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# Induction of Glutathione s-Transferase-π in Barrett's Metaplasia and Barrett's Adenocarcinoma Cell Lines

**Keith R. Compton, Mark B. Orringer, and David G. Beer***

*Department of Surgery, Section of Thoracic Surgery, University of Michigan Medical School, Ann Arbor, Michigan*

Barrett's metaplasia consists of columnar epithelium that replaces the normal esophageal mucosa in patients with chronic gastroesophageal reflux. Because intestinal-type Barrett's metaplasia is the major risk factor for adenocarcinoma development, understanding the mechanisms that predispose the esophageal mucosa to malignant degeneration is clinically important. Glutathione s-transferase (GST)-π belongs to a class of protective enzymes whose activity has been shown to be much lower in Barrett's metaplasia than in the normal esophagus, where this form of GST is predominant. In the studies described here, using immunocytochemical analysis, we observed higher levels of cytoplasmic GST-π protein in normal esophageal mucosa than in Barrett's metaplasia. Using northern blot analysis, we also observed lower *GST-π* mRNA levels in Barrett's metaplasia than in normal esophagus or adenocarcinomas from the same patients. Using as model systems three Barrett's adenocarcinoma cell lines and short-term organ culture of freshly resected normal esophagus and Barrett's metaplasia, dose-dependent induction of *GST-π* mRNA was observed by using butylated hydroxyanisole and dexamethasone. *GST-π* mRNA in Barrett's metaplasia was induced up to 2.5-fold with 60 μM butylated hydroxyanisole and nearly fivefold with 320 nM dexamethasone after 24 h. These studies demonstrate the ability to induce protective GST-π in Barrett's metaplasia and may suggest a mechanism for future chemoprevention studies in patients with this type of epithelium, which is at high risk for malignant degeneration. *Mol. Carcinog. 24:128–136, 1999.* 

Key words: chemoprevention; esophageal cancer; butylated hydroxyanisole; dexamethasone; chronic gastroesophageal reflux

## INTRODUCTION

Esophageal cancer is one of the 10 most common cancers worldwide. Squamous cell carcinoma and adenocarcinoma are the two main types. The incidence of esophageal adenocarcinoma is increasing [1], and the major risk factor for the development of esophageal adenocarcinoma is the presence of intestinal-type Barrett's metaplasia, a specialized condition in which the distal squamous esophageal mucosa is replaced by columnar epithelium resembling that of the normal intestine [2]. Development of Barrett's metaplasia is a consequence of chronic gastroesophageal reflux disease [3]; approximately 12% of patients who suffer from gastroesophageal reflux disease develop Barrett's metaplasia [4]. Patients with Barrett's metaplasia have a 30- to 40-fold higher incidence of esophageal adenocarcinoma than the general population [2,5], and they are often enrolled in surveillance programs to facilitate early cancer detection. The presence of Barrett's metaplasia is considered precancerous, and progression from low- to high-grade dysplasia and finally to esophageal adenocarcinoma is often observed [2,6]. Similar genetic alterations are detected in Barrett's metaplasia and associated adenocarcinomas [7–9], indicating that this type of epithelium may be susceptible to critical DNA damage that leads to cancer development.

The mechanisms underlying the dramatic increase in the incidence of Barrett's adenocarcinoma are unknown but may be related to specific properties of premalignant Barrett's metaplasia. The intestinal-type Barrett's epithelium expresses cytochrome P450 isoenzymes, which can metabolically activate chemical carcinogens [10] (Hughes et al., manuscript submitted for publication). The specific cells expressing cytochrome P450 isoenzymes are present in the glandular, proliferative regions of the epithelium. The dual ability to metabolically activate carcinogens and retain proliferative capabilities may lead to the accumulation of DNA alterations. Relative to the normal esophageal mucosa, Barrett's epithelium also has reduced levels of glutathione s-transferases (GSTs) [11], which are enzymes that play an important role in detoxifying reactive electrophilic compounds created by carcinogen metabolism within the cell [12]. GSTs are phase II enzymes that detoxify activated carcinogens by enzymatic conjugation to glutathione, thus rendering reactive compounds inac-

*Correspondence to: B560 MSRB II, Box 0686, Department of Surgery, Section of Thoracic Surgery, University of Michigan Medical School, Ann Arbor, MI 48109.

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Abbreviations: GST, glutathione s-transferase; BHA, butylated hydroxyanisole; Dex, dexamethasone; RT, reverse transcription; PCR, polymerase chain reaction; NSAID, nonsteroidal anti-inflammatory drug.

tive, targeting them for excretion out of the cell, and reducing the potential for genetic damage [13].

There are four GST isoforms in humans (α, μ, θ and π) based on nucleotide homology and biochemical and immunologic criteria [14]. GST activity is higher in the normal esophagus than in the gastric epithelium [15], with the predominant form being the GST-π isoenzyme. GST-π enzyme activity and content are significantly lower in Barrett's metaplasia, stomach, and duodenum than in normal esophageal mucosa [11]. Examination of tumor incidence in tissues at several sites of the gastrointestinal tract and their respective GST activities indicated that where GST activity is lowest, tumor incidence is highest [11].

Elevating the relatively low levels of GST in Barrett's metaplasia, especially the levels of GST-π, may be one way to help prevent further neoplastic development in this tissue. GSTs within different tissues have been induced in rodent models [16–22]. Two compounds that have shown reasonable ability to induce GST in murine tissues are the food additive and antioxidant butylated hydroxyanisole (BHA) and the corticosteroid dexamethasone (Dex) [12,13,16,23]. The ability of either BHA or Dex to induce GSTs in human Barrett's metaplasia has not been previously examined. The aim of this study was to examine the localization of the GST-π protein in Barrett's metaplasia and to determine whether BHA or Dex could induce *GST-π* mRNA in human Barrett's metaplasia, normal esophageal mucosa, and esophageal Barrett's adenocarcinoma cell lines.

## MATERIALS AND METHODS

### Human Tissues

After informed consent was obtained, normal esophagus and Barrett's metaplasia tissue samples were taken from patients undergoing esophagectomy for cancer at the University of Michigan Medical Center. Only patients who had not received prior chemotherapy or radiotherapy were included. Freshly resected tissue samples were transported to the laboratory in Dulbecco's modified Eagle's medium (Life Technologies, Gaithersburg, MD) on ice. A portion of each normal esophagus and Barrett's metaplasia samples was used immediately for short-term organ culture, an additional portion was snap frozen in liquid nitrogen and stored at –70°C for later RNA isolation, and the remaining portion was placed in 10% buffered formalin for 24 h at 4°C before processing for paraffin embedding.

### Immunohistochemical Analysis

The cellular localization and staining pattern of the GST-π protein were examined by immunocytochemical analysis of 5-μm formalin-fixed, paraffin-embedded sections of Barrett's metaplasia and normal esophageal tissue. A polyclonal rabbit anti–human GST-π antibody (Novocastra Laboratories, Newcastle upon Tyne, UK) was used at a 1:100 dilution in phosphate-buffered saline containing 1% bovine serum albumin. For the control reactions, horse serum blocking solution was used instead of the GST-π antibody. The avidin-biotin-peroxidase complex method of antibody detection with 3,3′-diaminobenzidine as the chromogen was used according to the manufacturer's recommendations (Vecta-Stain-Elite; Vector Laboratories, Burlingame, CA). The slides were lightly counterstained with hematoxylin, and the cellular localization and staining pattern of the GST-π protein was assessed by two independent observers.

### Short-Term Organ Culture

Barrett's metaplasia and normal esophageal tissues were cultured according to methods used for the short-term organ culture of human and rodent small intestine [24,25]. Immediately upon resection, a portion of Barrett's metaplasia or normal esophageal mucosa was cut into approximately five or six 1-$mm^3$ pieces and placed mucosa-side up onto stainless steel mesh within the inner wells of 60-mm polystyrene organ culture dishes (Becton Dickinson and Co., Franklin Lakes, NJ). The tissues were partially submerged in 2.3 mL of growth medium containing BHA, Dex, or the appropriate amount of ethanol (vehicle control). The medium consisted of eight parts of CMRL 1066 (Life Technologies) and two parts of Ham's F-12 nutrient mixture (Sigma Chemical Co., St. Louis, MO) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Norcross, GA), 25 mM glucose, 2 mM L-glutamine, 5 μM ferrous sulfate, 0.1 μM sodium selenite, 100 μM 5-aminovulinic acid, 3 μM zinc sulfate, 145 nM menadione sodium bisulfite, 100 U/mL penicillin G, and 100 μg/mL streptomycin sulfate. Sterile 0.9% NaCl solution was added to the outer wells of the culture dishes, which were then placed in a sealable tissue-culture incubation chamber (Billups-Rothenburg Inc., Del Mar, CA). The dishes were gassed for 20 min with 50% $O_2$, 45% $N_2$, and 5% $CO_2$ at 1700 mL/min, providing a minimum of 14 volume exchanges of gas. The chamber with the dishes was then sealed, and the entire unit was placed in an orbital shaking incubator at 37°C and 40 rpm to provide gentle mixing and washing of medium over the mucosa. The tissues were maintained under these conditions for 24 h and were then collected, snap-frozen, and stored at –70°C until processed for RNA extraction.

### Tissue Culture of Human Cell Lines

Human esophagus (Het-1A) and Barrett's adenocarcinoma (Seg-1, Flo-1, and Bic-1) cell lines were used. The Seg-1, Flo-1, and Bic-1 cell lines were derived from human esophageal Barrett's adenocarcinomas. Het-1A is a normal human esophageal mucosa cell line immortalized by using simian virus

40 and has been previously described [26]. The cells were grown in 10-cm tissue culture dishes with Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 100 U/mL penicillin G, and 100 μg/mL streptomycin sulfate at 37°C in a 5% $CO_2$/95% air humidified atmosphere. For the induction experiments, 60 μM BHA, 320 nM Dex, or the appropriate amount of 100% ethanol (vehicle control) was added to fresh medium, and the cultures were incubated for 24 h under the conditions described above. After gentle washing in phosphate-buffered saline, the cells were collected by scraping with a rubber policeman and pelleted at 1200 rpm for 2 min in 1.5-mL centrifuge tubes. The medium was then removed, the cells were snap-frozen in liquid nitrogen, and the cell pellets were stored at –70°C until processed for RNA isolation.

### Total RNA Isolation

Frozen cell pellets and tissue pieces were homogenized with 1 mL of TRIzol Reagent (Life Technologies) by using the procedure recommended by the manufacturer. The RNA pellets were then dissolved in 1 mM EDTA, pH 7.0, in diethyl pyrocarbonate–treated water. The RNA concentrations were determined by spectrophotometry at 260 nm, and 10-μg aliquots were used for northern blot analysis.

### Northern Blot Analysis

Northern blot analysis was performed as previously described [27]. Total cellular RNA (10 μg) was separated in 1.2% gels containing 2.2 M formaldehyde and then vacuum-transferred to nylon membranes (Gene Screen Plus; New England Nuclear, Wilmington, DE). The membranes were prehybridized in 5× standard saline phosphate with EDTA, 5× Denhardt's solution, 50% formamide, 3% sodium dodecyl sulfate, 5% dextran sulfate, 5 μg/mL heat-denatured salmon-sperm DNA, and 3 μg/mL yeast tRNA for 1 h at 48°C. Probes were labeled with [$^{32}$P]dCTP by the random-primer labeling method (Prime-It II; Stratagene, La Jolla, CA), and unincorporated [$^{32}$P]dCTP was removed by Sephadex G-50 size-exclusion chromatography. The membranes were hybridized with $1.5 \times 10^6$ cpm/mL heat-denatured $^{32}$P-labeled probe for 16 h in a 48°C shaking water bath. Then, the membranes were washed according to the manufacturer's recommendations, and autoradiograms were prepared (Hyperfilm-MP; Amersham Corp., Arlington Heights, IL). Loading and transfer of RNA was normalized by using a probe for 28S rRNA as previously described [28]. Autoradiographic signals were quantified by scanning laser densitometry (Molecular Dynamics, Sunnyvale, CA).

### cDNA Probe Synthesis

A *GST-π* probe was designed by using the nucleotide sequence of the human *GST-π* cDNA [15]. Oligonucleotide primers were designed to amplify the *GST-π* cDNA by reverse transcription (RT)–polymerase chain reaction (PCR) of total RNA from normal esophageal squamous epithelium. Total RNA (2 μg) was reverse transcribed into cDNA by using random hexamer primers (125 pmol/reaction; Promega Corp., Madison, WI) in a solution containing 50 mM Tris HCl, pH 8.3; 50 mM KCl; 8 mM $MgCl_2$; 10 mM dithiothreitol; and 1 mM each dATP, dCTP, dGTP, and dTTP in a total volume of 25 μL. The reaction mixture was heated to 65°C for 5 min and cooled to 41°C before avian myeloblastosis virus reverse transcriptase was added (7 U/reaction; Boehringer Mannheim, Indianapolis, IN). The reaction was allowed to proceed for 1 h at 41°C. PCR of *GST-π* was accomplished by adding 16 μL of the above RT mixture to a 100-mL reaction containing 10 mM Tris HCL, pH 8.3, 50 mM KCL; 1.5 mM $MgCl_2$; 2 mM dithiothreitol; 0.5 mM dNTPs; 12.5 pmol of each GST primer, and 0.5 mL of Taq polymerase (Promega Corp.). The sequence of the sense primer was 5′ CTC AAA GCC TCC TGC CTA TAC 3′; the sequence of the antisense primer was 5′ GGT AGT TAC CGT TGC CCT TTG 3′. Amplification was done with an initial cycle of 94°C for 1 min and then 35 cycles of 94°C for 1 min, 57°C for 2 min, and 72°C for 3 min. Amplification produced the expected 498-bp *GST-π* PCR product identified by using ethidium bromide–stained 2% agarose gels, and then the band was cut out of the gel, purified, and labeled for use as a probe for northern blot analysis. Confirmation of the *GST-π* PCR product was performed by automated DNA sequencing at the University of Michigan.

## RESULTS

### Localization of GST-π Protein

Immunohistochemical analyses were performed on normal esophageal and Barrett's metaplasia tissue sections. The localization and staining pattern of GST-π protein within the Barrett's metaplasia mucosa differed from that of the normal esophageal mucosa. Eight of 11 of the normal esophageal tissue specimens examined displayed predominantly cytoplasmic GST-π protein expression (Figure 1A and B and Table 1). A gradient of cytoplasmic GST-π protein was observed within the stratified squamous epithelium, with higher concentrations nearer the luminal surface and lesser amounts nearer the proliferative basal areas. A combination of nuclear and cytoplasmic GST-π staining was present in only three of 11 specimens. In these cases, nuclear staining appeared in the cells closest to the basal zone of the squamous epithelium. In contrast, 11 of 15 Barrett's metaplasia tissue specimens demonstrated staining primarily in the basal glandular regions of the mucosa (Figure 1C and Table 1). More than half (seven of 11) of the basal stained specimens displayed strong nuclear staining of the GST-π protein; the other cases showed equal cytoplasmic and nuclear staining. The

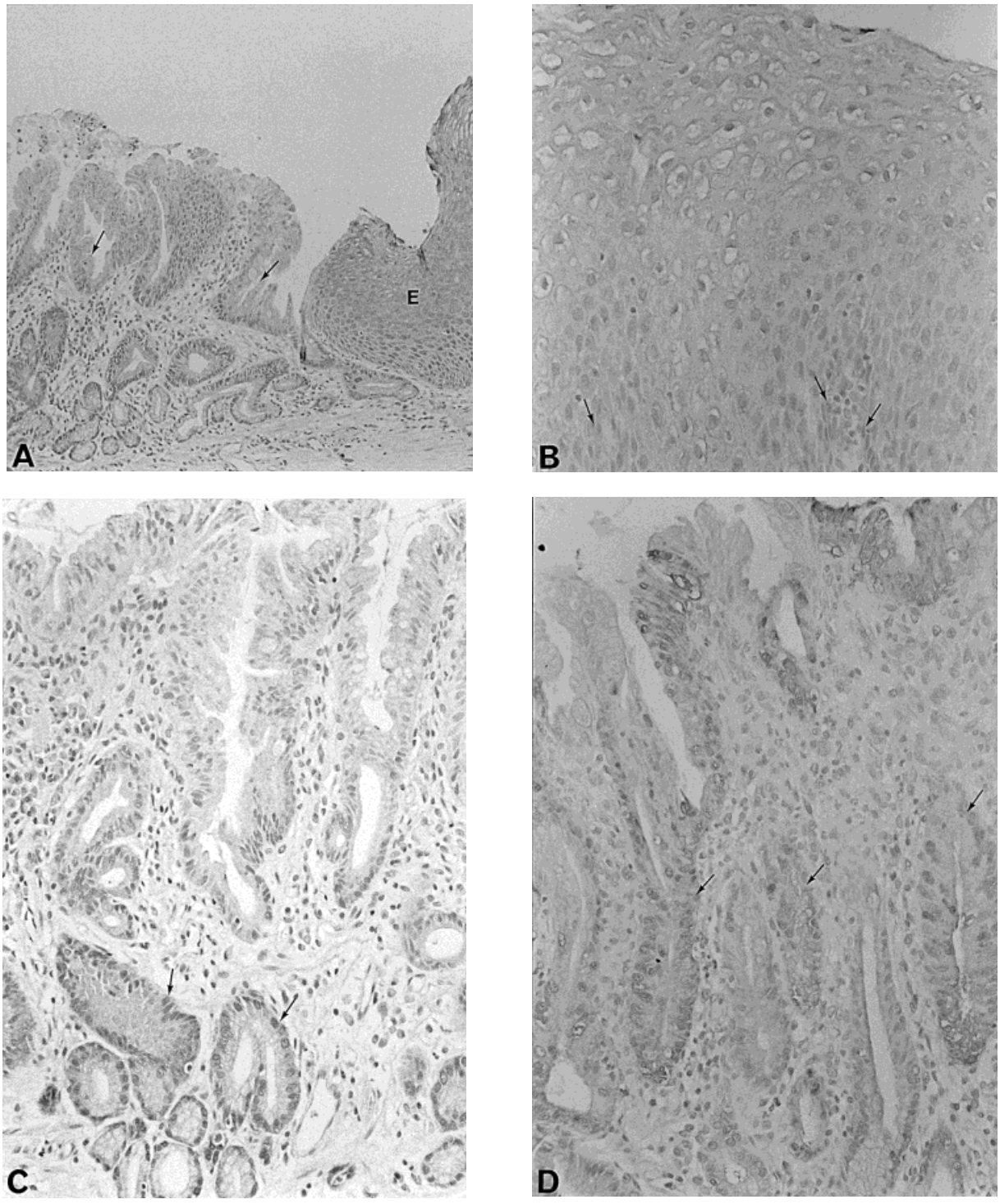


Figure 1. GST-π protein expression in normal esophageal tissue, non-dysplastic Barrett's metaplasia, and dysplastic Barrett's mucosa. (A) Area of normal esophageal tissue (E) adjacent to intestinal-type Barrett's metaplasia mucosa. The abundance of cytoplasmic GST-π protein expression in normal esophageal tissue is contrasted with the lower levels of nuclear GST-π staining in the Barrett's metaplasia mucosa (arrows). (B) Higher magnification of esophageal epithelium showing predominately cytoplasmic GST-π protein expression that is greatest nearer the luminal surface and less near the basal proliferative zone (arrows). (C) Predominant GST-π nuclear staining detected in the nuclei of most basal glandular regions (arrows) of non-dysplastic Barrett's metaplasia. (D) Increased cytoplasmic GST-π staining in areas of dysplasia (arrows) within the Barrett's mucosa but not in the non-dysplastic areas. Original magnifications: panel A, 100×; panels B–D, 200×.

Table 1. Localization of GST-π in Esophageal Tissues

| Tissue | No. of specimens examined | Location within tissue | | |
|---|---|---|---|---|
| | | Basal region | Upper region | Both regions |
| Normal esophagus | 11 | 0 (0%) | 9 (82%) | 2 (18%) |
| Barrett's metaplasia* | 15 | 11 (73%) | 0 (0%) | 4 (27%) |

*Includes only non-dysplastic Barrett's metaplasia.

basal glandular regions of Barrett's metaplasia are areas of active cell proliferation [29]. In contrast to the levels in the non-dysplastic Barrett's metaplasia, the levels of GST-π staining increased dramatically in dysplastic Barrett's mucosa (Figure 1D) and appeared to be both cytoplasmic and nuclear. These results indicate a different pattern of GST-π localization and staining in normal esophageal tissue and Barrett's metaplasia tissues.

### *GST-π* mRNA Expression in Normal Esophagus, Barrett's Metaplasia, and Adenocarcinoma Tissue

*GST-π* mRNA levels were examined in the normal esophageal mucosa, Barrett's metaplasia, and adenocarcinoma tissues of five patients by using northern blot analysis (Figure 2A). A *GST-π* mRNA transcript of 0.6 kb was detected in all samples; however, in four of the five patients, lower levels of *GST-π* mRNA were present in Barrett's metaplasia specimens than in the normal esophagus. A modest increase in *GST-π* mRNA above, the levels seen in corresponding Barrett's metaplasia specimens was present in the adenocarcinomas in four of five patients. The ratio of *GST-π* mRNA to 28S rRNA for each sample is shown in Figure 2B. The *GST-π* mRNA levels in the Barrett's metaplasia samples were approximately 24% less than the levels in the normal esophagus from the same patients. The exception was patient PA-560, who expressed more *GST-π* mRNA in the Barrett's metaplasia tissue; however, this patient's Barrett's mucosa contained areas of dysplastic mucosa, which may explain the higher levels of *GST-π* mRNA.

### Induction of *GST-π* mRNA in Barrett's Adenocarcinoma and Normal Esophageal Cell Lines

To determine whether lower levels of protective GST-π may increase the risk for adenocarcinoma development in patients with Barrett's metaplasia, we examined the inducibility of *GST-π* in Barrett's metaplasia. Cell lines of Barrett's metaplasia are not available, so *GST-π* mRNA inducibility was examined by northern blot analysis using three Barrett's adenocarcinoma cell lines (Seg-1, Bic-1, and Flo-1) and the immortalized esophageal squamous epithelium cell line Het-1A (Figure 3A). Two of the three Barrett's adenocarcinoma cell lines (Seg-1 and Flo-1) demonstrated increases in *GST-π* mRNA when treated with 30 μM BHA for 24 h (Table 2). The third cell line, Bic-1, showed only modest induction over the basal levels in three of four experiments with this compound. The Bic-1 cells also had the highest basal levels of *GST-π* mRNA of the three cell lines. Seg-1 and Flo-1 had lower basal levels of *GST-π* mRNA and showed greater induction with BHA. These results may suggest that when physiological levels of *GST-π* mRNA are lower, induction will be greater. Consistent with this, *GST-π* mRNA was induced in Het-1A cells with 30 μM BHA at somewhat lower levels than those observed with Seg-1 and Flo-1 cells, and Het-1A cells express relatively higher levels of *GST-π* mRNA. Induction with Dex was also examined in Seg-1 and Het-1A cell lines. Both cell lines demonstrated low-level induction of *GST-π* mRNA with Dex (Table 3).

The effects of increasing incubation times and BHA concentration on *GST-π* mRNA induction were investigated by using Seg-1 and the normal squamous cell line Het-1A, both of which showed the highest levels of induction by BHA in our previous experiments (Figure 3B). At 24 h of incubation, Seg-1 displayed a maximum level of *GST-π* mRNA induction at 60 mM BHA, whereas Het-1A achieved its maximum induction at 30 mM BHA. Interestingly, the Seg-1 cell line maintained near-maximal induction of *GST-π* mRNA, whereas in the Het-1A cell line, the mRNA levels began to taper off after 48 h.

### *GST-π* mRNA Induction in Normal Esophageal and Barrett's Metaplasia

Although induction of *GST-π* mRNA was observed in the esophageal and Barrett's adenocarcinoma cell lines, the induction of this mRNA also was examined in freshly resected normal esophagus and Barrett's mucosa by using organ culture (Figure 4). After treatment of normal esophageal tissue with 60 μM BHA for 24 h, an average induction of 72% above control levels was observed in three separate trials (Table 2). A 64% induction of *GST-π* mRNA was observed with Barrett's metaplasia tissue after treatment with BHA (Table 2). An even greater level of *GST-π* mRNA induction of 123% was seen in Barrett's metaplasia specimens treated with Dex (Table 3). These results clearly demonstrate the ability of both compounds to induce *GST-π* mRNA in Barrett's metaplastic tissue. The localization of GST-π protein after induction was examined in seven patients' Barrett's metaplasia and normal esophagus samples that had been organ-cultured in the presence of either 80 nM or 320 nM Dex for 24 h. Although these analyses are not quantitative, an increased but relatively similar

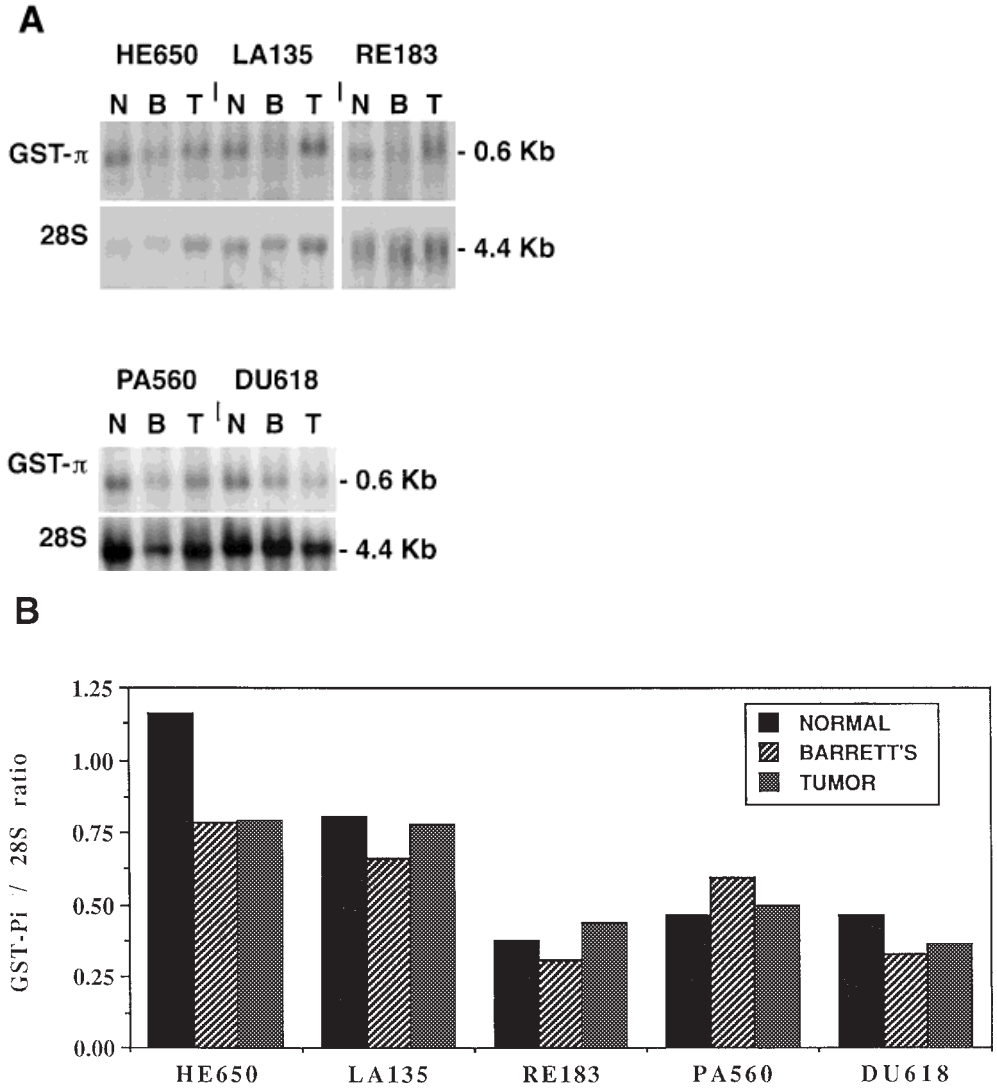


Figure 2. (A) Northern blot analysis demonstrating *GST-π* mRNA (0.6 kb) in normal esophageal tissue (N), Barrett's metaplasia (B), and adenocarcinoma tissue (T) from five patients. The 28S rRNA signals (4.4 kb) are shown in the lower panels. (B) The ratio of *GST-π* mRNA to 28S rRNA for the tissue samples from these five patients.

distribution of the GST-π protein in uncultured and control samples was observed in the Barrett's mucosa (data not shown). A predominance of nuclear staining in the glandular regions was observed in the Barrett's mucosa, whereas normal esophagus showed increased cytoplasmic staining in the cellular layers above the basal zone of the squamous mucosa.

## DISCUSSION

Barrett's metaplasia is a major risk factor for esophageal adenocarcinoma development. The mechanisms underlying increased cancer development in this tissue are not known. However, lower levels of certain protective enzymes such as GSTs may increase the potential for this mucosa to accumulate genetic alterations. Of the GSTs, GST-π is the most prevalent isoform in the esophagus, and studies by Peters et al. [11] demonstrated that Barrett's metaplasia has significantly less GST-π enzyme activity and content than normal esophageal tissue. *GST-π* mRNA content has also been previously reported to be proportional to GST-π enzyme activity [30]. We observed *GST-π* mRNA levels in Barrett's metaplasia an average of 24% lower than those of the normal esophageal specimens from the same patients, which confirms the results of Peters et al. [11]. These results suggest that this difference is controlled at the level of *GST-π* transcription or mRNA abundance. The difference in GST-π expression in these tissues does not appear to be due simply to the presence of squamous versus columnar cells, because small-intestine cells have high GST levels, whereas gastric cells have low levels,

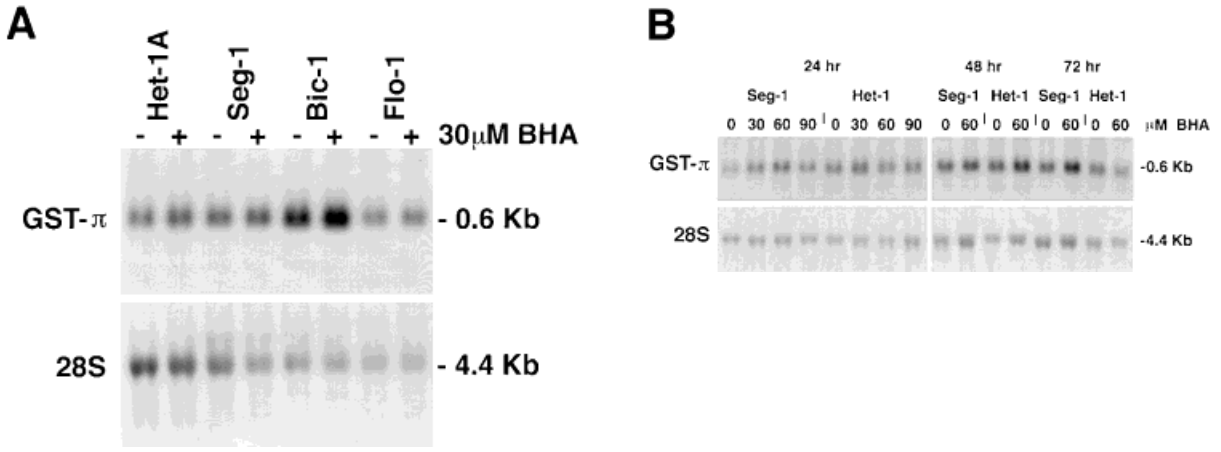


Figure 3. Northern blot analyses demonstrating induction of *GST-π* mRNA in three Barrett's adenocarcinoma cell lines (Seg-1, Bic-1, and Flo-1) and the immortalized esophageal cell line Het-1A. (A) The cell lines were incubated for 24 h with (+) and without (–) 30 μM BHA. (B) The cells were treated with increasing concentrations of BHA for the incubation times shown. The 28S rRNA signals are shown in the lower panels.

yet both are columnar [11]. We also observed a difference in the localization of the GST-π protein between the normal esophagus and Barrett's metaplasia. The GST-π protein was primarily cytoplasmic in the stratified squamous esophageal epithelium, whereas the GST-π protein was mostly localized to the nuclei of the cells within the basal, glandular regions of Barrett's mucosa. The significance of this difference is uncertain, but a relationship between nuclear GST-π protein expression and cell proliferation may be involved. However, the GST-π protein is known to exist in both nuclear and cytoplasmic forms [31]. Lower overall levels of GST-π in the actively proliferating cells of the glandular regions of Barrett's mucosa may make these cells prone to the accumulation of genetic damage caused by activated carcinogens. We have found that intestinal-type Barrett's metaplasia expresses a number of cytochrome P450s known to activate chemical carcinogens, and the cells expressing these enzymes are in the proliferating cellular regions of the metaplastic epithelium (Hughes et al., manuscript submitted for publication). Interestingly, an increase in GST-π protein was observed in some dysplastic Barrett's mucosa (Figure 1D), and an increase in *GST-π* mRNA was detected in some adenocarcinomas (Figure 2). Increased GST is known to occur in some tumors and preneoplastic lesions [12,15,17] and may be associated with selective chemoresistance in some cancers.

We observed induction of *GST-π* mRNA in both the squamous esophageal and Barrett's metaplasia tissues after treatment with BHA and Dex. Treatment of Barrett's tissue with 60 μM BHA in four separate experiments produced increases in *GST-π* mRNA as high as 2.55-fold, and even greater increases in *GST-π* mRNA were observed with 320 nM Dex. In addition, two of three Barrett's adenocarcinoma cell lines displayed increases in *GST-π* mRNA after treatment with BHA (Seg-1, 60%; Flo-1, 23%). Seg-1 also showed a modest induction in *GST-π* mRNA with Dex. Because these Barrett's adenocarcinoma cell lines demonstrated inducibilities nearly similar to those of resected Barrett's mucosa, they may be an adequate model for the analysis of additional agents capable of inducing these important protective proteins. At present, there are no Barrett's metaplasia cell lines.

We examined the induction of *GST-π* by BHA and

Table 2. BHA Induction of *GST-π* mRNA in Esophageal Cell Lines and Barrett's metaplasia*

| | % Induction | | | | | |
|---|---|---|---|---|---|---|
| Cell line or tissue | Exp. 1 | Exp. 2 | Exp. 3 | Exp. 4 | Average induction* | |
| Het-1A | 24% | 23% | 7% | NA | 18% | (3/3) |
| Seg-1 | 80% | 67% | 34% | NA | 60% | (3/3) |
| Bic-1 | 31% | 0% | 0% | 0% | 8% | (1/4) |
| Flo-1 | 6% | 36% | 0% | 50% | 23% | (3/4) |
| Esophageal mucosa | 42% | 13% | 161% | NA | 72% | (3/3) |
| Barrett's metaplasia | 47% | 155% | 52% | 0% | 64% | (3/4) |

*Exp., experiment; NA, not analyzed. The numbers in parenthesis are the number of experiments in which results for a particular cell line were obtained/the number of experiments performed.

Table 3. Dex Induction of *GST-π* mRNA in Esophageal Cell Lines and Barett's Metaplasia*

| Cell line or tissue | Exp. 1 | Exp. 2 | Average induction |
|---|---|---|---|
| Het-1A | 3% | 0% | 3% (1/2) |
| Seg-1 | 0% | 15% | 15% (1/2) |
| Barrett's metaplasia | 59% | 186% | 123% (2/2) |

*Exp., experiment. The numbers in parentheses are the number of experiments in which results for a particular cell line were obtained/the number of experiments performed.

Dex because of their known ability to increase GSTs in rodent models [12,13,16,23], and we demonstrated here that these compounds also can induce *GST-π* mRNA in Barrett's metaplasia tissue in short-term organ culture. The inducibility of GST in Barrett's mucosa may be important for future chemopreventative agents in humans. Examples include the natural anticarcinogens coumarin, flavone, phenethylisothiocyanate and oltipraz [20–22] as well as nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin and ibuprofen [19]. These compounds were shown to increase total GST enzyme activity, to increase specific GSTs in the rodent esophagus, or to have both effects. Recent analyses indicated that the risk of human esophageal cancers, including adenocarcinomas, is reduced in users of NSAIDs [32]. Although the exact mechanism by which NSAIDs reduce esophageal cancer risk is unknown, one mechanism may be by induction of protective GST enzymes, as has been demonstrated in rodents.

In summary, *GST-π* mRNA levels were reduced in Barrett's metaplasia compared with the normal esophageal mucosa, consistent with the lower GST-π enzyme activity and content reported in Barrett's metaplasia [11]. A difference in GST-π protein localization between Barrett's metaplasia and normal esophagus was also observed. Most importantly, we have shown, using short-term organ culture, the ability of BHA and Dex to induce GST-π in Barrett's metaplasia. Further studies are necessary to determine whether increasing protective GST-π may help reduce adenocarcinoma development in patients with Barrett's metaplasia.

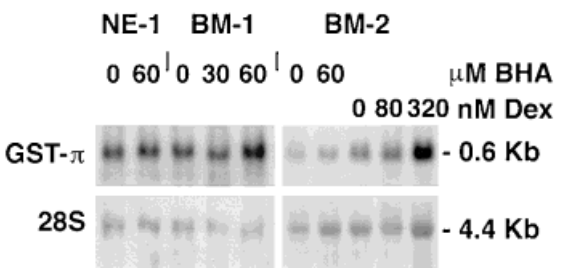


Figure 4. Northern blot analysis of *GST-π* mRNA induction by BHA and Dex in organ-cultured normal esophagus (NE-1) and Barrett's mucosa (BM-1 and BM-2). The 28S rRNA signals are shown in the lower panel.

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