considered to be a premalignant condition: patients suffering from this metaplastic condition have a 30- to 125-fold increased risk of developing adenocarcinoma of the esophagus as compared to the general population.[5–9] In the last 20 years, the incidence of adenocarcinoma of the esophagus has increased 5- to 6-fold in Western Europe and the United States.[8, 10]

Glutathione S-transferases (GSTs; EC 2.5.1.18) are a supergene family of enzymes involved in the detoxification of toxins and carcinogens. GSTs catalyze the binding of a large variety of electrophiles to the sulfhydryl group of glutathione (GSH), generally resulting in less harmful and more water-soluble molecules which can then be excreted via bile or urine.[11, 12] Since most chemical carcinogens are electrophiles, GSTs play a critical role in the detoxification of xenobiotics and carcinogens.[12] Human cytoplasmic GSTs have been grouped into four main classes; α, μ, π and θ.[12–14] The isoenzymes of these classes have a different but partly overlapping substrate specificity and a tissue-specific expression; for instance, GST α predominates in the adult liver and small intestine, whereas GST π is mainly found in erythrocytes, placenta and fetal liver.[12, 15]

GSTs are present in most epithelial tissues of the human gastrointestinal tract as assessed by biochemical methods.[16–19] GST enzyme activity and the incidence of gastrointestinal malignancies are inversely correlated; high GST activity is present in organs with low tumor incidence and low activity is found at sites with high tumor incidence.[19] As compared with adjacent squamous epithelium, Barrett's epithelium showed a significantly lower GST enzyme activity, and GST μ, GST π and GSH levels, whereas the minor GST α and GST θ levels in Barrett's epithelium were higher.[19, 20] No significant differences in mean GST activity and isoenzyme levels were found between normal esophagus and carcinoma.[21]

Biochemical data on GST expression in esophageal tissues merely reflect overall levels, and do not give information on cellular localization or heterogeneity within the

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specimens. We therefore studied the immunohistochemical localization of GSTs α and π in normal squamous esophageal epithelium, Barrett's metaplastic epithelium, adenocarcinoma and squamous cell carcinoma.

MATERIALS AND METHODS

Barrett's as well as squamous epithelium from 12 patients with Barrett's esophagus, and tumor samples from 11 patients with squamous cell carcinoma and 20 patients with adenocarcinoma are included in this study. Patient and tissue characteristics are shown in Table I. Specimens were fixed in formalin immediately after excision and embedded in paraffin.

From each specimen one tissue section (4 μm) was used for standard hematoxylin and eosin staining and two for immunohistochemical determination of GST α and π as described previously.[22] Incubation with primary antibodies was performed overnight (4°C) with either mouse monoclonal antibody against human GST α (1A11)[23] or rabbit polyclonal antibody against human GST P1-1 (Biotrin International, Dublin, Ireland), diluted 1:5000 and 1:1750, respectively. Class α antibody reacts with GST A1-1, A1-2 and A2-2. Peroxidase-conjugated rabbit anti-mouse (Dakopatts, Glostrup, Denmark; diluted 1:100) and swine anti-rabbit immunoglobulins (Dakopatts; diluted 1:40) served as second and third antibodies. Antibodies were diluted in phosphate-buffered saline (PBS) containing 4% bovine serum albumin (Boehringer Mannheim, Mannheim, Germany) and 0.1% Triton X-100 (BDH Chemicals Ltd., Poole, England). Staining was performed using 0.1% 3,3′-diaminobenzidine (Sigma Chemical Co., St. Louis, MO) in PBS containing 0.01% hydrogen peroxide (3 min) and enhanced in 0.5% $CuSO_4$ (5 min). The sections were counterstained with hematoxylin and examined by light microscopy.

Liver and colon tissues were used as positive controls for GST α and π, respectively. Omission of primary antibody and incubation with preimmune serum served as negative controls.

RESULTS

GST α immunoreactivity was absent in squamous epithelium, whereas 75% of Barrett's specimens showed positive staining. GST π was present in 75% and 100% of normal squamous and Barrett's epithelium, respectively. The results of scoring the staining intensity are summarized in Table II. The staining intensities for GST α and π were higher in Barrett's epithelium compared to those in adjacent normal squamous epithelium. Fig. 1 shows representative examples of immunostaining for GST α and π in squamous epithelium, Barrett's tissue, esophageal adeno-

Table I. Characteristics of Patients

	Barrett's esophagus	Adenocarcinoma	Squamous cell carcinoma
Number	12	20	11
Age (years)			
Mean±SE	59±5	68±3	59±4
Range	26–78	43–88	37–75
Sex			
Male	4	15	7
Female	8	5	4
Dysplasia			
No	2	NA	NA
Mild	3	NA	NA
Moderate	6	NA	NA
Severe	1	NA	NA
Differentiation			
Well	NA	3	1
Moderate	NA	7	7
Poor	NA	10	3

NA, not applicable.

Table II. Intensity of Staining for GST Class α and π in Normal Esophageal Tissue, Barrett's Epithelium and Esophageal Carcinoma

Intensity of staining		Normal squamous epithelium (*n*=12)	Barrett's epithelium (*n*=12)	Adenocarcinoma (*n*=20)	Squamous cell carcinoma (*n*=11)
GST α	Negative	12 (100%)	3 (25%)	15 (75%)	8 (73%)
	Weak	0 (0%)	3 (25%)	3 (15%)	1 (9%)
	Moderate	0 (0%)	3 (25%)	1 (5%)	2 (18%)
	Strong	0 (0%)	3 (25%)	1 (5%)	0 (0%)
GST π	Negative	3 (25%)	0 (0%)	0 (0%)	1 (9%)
	Weak	0 (0%)	0 (0%)	1 (5%)	1 (9%)
	Moderate	3 (25%)	2 (17%)	10 (50%)	2 (18%)
	Strong	6 (50%)	10 (83%)	9 (45%)	7 (64%)

Immunostaining for GST α and π was performed as described in "Materials and Methods."

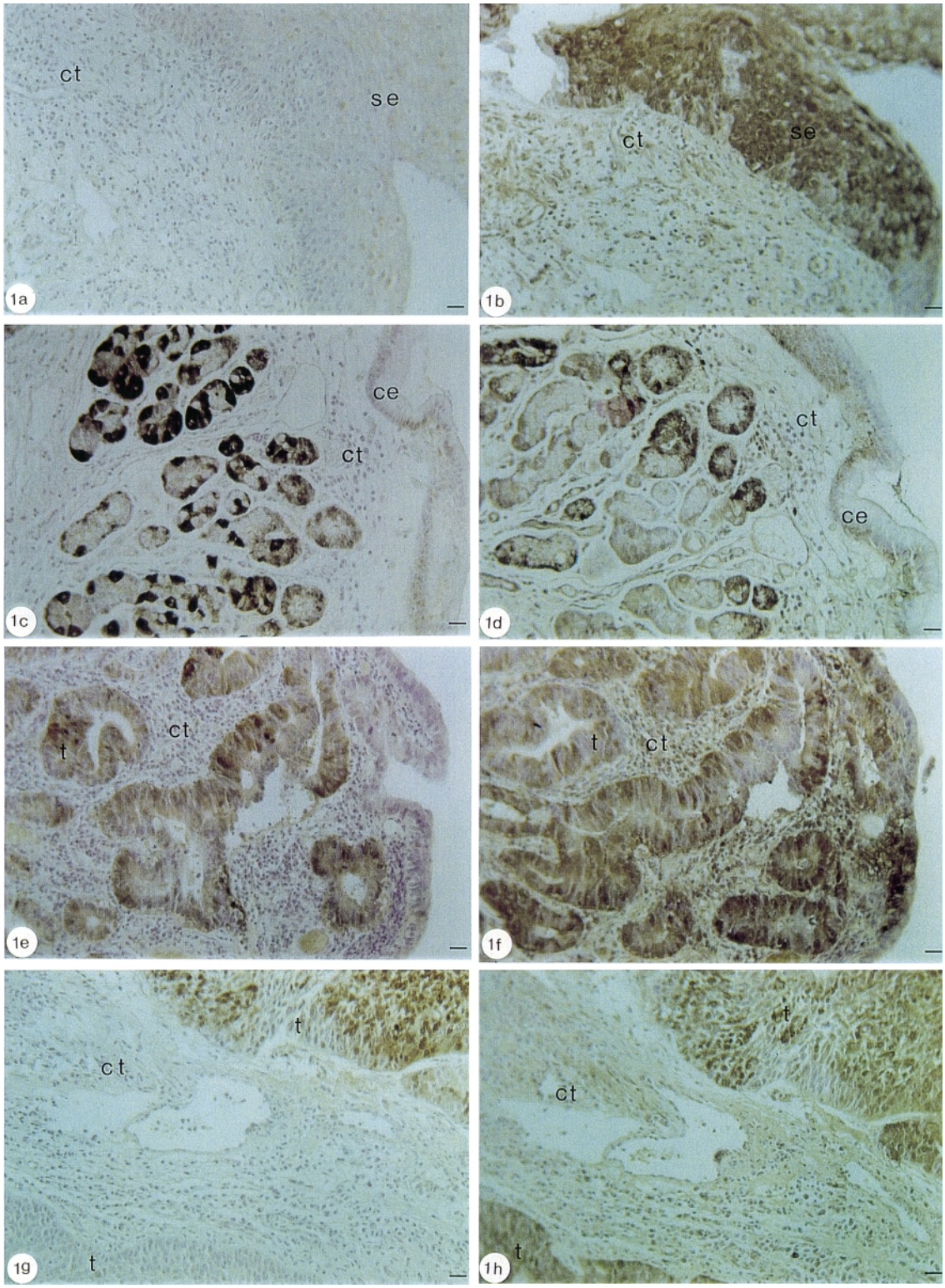

Fig. 1. Localization of GST α (a, c, e, g) and GST π (b, d, f, h) in squamous epithelium (a, b), Barrett's metaplasia (c, d), adenocarcinoma (e, f) and squamous cell carcinoma (g, h) of the esophagus. ce, columnar epithelium; ct, connective tissue; se, squamous epithelium; t, tumor. Bars indicate 4 μm.

carcinoma and squamous cell carcinoma. GST α immunoreactivity in Barrett's epithelium was seen only focally and it was localized in both superficial and glandular columnar epithelial cells. GST π immunoreactivity was much stronger and showed a similar staining pattern. In addition to cytoplasmic staining, some nuclear staining was found with the GST π antibody. In squamous epithelium, GST π immunoreactivity was seen throughout the epithelium, but mainly in the basal two-thirds.

In adenocarcinoma only 25% of the specimens showed focal staining for GST α, whereas GST π was present in the cytoplasm and some nuclei of all specimens. In squamous cell carcinoma 27% of specimens showed a weak to moderate cytoplasmic localization of GST α, whereas GST π staining was seen in tumor cells in almost all specimens, which were mainly differentiated carcinomas.

No immunoreactivity was observed in sections incubated with PBS, normal rabbit or mouse sera.

DISCUSSION

GSTs form part of an inherent protective mechanism against toxic or carcinogenic chemical compounds. These compounds may be taken up from the environment (food, drugs, medication, air pollution, etc.) or may be formed intracellularly by normal physiological processes. The immunohistochemical analysis of GST α and π in tissues from patients with Barrett's epithelium, adenocarcinoma and squamous cell carcinoma may provide insight into the variation in expression of these isoenzymes in different tissues and cell types and ultimately may lead to a better understanding of their role in protecting cells from damage by cytotoxic or carcinogenic agents. No data on cell-specific expression of GSTs in Barrett's epithelium have been published; only biochemical data on tissue homogenates are available.[19, 20] In addition to the distribution of GST α and π isoenzymes in Barrett's esophagus, we also studied esophageal adenocarcinoma, squamous cell carcinoma, and adjacent squamous epithelium.

Barrett's columnar epithelium contained GST α immunoreactivity, whereas squamous epithelium did not. This matches well with the GST α contents of these tissues as found by immunoblot analyses.[19, 20] All Barrett's epithelial samples contained a moderate to strong GST π immunoreactivity, whereas 25% of the squamous epithelial samples did not. Staining with the GST π antibody in squamous epithelium generally was weaker as compared to that in Barrett's epithelium. Earlier, however, we showed by immunoblot analyses that Barrett's epithelium contained less GST π than squamous epithelium.[19, 20] Such a discrepancy between biochemical and immunohistochemical data on GST expression has been noted previously after analysis of gastric tissue specimens.[24]

Patients with Barrett's esophagus have an increased risk of developing esophageal adenocarcinoma.[5–9] One of the possible reasons for this might be an impaired detoxification capacity due to reduced GST activity and GSH levels.[19, 20] Development of esophageal squamous cell carcinomas has been associated with increased exposure to exogenous factors, such as smoking and drinking of alcohol.[25–27] Many carcinogens are detoxified by conjugation with GSH, catalyzed by GSTs. The etiology of squamous cell carcinoma is complex and multifactorial and there is little evidence for progression to cancer via intermediate premalignant lesions such as dysplasia, which hampers early detection.[28, 29]

GST α immunoreactivity was absent in three out of five adeno- or squamous cell carcinomas, whereas GST π was present in most cases. Morphologically normal esophageal mucosa expressed approximately the same GST π levels as did carcinoma cells, suggesting that GST π overexpression is not associated with esophageal malignancy. Similar results were seen on immunoblots: GST isoenzyme patterns did not differ between normal esophageal mucosa and tumor.[21] Other studies showed increased GST π mRNA and protein levels in esophageal squamous cell carcinoma compared with corresponding normal squamous epithelium.[30–32] Niitsu *et al.*[15] documented increased serum GST π levels in 53% of patients with esophageal cancer. Altered expression of GST π has previously been associated with the progression to cancer after exposure to carcinogens.[28] GST π may be a marker of increased carcinogen exposure in esophageal mucosa rather than of malignant transformation. To address this issue, squamous esophageal epithelium from healthy people should be studied, though this would be difficult to do.

Nakajima *et al.*[33] showed that GST α was expressed on immunoblots in 32% of the patients with squamous cell carcinoma (total $n=41$), whereas GST π was found in all samples. This correlates well with our immunohistochemical results. We found no apparent differences in staining intensity for GST α between adenocarcinoma and squamous cell carcinoma. This contrasts with a study by Murray *et al.*,[34] who demonstrated that the expression of GST α was significantly less in adenocarcinoma (32% of 25 patients) than in squamous cell carcinoma (76% of 25 patients). This may be due to the limited number of subjects in our study. With respect to GST π, Murray *et al.*[34] were unable to demonstrate differences in immunoreactivity between adenocarcinoma and squamous cell carcinoma, which is in accordance with our results.

The nuclear staining of GST π as found here, has been previously reported in health and disease of the esophagus,[35] stomach[22] and uterine cervix.[36] The significance of this nuclear staining is still unclear, but besides a function in prevention of DNA damage, it has been postulated that nuclear GSTs are involved in RNA processing.[37]

In conclusion, we have shown large variations in GST α and π immunoreactivity in normal squamous epithelium and Barrett's epithelium, as well as in adeno- and squamous cell carcinoma. The results demonstrate the tissue specificity and marked inter- and intra-individual variation of important drug-metabolizing enzymes.

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