Glutathione Peroxidase 3, Deleted or Methylated in Prostate Cancer, Suppresses Prostate Cancer Growth and Metastasis

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Abstract
Glutathione peroxidase 3 is a selenium-dependent enzyme playing a critical role in detoxifying reactive oxidative species and maintaining the genetic integrity of mammalian cells. In this report, we found that the expression of glutathione peroxidase 3 (GPx3) was widely inactivated in prostate cancers. Complete inactivation of GPx3 correlates with a poor clinical outcome. Deletions (hemizygous and homozygous) of GPx3 gene are frequent in prostate cancer samples, occurring in 39% of the samples studied. The rate of methylation of the GPx3 exon 1 region in prostate cancer samples reaches 90%. Overexpression of GPx3 in prostate cancer cell lines induced the suppression of colony formation and anchorage-independent growth of PC3, LNCaP, and Du145 cells. PC3 cells overexpressing GPx3 reduced invasiveness in Matrigel transmigration analysis by an average of 2.7-fold. Xenografted PC3 cells expressing GPx3 showed reduction in tumor volume by 4.8-fold, elimination of metastasis (0/16 versus 7/16), and reduction of animal death (3/16 versus 16/16). The tumor suppressor activity of GPx3 seems to relate to its ability to suppress the expression of c-met. The present findings suggest that GPx3 is a novel tumor suppressor gene. [Cancer Res 2007;67(17):8043–50]

Introduction
Prostate cancer is one of the leading causes of deaths for men in the United States (1, 2). The clinical detection rate of prostate cancer has been increased steadily due to the much improved detection of micro-adenocarcinomas in the prostate gland. Although many of these prostate cancers are very slow growing and are likely not to be clinically relevant for many patients, nearly 30,000 patients die of prostate cancer annually (1, 2). Despite the tremendous advance in our knowledge about prostate cancer in recent years, the molecular mechanism by which a relatively indolent disease is converted into a highly aggressive and lethal one remains unclear.

Our recent comprehensive gene expression analysis study of prostate cancer tissue revealed that the expression levels of 671 genes and expressed sequence tags (ESTs) were significantly altered in prostate cancer tissues relative to control tissues (3). In this study, we identified that one of the consistently down-regulated genes, called glutathione peroxidase 3 (GPx3), located in 5q23, was frequently deleted in prostate cancer samples. GPx3 is a member of glutathione peroxidases and plays a critical role in the detoxification of hydrogen peroxide and other oxygen free radicals. GPx3 is secreted and is present in plasma. A study indicated that mice with compound Nnx3.1 and pTEN mutations suppressed the expression of GPx3 in prostate gland and induced prostate cancer (4). Our large-scale analysis of GPx3 expression revealed that GPx3 was normally present in the cytoplasm of prostate epithelial cells. However, the down-regulation of GPx3 expression in prostate cancer was widespread and was associated with higher rate of post-prostatectomy metastasis. Forced expression of GPx3 in prostate cancer cell lines seemed to suppress tumor growth and metastasis both in vitro and in vivo. Expression of GPx3 down-regulated the expression of c-met, a receptor tyrosine tumor-transforming gene involved in a variety of cellular processes (5, 6). Expression of c-met partially reversed the colony suppression activity of GPx3. This study suggests that GPx3 contains tumor suppressor activity. Down-regulation of c-met is one of the mechanisms in mediating GPx3’s tumor suppressor function.

Materials and Methods

Cells culture conditions. All cell lines, including PC-3, Du145, and LNCaP, were purchased from American Type Culture Collection. PC-3 cells were cultured with F12K medium supplemented with 10% fetal bovine serum (Invitrogen) and sodium selenite (1 μmol/L final concentration). Du145 cells were cultured with modified Eagle’s medium supplemented with 10% fetal bovine serum (Invitrogen) and similarly supplemented with sodium selenite. LNCaP were cultured with RPMI 1640 supplemented with 10% fetal bovine serum (Invitrogen) and similarly supplemented with sodium selenite.

Sample preparation. Fresh prostate cancer tissues, recovered immediately from the operating room after removal, were dissected and trimmed to obtain tumor largely free of normal prostate acinar cells. For fluorescence in situ hybridization (FISH) analysis, formalin-fixed paraffin-embedded tissues were used. FISH hybridization was done on a 5-μm thin section of selected tissue blocks. Forty-seven samples, including 27 tumors tissues, were used. For semiquantitative reverse transcription-PCR, tissues were obtained fresh within 30 min of surgical resection. These tissues were overlaid with OCT and snapped frozen. Microdissections were done on sections of frozen tissues. A total of 100,000 to 500,000 cells were obtained for each sample, depending on the size and dissectability of the samples. DNA was purified with QiAmp DNA mini kit (Qiagen). The procedure of DNA extraction followed the detailed manuals provided by the manufacturer. A total of 65 samples including 51 prostate cancer and 14 normal prostate samples were examined. For single nucleotide polymorphism (SNP) analysis, 20 matched normal blood or non-prostate tissues were used as controls using primers AAGGGATCTACCTAGACAG/CAGAAATGAA-GACCCAAAGACTC and ATGCTATATTGAGGAAAAGCCCCAGTC and ATGCTATATTGAGGAAAAGCCCCAGTC. For immunostaining, 6 tissue array slides and 40 thin sections of formalin-fixed paraffin-embedded tissues were used. Tissue arrays of prostate tissues were constructed by arraying a total of 611 formalin-fixed, paraffin-embedded tissue blocks using a 1-mm-diameter array donor needle into six receiver paraffin blocks by a semiautomatic
tissue arrayer (Chemicon). Each block contains 150 to 160 tissue cores. Thin sections (3 μm) of tissue array blocks were made for immunostaining assays. The procedure of archived-tissue collection, de-identification, and experimental protocols were approved by the Institutional Review Board.

**FISH.** The probe for FISH analysis was obtained through screening bacterial artificial chromosome (BAC) three-level paneled library (Invitrogen) using two sets of primers specific for GPx3 exon 1 (CGATTGGCTGCAAGGGTCTCGGCT/GTCGCCTGAGCCGCTGCCTGATCC) and exon 5 (ACTGACGTCCCCAGAAGTTTCTGG/CCTTGGTTGATCTCAGGGA-GGTTG). The DNA from the selected clone was extracted using Nucleobond Ax kit (Macherey-Nagel). The procedures of probe preparation, hybridization, wash, and counter staining were described previously (7, 8). Analysis was done using a Nikon Optiphot-2 and Quips Genetic Workstation equipped with Chroma Technology 83000 filterset with single-band excitors for Texas Red/rhodamine, FITC, and 4',6-diamidino-2-phenylindole (DAPI; UV 360 nm). Only individual and well-delineated cells with two hybridization signals were scored. Overlapping cells were excluded from the analysis. A total of 50 to 100 cells per sample were scored to obtain an average of signals.

**Construction of constitutive GPx3-expressing cell lines.** GPx3 cDNA was obtained by extended long PCR (Roche Applied Science) using primers (GCATTGCCCTTTCTTCTAACGGAA/ATAGCAGATTGAGACTACAGC) encompassing the coding region on cDNA templates from a donor prostate sample. The procedures of RNA extraction and cDNA synthesis were similar to those described previously (7). The PCR was set as the following condition: 94°C for 1 min, then 94°C for 15 s, 61°C for 2 min, and 68°C for 8 min for 35 cycles. The 2.4-kb PCR product was ligated into a TA cloning vector using TOPO TA cloning kit (Invitrogen). Six clones were picked for plasmid DNA analysis. The selected GPx3-containing plasmid was digested with HindIII and XhoI, and the insert was ligated into a similarly digested pCMVscript vector. The final pCMV-GPx3 construct was sequenced (automated sequencing by the University of Pittsburgh Biotechnology Support Center) to confirm that no mutations had been introduced into the cDNA. This construct was transfected into PC3, Du145, and LNCaP cells, using Superfect kit (Invitrogen). Colonies containing the pCMV-GPx3 construct were selected with F12K (PC3), MEM (Du145 and LNCaP), 10% fetal bovine serum (FBS), and 400 μg/mL G418. Eleven colonies of pCMV-GPx3–transformed PC3 cells, 12 for DU145, and 10 for LNCaP cells were picked. To construct pSG-GPx3, pSG vector was digested with EcoRI and ligated with similarly digested GPx3 cDNA from TA cloning vector.

**Peptide antibodies against GPx3.** GPx3 antisera were raised through immunizing rabbits with peptides corresponding to regions of GPx3 coding sequences (KMDILSYMRQAAALGVKRK). These antisera were peptide affinity purified using the aminolink kit from Pierce. The purified antisera

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**Figure 1.** Down-regulation of GPx3 mRNA in prostate cancer. A, meta-analysis of microarray data of GPx3 mRNA expression. B, semiquantitative RT-PCRs on total RNA (1 μg) of normal prostate tissues (OD15, OD22, OD23, OD19, and OD2), primary prostate cancer samples (88T, PT13-39, 93T, 76T, 42T, 14T, 46T, 47T, 101T, 68T, 85T, 83T, and 91T), and benign tissues adjacent to prostate cancer (68N, 85N, 83N, and 91N) were reverse transcribed. PCR was done on diluted templates using primers specific to GPx3 (top) and β-actin (bottom).
were tested for specificity for GPx3 in Western blot with protein extracts from PC-3 and 1573 cells. GPx3 bands were specifically detected in extracts from both 1573 and PC-3 cells with anti-GPx3 antiserum, but no visible GPx3 band was detected with either preimmune serum or antiserum depleted of GPx3 peptide antibodies (see Supplementary Fig. S1A).

**Immunohistochemistry and immunoblot detection of GPx3.** The anti-GPx3 sera were peptide purified using the aminolink kit from Pierce and was prepared at a 1:1,000 dilution. The immunohistochemistry procedure was done as described previously (7). Immunolabeling specificity was verified by incubating similar slides without a primary antibody. The expression of GPx3 was graded as 0 if no cells within the tumor were stained, 0.5+/− if focally positive cells for GPx3 were found, 1+ if cells were weakly positive, 2+ if cells were strongly positive. The overall score for each sample represents a consensus of scores by three observers who were blinded to the patients’ clinical outcomes. For immunoblot detection of GPx3 expression, the procedures are similar to those previously described (7). The GPx3 expression was detected with the enhanced chemiluminescence (ECL) system (Amersham Life Science) according to the manufacturer’s protocols.

**Matrigel transmigration analysis.** The procedure of Matrigel transmigration analysis was previously described (7). Briefly, cells from each clone were suspended in DMEM containing 0.1% bovine serum albumin added to the upper chamber at a density of 1 × 10^5 cells per insert. A conditioned medium obtained by incubating NIH 3T3 cells for 24 h in serum-free DMEM in the presence of 50 μg/mL ascorbic acid were placed in the lower compartment of the invasion chambers (Becton Dickinson) as chemoattractants. After 24 h of culture, the upper surfaces of the inserts were wiped with cotton swabs for subsequent H&E staining. Each experiment was done twice with each sample in triplicate. The cells that migrated through the Matrigel and the filter pores to the lower surface were counted under a light microscope with five random high-power fields per insert.

**Tumor growth and spontaneous metastasis.** Severe combined immunodeficient (SCID) mice (Taconic) were s.c. implanted at the abdominal flank with ~1 × 10^7 viable cells, suspended in 0.2 mL of HBSS. The animals were observed daily. Body weight, tumor size, and other special findings, including lymph-node enlargement, were recorded weekly. After 6 weeks or when the mice became moribund, they were sacrificed, and autopsies were done. Serial sections of lung, brain, liver, kidneys, vertebra, and lymph nodes were collected. These tissues were formalin fixed and paraffin embedded. The sections were stained with H&E and subject to histology examination. All animal procedures were approved by the University of Pittsburgh Institutional Animal Care and Utility Committee.

**Bisulfite treatment and methylation-specific PCR.** Microdissection was done using a laser capture microdissection microscope (PixCell II LCM System, Arcturus Engineering). The protocols for tissue collection and experiments were approved by our Institutional Review Board. Each cell sample was considered to be at least 95% homogeneously malignant, as determined by a careful microscopic visualization of the cells captured. DNA was extracted using QIAamp DNA Mini Kit. About 2 μg of DNA were similarly denatured, treated with sodium bisulfite, and purified as previously described (9, 10). The modified DNA was used as a template for methylation-specific PCR (MSP). PCR was carried out in a final 50-μL.
reaction mixture containing 10 mmol/L Tris-HCl (pH 8.3), 1.5 mmol/L MgCl₂, 50 mmol/L KCl, 100 μmol/L each of deoxynucleotide triphosphate, 0.5 μmol/L each primer, and 1.25 units of Taq DNA polymerase (Perkin-Elmer). The mixture was heated at 94°C for 5 min and then subjected to 30 cycles of the following program: 94°C for 30 s, 62°C for 1 min, and 72°C for 2 min. The primers used for methylated GPx3 were CGATTGGTTGTAAGGGTTTCGGTT and CTCAAAATCGCCTAAACCGCTAC and, for unmethylated GPx3, TGATTGGTTGTAAGGGTTTTGGTT and CTCAAAATCACCTAAACCGCTAC.

Results

Analysis of three data sets (3, 11, 12) of Affymetrix oligo array suggests that GPx3 is down-regulated in prostate cancer (Fig. 1A). The higher differential data of the most recent analysis are a result of using completely normal prostate tissues as the controls. To validate the microarray analysis, semiquantitative RT-PCRs on 22 samples were done. The results consistently showed a 5- to 10-fold difference between normal and cancerous prostate tissues (Fig. 1B). Benign prostate tissues adjacent to prostate cancer generally have higher level expressions of GPx3 than their matched tumor tissues. However, it seems that organ donor prostates have the highest level of expression among these samples. To investigate whether the protein expression of GPx3 was similarly down-regulated in prostate cancer, we did an immunohistochemistry analysis on 611 samples of prostate tissues using tissue array slides. These slides include 285 prostate cancer and 326 normal prostate tissues. To quantify the relationship between GPx3 expression and prostate cancer, the expression of GPx3 was graded as strong (2 point), weak (1 point), or negative (0 point) for each sample. The overall score for each sample represented the average of scores of three observers of the same sample. Thus, the score ranging from 0 to 0.5 is considered negative, 0.6 to 1.5 weakly positive, and 1.6 to 2.0 strongly positive. The GPx3 expression was detected in all cases of normal prostate tissues, including 67% strong positive,
whereas 67% of cancer cases with aggressive features had down-regulation (score < 0.5) of GPx3 expression. As shown in Fig. 2A and Supplementary Fig. S1B and C, GPx3 is both an intracellular and secreted protein. Intracellularly, expression of GPx3 was primarily located in the cytosol and plasma membrane in normal prostate epithelial cells. Stromal cells such as fibroblast or lymphocytes are weakly positive. Prostate cancer cells have clearly reduced GPx3 protein level (Fig. 2B) in comparison with normal prostate tissues (0.51 versus 1.62; P < 0.0001). We subsequently divided the prostate cancer samples into those relapsed within 5 years of prostatectomy, those nonrelapsed, and those without adequate clinical follow-up. Down-regulation of GPx3 protein expression was most obvious in the relapsed prostate cancer. Sixty-four percent (49/77) prostate cancer samples that were found to relapse in 5 years had negative GPx3 expression. Only 6 of 77 (7.8%) aggressive prostate cancer cases had strong GPx3 expression. The level of GPx3 expression was statistically analyzed. Although the down-regulation of GPx3 was not associated with Gleason’s grade (P = 0.38), strong suppression of GPx3 expression was associated with the relapse of prostate cancer (Wilcoxon rank-sum test P < 0.0001). Comparison of metastasis-free survival curves by log-rank test indicated that the negative expression of GPx3 was associated with significantly higher rate of clinical relapse versus those with the least focal expression of GPx3 (62% versus 24%; P < 0.0001; Fig. 2C). These results suggested an inverse correlation between the progression of prostate cancer and the expression of GPx3. In comparison with Gleason’s grading, GPx3 score is slightly more accurate in predicting the clinical outcome (70% accurate for GPx3 versus 65% for Gleason’s grade with a cutoff combined score of 8).

Genomic deletion is the main genomic event for GPx3 in prostate cancer. GPx3 is located in 5q33-34, a genetic locus frequently linked to advanced prostate cancer. To examine whether GPx3 is deleted in prostate cancer, PCRs were done on 51 microdissected prostate cancer samples using primers specific to exons 2 and 5 regions. The results indicated that GPx3 sequences were homozygously deleted in 10% (5/51) prostate cancer samples (Fig. 3). To verify the PCR results, FISH was done on 27 prostate cancer samples. About 5 of 27 samples (GPx3/centromere = 0, 18.5%) were shown similar homozygous deletion (Supplementary Table S1), whereas 14 of these samples were implicated as hemizygous deletion (GPx3/centromere <0.4). To characterize these hemizygous deletions, PCR was done on 20 pairs of prostate cancer/matched normal samples with primers specific to regions of GPx3 genome rich in SNP, the results confirmed a loss of heterozygosity in the GPx3 gene (Fig. 3C and Supplementary Table S1), indicating single allele deletion of GPx3 in these samples.

Figure 4. Demethylation of genomes of cancer cells produces limited expression alteration of GPx3 in prostate cancer cell line. A, methylation-specific PCR in exon 1 of GPx3 in 30 primary prostate cancer samples, PC3, DU145, OD (organ donor), LNCaP, and 1573 cells. M, methylated GPx3; U, unmethylated GPx3. B, treatment of cells with 5-aza-2-deoxycytidine increased GPx3 mRNA levels in PC-3, Du145, LNCaP, and 1573 cells. Total RNA of PC3, Du145, LNCaP, and 1573 cells were isolated and reversed transcribed. The cDNA templates were serially diluted, and PCR were done using primers specific to GPx3 (top) and β-actin (bottom).
A CpG island was identified in the exon 1/intron 1 region of GPx3, spanning from +53 to +793 of the mRNA start site. However, no CpG island was identified in the promoter region up to −3,000 bp of GPx3. Methylation-specific PCRs were done on 30 samples from prostate cancer without homozygous deletion, 4 cancer cell lines, and 1 normal donor prostate epithelia using primers encompassing +151 to +280 (within exon 1; Fig. 4A). The results indicated that methylation in the exon 1 region of GPx3 occurred in 90% (27/30) of prostate cancer samples. All cancer cell lines including three prostate cancer cell lines (PC-3, Du145, and LNCaP) were positive for methylation (Fig. 4). These MS-PCR products were subsequently cloned into TA cloning vector and sequenced. The results supported the methylation status of GPx3 in these samples (data not shown). To investigate whether demethylation of GPx3 in prostate cancer cell lines de-represses its expression, each of these cell lines (1573, PC-3, Du145, and LNCaP) was treated with azacitidine for 24 h to demethylate the gene. The results indicated that demethylation of GPx3 resulted in less than 3-fold increase on the mRNA level of this gene in three of these cell lines (Fig. 4B). Based on these results, we concluded that methylation of GPx3 is functional, although the CpG island of GPx3 is not located in an optimal area for gene regulation.

Overexpression of GPx3 inhibits invasiveness of prostate cancer. Examination of GPx3 expression in the available prostatic cancer cell lines PC-3, Du145, and LNCaP by RT-PCR revealed that a small amount of GPx3 mRNA was present in all these cell lines. GPx3 protein expression in these cell lines was also examined using rabbit anti-GPx3 sera. Minimal protein expressions were seen in all three cell lines. To examine the role of GPx3 in tumorigenesis of prostate cancer cells, cDNA of GPx3 was inserted into the eukaryotic expression vector pCMVscript. PC-3, Du145, and LNCaP cells were transfected with the pCMVscript and pCMV-GPx3 vectors. A total of 10 to 12 colonies of each cell line were picked and maintained. Two of these colonies were selected for further analysis based on their level of GPx3 overexpression (Fig. 5A). These cell lines were tested with regard to their ability of colony formation and anchorage-independent growth in soft agar. As shown in Fig. 5A and B, cell lines transformed with pCMV-GPx3 exhibited 2- to 2.5-fold decreases in colony formation in comparison with vector controls. Similarly, tumor cells transformed with pCMV-GPx3 displayed 2- to 4-fold reduction in anchorage-independent growth in soft agar. This observation supports the hypothesis that GPx3 has tumor suppressor activity.

Figure 5. Expression of GPx3 suppresses tumorigenesis. A, suppression of colony formation with GPx3 expression in pCMVscript- (CP1 and CP2) or pCMV-GPx3 (PMG8 and PMG9)–transfected PC3 cells, or pCMVscript- (DP1 and DP2) or pCMV-GPx3 (DMG7 and DMG8)–transfected DU145 cells, and pCMVscript- (LP1 and LP2) or pCMV-GPx3 (LMG2 and LMG3)–transfected LNCaP cells. Right, suppression of colony formation with inducible GPx3 expression (pCDNA4-GPx3) treated with or without tetracycline (PDG10, PDG21, and PDG24). SDs are indicated. B, suppression of anchorage-independent growth in soft agar by GPx3 expression in PC3 cells, DU145, and LNCaP cells. Same cell colonies as (A) were tested. SDs are indicated. C, suppression of tumor growth and metastasis of PC3 and DU145-xeongrafted tumors in SCID mice with GPx3-expressing cells. SDs are indicated. D, Kaplan-Meier curves of survival for mice xenografted with tumor cells harboring pCMV-GPx3 or control vector pCMVscript.
To test whether GPx3 tumor suppressor activity is instantaneous and can be induced by transient vector, a tetracycline-inducible eukaryotic expression vector (pCDNA4) overexpressing GPx3 was constructed and transfected into PC-3 cell line. Twenty-two colonies of each transfection were picked and examined for the GPx3 expression levels. Fourteen of these clones were found to overexpress GPx3 by 2- to 6-fold in protein level over the uninduced transformed cells. After expression of GPx3 protein in transformed cells was confirmed, three of these colonies were selected for colony formation and anchor-ajeindele growth analysis. As shown in Fig. 5, expression of GPx3 in PC-3 cells reduced the number of colony formation by 40% and colony formation in soft agar by 80%.

We tested PC-3 cell lines overexpressing GPx3 in prostate cancer cell invasiveness in in vitro Matrigel transmigration experiments and in in vivo mouse tumorigenesis experiments. More than 2.7-fold decrease in invasiveness was found in GPx3-expressing PC3 cells in comparison with the controls (Supplementary Table S2). Moreover, the propensity for PC3 cells injected into mice to develop into tumors was suppressed by GPx3 expression (Fig. 5C). Relative to the pCMVscript controls, mice injected with GPx3-expressing cells developed smaller tumors by >4.8-fold (mean 2.45 cm² versus 0.51 cm²), displayed a lower incidence of metastasis (0/16 versus 7/16 mice), and had a markedly reduced 6-week mortality (3/16 versus 16/16 mice; see Kaplan-Meier curves in Fig. 5D). These experiments clearly indicate that GPx3 suppresses tumor growth, decreases metastasis, and reduces cancer-related mortality.

**Overexpression of GPx3 down-regulates c-met expression.** To understand the mechanism of GPx3-mediated tumor suppression, we did Affymetrix oligonucleotide microarray analysis on cells with induced GPx3 expression using u133 2.0 chip. There is a 3.5-fold induction of GPx3 mRNA in PDG10 cells 48 h after treatment with tetracycline (1 µg/ml) and a corresponding decrease of c-met mRNA by 3.8-fold (P < 0.01, baseline analysis, GCOS1.4). To verify this finding, two pCDN4-GPx3-transformed PC3 cells were induced with tetracycline and immunoblotted for the level of c-met. As shown in Fig. 6, an average of 2.5-fold decrease of c-met was identified. Subsequently, cotransfection of pPSG-GPx3 and PSG-c-met into PC3 cells were done to evaluate whether overexpression of c-met overrides the tumor suppressor activity of GPx3. As shown in Fig. 6B, overexpression of c-met at least partially reverses the tumor suppressor activity of GPx3.

**Discussion**

Survival of cells depends on the balance of reactive oxidative species, which was generated from metabolism or from exposure to a harmful environment. The antioxidant mechanism includes several GPx enzymes to convert ROS into the harmless oxidized form of glutathione and water and dismutase to reduce oxygen superoxide to hydrogen peroxide intermediates. All GPx enzymes are widely expressed. A unique feature of GPx3 is that it is readily detectable in the plasma, suggesting that it provides the first line of defense against reactive oxygen species (ROS) even before they enter the cells. There are numerous evidences suggesting that the enzymes involved in ROS metabolism are critical in protecting cells from DNA damage and ensuring the progenies of a cell genetically intact. Implication of GPx3 in tumorigenesis of prostate cancer comes from several microarray analyses, where GPx3 was found to be one of the most consistently down-regulated genes, even when the arrays were done using different chip sets and in different institutes. Methylation analyses indicate that GPx3 was widely methylated among prostate cancer samples (13, 14). Our findings indicate that the down-regulation of GPx3 occurred widely in prostate cancer. However, complete shutdown of GPx3 expression correlates with poorer prognosis. This may reflect the possibility that the absence of GPx3 makes cells extremely vulnerable to ROS damage and increases genome instability. Interestingly, decreased expression of GPx3 can be detected in morphologically benign prostate tissues adjacent to cancer albeit less severe as that of the tumor per se, implying that such a decrease is preceding the development of a frank malignancy.

A surprising finding is that GPx3 contains growth and invasion suppression activity in our analysis. The data are corroborated by both in vitro and animal model experiments. The role of GPx3 in prostate cancer suppression is further supported by a large body of microarray analyses and immunostaining analysis in this study. The open reading frame of GPx3 cDNA contains an TGA opal codon (15). To have a successful readthrough of GPx3 by translation complex, a selenium-cysteine must be incorporated into the protein to prevent a translational truncation (16). As a result, the expression of GPx3 is dependent on the availability of selenium, which has been successfully employed as a critical agent for chemoprevention against prostate cancer. The mechanism of GPx3-mediated tumor suppression is probably related to its ability to down-regulate the expression level of c-met, a tumor-transforming gene. c-met is a receptor tyrosine kinase responsible for a...
variety of cellular processes, including promoting growth, motility, and invasiveness in several malignancies (5, 6). It is the receptor for the ligand known as the hepatocyte growth factor (HGF). Mutation of c-met in rodents resulted in papillary renal cell carcinoma (17). Interestingly, c-met may be activated through the autocrine mechanism. Overexpression of c-met was found in primary prostate cancer (18, 19). Forced expression of c-met in the DU145 cell line was shown to induce invasiveness (20). Because the reversal of tumor suppressor activity of GPx3 by c-met is partial, other mechanisms might be possible.

Previous studies indicated that GPx3 is methylated in prostate and esophageal carcinoma (13, 21). Indeed, this study confirms widespread hypermethylation of GPx3 in prostate cancer. However, the methylation suppression of GPx3 expression resulted in only a 2- to 3-fold decrease in mRNA level. To have a complete inactivation of GPx3 expression, other mechanisms are necessary. In this study, we identified that deletions, hemizygous deletion in particular, of GPx3 are quite frequent (up to 39%) in prostate cancer. This implies that both mechanisms may contribute to the down-regulation of GPx3 in prostate cancer. Although all three prostate cancer cell lines are hypermethylated in the exon 1 area of GPx3, there are significant if not abundant GPx3 expressions both in mRNA and protein levels. This might argue that deletion, instead of methylation, plays a major role in down-regulating the expression of this gene.

The findings of this study suggest that the function of GPx3 is probably beyond a detoxification enzyme: it contains activity directly or indirectly regulating cell growth and proliferation. Because GPx3 converts reduced glutathione into oxidized glutathione (GSSG) to reduce ROS into harmless water, one might suspect that the oxidized form of glutathione plays a role in tumor suppression. However, repeated experiments using GSSG to treat prostate cancer cells fail to deliver the appreciable effect in cell growth and colony formation assays. These results suggested that GPx3 exerts its tumor suppressor activity through other yet-to-be discovered mechanisms. There are important clinical implications of GPx3 expression inactivation in prostate cancer cells. First, as a tumor suppressor gene implicated in metastasis, inactivation of GPx3 may have a negative impact on a patient’s clinical outcome. As a result, additional precautions in the treatment and monitoring plan for patients with complete inactivation of GPx3 expression in their tumor tissues should be considered. Assays on GPx3 expression may supplement Gleason’s grading as an added objective criteria in predicting prostate cancer behavior. Second, GPx3 may provide a potential target for clinical intervention. Because the restoration of wild-type GPx3 expression in tumor cells significantly modifies the aggressive behavior of these tumors, gene-targeted therapy to enhance the activity of GPx3 might be useful in the treatment or prevention of prostate cancer.

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