Immunohistochemical Expression of $\pi$-Class Glutathione S-Transferase Is Down-Regulated in Adenocarcinoma of the Prostate

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BACKGROUND. Glutathione S-transferase is often up-regulated in neoplastic tissues. A single previous study found a loss of expression associated with carcinogenesis of the prostate.

METHODS. To extend these results, the authors performed immunohistochemical staining for the $\pi$-class of glutathione S-transferase (GST $\pi$) on 74 archival sequential prostate specimens. The antibody used was derived from rabbits immunized against purified human GST $\pi$. Paraffin blocks containing both benign tissue and adenocarcinoma were studied.

RESULTS. Heterogeneous expression of GST $\pi$ in benign acini was found in 96% of cases, but GST $\pi$ was not expressed in 95% of invasive adenocarcinomas of the prostate, nor was it expressed in any of the foci of high grade prostatic intraepithelial neoplasia. Basal cells of benign acini showed strong, diffuse staining for GST $\pi$, whereas the secretory luminal epithelium expressed GST $\pi$ weakly and focally.

CONCLUSIONS. This study confirms the down-regulation of GST $\pi$ in adenocarcinoma of the prostate and shows that the loss of GST $\pi$ expression is a phenotype associated with malignant transformation. Cancer 1997;79:1595–9.

KEYWORDS: glutathione S-transferase, prostate carcinoma, prostatic intraepithelial neoplasia, immunohistochemistry.

Glutathione S-transferases (GSTs) are a family of enzymes involved in the metabolism of xenobiotic compounds.¹ There are at least four distinct classes of human GSTs ($\alpha$, $\mu$, $\pi$ and $\theta$) each of which conjugate reduced glutathione to a variety of electrophilic and hydrophobic compounds, changing them into more soluble and more easily excreted compounds.² The $\pi$-class of GSTs (GST $\pi$) is found in a number of malignant tumors including colorectal carcinoma, stomach carcinoma, breast carcinoma, nonsmall cell lung malignancies, endometrial carcinoma, cervical carcinoma, renal cell carcinoma, seminoma, teratoma, fibrosarcoma, and chondrosarcoma³–23 as well as in a wide range of normal human tissues.⁴,¹⁰,²⁴–²⁷ In general, these studies have shown that GST $\pi$ is expressed at higher levels in neoplastic tissue than in normal tissue. In patients with oral and gastrointestinal cancers, GST $\pi$ levels are so increased that elevated plasma levels of GST $\pi$ have been found, and in some patients these levels have normalized after resection of their tumors.²⁸

There are several lines of evidence that implicate GST $\pi$ as a possible mediator of the drug resistance of tumors to chemotherapeutic agents. GST $\pi$ has been shown to conjugate several chemotherapeutic agents in vitro. It is also associated with drug-resistant tissue culture
cell lines, and has been implicated in the development of drug resistance in several tumor types.\textsuperscript{23} However, it has been shown that GST\textsubscript{π} expression is increased in a large proportion of primary tumors in patients who have not received chemotherapy, and hence have not experienced selective pressure for resistant tumor clones.\textsuperscript{15,19,21,22} In addition, there are several animal models of neoplasia in which increased GST\textsubscript{π} expression is a marker for premalignant and malignant changes in tissues during tumor induction.\textsuperscript{17,29,30} These findings suggest that, at least in some situations, increased GST\textsubscript{π} expression is a phenotype associated with malignant transformation.

In contrast to the general trend of an increase in GST\textsubscript{π} expression in malignant transformation, a recent study showed loss of immunohistochemical expression of GST\textsubscript{π} in association with prostatic carcinogenesis.\textsuperscript{31} The loss of GST\textsubscript{π} expression was associated with hypermethylation of regulatory sequences near the gene locus. In this article, the authors report the results of an immunohistochemical survey that confirmed the loss of GST\textsubscript{π} expression in a series of prostate carcinomas, and provide an additional description of the pattern of GST\textsubscript{π} expression in benign prostate tissue.

**MATERIALS AND METHODS**

Surgical pathology specimens containing prostate carcinoma and/or benign prostate tissue were selected from the files of the National Cancer Institute. Hematoxylin and eosin stained sections from these cases were evaluated for the presence of adenocarcinoma of the prostate. The grade of primary intraprostatic tumors were assigned using Gleason’s grading scheme.\textsuperscript{32} Metastatic tumors were graded as well, moderately, or poorly differentiated tumors based on the presence of glandular architecture and cytologic atypia.

Immunohistochemistry was performed on 5-μm paraffin embedded tissue sections on poly-L-lysine-coated glass slides. The sections were pretreated with 0.3% hydrogen peroxide in methanol for 30 minutes. After an initial 20-minute incubation with 2% normal goat serum, the specimens were then incubated overnight at 4 °C with affinity-purified anti-GST\textsubscript{π} polyclonal rabbit antibody at a dilution of 1/5000. The purified antibodies were obtained from rabbits immunized with purified human GST\textsubscript{π} as previously described.\textsuperscript{31} After a 30-minute incubation with a secondary biotinylated goat anti-rabbit antibody, the sections were then incubated with an avidin-biotin-horseradish peroxidase complex (Vector Laboratories, Burlingame, CA) (reagents prepared according to the manufacturer’s specifications) for 45 minutes. 3,3-diaminobenzidine tetrahydrochloride was used as the peroxidase substrate at a concentration of 0.05% in the presence of 0.2% hydrogen peroxide. The slides were counterstained with hematoxylin. All steps were performed at room temperature unless otherwise specified, and all reagents were diluted in Tris-buffered saline (0.5 M Tris [pH 7.5] and 0.9% NaCl). Between incubations the sections were washed in 3 serial Tris-buffered saline baths for 3-minute intervals.

**RESULTS**

Seventy-four cases were obtained from the surgical pathology files of the National Cancer Institute. The prostate specimens were comprised of 4 total prostatectomies, 2 surgical biopsies, 23 transurethral prostatectomies and 32 fine-needle biopsies. Forty-two of the 61 cases contained prostatic adenocarcinoma, with glandular architecture ranging from Gleason grade 2 to 5. Sections containing both infiltrating adenocarcinoma and benign glandular prostate tissue were especially sought. An additional 13 specimens of prostate carcinoma were from extraprostatic sites, either by direct extension of tumor from the prostate or by distant metastasis (five cases to bladder, three to regional lymph nodes, two to bone, and one case each to rectum, urethra, and skin). Of the 13 metastatic tumors, 3 were moderately differentiated and 10 were poorly differentiated. Nineteen cases of benign prostatic hypertrophy without carcinoma were also included.

Fifty-five specimens contained benign prostate glands. Fifty-three (96%) of these specimens exhibited staining of basal cells and 49 exhibited staining of luminal secretory epithelium. Although there was a large variability in the strength and percentage of staining of benign acini between specimens, only two specimens revealed a complete absence of staining of benign epithelium. Staining was predominantly diffusely cytoplasmic. No plasma membrane staining was observed. Thirty-three of these 55 cases (60%) also exhibited focal nuclear staining, primarily in the basal epithelial layer. In general, staining for GST\textsubscript{π} was stronger and more diffuse throughout the benign samples in the basal layer than in the luminal secretory layer (Fig. 1). In no instance was secretory epithelial layer staining present in the absence of basal layer staining. Secretory layer staining was qualitatively increased in the presence of chronic inflammation, corpora amylacea, and glandular atrophy. In a few specimens that exhibited squamous metaplasia, there was a marked staining of the metaplastic epithelium.

Of the 55 specimens in the current series that contained adenocarcinoma of the prostate 52 failed to stain for GST\textsubscript{π}. The majority of these cases contained
FIGURE 1. Strong glutathione S-transferase-π expression in the basal epithelium of a benign, hyperplastic prostatic acinus. Note the focal weaker staining in the luminal cytoplasm (magnification ×400).

FIGURE 2. Immunohistochemical staining of prostatic tissue for glutathione S-transferase-π showing the most commonly found pattern: infiltrating adenocarcinoma (center of field) shows no staining, contrasting with strong staining of three benign acini (hematoxylin counterstain, magnification ×400).

FIGURE 3. Immunohistochemical staining for glutathione S-transferase-π showing positive staining of adenocarcinoma of the prostate. This result was found in only 5% of cases, (hematoxylin counterstain, magnification ×200).

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adjacent and residual benign tissue that showed positive staining that served as an internal control (Fig. 2). Only 3 cases (5%) revealed tumor that stained for GSTπ (Fig. 3). Two biopsy specimens were moderately differentiated tumors (Gleason score 3 + 3 = 6/10), and one was poorly differentiated tumor metastatic to a lymph node. This tumor was identified as prostatic in origin by positive immunohistochemical staining for prostate specific antigen. Twelve specimens were found to contain foci of high grade prostatic intraepithelial neoplasia (PIN). In each instance the PIN component was negative, whereas residual benign elements showed positive staining for GSTπ.

Structures commonly encountered within the prostate that also exhibited staining for GSTπ were nerves, ganglia, uroepithelium, and seminal vesicles. Some stromal cells showed variable staining, which contrasted with the lack of expression in the malignant cells. At sites of metastases, positive staining was found in squamous epithelium, osteoclasts, and osteoblasts.

DISCUSSION

The results of the current study confirm that immunohistochemical staining for GSTπ is present in benign prostate acini, notably the basal cells as reported by Lee et al.31 The results show that malignant transformation is associated with a complete loss of GSTπ staining in 95% of the cases studied. This finding contrasts with other malignant neoplasms, in which increased GSTπ expression is the expected phenotype. Thus, prostate carcinoma joins hepatocellular carcinoma as an exception to this trend.6

These results confirm that GSTπ staining is stronger and more diffuse in the basal epithelial layer, and is only focally found in the luminal secretory epithelium. Prior studies have reported GSTπ in benign prostatic luminal secretory cells,33 and a predominant pattern in benign acinar basal cells.26,31 Although increased GSTπ expression in the luminal layer was associated with chronic inflammation, atrophy, and metaplasia, staining in this layer was also found in areas with none of these complicating factors.

Although the majority of GSTπ is cytosolic, nuclear staining was observed in a subset of positive cases. Nuclear staining for GSTπ has been reported in a number of previous studies in other tissues.4,14,23,26 but the
significance of this finding is not known. In the current study no correlation between nuclear staining and any other histologic parameter was noted. In a previous report, increased nuclear staining was associated with cervical intraepithelial neoplasia. The authors found no similar preinvasive nuclear GST $\pi$ in prostate specimens because all these current study cases of PIN were negative for GST $\pi$ staining.

GST $\pi$ has been investigated as both a biologic marker of carcinogenesis in animal models and as a marker of malignant transformation in human cancers. In the current series, clinical follow-up was not available for half of the cases examined, including the three cases that exhibited positive staining for GST $\pi$. However, because 2 of these 3 tumors were moderately differentiated (both had Gleason scores of $3 + 3 = 6/10$), and because 12 of 13 metastatic tumors were negative for GST $\pi$, it does not appear that positive GST $\pi$ expression correlates with aggressive tumor behavior.

The authors conclude that loss of GST $\pi$ expression is a common phenotype of carcinoma of the prostate. This phenotype is associated with early stages of neoplastic transformation of prostatic epithelium, because GST $\pi$ was not present in the high grade premalignant PIN. This study of 74 cases, taken with that of Lee et al., confirms the loss of GST $\pi$ expression in prostatic carcinogenesis.

REFERENCES


