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The diet, prostate inflammation, and the development of prostate cancer

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Summary

Evidence that somatic inactivation of *GSTP1*, encoding the human π-class glutathione *S*-transferase, may initiate prostatic carcinogenesis is reviewed along with epidemiological evidence implicating several environment and lifestyle factors, including the diet and sexually transmitted diseases, as prostate cancer risk factors. An integrated model is presented featuring *GSTP1* function as a 'caretaker' gene during the pathogenesis of prostate cancer, in which the early loss of GSTP1 activity renders prostate cells vulnerable to genome damage associated with chronic prostatic inflammation and repeated exposure to carcinogens. The model predicts that the critical prostate carcinogens will be those that are substrates for GSTP1 detoxification and are associated with high prostate cancer risk diet and lifestyle habits.

Both genetic and environmental factors likely contribute to the pathogenesis of human prostate cancer. In support of a role for inheritance in the development of prostate cancer, familial clusters of the disease have been reported, and segregation analyses have suggested that prostate cancer in some of these families is likely attributable to inheritance of prostate cancer susceptibility genes [1–3]. Over the past few years, a number of genetic loci have been identified that have been postulated to be responsible for inherited susceptibility to prostate cancer [4–17]. How do such genes lead to prostate cancer development? Until the suspected genes have been identified and characterized, the manner by which the genes increase prostate cancer risk will remain to be established. Nevertheless, in a recent study of cancer risks among 44,788 pairs of twins in Sweden, Denmark, and Finland [18], a statistically significant effect of genotype was observed only for some 42% of prostate cancer cases (with a 95% confidence interval of 29–50%), indicating that environment and lifestyle likely play a more dominant role than inheritance in the development of most prostate cancers.

A dominant role for environment and lifestyle in the development of life-threatening prostate cancer is further supported by ecological epidemiology data [19]. Prostate cancer incidence and mortality are well-known to vary greatly in different geographic regions of the world, with low risks of prostate cancer mortality characteristic of Asia and high risks of prostate cancer mortality characteristic of the US and Western Europe [19,20]. In addition, Asian immigrants to the US tend to adopt higher prostate cancer risks, strong evidence that the environment and lifestyle may be the major cause of life-threatening prostate cancer in the US [21,22]. How do the environment and lifestyle promote prostatic carcinogenesis? Insights into the earliest steps in the molecular pathogenesis of human prostate cancer have begun to provide a clue: prostate cancer development appears to be initiated by somatic inactivation of *GSTP1*, encoding the human π-class glutathione *S*-transferase [23–26]. Cancer cell DNA typically contains many somatic alterations, including mutations, deletions, amplifications, translocations, and hypermethylated CpG islands, that affect the function of critical genes and contribute to the malignant phenotype [27,28]. Critical genes targeted include oncogenes, which promote transformation when activated, and tumor suppressor genes, which fail to prevent transformation when inactivated. During the development of prostate cancer, *GSTP1* does not appear to function either as an oncogene, or as a tumor suppressor gene [25]. Instead, *GSTP1* likely acts as a 'caretaker' gene in prostate cells, which when inactivated, fails to prevent further somatic genome alterations upon chronic exposure to genome-damaging stresses [25,29]. In

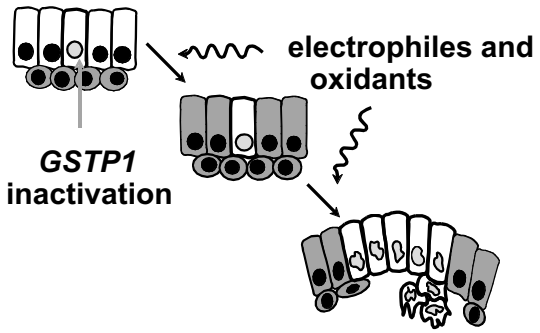

Figure 1. A new model for prostatic carcinogenesis. GSTP1 (gray) is constantly expressed in prostate basal epithelial cells, but can be induced in luminal epithelial cells upon exposure to reactive chemical species, including electrophiles and oxidants, that threaten genome damage. Loss of GSTP1 expression accompanying *GSTP1* CpG island hypermethylation increases vulnerability of luminal cells to neoplastic transformation and malignant progression.

this model of prostatic carcinogenesis, loss of *GSTP1* function increases the vulnerability of prostate cells to carcinogenic insults, contributed by environment and lifestyle, that promote prostate cancer development and progression (Figure 1).

Recognition that somatic inactivation of *GSTP1* may initiate prostatic carcinogenesis permits a reevaluation of the influence of environment and lifestyle on the development of life-threatening prostate cancer. Epidemiology studies have identified several environmental factors, including the diet, sexually transmitted diseases, and others, as candidate prostate cancer risk factors [30–32]. However, no specific prostate carcinogens have been identified. Glutathione *S*-transferases (GSTs), like GSTP1, are capable of detoxifying oxidant and electrophilic chemicals to prevent cell and genome damage [33]. If loss of *GSTP1* function plays a critical role in the pathogenesis of prostate cancer, we can expect that the relevant prostate carcinogens will be oxidants and electrophiles that (i) are substrates for GSTP1 detoxification, (ii) are associated with high prostate cancer risk diet and lifestyle habits, and (iii) are able to reach prostate cells with defective GSTP1 'caretaker' activity to inflict cell and genome injury. In this review, we consider the influence of environment and lifestyle on the development of life-threatening prostate cancer, focusing on the acquired vulnerability of prostate cells to carcinogens and other genome-damaging stresses associated with loss of *GSTP1* function.

The molecular pathogenesis of prostate cancer: Somatic inactivation of *GSTP1*

The pathogenesis of prostate cancer likely proceeds over at least 30 or more years. Small prostate cancers have been found in >30% of men aged 30–40 years, while diagnoses of clinically significant prostate cancers are typically made in men aged 60–70 years [34]. Prostate cancer cells typically contain a myriad of somatic genome alterations, including gene mutations, gene amplifications, gene deletions, chromosomal rearrangements, and changes in DNA methylation (see Figure 2) [35]. In addition, cancer cells from different prostate cancer cases, from different prostate cancer lesions in individual cases, or from different areas within the same prostate cancer lesions, often display marked differences in somatic genome changes [35,36]. This heterogeneity of somatic genome defects in prostate cancer cells, accumulating over decades, suggests that prostate cancers may arise as a consequence of either chronic or prolonged exposure to genome-damaging stresses, defective maintenance of genome integrity, or a combination of both processes. Furthermore, ongoing genomic instability in prostate cancer cells may be what leads to metastasis, progression to androgen independence after attempts at hormonal therapy, and other malignant behaviors [37–42].

Over the past several years, evidence has accumulated in support of the concept that somatic inactivation of *GSTP1*, most commonly by CpG island hypermethylation, may be the initiating somatic genome lesion for prostatic carcinogenesis, increasing the rate at which additional somatic genome alterations appear in response to exposure to genome-damaging stresses [23–26]. GSTs stereotypically provide an inducible defense against macromolecular damage by reactive chemical species [33]. In the normal prostate, GSTP1 polypeptides are selectively present in basal epithelial cells, with little, if any, enzyme detectable in non-stressed columnar epithelial cells (Figure 1). Nonetheless, *GSTP1* expression appears strikingly induced in columnar epithelial cells subjected to genome damaging stresses, such as those accompanying inflammation, commonly present in the human prostate. Proliferative inflammatory atrophy (PIA) lesions have been proposed to arise as a consequence of inflammatory injury to the prostate epithelium followed by exuberant epithelial cell proliferation/regeneration, and to give rise directly to prostatic intraepithelial neoplasia (PIN) lesions, known

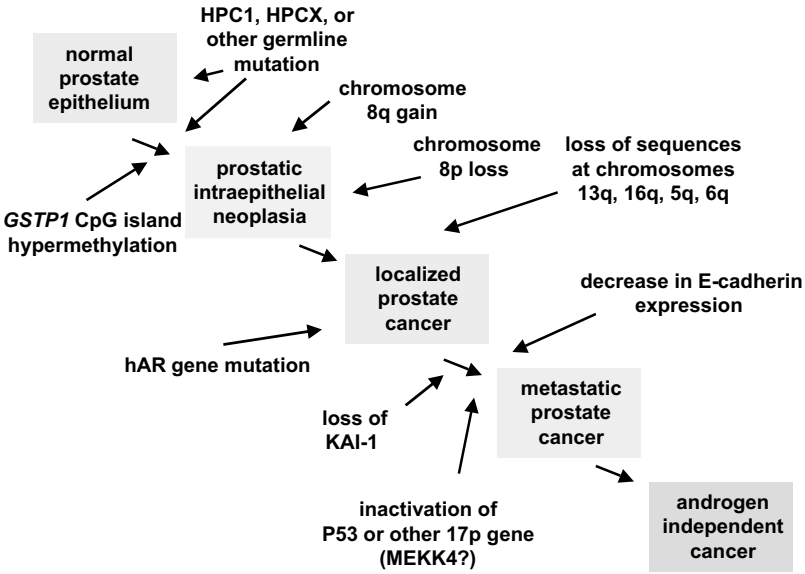

Figure 2. The molecular pathogenesis of prostate cancer. Somatic genome lesions accumulate during prostate cancer development. *GSTP1* CpG island hypermethylation appears to serve as the initiating somatic genome lesion.

precursors to prostate cancer [43,44]. In PIA lesions, atrophic luminal cells characteristically contain high levels of GSTP1 and other GSTs [43,45]. However, in PIN lesions, in contrast to PIA lesions, absent GSTP1 expression is a consistent finding [46]. Prostate cancer cells are also characteristically devoid of GSTP1. In almost all prostate cancer cases, the absence of GSTP1 expression can be attributed to somatic *GSTP1* CpG island hypermethylation, not present in DNA from normal tissues, but evident in DNA from some 70% of PIN lesions and >90% or prostate cancers [25,46]. As the most common somatic genome alteration yet reported for human prostate cancer, *GSTP1* CpG island hypermethylation, readily discriminated using various sensitive techniques, is an attractive candidate molecular biomarker for prostate cancer cells, under development for use in prostate cancer detection, diagnosis, and staging (Table 1) [23–25,46–54].

GSTP1 CpG island hypermethylation appears to prevent GSTP1 expression in prostate cancer cells via 'silencing' of *GSTP1* transcription. In support of this mechanism, LNCaP prostate cancer cells contain only hypermethylated *GSTP1* CpG island alleles, fail to express either *GSTP1* mRNA or GSTP1 polypeptides, and exhibit reduced *GSTP1* transcription, whereas treatment of LNCaP cells *in vitro* or *in vivo* with DNA methyltransferase inhibitors both reverses *GSTP1* CpG island hypermethylation and restores GSTP1 expression [24,25,55]. In addition, while unmethylated *GSTP1* transcriptional regulatory sequences readily promote abundant CAT reporter expression after transfection into LNCaP prostate cancer cells, *Sss*I CpG-methylase treatment of the *GSTP1* promoter before transfection leads to a marked reduction in CAT reporter expression [25,56]. Finally, detailed molecular pathology analyses have revealed, for almost all prostate cancer cases, that each of the cancer cells contains only hypermethylated *GSTP1* CpG island alleles, and each fails to express GSTP1 [25]. In contrast, for the two prostate cancer cases thus far described in which prostate cancer cells have been found to contain unmethylated *GSTP1* CpG island alleles, each of the prostate cancer cells displayed abundant GSTP1 expression [25,54]. CpG island hypermethylation has been proposed to repress gene transcription directly, via interference with transcriptional trans-activator binding, or indirectly, via the actions of $^{5\text{-m}}$C-binding proteins that affect chromatin structure, leading to a transcriptionally inactive chromatin conformation [57–63]. For the *GSTP1* CpG island, data collected thus far suggest that CpG island hypermethylation acts to repress *GSTP1* transcription indirectly [56]. Regardless of the mechanism by which CpG island hypermethylation prevents *GSTP1* transcription, because CpG methylation patterns are maintained through mitosis by the action of DNA methyltransferases,

Table 1. *GSTP1* CpG island hypermethylation as a molecular biomarker for prostate cancer

Reference	Assay technique	*GSTP1* CpG island hypermethylation*
Lee et al. (1994)	Southern blot	Tissue: 100% PCA; 0% BPH
Lee et al. (1997)	Restriction enzyme-PCR	Tissue: 91% PCA
Brooks et al. (1998)	Restriction enzyme-PCR	Tissue: 70% PIN
Santourlidis et al. (1999)	Restriction enzyme-PCR	Tissue: 75% PCA; 0% TCC
Millar et al. (1999)	Bisulfite genomic sequencing	Tissue: 83% PCA
Suh et al. (2000)	Restriction enzyme-PCR	Ejaculate: 44% PCA
Goessl et al. (2000)	Methylation-specific PCR	Tissue: 94% PCA; 0% BPH Urine: 36% PCA Ejaculate: 50% PCA Plasma: 50% PCA
Goessl et al. (2001)	Methylation-specific PCR	Urine: 73% PCA; 29% PIN; 2% BPH
Cairns et al. (2001)	Methylation-specific PCR	Tissue: 79% PCA Urine: 27% PCA
Lin et al. (2001)	Southern blot, restriction enzyme-PCR, bisulfite genomic sequencing	Tissue: 100% PCA
Goessl et al. (2001)	Methylation-specific PCR	Tissue: 90% PCA; 0% BPH Urine: 76% PCA Ejaculate: 50% PCA Plasma: 72% PCA
Jeronimo et al. (2001)	Quantitatitive methylation-specific PCR	Tissue: 91% PCA; 54% PIN; 29% BPH

*PCA-prostate cancer, BPH-benign prostatic hyperplasia, PIN-prostatic intraepithelial neoplasia, TCC-transitional cell carcinoma.

GSTP1 transcriptional 'silencing' associated with CpG island hypermethylation may be subject to selection during prostatic carcinogenesis. For this reason, the observation that most prostate cancers contain only prostate cancer cells with hypermethylated *GSTP1* CpG island alleles may be evidence that loss of *GSTP1* function likely provides some sort of selective growth or survival advantage at some point during the pathogenesis of prostate cancer.

Prostate cancer epidemiology: The diet, prostate inflammation, and prostate cancer risk

The etiology of prostate cancer has not been established. However, as mentioned previously, both prostate cancer incidence and mortality vary greatly in different geographic regions, with generally low risks of prostate cancer development characteristic of Asia, and generally high risks of prostate cancer development characteristic of the US and Western Europe [21,64–67]. Of note, prostate cancer risk among ethnic Asian immigrants to North America increases with duration of exposure to a Western lifestyle: Asian immigrants to North America have a higher risk of prostate cancer after living in North America for more than 25 years than after living in North America for less than 10 years [22]. Asian men born in the US have a risk for life-threatening prostate cancer development similar to Caucasian men. Of course, these epidemiological observations underscore the critical role for environment and lifestyle in fostering the epidemic of prostate cancer afflicting men in the US. The major environment and lifestyle factor modulating prostate cancer risk appears to be the diet. Clearly, stereotypical Asian diets are quite different from stereotypical Western diets. Unfortunately, whether the stereotypical Western diet makes an error of *commission* (e.g. over-consumption of dietary components increasing prostate cancer risks), an error of *omission* (e.g. under-consumption of dietary components decreasing prostate cancer risks), or *both* has been difficult to establish. Nonetheless, if specific dietary components could be demonstrated to change prostate cancer risks, the component could be *avoided* if it promoted prostate cancer and *provided* if it protected against prostate cancer as a rational prostate cancer prevention strategy.

Ecological, case-control, and cohort epidemiology studies all have long implicated fats and meats as candidate risks factors for prostate cancer (for a review, see [30]). The challenge for epidemiologists has been to ascertain whether increased prostate cancer risks might be attributable to total fat intake, to increased energy intake associated with high-fat diets, to intake of specific fats, or to intake of fats from specific sources, such as red meats. For example, in the Health Professionals

Follow-up Study, a prospective cohort of 51,529 men, total fat intake, adjusted for energy intake, appeared to confer increased risks (a relative risk of 1.79 for high *versus* low quintile of intake, with 95% confidence interval of 1.04–3.07) of prostate cancer development [68]. However, saturated fat intake *per se* did not appear to be responsible for the increased prostate cancer risks. Rather, animal fat intake (a relative risk of 1.63 with a 95% confidence interval of 0.95–2.78), particularly red meat intake (a relative risk of 2.64 with a 95% confidence interval of 1.21–5.77), may have been responsible for the observed prostate cancer risks associated with the high total fat diets. A deleterious effect of red meat consumption on prostate cancer risk has also been seen in the Physicians Health Study [69], a prospective cohort of 14,916 men (a relative risk of 2.5 for red meat consumption 5 times/week *versus* less than once/week with a 95% confidence interval of 0.9–6.7), and in a large cohort study in Hawaii [70], involving 20,316 men of varying ethnicities (a relative risk of 1.6 for highest *versus* lowest tertile of beef consumption with a 95% confidence interval of 1.1–2.4). Intriguingly, cooking of meats at high temperatures or on charcoal grills, is known to lead to the formation of heterocyclic amine carcinogens and/or polycyclic aromatic hydrocarbon carcinogens [71,72]. However, the level of intake of these carcinogenic substances has been difficult to estimate for epidemiologic studies of prostate cancer risk.

While chronic consumption of animal fats and red meats may promote prostate cancer, intake of vegetables may protect against prostate cancer development (for a review, see [31]). Among potentially protective vegetables, attention has been most intensively focused on tomatoes [73–76], which contain an α-carotenoid anti-oxidant, lycopene, and on cruciferous vegetables [76–78], which contain an anti-carcinogenic isothiocyanate, sulforaphane [79]. Lycopene, which appears in prostate tissues after tomato ingestion, can scavenge oxidant species, including nitric oxide, to prevent oxidative cell and genome damage [80–83]. In a study of 578 prostate cancer cases and 1294 controls from the Physician's Health Study cohort, high plasma lycopene levels were associated with a decrease in the risk of aggressive prostate cancer (a relative risk of 0.56 for high *versus* low quintile of lycopene plasma level with a 95% confidence interval of 0.34–0.91). Of interest, other anti-oxidants, selenium and vitamin E, have been found to attenuate prostate cancer development in randomized clinical trials [84–86]. Sulforaphane has been proposed to protect against cancer development by increasing the expression of carcinogen-detoxification enzymes, including GSTs and quinone oxidoreductases, that help prevent genome damage mediated by carcinogens [79,87–89]. In a study of 628 prostate cancer cases and 602 controls from King County, Washington, cruciferous vegetable consumption, adjusted for total vegetable intake, was associated with diminished prostate cancer risks (a relative risk of 0.59 for 3 or more servings/week *versus* less than one serving/week with a 95% confidence interval of 0.39–0.90) [77].

In addition to the diet, environment and lifestyle factors affecting prostate cancer risk include sexually transmitted diseases, risk factors that might be modified to prevent prostate cancer [32]. However, unlike cancer of the uterine cervix, nasopharyngeal carcinoma, and Kaposi's sarcoma, it has been difficult to identify a specific pathogen responsible for the direct transformation of prostatic cells. Instead, prostatic inflammation associated with sexually transmitted infections may play a more important role in prostatic carcinogenesis. In a recent population-based study involving 981 prostate cancer cases (479 black men, 502 white men) and 1315 controls (594 black men, 721 white men), prostate cancer risks were increased among men (i) who reported a history of gonorrhoea or syphilis (a relative risk of 1.6 with a 95% confidence internal of 1.2–2.1), (ii) who reported three or more episodes of gonorrhea (a relative risk of 3.3 with a 95% confidence interval of 1.4–7.8), and (iii) who displayed serological evidence of syphilis (a relative risk of 1.8 with a 95% confidence interval of 1.0–3.5) [90]. Inflammation, known to inflict oxidative damage, has been thought to contribute to the pathogenesis of many human cancers [91]. Prostatitis, whether the result of an identified infection or idiopathic, very commonly afflicts men as they grow older in the US [92]. Whether chronic or recurrent prostatic inflammation contributes to prostate cancer development, or might explain differences in prostate cancer risks between different geographic regions, has not been ascertained.

A 'caretaker' role for *GSTP1* during prostatic carcinogenesis: The integration of prostate cancer molecular biology and epidemiology into a unified model

Rather than function as a tumor suppressor during prostatic carcinogenesis, *GSTP1* appears most likely to act as a 'caretaker' [29], defending prostate cells

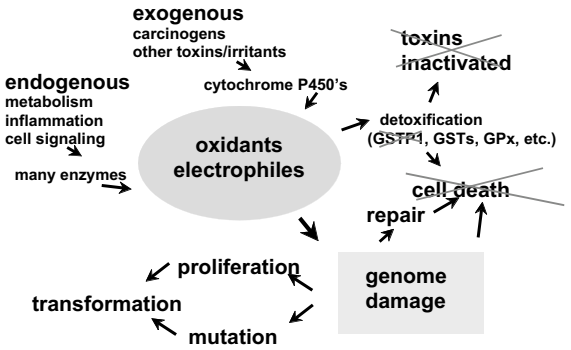

Figure 3. Loss of GSTP1 activity leads to a failure of cellular protection against oxidant and electrophilic carcinogens.

against genome damage mediated by carcinogens (Figure 3). GSTs have long been thought to protect against cancer development by catalyzing conjugation reactions between glutathione and a variety of reactive chemicals species, preventing carcinogen-induced cell and genome damage [33]. The enzymes are dimers composed of subunit polypeptides encoded by a complex collection of genes, organized into gene families α, μ, π, and θ. *GSTP1* encodes the single π-class GST subunit polypeptide; GSTP1-1 is a homodimer. The various different GSTs are normally expressed in different cells and tissues; however, GST activity can also be stereotypically induced in other cells and tissues in response to chemical stresses, via increases in *GST* subunit gene transcription [93,94]. The transcription factor Nrf2, binding to specific *cis*-regulatory sequences in *GST* subunit gene promoters, appears to play a critical role in activating *GST* subunit gene transcription in response to chemical stresses [95]. The inducible protection against reactive chemicals afforded by GSTs comprises a substantial barrier to chemical carcinogenesis. For example, mice carrying disrupted *Gstp1/2* genes, encoding the murine π-class GSTs, display increased skin tumorigenesis upon topical exposure to 7,12-dimethylbenz anthracene (DMBA) [96]. In addition, mice carrying disrupted *Nrf2* genes manifest increased gastric carcinogenesis upon exposure to benzo[a]pyrene [95]. By providing a barrier to cell and genome damage mediated by reactive chemicals, *GST* genes act as 'caretaker' genes for cancers arising as a consequence of carcinogen exposure.

As described above, prostate cancer epidemiology data have implicated heterocyclic amines (well-done meats), polycyclic aromatic hydrocarbons ('charbroiled' meats), and oxidants (inflammation) as candidate reactive chemicals that might threaten cell and genome damage leading to prostate cancer development. If *GSTP1* 'caretaker' function provides a defense against such carcinogens, then the reactive chemical species ought to be substrates for GSTP1 detoxification. Recent data have suggested that heterocyclic amine carcinogens present in well-done meats may be detoxified by GSTP1 [97]. The heterocyclic aromatic amine carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) is known to cause mutations by adduction to DNA bases after metabolic activation by various cellular enzymes [71,98–101]. Rats fed PhIP have been reported to display mutations in prostate DNA and develop prostate cancer [102–104]. However, when LNCaP human prostate cancer cells devoid of GSTP1 were exposed to metabolically activated PhIP, high levels of PhIP-DNA adducts were detected, while LNCaP prostate cancer cells genetically modified to express GSTP1 exhibited substantial resistance to the formation of pro-mutagenic PhIP-DNA adducts [97]. Like the heterocyclic amine carcinogens, metabolically activated polycyclic aromatic hydrocarbon carcinogens are also GSTP1 substrates [105]. Of interest, polymorphic GSTP1 variants that are homodimers with subunit polypeptides containing isoleucine at amino acid 105, or containing valine at amino acid 105, appear to exhibit different catalytic properties when confronted with polycyclic aromatic hydrocarbons [106]. Furthermore, genetic epidemiology studies have suggested that homozygosity for $GSTP1^{val\text{-}105}$ alleles may increase breast cancer risks (a relative risk of 1.97 for $GSTP1^{val\text{-}105/val\text{-}105}$ with a 95% confidence interval of 0.77–5.02) [107]. The prostate cancer risks associated with homozygosity for $GSTP1^{val\text{-}105}$ alleles have not been established. Finally, new preliminary data have suggested that oxidants may also be substrates for GSTP1: upon prolonged exposure to an oxidative stress, LNCaP prostate cancer cells genetically modified to express GSTP1 suffered less oxidative genome damage than LNCaP prostate cancer cells devoid of GSTP1 activity (DeWeese et al. unpublished data).

Remarkably, loss of *GSTP1* 'caretaker' function may provide a selective growth or survival advantage upon exposure to certain carcinogens. Usually, increased sensitivity to the genome damaging actions of chemical carcinogens, such as that seen upon metabolically activated PhIP treatment of LNCaP prostate cancer cells devoid of GSTP1 *versus* LNCaP cells expressing GSTP1, is accompanied by increased sensitivity to the cytotoxic effects of the same carcinogens [97]. However, new preliminary data have revealed that

when LNCaP prostate cancer cells, devoid of GSTP1 activity, are challenged by prolonged exposures to oxidant stresses, the cells appear to suffer much less cell death than LNCaP prostate cancer cells genetically modified to express GSTP1 (DeWeese et al. unpublished data). As a result, for LNCaP prostate cancer cells, loss of GSTP1 'caretaker' activity appeared to result in increased genome damage and decreased cell death upon oxidant exposure. Perhaps, this phenotype of oxidation damage 'tolerance' may provide PIN cells or prostate cancer cells a selective growth or survival advantage in the face of an oxidative stress like chronic prostate inflammation [26,43]. A similar phenotype of cell and genome damage 'tolerance' associated with loss of π-class GST activity has been reported for studies of mice exposed to acetaminophen overdoses, in which *Gstp1/2*$^{+/+}$ mice appeared to suffer markedly more hepatotoxicity than *Gstp1/2*$^{-/-}$ mice after administration of high doses of acetaminophen [108]. The mechanism(s) by which π-class GST activity might be coupled to cell death upon exposure to oxidants or to acetaminophen could potentially include glutathione 'bankruptcy' associated with π-class GST metabolism of reactive chemical species [108], toxification of the chemical species by π-class GST conjugation of the chemical species with glutathione [109], and/or potential modulation of stress-associated signal transduction pathways by π-class GSTs [110–113].

Thus, with loss of *GSTP1* 'caretaker' function as the initiating somatic genome lesion in the pathogenesis of prostate cancer, several features of the molecular pathogenesis of prostate cancer and several features of prostate cancer epidemiology can be integrated into a coherent model (Figure 4) [26]. In this model, the earliest steps in prostatic carcinogenesis occur as a result of prostatic inflammation, associated with prostatic infections or with idiopathic prostatitis, that leads to prostate cell oxidant injury and regeneration, resulting in the appearance of PIA lesions. The PIA cells, expressing high levels of GSTP1 and other oxidant protection enzymes, give rise to PIN cells, devoid of GSTP1, and ultimately to prostate cancer cells. With loss of *GSTP1* 'caretaker' function in the PIN cells and prostate cancer cells, decades of chronic oxidant stress, and of exposure to dietary heterocyclic amine and polycyclic aromatic hydrocarbons, lead to an accumulation of somatic genome alterations that drive malignant progression. Of interest, prostatic carcinogenesis may proceed via a similar pathway in rats, where both prostatic inflammation and heterocyclic aromatic amine exposures have been reported to lead to prostatic neoplasia [102,103,114,115].

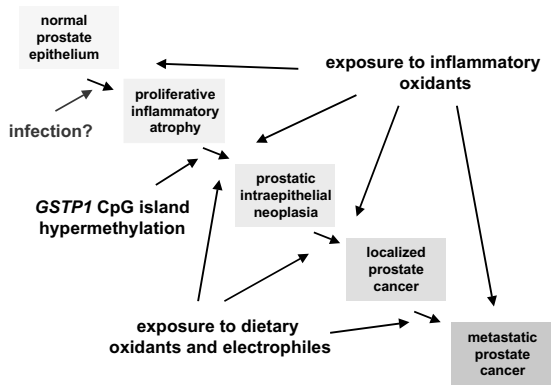

Figure 4. An integrated model for prostate cancer development featuring *GSTP1* inactivation in prostate cells in the face of exposure to inflammatory oxidants and dietary oxidants and electrophiles.

Rational strategies for prostate cancer prevention

To interrupt prostate cancer pathogenesis and prevent life-threatening prostate cancer, several rational approaches might be considered, including: (i) *attenuation* or *abrogation* of genome damaging stresses via avoidance of exogenous carcinogens (such as heterocyclic amines and polycyclic aromatic hydrocarbons) and/or reduction of endogenous carcinogenic stresses (such as inflammatory oxidants), (ii) *restoration* of *GSTP1* expression via treatment with inhibitors of CpG methylation, and (iii) *compensation* for inadequate GSTP1 activity via treatment with inducers of general GST activity. Some strategies for *attenuation* or *abrogation* of genome damaging stresses are already under evaluation for prostate cancer prevention. Antioxidant micronutrients, including vitamin E, selenium, and carotenoids, such as lycopene, might be expected to reduce oxidant damage to prostate cell DNA if used for prostate cancer prevention [73,74,116,117]. Each of these agents has reached human clinical studies. Selenium and vitamin E are to be tested in a large (n = 32,400) randomized trial (The Selenium and Vitamin E Chemoprevention Trial; SELECT) that is ongoing in the US to ascertain whether antioxidants selenium and vitamin E, alone or in combination, can reduce the incidence of prostate cancer in healthy men age 55 and older (age 50 and older for African–American men).

Early proof-of-principal clinical studies of lycopene, and/or tomato products, for prostate cancer have begun to be reported [118,119]. Anti-inflammatory agents might be expected to reduce inflammation-associated oxidant production in the prostate and reduce prostate cancer risks [120–122]. Several non-steroidal anti-inflammatory drugs are being examined as candidate drugs for prostate cancer prevention and/or treatment; 'proof-of-principle' clinical trials for prostate cancer are underway at Johns Hopkins featuring celecoxib (Celebrex®; Pharmacia) and sulindac.

Restoration of *GSTP1* function may be feasible as a prostate cancer prevention strategy (Figure 5). Silencing of *GSTP1* transcription in PIN and prostate cancer cells is likely maintained via the action of DNA methyltransferases, enzymes that could be targeted for therapeutic inhibition. We have collected data indicating that DNA methyltransferase inhibitors such as 5-aza-deoxycytidine and procainamide can restore GSTP1 expression in LNCaP prostate cancer cells *in vivo* [55]. These agents may be considered candidate prostate cancer prevention drugs. In addition, data have been reported revealing interactions both between $^{5-m}$C-binding proteins and histone deacetylases (HDACs), and between DNA methyltransferases and HDACs, in effecting transcriptional repression [60–62,123]. Perhaps, combinations of drugs active at inhibiting DNA methyltransferases and at inhibiting chromatin-remodeling enzymes might prove useful in restoring high level GSTP1 expression in PIN or prostate cancer cells to slow life-threatening prostate cancer progression [61]. The key issue for development of this approach, especially if it is to be used for prostate cancer prevention as well as for prostate cancer treatment, will be whether restoration of *GSTP1* expression (as well as the expression of other genes) can be accomplished with reasonable gene selectivity and with acceptable side effects. To this end, early phase I studies of combinations of the DNA methyltransferase inhibitor 5-aza-cytidine and the HDAC inhibitor phenylbutyrate have been initiated at Johns Hopkins.

Therapeutic *compensation* for inadequate 'caretaker' gene function may also hold promise for cancer prevention (Figure 6). Although *GSTP1* may be silenced early during the pathogenesis of prostate cancer, genes encoding other GST subunit polypeptides appear intact. As the genes encoding the other GST subunit polypeptides can be induced, via an Nrf2-dependent mechanism, this pathway can be exploited to better defend GSTP1-deficient prostate cells against injurious chemical stresses [93,94]. Augmentation of carcinogen-detoxification capacity, using a variety of such chemoprotective compounds, including isothiocyanates, 1,2-dithiole-3-thiones, terpenoids, etc., has been reported to prevent a variety of different cancers in different animal models by triggering the expression of carcinogen-detoxification enzymes [124]. Most or all of these compounds likely act to prevent cancer by activating carcinogen-detoxification enzyme gene expression via the Nrf2-dependent transcription induction pathway, as oltipraz, an anti-schistosomal 1,2-dithiole-3-thione compound known to protect against benzo[a]pyrene gastric carcinogenesis in murine models, had no effect on gastric tumor formation in mice carrying disrupted *Nrf2* alleles [95]. Induction of GST 'caretaker' activity in liver tissues, using oltipraz, a therapeutic inducer of GST activity, has been shown to reduce aflatoxin B_1

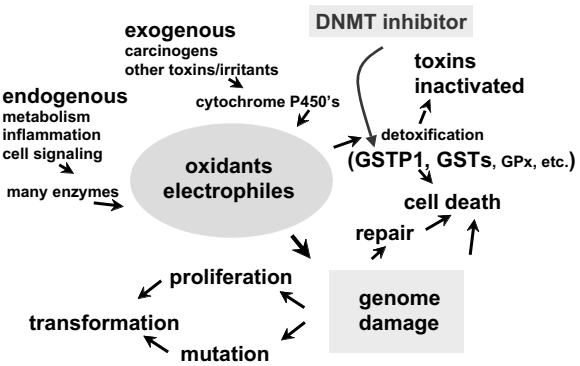

Figure 5. Therapeutic restoration of *GSTP1* function using DNA methyltransferase inhibitors.

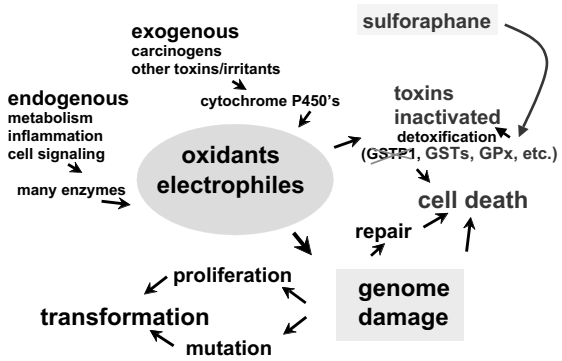

Figure 6. Therapeutic compensation for lack of GSTP1 activity using inducers of carcinogen detoxification enzymes, such as the isothiocyanate, sulforaphane, present in cruciferous vegetables.

damage when administered to a human clinical study cohort at high risk for aflatoxin exposure and liver cancer development in China [125–127]. Of greatest interest, many carcinogen-detoxification enzyme inducers have been detected in dietary components. For example, sulforaphane, an isothiocyanate that can trigger carcinogen-detoxification enzyme induction, is present in high amounts in cruciferous vegetables [79,87]. As described above, diets rich in carcinogen-inducers like sulforaphane have been associated with decreased cancer risks [77]. Early 'proof-of-principle' clinical trials of dietary carcinogen inducers as prevention for many different human cancers are currently underway [89,128].

Conclusions

Although both genes and the environment can contribute to the development of human prostate cancer, lifestyle factors, particularly the diet and possibly sexually transmitted diseases, likely play the dominant role in prostatic carcinogenesis. Loss of *GSTP1* 'caretaker' function, the earliest somatic genome alteration yet described for prostate cancer, appears to render prostate cells vulnerable to carcinogenic stresses. Heterocyclic amines, polycyclic aromatic hydrocarbons, and inflammatory oxidants, all carcinogens likely to be associated with high-risk prostate cancer diets and behaviors, are substrates for GSTP1 detoxification. In the absence of GSTP1 'caretaker' activity, chronic exposure to such carcinogens promotes an accumulation of somatic genome abnormalities, targeting oncogenes and tumor suppressor genes, that leads to life-threatening prostate cancer progression. Therapeutic strategies to *abrogate* genome damaging stresses, to *restore GSTP1* 'caretaker' function, or to *compensate* for inadequate *GSTP1* 'caretaker' gene function via induction of carcinogen-detoxification enzymes, might be expected to attenuate prostatic carcinogenesis.

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