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REVIEW

Glutathione S-transferase pi (GSTP1) hypermethylation in prostate cancer: review 2007

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Summary

Prostatic carcinoma is characterised by the silencing of the pi-class glutathione S-transferase gene (GSTP1), which encodes a detoxifying enzyme. The silencing of GSTP1 results from aberrant methylation at the CpG island in the promoter-5' and occurs in the vast majority of cases of high-grade prostatic intraepithelial neoplasia (PIN) and prostate cancers.

We review the potential novel role of GSTP1 and its related expression in prostate cancer.

The loss of expression (silencing) of the GSTP1 gene is the most common (>90%) genetic alteration reported to date in prostate cancer. Quantitative methylation-specific PCR assays allow detection of GSTP1 methylation in prostate biopsies and may improve the sensitivity of cancer detection. Advances in the epigenetic characterisation of prostate cancer have enabled the development of DNA methylation assays that may soon be used in diagnostic testing of serum and tissue for prostate cancer. Inhibition of aberrant promoter methylation could theoretically prevent carcinogenesis.

Key words: Prostate cancer, gluthathione-S-transferase, methylation analysis.

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INTRODUCTION

Prostate cancer is the most common non-cutaneous cancer and the second leading cause of cancer death in men in Western countries. In 2007, 27 050 Americans will die of prostate cancer, and 218 890 new cases will be diagnosed.[1] However, the pathogenesis of prostate cancer remains unknown. Previous studies showed that age was the most important risk factor, perhaps reflecting the shift toward a more oxidative cellular state as one gets older. Reactive oxygen species that are generated by physiological processes such as cellular respiration, exposure to chemical agents, or exposure to ionising radiation may overcome cellular antioxidant defense and cause DNA damage.[2] Such damage may result in mutations and alteration of oncogenes or tumour suppressor genes. The cytosolic isoenzyme glutathione S-transferase pi (GSTP1) is an important multifunctional detoxifying enzyme within the glutathione S-transferase family enzymes that inactivates electrophilic carcinogens by conjugation with glutathione.[3,4]

Several classes of GST, including alpha, mu, pi, and theta, have been previously found in human prostate tissue, GST pi being the most abundant.[5,6] However, most prostate cancers fail to express GSTP1,[7–11] and this stands in contrast with most other human cancers in which GST pi expression is increased, often greatly so, compared with benign tissue. For example, there is increased expression of GST pi in cancers of the breast, colon, stomach, pancreas, bladder, lung, head and neck, ovary, and cervix, as well as soft tissue sarcoma, testicular embryonal carcinoma, meningioma, and glioma.[3,12–21]

The regulatory sequence near the GST gene is commonly inactivated by hypermethylation during the early stages of prostatic carcinogenesis.[8–11,22] The extensive methylation of deoxycytidine nucleotides distributed throughout the 5' 'CG island' region of GSTP1 is not detected in benign prostatic epithelium, but has been detected in intraepithelial neoplasia, prostatic adenocarcinoma, and fluids (plasma, serum, ejaculate, and urine) of patients with prostate cancer by methylation-specific polymerase chain reaction assay, and may be useful as a cancer-specific molecular biomarker.[7,8,22–26]

In this review, we focus on the rationale for use of molecular assays for the detection of prostate cancer, emphasising the role of the identification of epigenetic alterations.

EPIGENETIC ALTERATIONS: EMERGING MOLECULAR MARKERS FOR PROSTATE CANCER DETECTION

Recent data suggest that cancer is a process fuelled both by genetic alterations and epigenetic mechanisms. Epigenetics refer to changes in gene expression that can be mitotically inherited, but are not associated with the changes in the coding sequence of the affected genes. In other words, epigenetics refer to the inheritance of information based on gene expression levels, in contrast to genetics that refer to transmission of information based on gene sequence.[27] DNA methylation, the best understood mechanism in epigenetics, is an enzyme-mediated chemical modification that adds methyl (CH3) groups at selected sites on DNA. In humans and most mammals, DNA methylation only affects the cytosine base (C), when it is followed by a guanosine (G). Methylation of the cytosine nucleotide residue located within the dinucleotide 5'-CpG-3' is the most frequent epigenetic alteration in humans. These CpG dinucleotides

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are not randomly distributed in the genome. Indeed, there are CpG-rich regions called 'CpG islands' frequently associated with the 5' regulatory regions of genes, including the promoter. DNA methylation in the promoter regions is a powerful mechanism for the suppression of gene activity.

Methylation of CpG islands in the promoter of the pi class of glutathione S-transferase occurs in prostatic intraepithelial neoplasia (PIN) and cancer.[28] Other hypermethylated regions relevant to prostate cancer include the retinoic acid receptor beta 2.[29] These findings in prostate cancer suggest that DNA methylation is among the early events in tumorigenesis, but it remains to be seen whether DNA methylation is a necessary or permissive event in tumorigenesis.

DNA METHYLATION ANALYSIS: CURRENTLY AVAILABLE METHODS

Methylation of CpG islands is of interest for diagnostic and prognostic reasons. Methylation of one or both alleles of a region can serve as a biomarker of cancer or silence gene expression when they are in a promoter region.[30] Assays for methylation are appealing for translational research since they can utilise amplification techniques, such as methylation-specific polymerase chain reaction (PCR), and thereby utilise small amounts of samples.

Due to its relative simplicity, safety, and sensitivity, methylation-specific PCR is the most commonly employed method for methylation analysis.[31]

The conventional methylation-specific PCR (CMSP) assay uses two sets of primers specifically designed to amplify the methylated or unmethylated sequence, and the PCR products are run in a gel.[31] The results of CMSP at a particular DNA region are simply reported as methylated or unmethylated, not allowing quantitation or identification of partial methylation. Methylation-specific PCR methods allow detection of GSTP1 methylation in virtually all body fluids from prostate cancer patients. The CMSP assay is mainly used for GSTP1 methylation detection in fluids. GSTP1 methylation in serum of men with localised prostate cancer prior to treatment carries a 4.4-fold increased risk of biochemical recurrence following surgery.[32]

The use of fluorescence-based real-time quantitative methylation-specific PCR (QMSP) assay in conjunction with histological review of biopsy specimens improved the sensitivity of tumour detection. Continuous monitoring of fluorescent signals during the PCR process enabled quantification of methylated alleles of a single region amongst unmethylated DNA because the fluorescence emission of the reporter represents the number of generated DNA fragments.[33] Quantitative GST pi promoter expression has high specificity for cancer detection biopsies with a theoretical sensitivity of 95%, a positive predictive value of 100%, and a negative predictive value of 83%.[7,34,35] However, this assay is not currently commercially available.

Table 1 summarises the most recent studies on GSTP1 hypermethylation as a molecular tool for prostate cancer detection.

GSTP1 HYPERMETHYLATION: SIGNIFICANCE AND INCIDENCE RELATED TO PROSTATE CANCER

Quantitative methylation-specific PCR (QMSP) reveals that the epigenetic silencing (loss of expression) of the GSTP1 gene is the most common genetic alteration in

TABLE 1 GSTP1 hypermethylation as a biomarker for prostate cancer detection

Publication date	Sample	Reference	Method	No. subjects positive/All (%)	Specificity
2000	Tissue	Goessl *et al.*[76]	CMSP	16/17 (94)	100
	Urine		CMSP	4/11 (36)	100
	Plasma/serum		CMSP	23/32 (72)	100
	Ejaculate		CMSP	4/8 (50)	100
2001	Tissue	Cairns *et al.*[77]	CMSP	22/28 (79)	–
	Urine		CMSP	6/28 (21.4)	–
2001	Urine	Goessl *et al.*[78]	CMSP	29/40 (73)	98
2001	Prostatectomy	Jerónimo *et al.*[7]	QMSP	59/69 (85.5)	96.8
	Biopsies		QMSP	10/11 (90.9)	100
2001	Urine	Goessl *et al.*[79]	QMSP	4/11 (36), normal DNA isolation kit; 22/29 (76), viral kit	100
	Plasma/serum		QMSP	23/32 (72)	100
	Ejaculate		QMSP	4/8 (50)	100
2002	Urine	Jerónimo *et al.*[80]	CMSP	21/69 (30.4)	95
	Urine		QMSP	13/69 (18.8)	93
	Plasma		CMSP	25/69 (36.2)	100
	Plasma		QMSP	9/69 (13)	100
2002	Biopsy washing	Goessl *et al.*[81]	CMSP	10/10 (100)	100
2003	Urine	Gonzalgo *et al.*[82]	CMSP	7/18 (38.9)	–
2003	Biopsies	Harden *et al.*[35]	QMSP	11/15 (73)	100
2003	Prostatectomy	Harden *et al.*[34]	QMSP	43/61 (70.5)	100
2004	Prostatic secretions	Gonzalgo *et al.*[83]	CMSP	86/100 (86)	–
2004	Biopsies	Bastian *et al.*[84]	QMSP	28/31 (90)	100
2004	Prostatectomy	Jerónimo *et al.*[22]	QMSP	112/118 (95)	100
2005	Urine	Hoque *et al.*[85]	QMSP	25/52 (48)	100

CMSP, conventional methylation-specific PCR; QMSP, quantitative methylation-specific PCR.