The expression of GST isoenzymes in acinar adenocarcinoma, intraepithelial neoplasia, and benign prostate tissue: correlation of clinical parameters with GST isoenzymes

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Abstract: This study investigated the immunohistochemical staining characteristics of glutathione-S-transferase (GST) alpha, pi, mu, and theta in prostatic acinar adenocarcinoma (PCA), prostatic intraepithelial neoplasia (PIN), and benign prostatic tissues from 19 patients. Relationships between GST isoenzyme expression in benign, PIN, and PCA tissue were examined by the Wilcoxon signed-rank test and clinicopathological data were examined by the Spearman correlation rank test. When the benign, PIN, and PCA tissues from these cases were compared according to their staining intensity, GST alpha, pi, mu, and theta expressions in tumor cells were significantly lower than in benign epithelial cells (P < 0.05). The GST alpha class displayed the lowest level of expression in PIN and PCA. Expression of GST pi was lower in PCA tissue than in PIN and benign epithelial tissue (P < 0.05). We hypothesize that carcinogenesis in the prostate results from impaired cellular handling of mutagenic agents owing to reduction or loss of expression of multiple GST isoenzymes and other detoxifying and antimutagenesis agents. This study confirms the down-regulation of GST isoenzymes in PCA of the prostate and shows that the loss of GST isoenzyme expression is a phenotype associated with malignant transformation. There was no statistical relationship between GST isoenzyme expression and the clinicopathological data (age, Gleason score, and total serum prostate-specific antigen levels) (P > 0.05).

Key words: Prostate acinar adenocarcinoma, prostatic intraepithelial neoplasia, glutathione-S-transferase, immunohistochemistry

Introduction

Glutathione-S-transferases (GSTs) are a family of enzymes that detoxify intracellular xenobiotics, primarily by catalysis of the nucleophilic attack of reduced glutathione on electrophilic compounds (1,2). Since conjugation to glutathione renders these potential carcinogens chemically inactive and hence incapable of forming DNA adducts, it has been hypothesized that GSTs protect against neoplastic transformation (3). In humans, the cytosolic GSTs can be divided into 7 major classes: alpha (A), mu (M), pi (P), sigma (S), theta (T), omega (O), and zeta (Z). These isoenzymes are found in a wide range of normal tissues, and their expression has significant biological and clinical implications,
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including drug resistance and carcinogenesis (4,5). Although GSTP can be detected in normal prostatic epithelium, prostate cancer cells fail to express GSTP polypeptides. Loss of GSTP function also appears to be characteristic of prostatic intraepithelial neoplasia (PIN) lesions (6). The loss of GSTP expression was associated with hypermethylation of regulatory sequences near the gene locus. As GSTs are inducible enzymes, enzymatic activities were thought to be linked to genetic variations and involvement of several cytochrome P450 (CYP) and/or GST genetic polymorphisms, especially for tobacco-related cancers (7). The neoplasms express higher levels of GST than normal tissues. In this study, we assessed the cellular prevalence and distribution of GSTP, GSTM4, GSTA, and GSTT1 isoenzymes in matched human tissue samples of benign prostate, PIN, and acinar adenocarcinoma of the prostate (PCA).

Materials and methods

Patients

For immunohistochemical studies, radical prostatectomy tissues from 19 Keçiören Education and Research Hospital patients with PCA were used. For all patients, total serum prostate-specific antigen (PSA) levels, patient age, and Gleason score were included. Operation material was examined macroscopically by 2 pathologists in each case. Radical prostatectomy materials were fixed overnight, and serial tissue samples were taken at 2-mm intervals from apex to base. If the tumor could be grossly identified as a gray, firm area, 2 sections were taken: 1 from the tumor tissue, and 1 from the macroscopically normal tissue and tissue peripheral to the tumor tissue. If it was difficult to see macroscopically, PCA, PIN, and benign prostatic areas were detected microscopically and paraffin blocks were chosen.

Immunohistochemical staining

The tissues were fixed in 10% buffered formalin and embedded in paraffin blocks. Sections 4 µm thick were cut, and 1 section was stained with hematoxylin and eosin to observe the tissue morphology and tumor score. For immunohistochemistry, endogenous peroxidase activity was blocked by incubating the sections in 1% hydrogen peroxide (v/v) in methanol for 10 min at room temperature (RT). The sections were subsequently washed in distilled water for 5 min, and antigen retrieval was performed for 3 min using 0.01 M citrate buffer (pH 6.0) in a domestic pressure cooker. The sections were transferred in 0.05 M Tris-HCl (pH 7.6) containing 0.15 M sodium chloride (TBS). After washing in water, the sections were incubated at RT for 30 min with either normal swine serum (for anti-GSTA, anti-GSTP, and anti-GSTM4 at 1:20) or normal goat serum (for anti-GSTT1 at 1:20) diluted in TBS to block nonspecific binding. The sections were then covered with the primary antibodies and diluted at 1:100 for anti-GSTA and GSTP, 1:50 for anti-GSTM4, and 1:500 for anti-GSTT1 in 4 °C overnight (monoclonal antibody against hGSTT1 raised in mouse, Labas International Limited, Estonia; polyclonal antibodies against GSTA, GSTM4, and GSTP raised in rabbit, Lab Vision, Thermo Shandon, USA). After washing in TBS (15 min), sections were incubated at RT for 1 h with secondary antibody (swine-antirabbit Ig-biotinylated for anti-GSTA, GSTP, and GSTM4, or goat-antimouse Ig-biotinylated for anti-GSTT1) at a dilution of 1:100. This was followed by treatment with avidin–biotin peroxidase complex (Dakopatts, Denmark). Diaminobenzidine was used to visualize peroxidase activity in the tissues. Nuclei were lightly counterstained with hematoxylin, and then the sections were dehydrated and mounted. Both positive and negative controls were included in each run. Positive controls consisted of sections of normal human liver for GSTA, GSTM4, and GSTT1 and normal human small intestine for GSTP. TBS was used in place of the primary antibody for negative controls. Immunohistochemically stained sections were examined by light microscopy, and distribution, localization, and the intensity of immunostaining were recorded. Brown color in the cytoplasm and/ or nucleus of epithelial cells was evaluated as positive staining. Scoring was performed by 2 observers without knowledge of patient data. Scoring differences between observers were resolved by consensus. For each antibody, the intensity of staining was graded semiquantitatively as follows: score 0, negative
staining; score 1, weak staining; and score 2, strong staining.

Statistical analysis

For each isoenzyme, staining scores in benign tissue, intraepithelial neoplasia, and cancer epithelium were compared statistically. The relationship among expression of GST isoenzymes in benign tissue, intraepithelial neoplasia, and cancer epithelium was analyzed using the Wilcoxon signed-rank test. The correlation between expressions of GST isoenzymes and clinicopathological data was also examined using Spearman’s correlation rank tests. Data analysis was performed using SPSS 11.5 for Windows (SPSS Inc., Chicago, IL, USA). A P-value of less than 0.05 was considered statistically significant; however, for all possible multiple comparison tests, the Bonferroni adjustment was applied to control type I error.

Results

The median patient age was 66 years (minimum: 54, maximum: 77), the median value of patient Gleason scores was 6 (minimum: 5, maximum: 9), and the median value of total PSA was 9.9 (minimum: 0.68, maximum: 28.03).

From 19 patients, 20 samples of PCA, PIN, and benign tissue from the peripheral prostate were examined (Table). GSTA expressions were stronger in benign epithelium than in prostate tumor epithelium (Table). Of positive GSTA expression, 68% occurred in benign epithelium; 37% of PIN and 11% of tumors were considered to have GSTA expression (Table). Figure 1 shows that strong nuclear and cytoplasmic staining with GSTA was observed in the basal and secretory cells of PIN cells (Figure 1).

There were no statistically significant differences in GSTT1 and GSTM4 expression between benign and tumor epithelium (Table). Of benign epithelium, 53% was GSTM4-positive; however, 58% of PIN and 37% of tumors were considered to have GSTM4 expression (Figure 2). Similarly, both benign epithelium and PIN showed 79% GSTT1-positive expression; however, there was no statistically significant difference (Table; Figure 3).

There was a statistically significant difference in the GSTP expression between cancer and benign epithelium and PIN (P < 0.05). GSTP expression was seen at 32% in tumor epithelium (Figure 4), but 89% of the benign samples were considered to have GSTP expression. Thus, stronger GSTP expression was observed in benign epithelium than in tumor epithelium in human prostate cancers.

According to the Bonferroni correction method, there was no statistically significant difference in the staining levels of other GST isoenzymes among benign, PIN, and PCA tissues (P > 0.05).

The clinical and pathologic characteristics of the prostate cancers and the levels of GSTA, GSTP, GSTM4, and GSTT1 expression were correlated separately. There was a significant negative association between GSTT1 expression and patient age (r = −0.570; P = 0.011). There were no significant associations among patient age, Gleason score, total serum PSA, and GSTs in tumor tissue.

Discussion

In this study, we investigated the association of neoplastic transformation in prostate tissue and the protein expression of GSTP, GSTM4, GSTA, and the levels of GSTT1 and GSTM4 expression between benign and tumor epithelium. The results showed that GSTP expression was higher in benign epithelium compared to tumor epithelium, while GSTA expression was higher in benign epithelium compared to PIN and tumor epithelium. There was no statistically significant difference in the levels of GSTT1 and GSTM4 expression between benign and tumor epithelium. The correlation analysis revealed a significant negative association between GSTT1 expression and patient age, but no significant associations were found between patient age, Gleason score, total serum PSA, and GST expression in tumor tissue.
GSTT1 isoenzymes. GSTs are a family of isoenzymes that play an important role in protecting cells from cytotoxic and carcinogenic agents. GSTs are divided into 7 subclasses: GSTA, GSTM, GSTP, GSTT, GSTS, GSTO, and GSTZ (8). The alpha, mu, pi, and theta subclasses are mostly expressed in mammalian tissues, with GSTP (the major class of the GSTs) being the most abundant in the urinary, respiratory, and gastrointestinal systems and thus in the prostate (9,10). GSTP protein plays a role in toxin excretion and metabolism. Loss of GSTP function may render prostate cells vulnerable to genome damage mediated by environmental carcinogens that may be GSTP substrates, including oxidants, such as those arising from prostate inflammation, and electrophiles, which may be contributed via dietary exposure to heterocyclic aromatic amine carcinogens (6). To the best of our knowledge, this is the first study offering a comprehensive description of the 4 classes of GST isoenzymes in PCA tissues.

Our findings were consistent with those of Bostwick et al. (11); most of the benign prostate tissues expressed GSTP (P < 0.05). Contrary to these findings, there was increased expression of GSTP in other cancers such as breast, colon, stomach, pancreas, bladder, lung, and larynx (12–15). Induction of the enzymes in these cancers could be an adaptive response to stress or to chemical agents, or they could have an increased half-life of hypermethylated gene products.

In the current study, GSTP expression decreased with the neoplastic transformation of the prostate, as was also found by Moskaluk et al. (16), and 32% of the tumors stained with GSTP in our study. GSTP-positive tumors were moderately differentiated tumors in this study.

Our results for GSTA and GSTM4 are in agreement with those of previous studies (10,11,17,18), whereas Parsons et al. found that GSTA was nearly absent in normal epithelium and was stained strongly in prostatic inflammatory atrophy (3). GSTA was identified in both normal prostate tissue and prostate cancer by Murray et al. (17). In a study by Bostwick et al. (11), GSTA displayed the lowest level of expression, with diffuse weak staining in scattered cells (<1%) in high-grade PIN and PCA. Only 11% of carcinomas were positive for GSTA in this work. It was reported that GSTP expression may also be predictive of patient outcome. For example, in breast cancer, GSTP expression was inversely related to grade (19) and probably related to drug resistance; other studies found that GSTP expression was a prognostic indicator (20). We found no relationship between GSTP expression and Gleason grade in prostate cancer and no statistically significant difference between other GSTs and Gleason grade, age, and serum PSA. There are very few studies about the correlation of GSTs with these clinical parameters in the literature.

Figure 1. Nuclear and cytoplasmic positive staining with GSTA was observed in secretory cells (GSTA, 400×).

Figure 2. Nuclear and cytoplasmic positive staining with GSTM4 was observed in basal and secretory cells of PIN cells (GSTM4, 200×).
Through genetic research, the loss of expression of GSTP was found to be the most common genetic alteration in prostate cancer (21−24). In our work, GSTP expression in PIN was higher than the expression in carcinoma, and this was statistically significant (P < 0.05). The results of Srivastava et al. indicated that the null genotypes of GSTM1 and GSTT1 and the G allele of GSTP1 are associated with a higher risk of prostate cancer than controls (25).

Some genetic analytical studies could not address whether GST polymorphisms have an effect on the clinical behavior of prostate cancer or other clinicopathologic attributes. The metaanalysis cannot exclude the possibility that other polymorphisms in GST genes may still be useful to pursue. Moreover, these studies could not address gene−gene or gene−environmental interactions. Some other studies showed a significant relation between prostate carcinoma and genetic polymorphisms (7,26−37). Interindividual differences in cancer susceptibility may be mediated partially through polymorphic variability in the bioactivation and detoxification of carcinogens.

Rebbeck et al. reported that men who did not have homozygous deletions at GSTT1 were at increased risk of prostate carcinoma (1). Other studies suggest that individuals homozygous for the polymorphic GSTP1 genotype had a decreased risk of prostate cancer (38). Loss of GSTP1 gene expression is associated with hypermethylation of deoxycytidine residues (CG islands) in the 5′-regulatory region of the gene. Hypermethylation in the promoter region of GSTP1 appears to be a frequent and early event in prostate cancer development (6,39−44).

In our study, the GSTA class displayed the lowest level of expression in PIN and PCA. GSTP expression was lower in PCA tissue than in PIN (P < 0.05). There was a significant negative association between GSTT1 expression and patient age; however, we did not find a statistically significant association among patient age, Gleason score, and total serum PSA and GSTs in tumor tissue. These results suggest that the GST population, owing to reduction or loss of expression of multiple GSTs, especially GSTP and the alpha class, plays a role in tumor growth and carcinogenesis of the prostate.

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