Original Article

GSTP1 expression and promoter methylation in epithelial ovarian carcinoma

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ABSTRACT

Context: *GSTP1* is a subgroup of glutathione-S-transferase family, which provides cellular protection against free radical and carcinogenic compounds due to its detoxifying function. Altered *GSTP1* activity due to down regulation of enzyme activity and DNA methylation has been reported in many tumors, although data for ovarian cancer are few. In this study, we aimed at determining the expression of *GSTP1* in relation to the methylation of the *GSTP1* promoter in epithelial ovarian cancer (EOC). **Materials and Methods:** *GSTP1* mRNA expression and *GSTP1* enzyme concentration were assessed by quantitative reverse transcriptase polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay, respectively, in 88 EOCs, 14 low malignant potential (LMP) tumors, and 20 benign tumors. The promoter methylation of *GSTP1* gene was evaluated by methylation-specific PCR. **Results:** Reduced *GSTP1* mRNA expression was observed in 49% EOCs, 21.4% LMP, and 45% benign tumors. Significantly lower levels of plasma *GSTP1* were observed in all tumor samples compared to normal. *GSTP1* promoter methylation was detected in 10 (11.4%) EOCs and 1 (7.3%) LMP tumors. No methylation was observed in benign tumors and normal ovaries. **Conclusions:** Our results show that there is a significant down regulation of *GSTP1* expression while hypermethylation of the *GSTP1* gene promoter is not very frequent in EOC. Further studies are needed to study underlying mechanisms leading to decreased expression.

Key words: *GSTP1*, *GSTP1* enzyme, mRNA expression, ovarian carcinoma, promoter methylation

INTRODUCTION

Ovarian cancer is the most lethal tumor of the female genital tract and the second most frequent gynecological cancer.[1] Most cases are diagnosed in the late stages, if diagnosed early may be curable. Although 65-80% of the patients respond to first-line chemotherapy, most patients will relapse with drug-resistant disease.[2] Current prognostic indicators neither accurately predict clinical outcome nor provide biological insight into the disease. Thus, a better understanding of the molecular mechanisms responsible for ovarian cancer development and progression is likely to aid the improvement of the diagnosis and treatment of the disease.

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Glutathione-S-transferases (GSTs) (2.5.1.18) are a family of enzymes that detoxify intracellular xenobiotics, primarily by catalysis of the nucleophilic attack of reduced glutathione on electrophilic compounds. In humans, the cytosolic GSTs can be divided into seven major classes: α (A), μ (M), π (P), σ (S), θ (T), ω (O), and ζ (Z). GST Pi (*GSTP1*) is the predominant isoform present in normal human tissues, and its expression has significant biological and clinical implications including drug-resistance and carcinogenesis. The loss of *GSTP1* expression is associated with promoter hypermethylation and has been identified in prostate, breast, liver, renal, urinary bladder, and endometrial cancers.[3]

Malignancies of the colon, stomach, urinary bladder, uterus, cervix, lung, and larynx often contain increased amounts of *GSTP1* compared to adjacent normal tissue.[4] Three studies published in 1989 have all indicated a considerable elevation of serum *GSTP1* levels in gastrointestinal malignancies.[5-7] The results for ovarian cancer are not very conclusive.

There is a strong need for the development of biomarkers that can spot the disease at an early stage which in turn would improve the survival rates.[8] Hypermethylation is

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assumed to be an early event of carcinogenesis and a possible biomarker for cancer detection, prognosis and potential chemoprevention, and a therapeutic target.[9] Aberrant promoter hypermethylation associated with an epigenetically mediated gene silencing constitutes an alternative to coding region mutation for loss of gene function in cancer.[10] The methylation-specific polymerase chain reaction (PCR) (MSP) protocol designed by Herman and Baylin was able to assay methylation status of CpG islands within a gene promoter that correlates with loss of transcription.[10]

In this study, we aimed at evaluating the mRNA expression, plasma *GSTP1* levels, and methylation status of *GSTP1* promoter in ovarian carcinoma and its association with clinicopathological features.

MATERIALS AND METHODS

Sample collection

A total of 122 consecutive patients which included 88 epithelial ovarian carcinoma (EOC), 14 low malignant potential (LMP) tumors, and 20 benign tumors, who underwent potentially curative surgery between October 2010 and September 2012, were enrolled in this study. 15 normal ovarian tissues were collected at the time of oophorectomy from women without any family history of breast and ovarian cancer. The median age at diagnosis was 48 years (range: 23-72 years). No preoperative chemotherapy was given. All tumors were graded according to WHO criteria and staged according to the Federation of Gynecology and Obstetrics (FIGO) classification. 57 (65%) were grade 3 tumors and 64 (73%) cases had clinical stage III disease. Serous tumors were the most common histological subtype (52 [59%]).

Patients' information included FIGO stage, histological grade, and subtype. Study approval was given by the Institutional Review Board and Medical Ethics Committee, and written informed consent was obtained from all participants.

Specimens removed from surgery were snap frozen at − 80°C until the extraction of DNA. Histology reports and slides as well as clinical data of these patients were reviewed by a Gynecological Oncologist and a Pathologist before the study. The patients' plasma sample for the analysis of *GSTP1* was collected in EDTA vacutainer. Immediately, after collection of the sample, the tubes were centrifuged for 15 min at 5000 rpm at room temperature. The upper two-third of the plasma was collected, and care was taken not to aspirate the platelets on top of the cell layer. Plasma samples were stored in aliquots at −20°C until the assay. The sampling, handling, storage, and assay procedures were identical for all patients and controls.

DNA and RNA extraction

Genomic DNA was extracted from 25 mg of ovarian tissue using QIAamp DNA mini kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. Total RNA was isolated from 30 mg of tissue preserved in RNA Later™ (Applied Biosystems, Foster city, CA, USA) using RNeasy mini kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. The quality and quantity of the extracted DNA and RNA were determined using Eppendorf Biospectrophotometer Kinetics™ and electrophoretically on a 2% agarose gel.

cDNA synthesis and quantitative reverse transcriptase-polymerase chain reaction

cDNA synthesis from total RNA was performed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) following the company protocol. The gene expression levels of *GSTP1* were quantified using TaqMan technology on a StepOnePlus™ real time PCR system (Applied Biosystem, Foster City CA, USA). Gene specific primers and probe of *GSTP1* (assay ID Hs002512067_s1) were available as TaqMan gene expression assays (Applied Biosystems, Foster City, CA, USA). The 18 s ribosomal RNA (18S rRNA) (assay ID Hs99999901_s1) was used as an endogenous control in the quantification.

The real time PCR was performed in 20 µl of reaction volume containing 10 µl TaqMan gene expression master mix, 1 µl TaqMan gene expression assay, 2 µl cDNA and 7 µl of nuclease free water. The thermal cycling conditions were 50°C for 2 min, 95°C for 10 min followed by 50 cycles of 95°C for 15 s and 60°C for 1 min. A no template control was included in each reaction, and all reactions were performed in triplicate. Serial dilutions of cDNA from normal ovarian tissues were amplified in parallel as a control of amplification efficiency with each experiment and for the establishment of a standard curve for relative quantification. Expression of *GSTP1* mRNA was normalized for 18S rRNA as an internal reference. Relative expression levels were calculated as *GSTP1*/18S rRNA in tumor and normal tissues, respectively, using delta delta Ct method ($^{\Delta\Delta}C_T$).

Plasma *GSTP1* estimation

Plasma *GSTP1* level was estimated in tumors and normal ovarian samples using an enzyme-linked immunosorbent assay (ELISA) kit procured from Cusabio (Cat No CSB–EL009989M0) following the manufacturer's instructions. The sandwich ELISA used in the current study had a detection limit and an intra and inter assay coefficient of variation comparable to previously published immunoassays for *GSTP1*. All standards and samples were measured in duplicate.

Methylation-specific polymerase chain reaction and bisulfite sequencing

Sodium bisulfite conversion of unmethylated cytosine residues to uracil in genomic DNA samples was performed with 600 ng of DNA using the EZ DNA methylation kit (D5005, Zymoresearch Corp, CA, USA).

The promoter methylation status of *GSTP1* was investigated by nested MSP. The primer sequences used for the first round PCR were 5′ GGGATTTTAGGGYGTTTTTTTG 3′ (forward) and 5′ ACCTCCRAACCTTATAAAAATAAT 3′ (reverse). The region chosen for *GSTP1* spans the area of the greatest CpG density immediately 5′ to the transcription start site, in an area studied previously for methylation changes. The PCR reaction was performed with 2 μL bisulfite modified DNA template in 50 μL reaction mixture containing 1.5 mM/L $MgCl_2$, 10 pM/L of each forward and reverse primer, 0.2 mM/L of each of the four dNTPs, 5 μL 10x PCR buffer and 1U Taq polymerase (New England Biolabs Inc., England). The primer sets used were designed to bind to both methylated and unmethylated DNA. The resulting 159 bp PCR product was diluted ten folds and subjected to the second round PCR with primer sets 5′TTCGGGGTGTAGCGGTCGTC3′ (forward) and 5′GCCCCAATACTAAATCACG ACG3′ (reverse) for methylated DNA and 5′GATGTTTGGGGTGTAGTGGTTGTT3′ (forward) and 5′CCACCCCAATACTAAATCACAACA3′ (reverse) for unmethylated DNA, respectively.

The PCR reaction was performed in duplicate in a Veriti™ thermal cycler (Applied Biosystems, Foster City, CA, USA) and was subjected to denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s and extension at 72°C for 30 s and the final extension of 5 min at 72°C. The second step PCR was performed using an identical PCR program with the exception of annealing temperature, which was 53°C for methylated and unmethylated sequences. The resulting PCR products were 91 bp and 97 bp for methylated and unmethylated alleles, respectively. For each step of bisulfite modification and PCR, a positive and negative control for methylated and methylated DNA, CpGenome Universal Methylated DNA (Zymoresearch Corp, CA, USA) and peripheral blood lymphocyte DNA were used, respectively. Water with no DNA template was included as a control for possible contamination. 10 μL of each PCR reaction was loaded onto 2% agarose gel stained with 0.1% ethidium bromide and analyzed using a gel documentation system (G Box F3, Syngene) with a 100 bp DNA ladder (Fermentas, Germany) as a molecular weight standard.

The results obtained from MSP were validated using direct bisulfite sequencing. Four methylated, and two unmethylated PCR products were selected randomly and purified for direct sequencing using the Big Dye Terminator V1.1 sequencing kit cycler (Applied Biosystems, Foster City, CA, USA) and the ABI PRISM 310 Genetic Analyzer cycler (Applied Biosystems, Foster City, CA, USA). The PCR products were sequenced from both ends. The sequenced region included 12 CpG sites.

Statistics

Chi-square test or Fischer's exact probability test was used to analyze methylation frequency and mRNA expression of *GSTP1* gene. Student's *t*-test was used to analyze plasma *GSTP1* levels using SPSS software package (22.0 version, Chicago, IL, USA). Correlation with clinicopathological parameters was analyzed using the Chi-square test or Fischer's exact probability test. All tests were carried out two sided. $P < 0.05$ was considered statistically significant.

RESULTS

mRNA expression of *GSTP1* gene

Results of *GSTP1* gene expression are given in Table 1. The median relative expression level in the control was used as the cut-off value. Reduced expression was seen in 49% EOCs, 21.4% LMP tumors, and 45% benign tumors whereas all normal ovarian tissues showed normal *GSTP1* mRNA expression. *GSTP1* expression was significantly lower in EOCs and benign tumors as compared to normal samples (P - 0.001 and P - 0.004, respectively). Reduced *GSTP1* expression showed an association with the stage of the tumor and the presence of ascites (P - 0.001 and 0.09, respectively) (data not shown).

Plasma *GSTP1*

GSTP1 is a cytosolic enzyme and plasma levels of *GSTP1* enzyme were assessed, and the results obtained are shown in Table 2. Ovarian tumors had lower plasma *GSTP1* levels

Table 1: mRNA expression of *GSTP1* gene

Expression	Tumor type (%)			
	Malignant (88)	LMP (14)	Benign (20)	Normal (15)
Normal	45 (51)	11 (78.6)	11 (55)	5 (100)
Reduced	43 (49)	3 (21.4)	9 (45)	0 (0)
P value	0.001*	0.099	0.004*	

*Significant (P>0.05). *GSTP1*: Glutathione-S-transferase π1

Table 2: Plasma levels of *GSTP1* enzyme in the study cohort

Tumor type	*n*	Mean plasma GSTP1 levels (ng/mL)
Carcinomas	88	12.2±2.8
P		0.136
LMP	14	15.1±4.0
P		0.152
Benign	20	13.4±3.6
P		0.074
Normal	15	22.6±3.3

LMP: Low malignant potential, *GSTP1*: Glutathione-S-transferase π1

compared to normal subjects (*P* - 0.136, *P* - 0.152, and *P* - 0.0749 in EOCs, LMPs and benign tumors, respectively).

Promoter methylation of *GSTP1* gene

Table 3 shows the methylation frequency of *GSTP1* gene. Hypermethylation of the promoter region of *GSTP1* was detected in 10 of 88 (11.4%) EOCs and 1 of 14 (7.1%) LMP tumors. No hypermethylation was observed in benign and normal ovarian tissues. There was no significant correlation between *GSTP1* methylation and clinicopathological characteristics of the patients. Figure 1 shows the representative agarose gel pattern of MSP product of *GSTP1* promoter region.

The results obtained by MSP were validated by direct bisulfite sequencing, and the results were concordant. Figure 2 shows the results obtained by direct bisulfite sequencing. In unmethylated MSP products, all cytosine nucleotides including those in the CpG islands are changed to thymine and in methylated MSP products, cytosine nucleotides in the CpG islands remain as cytosine.

Correlation between *GSTP1* promoter hypermethylation and *GSTP1* expression

We analyzed the relationship between *GSTP1* mRNA expression and methylation status in the study cohort. Of the 10 cases of EOCs which showed hypermethylation of the gene promoter, 6 cases (60%) showed reduced mRNA expression of the *GSTP1* gene [Table 4]. However, since the numbers were little no statistical significance was observed (*P* - 0.516). Low levels of plasma *GSTP1* also correlated with *GSTP1* promoter methylation and the corresponding reduced mRNA expression in EOCs.

DISCUSSION

GSTP1 is a major member of the cytosolic GST super family expressed in several tissues and generally serves as a protector of cellular macromolecules from the damage caused by cytotoxic and carcinogenic agents that are thought to function in the xenobiotic metabolism.[11] Little is known about the mechanism of release of *GSTP1* from tumor tissue. *GSTP1* has several roles in tumorigenesis. First, *GSTP1* might act as a tumor suppressor gene, which leads to tumor growth, when activated. Second, *GSTP1* might act like a caretaker gene, which leads to somatic genome alterations that promote tumor growth when inactivated.

We studied the expression of *GSTP1* by measuring the mRNA and plasma levels of *GSTP1*. The data obtained from relative gene expression through quantitative reverse transcriptase-PCR from our study, shows a significant reduction in mRNA expression levels in 49% EOCs and 45% benign tumors. We found significantly lower levels of plasma *GSTP1* in EOC, LMP, and benign tumors. The down regulation of *GSTP1* enzyme activity may be secondary to decrease *GSTP1* mRNA level as almost 50% of EOCs, and

Table 3: Methylation frequency of *GSTP1* promoter

Genes	Tumor type (%)			
	Malignant (88)	LMP (14)	Benign (20)	Normal (15)
GSTP1 (U)	78 (88.6)	13 (92.9)	20 (100)	15 (100)
GSTP1 (M)	10 (11.4)	1 (7.1)	0 (0)	0 (0)
P	0.351	0.483	-	-

U: Unmethylated, M: Methylated, *GSTP1*: Glutathione-S-transferase π1

Table 4: Correlation of *GSTP1* promoter methylation with gene expression

Tumor type	Methylation status	Gene expression (%)	
		Normal	Reduced
Malignant (88)	U (78)	41 (52.6)	37 (47.4)
	M (10)	4 (40)	6 (60)
P		0.516	
LMP (9)	U (13)	7 (77.3)	2 (22.2)
	M (1)	1 (100)	0 (0)
P		1.000	
Benign (20)	U (20)	11 (55)	9 (45)
Normal (15)	U (15)	5 (100)	0 (0)

U: Unmethylated, M: Methylated, *GSTP1*: Glutathione-S-transferase π1

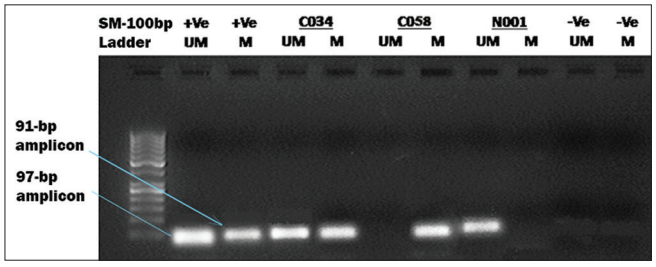

Figure 1: Methylation analysis of *GSTP1* gene. Agarose gel showing representative product of methylation-specific polymerase chain reaction analysis of *GSTP1* gene in epithelial ovarian tumors. UM: Unmethylated allele, M: Methylated allele, +UM: Positive control for unmethylated allele, +M: Positive control for methylated allele, −ve UM: Negative control for unmethylated allele, −ve M: Negative control for methylated allele, C034 and C058: Carcinomas, N001: Normal ovarian tissue

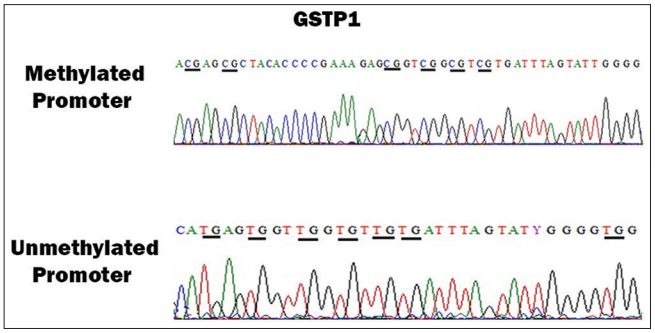

Figure 2: Bisulfite sequencing of the *GSTP1* promoter. Two representative sequences showing methylation and unmethylation status of cytosine at the target sites of methylation-specific-polymerase chain reaction primers. Methylated cytosine found in tumor cases remained unchanged as cytosine after bisulfite modification, whereas unmethylated cytosine was converted and sequenced as thymine

benign tumors showed reduced mRNA expression. *GSTP1* polymorphism has also been suggested to be involved in the alteration of *GSTP1* enzyme activity. We postulate that reduced enzyme levels may play a role in the development and progression of cancer.

The postulation that *GSTP1* reflects the sum of *GSTP1* expression may be supported by the fact that *GSTP1* concentrations in the culture media of various tumor cell lines correlate well with these of their representative cell lysates.[12] The release of *GSTP1* from the cells may not be a simple leakage because *GSTP1* in the medium is a monomer whereas the intracellular form is a homodimer.

Previously, *GSTP1* expression in tissue and serum was suggested as a cancer marker in several earlier studies with results showing inconsistent expression patterns in various cancers particularly gastric cancers. Niitsu *et al*. and Fan *et al*. have both showed that the plasma *GSTP1* levels were higher in patients with gastric cancer and were associated with advanced stage disease.[7,13] Similar results have been reported for patients with nonsmall cell lung cancer and patients with oral cancer.[14,15] Down regulation of *GSTP1* with loss of *GSTP1* mRNA expression was regarded as a phenotype associated with malignant transformation in prostate carcinoma, esophageal carcinoma, breast cancer, and hepatocellular cancer.[16-19]

The results for ovarian cancer have been inconsistent. Some researchers found a relationship between over expression of *GSTP1* in malignant ovarian tissue and poor prognosis or bad response to chemotherapy whereas others could not detect such an association.[20] Less attention has been paid to the analysis of *GSTP1* in body fluids of patients with ovarian cancer. Kolwijck *et al*. have observed high levels of *GSTP1* in ovarian cyst fluid in EOC patients compared to benign tumors which significantly correlated with clinicopathological variables.[21]

Gene silencing of tumor suppressor genes is an important mechanism in tumorigenesis. It is hypothesized that promoter methylation may play a role in gene silencing and has been studied in a variety of tumors. We examined the methylation status of the CpG island in the *GSTP1* promoter region to find any association of methylation with reduced *GSTP1* mRNA expression. Our results showed hypermethylation in 11.4% EOCs and 7.1% LMP tumors, while none of the benign tumors and normal ovarian tissues expressed any methylated alleles.

Of the 10 cases of EOC, which were hypermethylated at the promoter region, reduced mRNA expression was seen in 6 cases while the one methylated case of LMP tumor showed normal expression showing that hypermethylation of the gene promoter is associated with gene silencing. Due to few methylated cases, reduced *GSTP1* mRNA expression was not found to be significant and hence methylation may not be a major regulator in *GSTP1* gene silencing. Reduced mRNA expression due to promoter methylation of *GSTP1* has been reported in prostate and other cancers.[22,23] However, the finding of reduced mRNA expression with no demonstrable methylated alleles also suggest that along with promoter methylation there may be other predisposing factors such as histone deacetylation, nonCpG methylation, methylation of a broader chromosomal region, or loss of transcription factors necessary for the maintenance of *GSTP1* expression. Compton *et al*. have suggested that down regulation may also happen in the transcription level due to various gene interactions.[24]

In our study, we also observed that the methylated tumor tissue always showed a heterogeneous methylation pattern with both methylated and unmethylated alleles alternating each other. There was no significant correlation between *GSTP1* methylation and patient age, thus, methylation of *GSTP1* in ovarian cancer is not attributable to the aging process.

There are not many published studies which have studied promoter methylation of *GSTP1* in EOC and no reported data for the Indian population. A study by Wiley *et al*. in ovarian cancer did not report any methylation while Makarla *et al*. have reported the frequency of 9% which was mainly observed in serous ovarian carcinomas.[25,26] Our results of 11.4% promoter methylation in EOCs are similar to the findings of Makarla *et al*. and serous tumors were the most common histological subtype (59%) in our study group also.[25]

In summary, our results indicate that there is a significant down regulation in the expression of *GSTP1* in EOC and an imbalance between redox and GST enzyme which may further damage DNA and promote malignancy increasing the mutation rate. Further, studies are clearly warranted to explore the underlying mechanisms leading to decreased *GSTP1* expression and to understand the complexity of cellular detoxification mechanisms.

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REFERENCES

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin 2013;63:11-30.
2. Colombo N, Van Gorp T, Parma G, Amant F, Gatta G, Sessa C, *et al.* Ovarian cancer. Crit Rev Oncol Hematol 2006;60:159-79.

3. Coles BF, Kadlubar FF. Detoxification of electrophilic compounds by glutathione S-transferase catalysis: Determinants of individual response to chemical carcinogens and chemotherapeutic drugs? Biofactors 2003;17:115-30.
4. Hopkins TG, Burns PA, Routledge MN. DNA methylation of GSTP1 as biomarker in diagnosis of prostate cancer. Urology 2007;69:11-6.
5. Takahashi Y, Hirata Y, Saito T, *et al*. Enzyme immunoassay for human serum glutathione S-transferase pi using monoclonal and polyclonal antibodies. Cancer J 1989;2:225-9.
6. Tsuchida S, Sekine Y, Shineha R, Nishihira T, Sato K. Elevation of the placental glutathione S-transferase form (GST-pi) in tumor tissues and the levels in sera of patients with cancer. Cancer Res 1989;49:5225-9.
7. Niitsu Y, Takahashi Y, Saito T, Hirata Y, Arisato N, Maruyama H, *et al.* Serum glutathione-S-transferase-pi as a tumor marker for gastrointestinal malignancies. Cancer 1989;63:317-23.
8. Laird PW. The power and the promise of DNA methylation markers. Nat Rev Cancer 2003;3:253-66.
9. Widschwendter M, Jones PA. DNA methylation and breast carcinogenesis. Oncogene 2002;21:5462-82.
10. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. N Engl J Med 2003;349:2042-54.
11. Brabender J, Lord RV, Wickramasinghe K, Metzger R, Schneider PM, Park JM, *et al.* Glutathione S-transferase-pi expression is downregulated in patients with Barrett's esophagus and esophageal adenocarcinoma. J Gastrointest Surg 2002;6:359-67.
12. Katahira T, Takayama T, Miyanishi K, Hayashi T, Ikeda T, Takahashi Y, *et al.* Plasma glutathione S-Transferase P1-1 as a prognostic factor in patients with advanced non-Hodgkin's lymphoma (stages III and IV). Clin Cancer Res 2004;10:7934-40.
13. Fan KC, Huang YC, Li CH. Radioimmunoassay for plasma glutathione S-transferase-pi and its clinical application in gastrointestinal cancer. Cancer 1995;76:1363-7.
14. Howie AF, Douglas JG, Fergusson RJ, Beckett GJ. Measurement of glutathione S-transferase pi isoenzyme in plasma, a possible marker for adenocarcinoma of the lung. Clin Chem 1990;36:453-6.
15. Hirata S, Odajima T, Kohama G, Ishigaki S, Niitsu Y. Significance of glutathione S-transferase-pi as a tumor marker in patients with oral cancer. Cancer 1992;70:2381-7.
16. Wang Z, He W, Yang G, Wang J, Wang Z, Nesland JM, *et al.* Decreased expression of GST pi is correlated with a poor prognosis in human esophageal squamous carcinoma. BMC Cancer 2010;10:352.
17. Okino ST, Pookot D, Majid S, Zhao H, Li LC, Place RF, *et al.* Chromatin changes on the GSTP1 promoter associated with its inactivation in prostate cancer. Mol Carcinog 2007;46:839-46.
18. Mavis CK, Morey Kinney SR, Foster BA, Karpf AR. Expression level and DNA methylation status of glutathione-S-transferase genes in normal murine prostate and TRAMP tumors. Prostate 2009;69:1312-24.
19. Li T, Zhao XP, Wang LY, Gao S, Zhao J, Fan YC, *et al.* Glutathione S-transferase P1 correlated with oxidative stress in hepatocellular carcinoma. Int J Med Sci 2013;10:683-90.
20. Saip P, Tuzlali S, Demir K, Sakar B, Yavuz E, Berkman S, *et al.* Value of glutathion-S transferase pi as a prognostic factor in epithelial ovarian carcinoma. Eur J Gynaecol Oncol 2005;26:90-4.
21. Kolwijck E, Zusterzeel PL, Roelofs HM, Hendriks JC, Peters WH, Massuger LF. GSTP1-1 in ovarian cyst fluid and disease outcome of patients with ovarian cancer. Cancer Epidemiol Biomarkers Prev 2009;18:2176-81.
22. Xue WC, Feng HC, Tsao SW, Chan KY, Ngan HY, Chiu PM, *et al.* Methylation status and expression of E-cadherin and cadherin-11 in gestational trophoblastic diseases. Int J Gynecol Cancer 2003;13:879-88.
23. Chan QK, Khoo US, Chan KY, Ngan HY, Li SS, Chiu PM, *et al.* Promoter methylation and differential expression of pi-class glutathione S-transferase in endometrial carcinoma. J Mol Diagn 2005;7:8-16.
24. Compton KR, Orringer MB, Beer DG. Induction of glutathione s-transferase-pi in Barrett's metaplasia and Barrett's adenocarcinoma cell lines. Mol Carcinog 1999;24:128-36.
25. Wiley A, Katsaros D, Chen H, Rigault de la Longrais IA, Beeghly A, Puopolo M, *et al.* Aberrant promoter methylation of multiple genes in malignant ovarian tumors and in ovarian tumors with low malignant potential. Cancer 2006;107:299-308.
26. Makarla PB, Saboorian MH, Ashfaq R, Toyooka KO, Toyooka S, Minna JD, *et al.* Promoter hypermethylation profile of ovarian epithelial neoplasms. Clin Cancer Res 2005;11:5365-9.

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