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GSTP1 CpG Island Hypermethylation as a Molecular Biomarker for Prostate Cancer

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Abstract Somatic hypermethylation of CpG island sequences at *GSTP1*, the gene encoding the π-class glutathione S-transferase, appears to be characteristic of human prostatic carcinogenesis. To consider the potential utility of this epigenetic alteration as a biomarker for prostate cancer, we present here a comprehensive review of the literature describing somatic *GSTP1* changes in DNA from prostate cells and tissues. *GSTP1* CpG island hypermethylation has been detected in prostate cancer DNA using a variety of assay techniques, including (i) Southern blot analysis (SB), after treatment with $^{5\text{-m}}$C-sensitive restriction endonucleases, (ii) the polymerase chain reaction, following treatment with $^{5\text{-m}}$C-sensitive restriction endonucleases (RE-PCR), (iii) bisulfite genomic sequencing (BGS), and (iv) bisulfite modification followed by the polymerase chain reaction, using primers selective for target sequences containing $^{5\text{-m}}$C (MSP). In the majority of the case series so far reported, *GSTP1* CpG island hypermethylation was present in DNA from at least 90% of prostate cancer cases. When analyses have been carefully conducted, *GSTP1* CpG island hypermethylation has not been found in DNA from normal prostate tissues, or from benign prostatic hyperplasia (BPH) tissues, though *GSTP1* CpG island hypermethylation changes have been detected in DNA from candidate prostate cancer precursor lesions proliferative inflammatory atrophy (PIA) and prostatic intraepithelial neoplasia (PIN). Using PCR methods, *GSTP1* CpG island hypermethylation has also been detected in urine, ejaculate, and plasma from men with prostate cancer. *GSTP1* CpG island hypermethylation, a somatic epigenetic alteration, appears poised to serve as a molecular biomarker useful for prostate cancer screening, detection, and diagnosis. J. Cell. Biochem. 91: 540–552, 2004.

Key words: glutathione *S*-transferase; CpG island; DNA methylation; prostate cancer

THE NEED FOR NEW PROSTATE CANCER BIOMARKERS

Prostate cancer is the most commonly diagnosed cancer, and the second leading cause of cancer death, in men over the age of 40 years in the United States (US). An estimated 220,900 men in the US will be diagnosed with prostate cancer in 2003, accompanied by an estimated 28,900 prostate cancer deaths [Jemal et al., 2003]. Prostate cancer screening, using serum prostate-specific antigen (PSA) testing and digital rectal examination, clearly detects prostate cancer at an early stage, permitting more men with prostate cancer to be treated with curative intent using surgery or radiation therapy, though whether population screening reduces prostate cancer mortality has been debated [de Koning et al., 2002; Harris and Lohr, 2002; Frankel et al., 2003]. Nonetheless, the contribution of serum PSA testing to prostate cancer screening and early detection has been dramatic: the predictive value of an elevated serum PSA for prostate cancer is greater than that of digital rectal examination or any other clinical test [Cooner et al., 1990; Catalona et al., 1994; Ellis et al., 1994].

Men with an abnormally elevated serum PSA (or with an abnormal digital rectal examination)

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are typically subjected to prostate core needle biopsies, in which prostate tissues are systematically sampled to discern the presence or absence of prostate cancer. Traditionally, a serum PSA of 4 ng/ml has been used as the threshold or "cut-point" value to prompt prostate biopsy, though the use of lower threshold values has been proposed [Catalona et al., 1997b, 2000; Carter, 2000]. The difficulty is that for any given PSA threshold value, many men with prostate cancer will have serum PSA values below the threshold and not be diagnosed, while many men without prostate cancer will have serum PSA values above the threshold and be needlessly subjected to prostate biopsy procedures. The source of this difficulty is that an elevated serum PSA is not specific for prostate cancer. PSA, produced by prostatic epithelial cells in response to androgenic stimulation of the prostate, is normally secreted into the ejaculate. The appearance of PSA in the bloodstream, reflecting distortion of the normal glandular architecture of the prostate, occurs not only in men with prostate cancer, but also in men with infection and/or inflammation of the prostate or with benign prostatic hyperplasia (BPH) [Stamey et al., 1987]. Several new strategies for selectively detecting various molecular forms of PSA in the bloodstream ("free" PSA, "complexed" PSA, and "pro-PSA") are under development to improve the specificity of PSA testing for prostate cancer detection [Christensson et al., 1993; Mikolajczyk et al., 2000; Djavan et al., 2002]. However, new molecular biomarkers of prostate cancer, capable of improving both the sensitivity and specificity of prostate cancer detection, are desperately needed.

The accurate diagnosis of prostate cancer using systematic core needle biopsies can also be improved. Currently, trans-rectal ultrasound (TRUS) is used to ensure adequate sampling of prostate tissues by biopsies in men suspected to have prostate cancer based on serum PSA levels or a suspicious digital rectal examination. Most often, hypoechoic regions in the prostate are biopsied, along with systematic sampling of the peripheral zone of the prostate, where most significant cancers arise. The optimal biopsy strategy, including the number and placement of biopsies, that avoids missing significant cancers, remains controversial [Babaian et al., 2000; Terris, 2000]. Another challenge for prostate cancer diagnosis by core needle biopsy is that because only small amounts of prostate tissues are sampled by core needle biopsies, many prostate cancers can be difficult to diagnose by surgical pathologists. Tiny fragments of prostate cancer may appear as "small foci of atypical glands suspicious for, but not diagnostic of, prostate cancer" in a prostate biopsy; a variety of non-neoplastic conditions seen in prostate biopsies may mimick prostate cancer [Epstein and Yang, 2002; DeMarzo et al., 2003]. To aid in better recognizing prostate cancer when it is present in prostate biopsy specimens, a variety of immunohistochemistry tools have been developed and applied, including antibodies against cytokeratins, α-methyl-acyl-CoA racemase (AMACR), and p63 [Signoretti et al., 2000; Jiang et al., 2001; Parsons et al., 2001; Luo et al., 2002]. Unfortunately, none of these tools used alone provides a definitive prostate cancer diagnosis. Most pathologists use a combination of findings to make an accurate diagnosis of prostate cancer on a core needle biopsy specimen. Clearly, new biomarkers of prostate cancer, capable of distinguishing prostate cancer from other prostatic abnormalities, will aid in prostate cancer diagnosis.

In this review, the potential utility of somatic *GSTP1* CpG island hypermethylation change as a molecular biomarker for prostate cancer screening, detection, and diagnosis, is considered.

GSTP1 AND CARCINOGEN DETOXIFICATION

Glutathione *S*-transferases (GSTs) are a large family of enzymes that can detoxify reactive chemical species by catalyzing conjugation reactions with reduced glutathione [Hayes and Pulford, 1995]. The enzymes function as dimers composed of subunit polypeptides from four main classes: α, μ, π, and θ. *GSTP1* encodes the single π-class GST subunit polypeptide; GSTP1-1 is homo-dimeric enzyme. In most cell types, GST expression can be induced to high levels by exposure to reactive chemical species, a process involving increased *GST* gene transcription, mediated by activation of the transcriptional trans-activator Nrf2 via release from Keap1 complexes in the cytoplasm [Dinkova-Kostova et al., 2002]. This induction of GST activity likely prevents or attenuates the development of cancer upon exposure to carcinogens [Ramos-Gomez et al., 2001;

Thimmulappa et al., 2002]. A critical role for π-class GSTs as a barrier to cancer development is supported by studies of mice carrying disrupted *Gstp1/2* genes, which when compared to wild-type mice, display increased skin tumors upon topical exposure to the carcinogen 7,12 dimethylbenz[a]anthracene (DMBA) [Henderson et al., 1998].

Inducible GST activity also appears to protect against liver cancer development in response to carcinogen exposures. For example, when rats are treated with hepatocarcinogens, hyperplastic liver nodules composed of cells containing very high levels of the rat π-class GST, GST-P, appear [Farber and Cameron, 1980; Sato et al., 1984; Roomi et al., 1985; Satoh et al., 1985; Bannasch, 1986; Farber and Sarma, 1987]. The majority of these hyperplastic liver nodules ultimately regress and disappear, perhaps as a result of increased protection against further cell and genome damage afforded by high level GST-P expression. A few of the lesions progress to hepatocellular carcinoma, indicating that the high GST-P-expressing hyperplastic liver nodules are bona fide cancer precursor lesions. Rainbow trout (*Onchorhynchus mykiss*), which display defects in GST regulation in the liver following exposure to aflatoxin B_1 or 1,2-dimethylbenzanthracene, develop both high GST-expressing and low GST-expressing hyperplastic liver nodules upon carcinogen treatment, but only the low GST-expressing hyperplastic nodules appear to progress to hepatocellular carcinoma [Kirby et al., 1990]. Thus, inadequate GST expression in liver cancer precursor lesions likely increases the risk of progression to cancer [Hayes et al., 1990; Kirby et al., 1990]. Prophylactic induction of GST activity may protect against carcinogen damage in the liver. Oltipraz, a therapeutic inducer of GST activity, reduced aflatoxin B_1 damage when administered to a human clinical study cohort at high risk for aflatoxin exposure and liver cancer development in China [Kensler et al., 1998].

π-class GSTs may help detoxify heterocyclic amines, candidate dietary prostate carcinogens present in well-done or charred meats, in prostate cells [Nelson et al., 2001]. The best studied of these carcinogens, the heterocyclic aromatic amine 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), triggers mutations by adduction to DNA bases after metabolic activation by various cellular enzymes [Gross et al., 1993; Morgenthaler and Holzhauser, 1995; Knize et al., 1997]. For rats, PhIP is a prostate carcinogen: rats fed PhIP have been reported to accumulate mutations in prostate DNA and to develop prostate cancer [Shirai et al., 1997, 1999; Stuart et al., 2000]. Whether PhIP consumption leads to human prostate cancer development has not been established. Nonetheless, in human prostate cancer cells, GSTP1 activity has been found to provide protection against PhIP genotoxicity. When LNCaP human prostate cancer cells, known to be devoid of GSTP1, were exposed to metabolically-activated PhIP, high levels of PhIP–DNA adducts were detected, while LNCaP prostate cancer cells modified to express GSTP1 were resistant to the formation of pro-mutagenic PhIP–DNA adducts [Nelson et al., 2001].

SOMATIC *GSTP1* CpG ISLAND HYPERMETHYLATION AND TRANSCRIPTIONAL SILENCING

The self-complementary dinucleotide sequence CpG, which is under-represented in the human genome, frequently carries $^{5\text{-m}}$C, a modification that can be maintained through DNA replication via the action of DNA methyltransferases. CpG islands, clusters of CpG dinucleotides that do not carry $^{5\text{-m}}$C modifications, ranging in size from ~400–2,000 bp, encompass the transcriptional regulatory region of many genes [Bird, 1986]. Aberrant methylation of CpG dinucleotides in these CpG island sequences has emerged as one of the most common somatic genome alterations in human cancers [Jones and Baylin, 2002]. Hypermethylation of CpG island sequences leads to gene silencing by preventing gene transcription. The *GSTP1* CpG island (see Fig. 1), a region extending from a pentad $[ATAAA]_n$ repeat sequence located at −414 of the GSTP1 transcription start site to an area between +296 and +625, is unmethylated in all normal human cells and tissues [Millar et al., 2000]. However, in human prostate cancer cells, somatic *GSTP1* CpG island hypermethylation and loss of GSTP1 expression appears to be the most common and consistent genome abnormality [Lee et al., 1994a; Lin et al., 2001]. Somatic *GSTP1* CpG island hypermethylation has also been reported in >80% of hepatocellular carcinoma cases and ~30% of breast cancer cases [Esteller et al., 1998; Tchou et al., 2000].

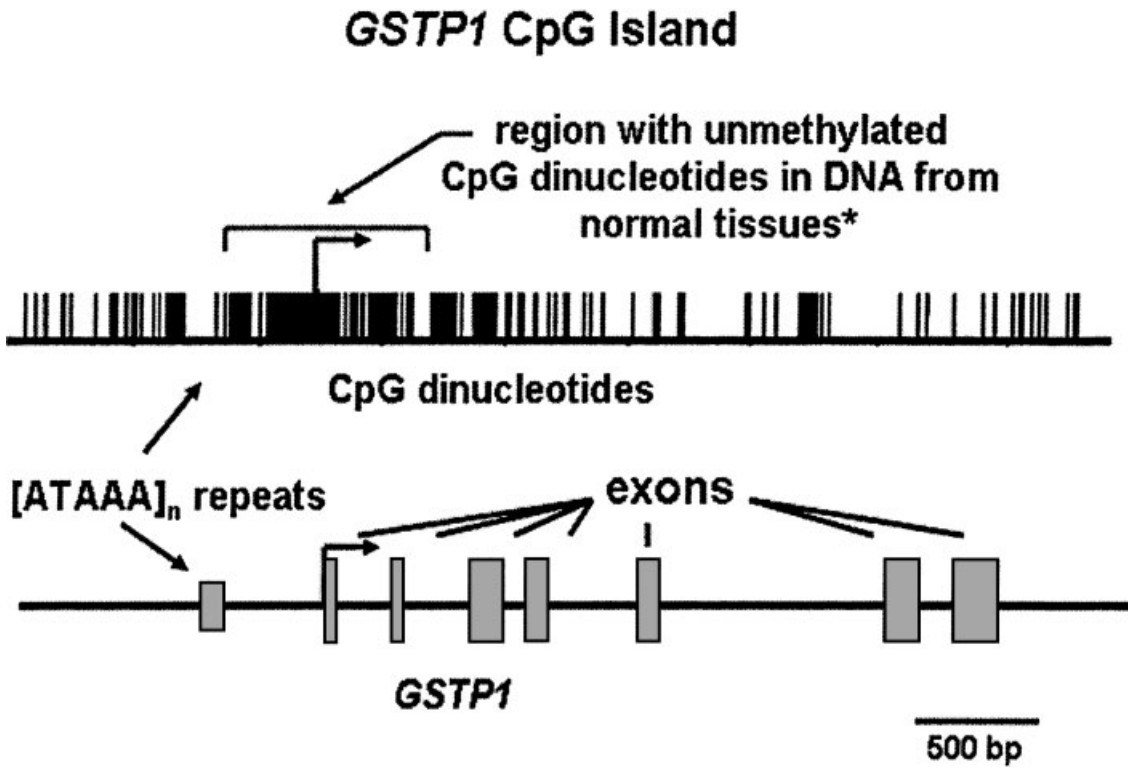

Fig. 1. The *GSTP1* CpG island (adapted from Millar et al., J Biol Chem 275:24893–24899, 2000).

The mechanism by which an accumulation of $^{5\text{-m}}$CpG dinucleotides in the *GSTP1* promoter region leads to inhibition of *GSTP1* transcription involves $^{5\text{-m}}$C-binding domain (MBD) family proteins. All MBD family proteins contain sequences similar to a 60–80 amino acid motif shown in MeCP2 to be responsible for $^{5\text{-m}}$CpG binding binding [Nan et al., 1993; Hendrich and Bird, 1998]. MeCP2, the first of these proteins to be identified, acts as a transcriptional *trans*-repressor through its interactions with Sin3A and histone deacetylases [Meehan et al., 1992; Jones et al., 1998; Nan et al., 1998]. However, MeCP2 does not appear to participate in the silencing of *GSTP1* carrying hypermethylated CpG island sequences in human cancer cells. Instead, MeCP1, a multicomponent transcriptional repression complex that contains MBD2,[144] is more likely responsible: in experiments using chromatin immunoprecipitation (ChIP) and siRNA "knock-down" analyses, MBD2 has been identified as a critical mediator of transcription repression associated with *GSTP1* CpG island hypermethylation (see Fig. 2) [Feng and Zhang, 2001; Bakker et al., 2002; Lin and Nelson, 2003]. In addition to MBD2, MeCP1 contains proteins, such as the SWI/SNF helicase Mi-2 and HDACs, that may contribute to transcription repression [Feng and Zhang, 2001].

ASSAYS FOR THE DETECTION OF SOMATIC CpG ISLAND HYPERMETHYLATION

A number of strategies for the detection of CpG island hypermethylation have been developed, including Southern blot (SB) analysis or

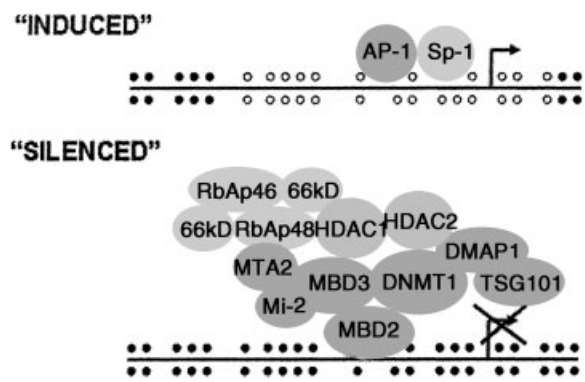

Fig. 2. *GSTP1* silencing mediated by recruitment of $^{5\text{-m}}$C-binding domain (MBD) family proteins to hypermethylated *GSTP1* CpG island alleles.

polymerase chain reaction amplification of DNA treated with $^{5\text{-m}}$C-sensitive restriction endonucleases (RE-PCR), bisulfite genomic sequencing (BGS) [Clark et al., 1994], and bisulfite modification of DNA followed by selective polymerase chain reaction amplification of target DNA sequences containing $^{5\text{-m}}$C (MS-PCR) [Herman et al., 1996]. Historically, SB analysis was the first assay systematically applied to the study of DNA methylation. More recently, PCR-based approaches, with greater sensitivity for $^{5\text{-m}}$CpG-containing DNA sequences, have more or less replaced SB analysis. RE-PCR is a very sensitive technique, capable of detecting a single hypermethylated CpG island allele [Lee et al., 1997]. However, this assay is prone to "false positive" detection of CpG island as incomplete destruction of unmethylated CpG island sequences by $^{5\text{-m}}$C-sensitive restriction endonucleases tends to lead to "false positive" detection of CpG island hypermethylation. Currently, the most popular approach used is MS-PCR [Herman et al., 1996]. In this assay, genomic DNA is subjected to treatment with bisulfite, which reacts with C bases in preference to $^{5\text{-m}}$C bases, facilitating the deamination of C to produce U while $^{5\text{-m}}$C remains unchanged. As a consequence, differences in DNA methylation become manifest as differences in DNA sequence. To selectively detect target CpG island DNA carrying $^{5\text{-m}}$CpG, PCR primers specific for target sequences resulting from bisulfite modification of $^{5\text{-m}}$CpG-containing DNA are used for PCR. Of importance, if the primers are appropriately designed, MS-PCR specifically detects CpG island alleles carrying $^{5\text{-m}}$CpG within the PCR primer annealing sites, leading to low "false-positive" detection of CpG island hypermethylation. However, CpG island alleles carrying $^{5\text{-m}}$CpG at various sites other than the primer annealing sites are often not detected, representing "false-negatives" (see Fig. 3). Both RE-PCR and MS-PCR can be performed using quantitative "real-time" PCR amplification methods. BGS is the only one of the commonly used DNA methylation assays which can discern the pattern of CpG dinucleotide methylation at target sequences in specific alleles. Nevertheless, because BGS has limited sensitivity for the detection of somatic CpG dinucleotide methylation changes, it is not under development as a clinical test.

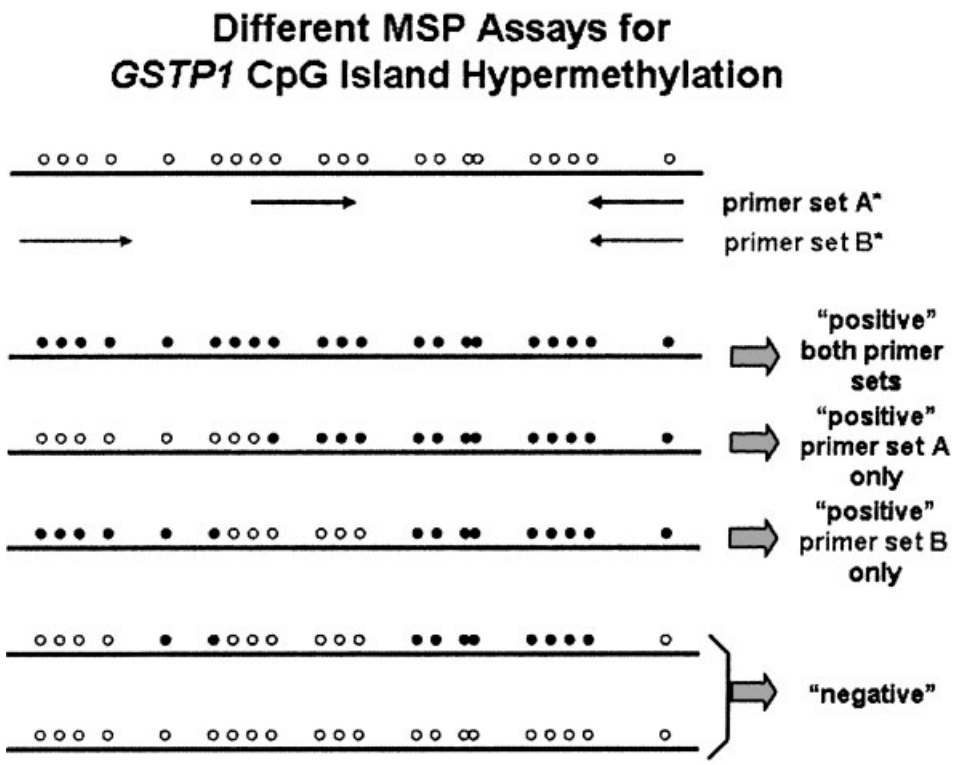

Fig. 3. Methylation-specific PCR (MSP or MS-PCR) is prone to ''false-negative'' detection of *GSTP1* CpG island hypermethylation.

GSTP1 CpG ISLAND HYPERMETHYLATION IN NORMAL AND NEOPLASTIC PROSTATE TISSUES

Several studies assessing *GSTP1* CpG island hypermethylation in prostate cancer, and other human cancers, have been reported (see Table I). In general, independent of the assay technique used, somatic *GSTP1* CpG island hypermethylation has been detected in DNA from >90% of prostate cancers and ~70% of prostatic intraepithelial neoplasia (PIN) lesions. In addition, *GSTP1* CpG island methylation changes have been found in >80% of liver cancers, ~30% of breast cancers, and 10% or less of other human cancers [Esteller et al., 1998; Tchou et al., 2000]. As described above, most of the *GSTP1* CpG island methylation assay techniques used in case series of prostate cancer are prone to "false-negatives." The fraction of prostate cancers harboring genomic DNA with *GSTP1* CpG island hypermethylation may actually be much higher than 90%. In the two case series that have featured BGS analyses of GSTP1 CpG island hypermethylation, unmethylated *GSTP1* CpG island alleles were only found in two prostate cancer cases exhibiting high level GSTP1 expression, and in one of these cases, one *GSTP1* allele carried *GSTP1* CpG island hypermethylation changes while the other did not [Millar et al., 1999; Lin et al., 2001]. GSTP1 expression has been detected in only 3% of prostate cancer cases or less [Lee et al., 1994b]. As proof that *GSTP1* CpG hypermethylation leads to gene silencing, all of the prostate cancer cases devoid of GSTP1 polypeptide expression that have been studied using BGS have exhibited *GSTP1* CpG island hypermethylation [Millar et al., 1999; Lin et al., 2001].

Some studies have claimed to have detected *GSTP1* CpG island hypermethylation in DNA from normal prostate tissues or from benign prostatic hyperplasia (BPH) tissues. For example, Jeronimo et al. [2001] found *GSTP1* CpG

TABLE I. *GSTP1* CpG Island Hypermethylation in DNA From Normal and Neoplastic Prostate Cells and Tissues

Study	Detection technique[a]	Specimen	Results[b] (% with *GSTP1* CpG island hypermethylation)			
			Normal (%)	BPH (%)	PIN (%)	PCA (%)
Lee et al. [Lee et al., 1994b]	SB	Tissue	3	0		100
Lee et al. [Lee et al., 1997]	RE-PCR	Tissue	8.1			91
Brooks et al. [Brooks et al., 1998]	RE-PCR	Tissue			70	
Esteller et al. [Esteller et al., 1998]	MS-PCR	Tissue				83
Santourlidis et al. [Santourlidis et al., 1999]	RE-PCR	Tissue				75
Millar et al. [Millar et al., 1999]	BGS	Tissue				83
Suh et al. [Suh et al., 2000]	RE-PCR	Ejaculate				44
Goessl et al. [Goessl et al., 2000]	MS-PCR	Tissue		0		94
		Plasma		0		72
		Ejaculate		0		50
Goessl et al. [Goessl et al., 2001b]	MS-PCR		Urine	2	29	73
Cairns et al. [Cairns et al., 2001]	MS-PCR	Tissue				79
		Urine				27
Lin et al. [Lin et al., 2001]	SB, RE-PCR	Tissue	0			95?
Goessl et al. [Goessl et al., 2001a]	MS-PCR	Tissue		0		90
		Plasma		0		72
		Ejaculate		0		50
		Urine		3		76
Jeronimo et al. [Jeronimo et al., 2001]	Q-MS-PCR	Tissue		29	54	91
Murayama et al. [Maruyama et al., 2002]	MS-PCR	Tissue	3			36
Goessl et al. [Goessl et al., 2002]	MS-PCR	Biopsy washing		0	67	70
Chu et al. [Chu et al., 2002]	RE-PCR	Tissue		7		100
Jeronimo et al. [Jeronimo et al., 2002]	MS-PCR	Plasma		0		36
		Urine		3.2		30
Harden et al. [Harden et al., 2003]	Q-MS-PCR	Tissue (biopsy)		0		73
Gonzalgo et al. [Gonzalgo et al., 2003b]	Q-MS-PCR	Urine (after biopsy)	33		67	58
Nakayama et al. [Nakayama et al., 2003]	MS-PCR	Tissue (LCM[c])	0	0	69	91
Gonzalgo et al. [Gonzalgo et al., 2003a]	MS-PCR	Prostatic secretions				86

[a]SB, southern blot analysis: RE-PCR, $^{5\text{-m}}$CpG-sensitive restriction enzyme-PCR; Q-RE-PCR, quantitative "real-time" $^{5\text{-m}}$CpG-sensitive restriction enzyme-PCR; BGS, bisulfite genomic sequencing; MS-PCR, methylation-specific PCR, Q-MS-PCR, quantitative "real-time" methylation-specific PCR.
[b]PCA, prostate adenocarcinoma; PIN, high-grade prostatic intraepithelial neoplasia; BPH, benign prostatic hyperplasia.
[c]LCM, laser capture micro-dissection.

island hypermethylation in DNA from 29% of BPH specimens. However, in these studies, whether the tissues also harbored prostate cancer cells or PIN cells was not carefully assessed. To assess this issue directly, Nakayama et al. undertook a study of *GSTP1* CpG island hypermethylation in the prostate using laser capture microdissection to selectively recover cells from normal prostatic epithelium, from PIN, from prostate cancer, and from proliferative inflammatory atrophy (PIA), a common lesion that may at times be a precursor prostate cancer, from 27 different prostates [Nakayama et al., 2003]. After microdissection, genomic DNA was subjected to MS-PCR assay for *GSTP1* CpG island hypermethylation. In this study (see Fig. 4), *GSTP1* hypermethylation was not detected at all in DNA from normal prostatic epithelium (0 of 48 regions) or from BPH (0 of 22 nodules). *GSTP1* hypermethylation was detected in DNA from 6% (4 of 64 lesions) of PIA, 69% of PIN (22 of 32 lesions), and 91% of prostate cancer (30 of 33 lesions) [Nakayama et al., 2003].

The mechanism by which cells carrying hypermethylated *GSTP1* CpG island alleles accumulate during prostatic carcinogenesis has not been determined. One possibility is that GSTP1 may serve as a "caretaker" gene for prostatic carcinogenesis analogous to the role of DNA mismatch repair genes in the pathogenesis of colorectal cancer [Kinzler and Vogelstein, 1997; DeWeese and Nelson, 2003]. Unlike oncogenes and tumor suppressor genes, which when altered often modulate cancer cell growth and aggressiveness, abnormal "caretaker" genes tend to increase the rates at which somatic genome alterations appear, increasing the likelihood that cancer will arise [Kinzler and Vogelstein, 1997; DeWeese and Nelson, 2003]. "Caretaker" genes identified so far encode carcinogen-detoxification enzymes (like *GSTP1*), DNA mismatch repair enzymes, DNA damage recognition and repair enzymes, and proteins responsible for maintaining chromosome integrity and/or the fidelity of chromosome segregation during DNA replication and cell division [DeWeese and Nelson, 2003]. Defects in such "caretaker" genes often arise early during cancer development, or can be inherited in the germline, and can render cells vulnerable to genome damage mediated by environmental carcinogens, facilitating cancer development in response to such exposures. Another possibility

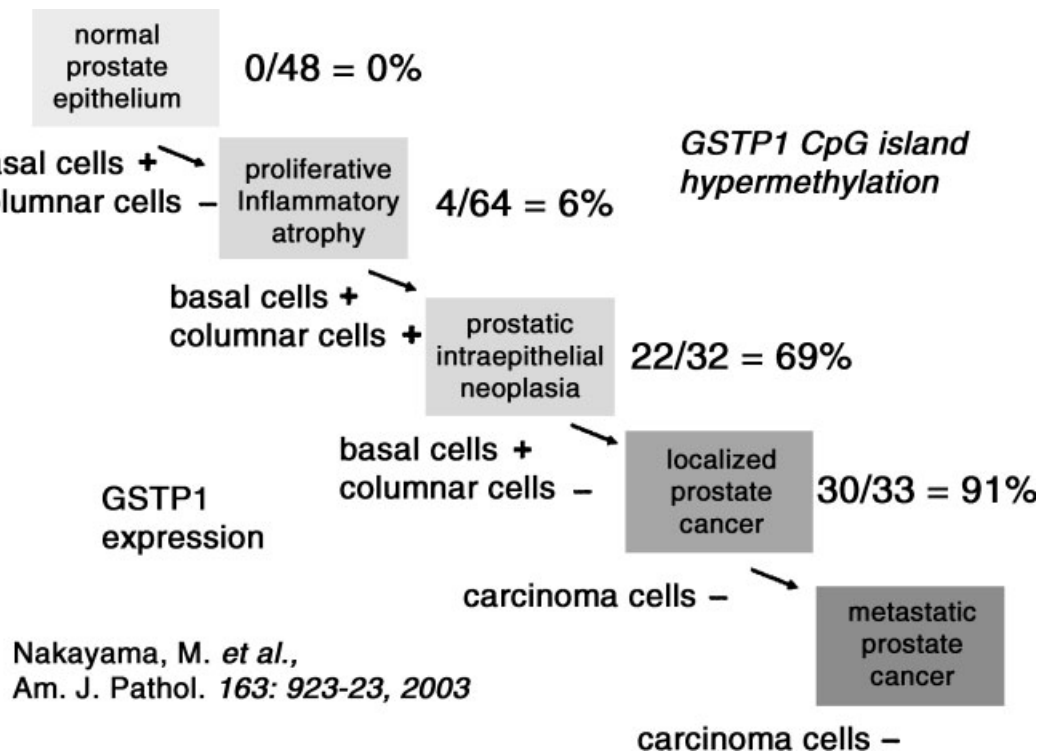

Fig. 4. Somatic GSTP1 CpG island hypermethylation accompanies prostatic carcinogenesis: results of a laser capture micro-dissection study (Nakayama et al. Am J Pathol 163:923–933, 2003).

to explain the accumulation of cells carrying hypermethylated *GSTP1* CpG island alleles during prostatic carcinogenesis is that GSTP1 may interfere with growth or survival signaling pathways, such as those activating N-terminal c-Jun kinase (JNK) [Adler et al., 1999; Ruscoe et al., 2001; Wang et al., 2001]. By this mechanism, it is possible that *GSTP1* may act as a tumor suppressor gene.

GSTP1 CpG ISLAND HYPERMETHYLATION AS A BIOMARKER FOR PROSTATE CANCER SCREENING AND DETECTION

To be useful for prostate cancer screening and early detection, assays for *GSTP1* CpG island hypermethylation must target readily available clinical specimens, such as peripheral blood, urine, ejaculate, or expressed prostatic secretions, and must have high sensitivity and specificity for prostate cancer when used to test such specimens. Analyses of DNA from prostate tissues suggest that only prostate cancers or prostate cancer precursor lesions contain hypermethylated *GSTP1* CpG island sequences, indicating a high specificity for prostatic carcinogenesis [Nakayama et al., 2003]. PCR-based detection strategies have proven to be extraordinarily sensitive at detecting DNA sequences containing *GSTP1* CpG island hypermethylation if present [Lee et al., 1997; Jeronimo et al., 2001]. The key determinant of assay specificity for prostate cancer (or prostate cancer precursor lesion) detection will likely be whether or not DNA sequences containing *GSTP1* CpG island hypermethylation are present in the clinical specimen of interest.

Peripheral blood specimens are easy to obtain and are the basis for most current prostate cancer screening and early detection, which feature serum PSA assays. Prostate cancer DNA, with GSTP1 CpG island hypermethylation changes, could appear in the peripheral blood as a result of (i) circulating prostate cancer cells contributing to prostate cancer metastases, of (ii) intravascular death of prostate cancer cells with release of free DNA or chromatin fragments, or of (iii) circulating phagocytic cells that have ingested prostate cancer cells. PCR methods have revealed that transcripts for prostate lineage-restricted genes, such as *PSA*, *hK2*, *PSMA*, and others, thought to be present in circulating prostate cancer cells, are commonly present in peripheral blood specimens from men with prostate cancer [Moreno et al., 1992; Katz et al., 1994; de la Taille et al., 1999; Shariat et al., 2002]. In addition, cell purification strategies have directly identified circulating prostate cancer cells in men with prostate cancer [Ts'o et al., 1997; Ellis et al., 2003]. Of course, DNA-based assays have several practical advantages over RNA-based or cell-based assays as clinical tests. Jeronimo et al. reported that *GSTP1* CpG island hypermethylation could be detected in plasma from some 36% of men with clinically localized prostate cancer [Jeronimo et al., 2002]. In another study, Goessl et al. claimed that DNA with *GSTP1* CpG island hypermethylation changes was present in plasma from 56% of men with stage $T_{2-3}N_0M_0$ prostate cancer and 93% of men with T_4, N_+, or M_+ prostate cancer [Goessl et al., 2000, 2001b]. Are the 36–56% of men with clinically localized prostate cancer and a "positive" test for DNA with *GSTP1* CpG island hypermethylation in plamsa at risk for prostate cancer recurrence or metastasis after surgery or radiation therapy? Prospective cohort studies, in which the correlation between plasma *GSTP1* CpG island hypermethylation and prostate cancer relapse or progression is tested, will need to be undertaken to address this question.

Urine, ejaculate, or expressed prostate fluid are likely obtainable from many men at risk for prostate cancer development. Presumably, prostate cancer DNA, with *GSTP1* CpG island hypermethylation changes, could appear in these specimens only via shedding of prostate cancer cells, or cell fragments, into prostatic ducts that communicate with the prostatic urethra. PIA and PIN lesions are entirely encompassed within prostatic ducts and might be expected to shed cells, with *GSTP1* CpG island hypermethylation changes into the prostatic urethra. However, prostate cancers tend to invade out of the prostatic ducts. Whether prostate cancers cells or cell fragments also appear in secretions from prostatic ducts was recently tested by Gonzalgo et al. [2003a], who reported that hypermethylated *GSTP1* CpG island sequences could be detected in secretions collected from 86% of radical prostatectomy specimens from men with prostate cancer. The abnormal DNA in these secretions may have come from prostate cancer cells, or from PIN cells, shed into prostate ducts. There is a high concordance between the presence of PIN and prostate cancer in prostate tissues [Sakr et al.,

1994]. Goessl et al. have found that some 76% of men with prostate cancer shed DNA with *GSTP1* CpG island hypermethylation into the urine after prostate massage expression of prostate secretions [Goessl et al., 2001b]. Spot urine samples collected without prostate massage from men with prostate cancer may be less likely (~30%) to contain prostate cancer DNA [Jeronimo et al., 2002]. Ejaculate specimens from men with prostate cancer have been found to carry DNA with hypermethylated *GSTP1* CpG island sequences in 44–50% of cases [Goessl et al., 2000, 2001a; Suh et al., 2000]. The predictive value of *GSTP1* CpG island hypermethylation assays for urine or ejaculate has not been assessed in prospective studies. Also, the specificity of urine *GSTP1* CpG island hypermethylation testing for prostate cancer may be undermined slightly be the propensity for as many as 20% of renal cell carcinoma cases to carry hypermethylated *GSTP1* CpG island alleles [Esteller et al., 1998].

GSTP1 CpG ISLAND HYPERMETHYLATION AS A BIOMARKER TO AID IN PROSTATE CANCER DIAGNOSIS

The diagnosis of prostate cancer using core needle biopsy specimens can be challenging for surgical pathologists, as there are many conditions that can mimick the histological appearance of prostate cancer. Because *GSTP1* CpG island hypermethylation changes are present only in prostate cancers, PIN lesions, and a small fraction of PIA lesions, assays for hypermethylated *GSTP1* CpG island alleles in DNA from tissue specimens might aid pathologists in establishing an accurate diagnosis [Nakayama et al., 2003]. Also, because prostate cancers are detected by a repeat biopsy procedure in as many as 30% of men without a prostate cancer diagnosis on an initial biopsy, highly sensitive *GSTP1* CpG island hypermethylation assays used at the time of the first biopsy might identify men harboring prostate cancer who might otherwise be missed [Catalona et al., 1997a; Chon et al., 2002]. Concomitantly, if *GSTP1* CpG island hypermethylation had a high negative predictive value, as many as 70% of men could be safely spared a second biopsy procedure.

Goessl et al. [2002] used MS-PCR to detect *GSTP1* CpG island hypermethylation in DNA from core biopsy needle washes, obtained by rinsing biopsy specimens in isotonic saline solutions. Via this approach *GSTP1* CpG island hypermethylation was detected in 0% of men without prostate cancer, 67% of men with PIN, and 70% of men with cancer. Harden et al. [2003] prepared DNA from paraffin-embedded prostate biopsy specimens and found that 0% of men without prostate cancer versus 73% of men with prostate cancer had hypermethylated GSTP1 CpG island alleles. Ideally, if a *GSTP1* CpG island hypermethylation assay could be adapted for use on tissue sections in situ, in such a way that histological appearance and DNA methylation changes could be assessed simultaneously, such an assay might serve as a more effective adjunctive tool for surgical pathologists [Nuovo et al., 1999]. Nonetheless, Gonzalgo et al. examined hypermethylation of the *GSTP1* CpG island in DNA from urine collected immediately after prostate biopsy, comparing the results of methylation assays to the histological diagnosis and finding *GSTP1* CpG island hypermethylation in 67% of men with PIN and 58% of men with prostate cancer [Gonzalgo et al., 2003b]. 33% of men without prostate cancer or PIN also exhibited *GSTP1* CpG island hypermethylation in post-biopsy urine DNA. For two of the men with hypermethylated *GSTP1* CpG island sequences in post-biopsy urine specimens but no clear prostate cancer diagnosis, a subsequent prostate biopsy procedure resulted in a prostate cancer diagnosis. An estimated 20–36% men without prostate cancer on an initial biopsy will have cancer detected on a subsequent biopsy [Catalona et al., 1997a; Chon et al., 2002]. Is it possible that men with missed prostate cancers at the time of initial prostate biopsy can be identified via *GSTP1* CpG island hypermethylation testing of post-biopsy urine specimens, providing an indication for early repeat biopsy procedures? Prospectively collected data are needed for the answer.

CONCLUSIONS

A somatic epigenetic change, hypermethylation of CpG island sequences encompassing the regulatory region of *GSTP1*, almost uniformly accompanies prostatic carcinogenesis. This genome alteration leads to gene silencing, mediated by the MBD family protein MBD2, and a phenotype of vulnerability to genome damaging species. New PCR-based methods to

specifically detect hypermethylated *GSTP1* CpG island sequences have great promise as molecular biomarkers for prostate cancer and PIN. When these assays are applied to clinical specimens, such as blood, urine, ejaculate, and prostate secretions for prostate cancer screening and early detection, or such as prostate biopsy specimens for aid in prostate cancer diagnosis, the have exhibited great promise as candidate clinical tests. A new series of prospective studies, critically assessing the predictive value of such tests, are needed.

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REFERENCES

Adler V, Yin Z, Fuchs SY, Benezra M, Rosario L, Tew KD, Pincus MR, Sardana M, Henderson CJ, Wolf CR, Davis RJ, Ronai Z. 1999. Regulation of JNK signaling by GSTp. Embo J 18:1321–1334.

Babaian RJ, Toi A, Kamoi K, Troncoso P, Sweet J, Evans R, Johnston D, Chen M. 2000. A comparative analysis of sextant and an extended 11-core multisite directed biopsy strategy. J Urol 163:152–157.

Bakker J, Lin X, Nelson WG. 2002. Methyl-CpG binding domain protein 2 represses transcription from hypermethylated pi-class glutathione S-transferase gene promoters in hepatocellular carcinoma cells. J Biol Chem 277:22573–22580.

Bannasch P. 1986. Preneoplastic lesions as end points in carcinogenicity testing. I. Hepatic preneoplasia. Carcinogenesis 7:689–695.

Bird AP. 1986. CpG-rich islands and the function of DNA methylation. Nature 321:209–213.

Brooks JD, Weinstein M, Lin X, Sun Y, Pin SS, Bova GS, Epstein JI, Isaacs WB, Nelson WG. 1998. CG island methylation changes near the *GSTP1* gene in prostatic intraepithelial neoplasia. Cancer Epidemiol Biomarkers Prev 7:531–536.

Cairns P, Esteller M, Herman JG, Schoenberg M, Jeronimo C, Sanchez-Cespedes M, Chow NH, Grasso M, Wu L, Westra WB, Sidransky D. 2001. Molecular detection of prostate cancer in urine by GSTP1 hypermethylation. Clin Cancer Res 7:2727–2730.

Carter HB. 2000. A PSA threshold of 4.0 ng/ml for early detection of prostate cancer: The only rational approach for men 50 years old and older. Urology 55:796–799.

Catalona WJ, Richie JP, Ahmann FR, Hudson MA, Scardino PT, Flanigan RC, deKernion JB, Ratliff TL, Kavoussi LR, Dalkin BL, et al. 1994. Comparison of digital rectal examination and serum prostate specific antigen in the early detection of prostate cancer: Results of a multicenter clinical trial of 6,630 men. J Urol 151: 1283–1290.

Catalona WJ, Beiser JA, Smith DS. 1997a. Serum free prostate specific antigen and prostate specific antigen density measurements for predicting cancer in men with prior negative prostatic biopsies. J Urol 158:2162–2167.

Catalona WJ, Smith DS, Ornstein DK. 1997b. Prostate cancer detection in men with serum PSA concentrations of 2.6 to 4.0 ng/ml and benign prostate examination. Enhancement of specificity with free PSA measurements. JAMA 277:1452–1455.

Catalona WJ, Ramos CG, Carvalhal GF, Yan Y. 2000. Lowering PSA cutoffs to enhance detection of curable prostate cancer. Urology 55:791–795.

Chon CH, Lai FC, McNeal JE, Presti JC, Jr. 2002. Use of extended systematic sampling in patients with a prior negative prostate needle biopsy. J Urol 167:2457–2460.

Christensson A, Bjork T, Nilsson O, Dahlen U, Matikainen MT, Cockett AT, Abrahamsson PA, Lilja H. 1993. Serum prostate specific antigen complexed to alpha 1-antichymotrypsin as an indicator of prostate cancer. J Urol 150:100–105.

Chu DC, Chuang CK, Fu JB, Huang HS, Tseng CP, Sun CF. 2002. The use of real-time quantitative polymerase chain reaction to detect hypermethylation of the CpG islands in the promoter region flanking the *GSTP1* gene to diagnose prostate carcinoma. J Urol 167:1854–1858.

Clark SJ, Harrison J, Paul CL, Frommer M. 1994. High sensitivity mapping of methylated cytosines. Nucleic Acids Res 22:2990–2997.

Cooner WH, Mosley BR, Rutherford CL, Jr., Beard JH, Pond HS, Terry WJ, Igel TC, Kidd DD. 1990. Prostate cancer detection in a clinical urological practice by ultrasonography, digital rectal examination, and prostate specific antigen. J Urol 143:1146–1152 (discussion).

de Koning HJ, Auvinen A, Berenguer Sanchez A, Calais da Silva F, Ciatto S, Denis L, Gohagan JK, Hakama M, Hugosson J, Kranse R, Nelen V, Prorok PC, Schroder FH. 2002. Large-scale randomized prostate cancer screening trials: Program performances in the European Randomized Screening for Prostate Cancer trial and the prostate, lung, colorectal, and ovary cancer trial. Int J Cancer 97:237–244.

de la Taille A, Olsson CA, Buttyan R, Benson MC, Bagiella E, Cao Y, Burchardt M, Chopin DK, Katz AE. 1999. Blood-based reverse transcriptase polymerase chain reaction assays for prostatic specific antigen: Long term follow-up confirms the potential utility of this assay in identifying patients more likely to have biochemical recurrence (rising PSA) following radical prostatectomy. Int J Cancer 84:360–364.

DeMarzo AM, Nelson WG, Isaacs WB, Epstein JI. 2003. Pathological and molecular aspects of prostate cancer. Lancet 361:955–964.

DeWeese TL, Nelson WG. 2003. Inadequate "caretaker" gene function and human cancer development. Methods Mol Biol 222:249–268.

Dinkova-Kostova AT, Holtzclaw WD, Cole RN, Itoh K, Wakabayashi N, Katoh Y, Yamamoto M, Talalay P. 2002. Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. Proc Natl Acad Sci USA 99:11908–11913.

Djavan B, Remzi M, Zlotta AR, Ravery V, Hammerer P, Reissigl A, Dobronski P, Kaisary A, Marberger M. 2002. Complexed prostate-specific antigen, complexed prostate-specific antigen density of total and transition zone, complexed/total prostate-specific antigen ratio, free-to-total

prostate-specific antigen ratio, density of total and transition zone prostate-specific antigen: Results of the prospective multicenter European trial. Urology 60:4–9.

Ellis WJ, Chetner MP, Preston SD, Brawer MK. 1994. Diagnosis of prostatic carcinoma: The yield of serum prostate specific antigen, digital rectal examination, and transrectal ultrasonography. J Urol 152:1520–1525.

Ellis WJ, Pfitzenmaier J, Colli J, Arfman E, Lange PH, Vessella RL. 2003. Detection and isolation of prostate cancer cells from peripheral blood and bone marrow. Urology 61:277–281.

Epstein JI, Yang XJ. 2002. Prostate biopsy interpretation. Philadelphia: Lippincott Williams & Wilkins.

Esteller M, Corn PG, Urena JM, Gabrielson E, Baylin SB, Herman JG. 1998. Inactivation of glutathione S-transferase P1 gene by promoter hypermethylation in human neoplasia. Cancer Res 58:4515–4518.

Farber E, Cameron R. 1980. The sequential analysis of cancer development. Adv Cancer Res 31:125–226.

Farber E, Sarma DS. 1987. Hepatocarcinogenesis: A dynamic cellular perspective. Lab Invest 56:4–22.

Feng Q, Zhang Y. 2001. The MeCP1 complex represses transcription through preferential binding, remodeling, and deacetylating methylated nucleosomes. Genes Dev 15:827–832.

Frankel S, Smith GD, Donovan J, Neal D. 2003. Screening for prostate cancer. Lancet 361:1122–1128.

Goessl C, Krause H, Muller M, Heicappell R, Schrader M, Sachsinger J, Miller K. 2000. Fluorescent methylation-specific polymerase chain reaction for DNA-based detection of prostate cancer in bodily fluids. Cancer Res 60: 5941–5945.

Goessl C, Muller M, Heicappell R, Krause H, Miller K. 2001a. DNA-based detection of prostate cancer in blood, urine, and ejaculates. Ann NY Acad Sci 945:51–58.

Goessl C, Muller M, Heicappell R, Krause H, Straub B, Schrader M, Miller K. 2001b. DNA-based detection of prostate cancer in urine after prostatic massage. Urology 58:335–338.

Goessl C, Muller M, Heicappell R, Krause H, Schostak M, Straub B, Miller K. 2002. Methylation-specific PCR for detection of neoplastic DNA in biopsy washings. J Pathol 196:331–334.

Gonzalgo ML, Nakayama M, Lee SM, De Marzo AM, Nelson WG. 2003a. Detection of GSTP1 methylation in prostatic secretions using combinatorial MSP analysis. Urology (in press).

Gonzalgo ML, Pavlovich CP, Lee SM, Nelson WG. 2003b. Prostate cancer detection by GSTP1 methylation analysis of postbiopsy urine specimens. Clin Cancer Res 9: 2673–2677.

Gross GA, Turesky RJ, Fay LB, Stillwell WG, Skipper PL, Tannenbaum SR. 1993. Heterocyclic aromatic amine formation in grilled bacon, beef, and fish and in grill scrapings. Carcinogenesis 14:2313–2318.

Harden SV, Guo Z, Epstein JI, Sidransky D. 2003. Quantitative GSTP1 methylation clearly distinguishes benign prostatic tissue and limited prostate adenocarcinoma. J Urol 169:1138–1142.

Harris R, Lohr KN. 2002. Screening for prostate cancer: An update of the evidence for the U.S. Preventive Services Task Force. Ann Intern Med 137:917–929.

Hayes JD, Pulford DJ. 1995. The glutathione S-transferase supergene family: Regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit Rev Biochem Mol Biol 30:445–600.

Hayes MA, Smith IR, Rushmore TH, Crane TL, Thorn C, Kocal TE, Ferguson HW. 1990. Pathogenesis of skin and liver neoplasms in white suckers from industrially polluted areas in Lake Ontario. Sci Total Environ 94: 105–123.

Henderson CJ, Smith AG, Ure J, Brown K, Bacon EJ, Wolf CR. 1998. Increased skin tumorigenesis in mice lacking pi class glutathione S-transferases. Proc Natl Acad Sci USA 95:5275–5280.

Hendrich B, Bird A. 1998. Identification and characterization of a family of mammalian methyl-CpG binding proteins. Mol Cell Biol 18:6538–6547.

Herman JG, Graff JR, Myohanen S, Nelkin BD, Baylin SB. 1996. Methylation-specific PCR: A novel PCR assay for methylation status of CpG islands. Proc Natl Acad Sci USA 93:9821–9826.

Jemal A, Murray T, Samuels A, Ghafoor A, Ward E, Thun MJ. 2003. Cancer statistics, 2003. CA Cancer J Clin 53: 5–26.

Jeronimo C, Usadel H, Henrique R, Oliveira J, Lopes C, Nelson WG, Sidransky D. 2001. Quantitation of GSTP1 methylation in non-neoplastic prostatic tissue and organ-confined prostate adenocarcinoma. J Natl Cancer Inst 93:1747–1752.

Jeronimo C, Usadel H, Henrique R, Silva C, Oliveira J, Lopes C, Sidransky D. 2002. Quantitative GSTP1 hypermethylation in bodily fluids of patients with prostate cancer. Urology 60:1131–1135.

Jiang Z, Woda BA, Rock KL, Xu Y, Savas L, Khan A, Pihan G, Cai F, Babcook JS, Rathanaswami P, Reed SG, Xu J, Fanger GR. 2001. P504S: A new molecular marker for the detection of prostate carcinoma. Am J Surg Pathol 25:1397–1404.

Jones PA, Baylin SB. 2002. The fundamental role of epigenetic events in cancer. Nat Rev Genet 3:415–428.

Jones PL, Veenstra GJ, Wade PA, Vermaak D, Kass SU, Landsberger N, Strouboulis J, Wolffe AP. 1998. Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. Nat Genet 19:187–191.

Katz AE, Olsson CA, Raffo AJ, Cama C, Perlman H, Seaman E, O'Toole KM, McMahon D, Benson MC, Buttyan R. 1994. Molecular staging of prostate cancer with the use of an enhanced reverse transcriptase-PCR assay. Urology 43:765–775.

Kensler TW, He X, Otieno M, Egner PA, Jacobson LP, Chen B, Wang JS, Zhu YR, Zhang BC, Wang JB, Wu Y, Zhang QN, Qian GS, Kuang SY, Fang X, Li YF, Yu LY, Prochaska HJ, Davidson NE, Gordon GB, Gorman MB, Zarba A, Enger C, Munoz A, Helzlsouer KJ, et al. 1998. Oltipraz chemoprevention trial in Qidong, People's Republic of China: Modulation of serum aflatoxin albumin adduct biomarkers. Cancer Epidemiol Biomarkers Prev 7:127–134.

Kinzler KW, Vogelstein B. 1997. Cancer-susceptibility genes. Gatekeepers and caretakers [news; comment] [see comments]. Nature 386:761, 763.

Kirby GM, Stalker M, Metcalfe C, Kocal T, Ferguson H, Hayes MA. 1990. Expression of immunoreactive glutathione S-transferases in hepatic neoplasms induced by aflatoxin B1 or 1,2-dimethylbenzanthracene in rainbow

trout (*Oncorhynchus mykiss*). Carcinogenesis 11:2255–2257.

Knize MG, Salmon CP, Mehta SS, Felton JS. 1997. Analysis of cooked muscle meats for heterocyclic aromatic amine carcinogens. Mutat Res 376:129–134.

Lee WH, Morton RA, Epstein JI, Brooks JD, Campbell PA, Bova GS, Hsieh WS, Isaacs WB, Nelson WG. 1994a. Cytidine methylation of regulatory sequences near the pi-class glutathione S-transferase gene accompanies human prostatic carcinogenesis. Proc Natl Acad Sci USA 91:11733–11737.

Lee WH, Morton RA, Epstein JI, Brooks JD, Campbell PA, Bova GS, Hsieh WS, Isaacs WB, Nelson WG. 1994b. Cytidine methylation of regulatory sequences near the pi-class glutathione S-transferase gene accompanies human prostatic carcinogenesis. Proc Natl Acad Sci USA 91:11733–11737.

Lee WH, Isaacs WB, Bova GS, Nelson WG. 1997. CG island methylation changes near the *GSTP1* gene in prostatic carcinoma cells detected using the polymerase chain reaction: A new prostate cancer biomarker. Cancer Epidemiol Biomarkers Prev 6:443–450.

Lin X, Nelson WG. 2003. Methyl-CpG-binding domain protein-2 mediates transcriptional repression associated with hypermethylated GSTP1 CpG islands in MCF-7 breast cancer cells. Cancer Res 63:498–504.

Lin X, Tascilar M, Lee WH, Vles WJ, Lee BH, Veeraswamy R, Asgari K, Freije D, van Rees B, Gage WR, Bova GS, Isaacs WB, Brooks JD, DeWeese TL, De Marzo AM, Nelson WG. 2001. GSTP1 CpG island hypermethylation is responsible for the absence of GSTP1 expression in human prostate cancer cells. Am J Pathol 159:1815–1826.

Luo J, Zha S, Gage WR, Dunn TA, Hicks JL, Bennett CJ, Ewing CM, Platz EA, Ferdinandusse S, Wanders RJ, Trent JM, Isaacs WB, De Marzo AM. 2002. Alpha-methylacyl-CoA racemase: A new molecular marker for prostate cancer. Cancer Res 62:2220–2226.

Maruyama R, Toyooka S, Toyooka KO, Virmani AK, Zochbauer-Muller S, Farinas AJ, Minna JD, McConnell J, Frenkel EP, Gazdar AF. 2002. Aberrant promoter methylation profile of prostate cancers and its relationship to clinicopathological features. Clin Cancer Res 8:514–519.

Meehan RR, Lewis JD, Bird AP. 1992. Characterization of MeCP2, a vertebrate DNA binding protein with affinity for methylated DNA. Nucleic Acids Res 20:5085–5092.

Mikolajczyk SD, Millar LS, Wang TJ, Rittenhouse HG, Marks LS, Song W, Wheeler TM, Slawin KM. 2000. A precursor form of prostate-specific antigen is more highly elevated in prostate cancer compared with benign transition zone prostate tissue. Cancer Res 60:756–759.

Millar DS, Ow KK, Paul CL, Russell PJ, Molloy PL, Clark SJ. 1999. Detailed methylation analysis of the glutathione S-transferase pi (*GSTP1*) gene in prostate cancer. Oncogene 18:1313–1324.

Millar DS, Paul CL, Molloy PL, Clark SJ. 2000. A distinct sequence (ATAAA)n separates methylated and unmethylated domains at the 5′-end of the GSTP1 CpG island. J Biol Chem 275:24893–24899.

Moreno JG, Croce CM, Fischer R, Monne M, Vihko P, Mulholland SG, Gomella LG. 1992. Detection of hematogenous micrometastasis in patients with prostate cancer. Cancer Res 52:6110–6112.

Morgenthaler PM, Holzhauser D. 1995. Analysis of mutations induced by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in human lymphoblastoid cells. Carcinogenesis 16:713–718.

Nakayama M, Bennett CJ, Hicks JL, Epstein JI, Platz EA, Nelson WG, De Marzo AM. 2003. Hypermethylation of the human glutathione S-transferase-pi gene (*GSTP1*) CpG island is present in a subset of proliferative inflammatory atrophy lesions but not in normal or hyperplastic epithelium of the prostate: A detailed study using laser-capture microdissection. Am J Pathol 163: 923–933.

Nan X, Meehan RR, Bird A. 1993. Dissection of the methyl-CpG binding domain from the chromosomal protein MeCP2. Nucleic Acids Res 21:4886–4892.

Nan X, Ng HH, Johnson CA, Laherty CD, Turner BM, Eisenman RN, Bird A. 1998. Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. Nature 393:386–389.

Nelson CP, Kidd LC, Sauvageot J, Isaacs WB, De Marzo AM, Groopman JD, Nelson WG, Kensler TW. 2001. Protection against 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-b]pyridine cytotoxicity and DNA adduct formation in human prostate by glutathione S-transferase P1. Cancer Res 61:103–109.

Nuovo GJ, Plaia TW, Belinsky SA, Baylin SB, Herman JG. 1999. In situ detection of the hypermethylation-induced inactivation of the *p16* gene as an early event in oncogenesis. Proc Natl Acad Sci USA 96:12754–12759.

Parsons JK, Gage WR, Nelson WG, De Marzo AM. 2001. p63 protein expression is rare in prostate adenocarcinoma: Implications for cancer diagnosis and carcinogenesis. Urology 58:619–624.

Ramos-Gomez M, Kwak MK, Dolan PM, Itoh K, Yamamoto M, Talalay P, Kensler TW. 2001. From the cover: Sensitivity to carcinogenesis is increased and chemoprotective efficacy of enzyme inducers is lost in nrf2 transcription factor-deficient mice. Proc Natl Acad Sci USA 98:3410–3415.

Roomi MW, Ho RK, Sarma DS, Farber E. 1985. A common biochemical pattern in preneoplastic hepatocyte nodules generated in four different models in the rat. Cancer Res 45:564–571.

Ruscoe JE, Rosario LA, Wang T, Gate L, Arifoglu P, Wolf CR, Henderson CJ, Ronai Z, Tew KD. 2001. Pharmacologic or genetic manipulation of glutathione S-transferase P1-1 (GSTpi) influences cell proliferation pathways. J Pharmacol Exp Ther 298:339–345.

Sakr WA, Grignon DJ, Crissman JD, Heilbrun LK, Cassin BJ, Pontes JJ, Haas GP. 1994. High grade prostatic intraepithelial neoplasia (HGPIN) and prostatic adenocarcinoma between the ages of 20–69: An autopsy study of 249 cases. In Vivo 8:439–443.

Santourlidis S, Florl A, Ackermann R, Wirtz HC, Schulz WA. 1999. High frequency of alterations in DNA methylation in adenocarcinoma of the prostate. Prostate 39:166–174.

Sato K, Kitahara A, Satoh K, Ishikawa T, Tatematsu M, Ito N. 1984. The placental form of glutathione S-transferase as a new marker protein for preneoplasia in rat chemical hepatocarcinogenesis. Gann 75:199–202.

Satoh K, Kitahara A, Soma Y, Inaba Y, Hatayama I, Sato K. 1985. Purification, induction, and distribution of placental glutathione transferase: A new marker enzyme for

preneoplastic cells in the rat chemical hepatocarcinogenesis. Proc Natl Acad Sci USA 82:3964–3968.

Shariat SF, Gottenger E, Nguyen C, Song W, Kattan MW, Andenoro J, Wheeler TM, Spencer DM, Slawin KM. 2002. Preoperative blood reverse transcriptase-PCR assays for prostate-specific antigen and human glandular kallikrein for prediction of prostate cancer progression after radical prostatectomy. Cancer Res 62:5974–5979.

Shirai T, Sano M, Tamano S, Takahashi S, Hirose M, Futakuchi M, Hasegawa R, Imaida K, Matsumoto K, Wakabayashi K, Sugimura T, Ito N. 1997. The prostate: A target for carcinogenicity of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) derived from cooked foods. Cancer Res 57:195–198.

Shirai T, Cui L, Takahashi S, Futakuchi M, Asamoto M, Kato K, Ito N. 1999. Carcinogenicity of 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine (PhIP) in the rat prostate and induction of invasive carcinomas by subsequent treatment with testosterone propionate. Cancer Lett 143:217–221.

Signoretti S, Waltregny D, Dilks J, Isaac B, Lin D, Garraway L, Yang A, Montironi R, McKeon F, Loda M. 2000. p63 is a prostate basal cell marker and is required for prostate development. Am J Pathol 157:1769–1775.

Stamey TA, Yang N, Hay AR, McNeal JE, Freiha FS, Redwine E. 1987. Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate. N Engl J Med 317:909–916.

Stuart GR, Holcroft J, de Boer JG, Glickman BW. 2000. Prostate mutations in rats induced by the suspected human carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine. Cancer Res 60:266–268.

Suh CI, Shanafelt T, May DJ, Shroyer KR, Bobak JB, Crawford ED, Miller GJ, Markham N, Glode LM. 2000. Comparison of telomerase activity and GSTP1 promoter methylation in ejaculate as potential screening tests for prostate cancer. Mol Cell Probes 14:211–217.

Tchou JC, Lin X, Freije D, Isaacs WB, Brooks JD, Rashid A, De Marzo AM, Kanai Y, Hirohashi S, Nelson WG. 2000. GSTP1 CpG island DNA hypermethylation in hepatocellular carcinomas. Int J Oncol 16:663–676.

Terris MK. 2000. Extended field prostate biopsies: Too much of a good thing? Urology 55:457–460.

Thimmulappa RK, Mai KH, Srisuma S, Kensler TW, Yamamoto M, Biswal S. 2002. Identification of Nrf2-regulated genes induced by the chemopreventive agent sulforaphane by oligonucleotide microarray. Cancer Res 62:5196–5203.

Ts'o PO, Pannek J, Wang ZP, Lesko SA, Bova GS, Partin AW. 1997. Detection of intact prostate cancer cells in the blood of men with prostate cancer. Urology 49:881–885.

Wang T, Arifoglu P, Ronai Z, Tew KD. 2001. Glutathione S-transferase P1-1 (GSTP1-1) inhibits c-Jun N-terminal kinase (JNK1) signaling through interaction with the C terminus. J Biol Chem 276:20999–21003.