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Regulation of transcription of the glutathione *S*-transferase P1 gene by methylation of the minimal promoter in human leukemia cells

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Abstract

To study the relationship between methylation and the transcriptional activity of the minimal promoter of the glutathione *S*-transferase *GSTP1* gene encoding glutathione *S*-transferase P1-1, *GSTP1* mRNA levels as well as basal promoter activity were compared in human leukemia cell lines. The K562 erythroleukemia cell line presented a strong *GSTP1* promoter activity, as measured in transient transfection assays using a luciferase reporter plasmid, and correlated with a high mRNA whereas in Raji cells no mRNA was expressed. In order to establish a relationship between the expression and the methylation status, we used *in vitro* bisulfite sequencing which indicated that both methylated and unmethylated *GSTP1* promoter alleles coexisted in K562 cells, whereas Raji lymphoma cells showed a nearly uniform hypermethylation of the promoter region. To determine the impact of methylation, we used *in vitro Sss*I methylation of the minimal *GSTP1* promoter, which led to the silencing of the promoter activity in transient transfection assays in expressing K562 as well as in non-expressing Raji cells. These data are in good agreement with previously obtained results and indicate that methylation of CpG sites of the basal promoter is an essential mechanism in the control of *GSTP1* gene expression in human leukemia.

Keywords: Gene expression; Glutathione *S*-transferase P1; DNA methylation; Transcription regulation; Promoter

1. Introduction

Methylation of cytosine residues in CpG dinucleotides affects the expression of many genes and is associated with reduced levels of transcription [1,2]. Over the last decade, numerous studies have demonstrated a close correlation between hypermethylated 5′ CpG islands and the inactivation of the corresponding downstream genes, including those coding for $p16^{INKA}$ [3], E-cadherin [4], von Hippel–Lindau protein [5], and estrogen-α receptor [6], suggesting that hypermethylation of CpG islands could be a general mechanism of transcriptional regulation.

GSTs are dimeric proteins encoded by a family of distinct genes. Humans express four classes of cytosolic GSTs and two membrane-bound GST enzymes with distinct but overlapping substrate specificities [7–9]. GSTs catalyze the conjugation of glutathione with a wide variety of hydrophobic compounds bearing an electrophilic center, including chemical carcinogens, mutagens, and anticancer agents [10]. GSTs also protect cells by preventing oxidative damage through intrinsic organic peroxidase activity [11]. The distribution of GST isoenzymes is tissue-specific. GSTA is mainly expressed in the liver and the kidney, while GSTP1-1 is expressed as a major form in organs such as lung, breast, or bladder [10,12]. In many human tumors and preneoplastic lesions, GSTP1-1 is overexpressed, even though in the corresponding normal tissues the protein is either absent or expressed at very low levels [13]. GSTP1-1 appears to be involved in the development of anticancer drug resistance, and elevated levels of *GSTP1* mRNA are found in cell lines resistant to a range of anticancer drugs [14,15].

Promoter methylation is believed to be one of the factors implicated in the regulation of *GSTP1* gene expression in a

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Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GST, glutathione *S*-transferase; and PCR, polymerase chain reaction.

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wide range of human tissues, as shown by Millar *et al*. [16]. In prostate cancer cells, DNA methylation is extensive throughout the CpG island of the *GSTP1* gene and leads to the loss of *GSTP1* expression [17,18]. Hepatic carcinoma cells also display abnormal *GSTP1* CpG island hypermethylation correlated with a loss of *GSTP1* expression [19]. The methylation of the *GSTP1* gene has been suggested to play an important role in the silencing of this gene in renal and breast carcinoma cells [20,21]. To test the hypothesis that CpG methylation modulates the regulatory activity of the *GSTP1* gene promoter in leukemia, we used bisulfite genomic sequencing to determine the methylation state of 15 CpG sites in the human *GSTP1* promoter in an expressing and a non-expressing leukemia cell line and determined the impact of methylation by *in vitro Sss*I methylation of the minimal *GSTP1* promoter in expressing K562 as well as in non-expressing Raji cells.

2. Materials and methods

2.1. Cells and medium

K562 (chronic myelogenous leukemia), U937 (histiocytic lymphoma), Jurkat (acute T cell leukemia), Raji (Burkitt's lymphoma), and Molt-3 (acute lymphoblastic leukemia) cells (all from the American Type Culture Collection) were cultured in RPMI medium containing 10% (v/v) fetal bovine serum and 1% (v/v) antibiotic–antimycotic (all from Life Technologies).

2.2. Northern blot analysis

Total RNA was isolated from approximately 5×10^6 human leukemia cells using the Trizol reagent (Life Technologies). Ten micrograms of total RNA was loaded per lane and separated on a 1% denaturing formaldehyde agarose gel. After electrophoresis, the RNA was transferred by capillary action to a Hybond N+ nylon membrane (Amersham/Pharmacia Biotech). Northern blots were hybridized to a 0.72-kb genomic ^{32}P-labeled genomic DNA fragment coding for human GSTP1-1 (American Type Culture Collection). A human GAPDH probe (Clontech) was used as a control for equal sample loading. Probes were ^{32}P-labeled by random priming using the RTS Radprime DNA Labeling System (Life Technologies). Prehybridization and hybridization were carried out at 42°. Northern blots were washed for 5 min with 2× SSC (sodium chloride/sodium citrate) and 0.5% SDS, then washed twice with 2× SSC and 0.1% SDS for 5 min, and finally for at least 30 min in a 1 × SSC buffer containing 0.1% SDS at 65°. Autoradiography was performed using X-OMAT AR film (Eastman Kodak Co.) and two intensifying screens at −70°. Quantifications were performed by scanning of the autoradiograms and the Image 1.62b7 software (NIH).

2.3. Construction of expression vectors

Human genomic DNA was extracted from human peripheral blood cells following standard procedures. Primers GST1 and GST2 (Eurogentec) were used to amplify a 136-bp product corresponding to the minimal promoter of the human *GSTP1* gene on the basis of sequences deposited in GenBank (Accession Number X08058) by Morrow *et al*. [22]. Primers correspond to the following regions: −97 to −79 5′-AAAAAGGATCCGGACCCTCCAGAAGAGCGG-3′ for GST1 and +21 to +39 5′-AAGCTTCGTACTCACTGGTGGCGAAG-3′ for GST2 relative to the transcriptional start site. PCR conditions were performed in 25-μL volumes at 95° for 5 min, 30 cycles at 95° for 1 min, 57.5° for 1 min, and 72° for 90 sec. The amplified product was subcloned into a pCRII-Topo (topoisomerase) plasmid (pCRII-GST1128) (Invitrogen), excised by *Kpn*I and *Hin*dIII, and religated into a *Kpn*I- and *Hin*dIII-linearized and -dephosphorylated pGL3-enhancer plasmid (Promega). The final construct was termed pGST1128. Relevant regions of the final constructs were confirmed by sequencing in both directions using a T7 sequencing kit (Amersham/Pharmacia Biotech).

2.4. Bisulfite modification of genomic DNA

DNA was obtained from the different cells and bisulfite modification was carried out as described by Frommer *et al*. [23] with modifications described by Feil *et al*. [24]. Briefly, 5 micrograms of genomic DNA from K562 cells was treated with 5 M sodium bisulfite and 100 mM hydroquinone pH 5.0 at 50° for 16 hr. After modification, the sense strand of the *GSTP1* promoter was amplified by PCR using the following primers, designed after taking into account the bisulfite conversion reaction −98 to −82 5′-GGGATTTTTTAGAAGAG-3′ for GST1' and +19 to +37. 5′-TACTCACTAATAACRAAAA-3′ with R = A or G) for GST2' relative to the transcriptional start site (Fig. 3A). PCRs were performed in 25-μL volumes at 95° for 5 min, 30 cycles at 94° for 30 sec, 51° for 1 min, and 72° for 90 sec. Amplified PCR fragments were subcloned into a pCRII-Topo plasmid (Invitrogen). Positive clones from at least three independent PCRs were sequenced to determine the methylation pattern of individual DNA molecules using a T7 sequencing kit (Amersham/Pharmacia Biotech). After modification, Cs derived from unmethylated Cs appear as Ts whereas methylated Cs appear as Cs.

2.5. Methylation of plasmid constructs

Whole plasmids were methylated using *Sss*I methylase (M.*Sss*I), which methylates all cytosine residues within the double-stranded dinucleotide recognition sequence 5′-CG-3′ (New England Biolabs). Ten micrograms of pGST1128 plasmid was incubated with 30 units of M.*Sss*I, and in a parallel control reaction 10 μg of the same plasmid

was mock-methylated in the absence of *S*-adenosylmethionine. Methylated and mock-methylated plasmids were digested with *Kpn*I/*Hin*dIII and religated.

Region-specific methylation was carried out by a *Kpn*I/HindIII digestion of methylated and mock-methylated pGST1128 constructs. After digestion, promoter DNA fragments were separated by agarose gel electrophoresis, purified, and ligated to either mock-methylated or methylated plasmid DNA to produce partially methylated constructs.

2.6. Transient transfection assays

Transfections of K562, U937, Jurkat, and Raji cells were done by electroporation using a Bio-Rad Gene Pulser (Bio-Rad Laboratories). For each experiment, 5×10^6 cells at a concentration of 1.8×10^7/mL were electroporated at the following settings: 625 V/cm and 500 μF (K562 and U937), 800 V/cm and 500 μF (Jurkat), 775 V/cm and 960 μF (Raji) in a final volume of 300 μL. Twenty micrograms of the reporter gene construct and 8 μg of a p cytomegalovirus (CMV) β-plasmid (Clontech) were used for each pulse. Results from all transfections were normalized to the β-galactosidase activity expressed by the co-transfected control plasmid pCMVβ. For methylation cassette assays, K562 cells were grown to early log phase, harvested, and resuspended in RPMI medium at 1×10^6 cells/mL. Cells (2×10^5) were transfected by transferrin-mediated endocytosis according to the instructions of the manufacturer (Bender Medsystems). Briefly, cells were transfected with 2 μg of each luciferase construct and 8 μg of an *Xho*I/*Hin*dIII-digested and -religated β-galactosidase-expressing control plasmid. After transfection, cells were cultured in 1.5 mL preheated medium. After 48 hr, the cells were harvested and resuspended in 300 μL of reporter lysis buffer (Promega). The light emission resulting from luciferase activity was measured in a Turner luminometer by integration of the peak light emission over 15 sec at 25°. β-galactosidase activities were measured in an assay buffer containing 100 mM sodium phosphate (pH 7.3), 1 mM $MgCl_2$, 50 mM 2-mercaptoethanol, 0.665 mg/mL of *o*-nitrophenyl-β-d-galactopyranoside, and 50 μL of cell lysate.

The ratio between arbitrary luciferase light units and β-galactosidase activity was normalized for each cell line separately relative to the cells transfected with pGL3-control (Promega). All results are expressed relative to the luciferase activity of the pGL3-control, arbitrarily set to 100%. Each value represents the average ± SD of three independent transfection experiments. Assays were repeated twice.

3. Results

3.1. Differential expression of GSTP1 mRNA in human leukemia cell lines

To characterize the expression of the *GSTP1* gene in human leukemia cells, we used Northern blot hybridization

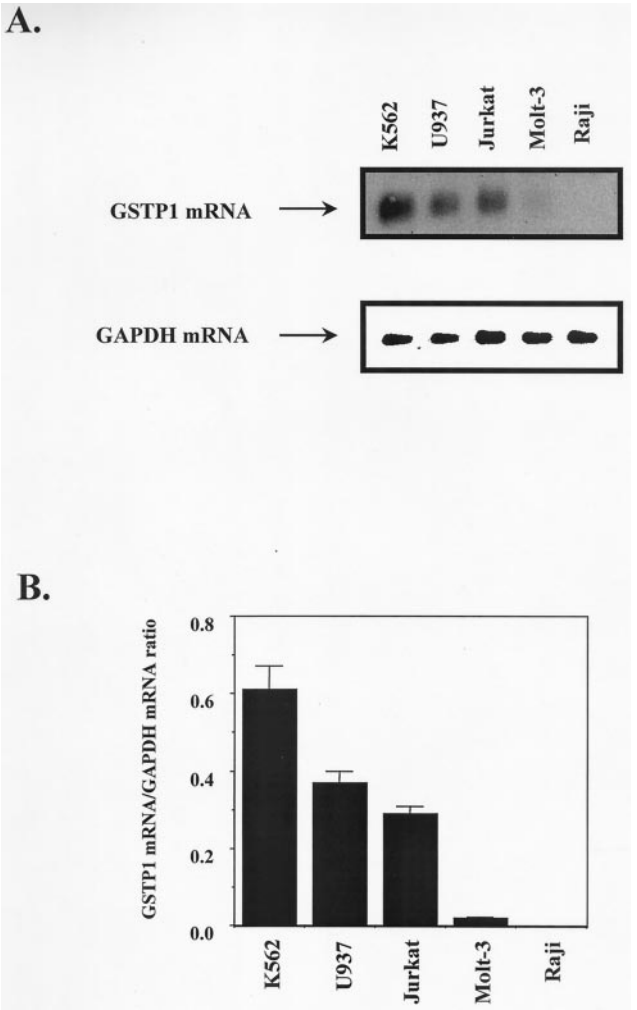

Fig. 1. (A) Northern blot analysis. The upper panel shows *GSTP1* RNA from five different human leukemia cell lines. As a control for sample loading, the blot was stripped and rehybridized with a GAPDH- specific probe, shown in the lower panel. (B) Quantitation of Northern blot data. Three different Northern blots were quantitated using the NIH Image 1.62b7 software. The ordinate represents the GSTP1 mRNA/GAPDH mRNA ratio for each cell line tested. Each bar represents the average ± SD of three independent Northern blots.

with a 0.72-kb genomic fragment of human *GSTP1*. K562, Jurkat, and U937 cells showed significantly higher mRNA levels than Molt-3, whereas no significant amount of *GSTP1* mRNA was detected in Raji cells (Fig. 1). Hybridization with a GAPDH-specific probe showed no difference in GAPDH mRNA contents for these cell lines.

To measure basal *GSTP1* promoter activity in these cells, we constructed an expression plasmid containing a 136-bp fragment of the *GSTP1* promoter fused to a luciferase reporter gene. Transfection of this construct into K562 chronic myelogenous leukemia cells resulted in a 10-fold increase in luciferase activity relative to cells transfected with a simian virus (SV) 40 promoter-driven pGL3-control plasmid (Fig. 2). Transfection of the same construct into Jurkat acute T cell leukemia cells and U937 histiocytic lymphoma cells yielded 7-fold and 3.5-fold increases, re-

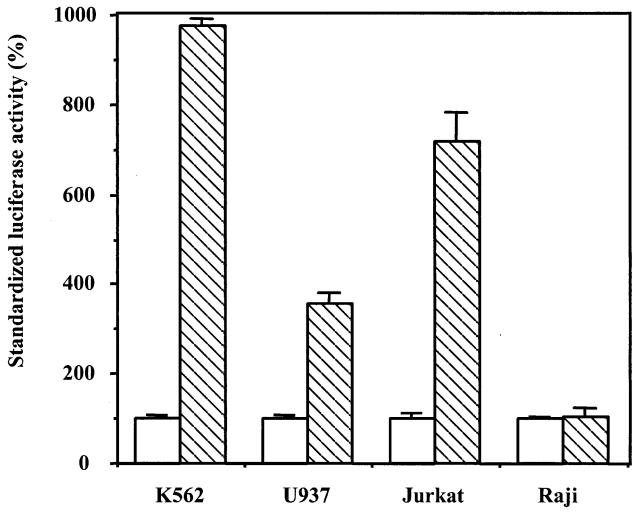

Fig. 2. Transcriptional activity of the minimal *GSTP1* promoter in human leukemia cells. Summary of luciferase assay results. Each cell line was transfected with a pGL3-control plasmid (white bars), with pGST1128 construct (shaded bars), and co-transfected with a β-galactosidase-expressing pCMVβ plasmid. The cells were harvested 48 hr later, and standardized luciferase activity was determined as described in Materials and Methods. Results were compared between cell lines taking into account the correction for the pGL3-control activity levels. Each bar represents the average ± SD of three independent transfection experiments. Assays were repeated twice.

spectively. In contrast, transfection of this reporter construct into Raji, a Burkitt's lymphoma cell line, produced no significant increase above pGL3 luciferase activity (Fig. 2). These results are in good agreement with our observations by Northern blot analysis as K562 cells expressed the highest *GSTP1* mRNA levels. These data suggest that expression of GSTP1-1 is cell line-specific.

3.2. Methylation status of CpG sites in the GSTP1 promoter

The central tenet of our hypothesis is that, if a relationship exists between CpG methylation and transcriptional activity of the *GSTP1* gene promoter in leukemia cell lines, we would expect *GSTP1* promoter methylation to result in lower levels of *GSTP1* gene expression. To analyze the precise methylation pattern of the *GSTP1* promoter, we chose the chronic myelogenous leukemia cell line, K562, which, as shown above, expresses high levels of *GSTP1* mRNA. Fifteen CpG dinucleotides can be recognized in the 136-bp fragment that we studied, spanning from position −98 to +37 relative to the transcriptional start site + 1 (Fig. 3A). This region contains numerous canonical binding sites for transcription factors including two Sp1 sites and one activator protein-1 (AP-1) site. PCR fragments resulting from amplification of bisulfite-modified K562 DNA were subcloned into pCRII-Topo (topoisomerase) plasmid vectors. After modification and PCR, unmethylated cytosines appear as thymines whereas methylcytosines appear as cytosines on the sequencing gel. Fig. 3B shows the tracks of a typical bisulfite sequencing autoradiograph. To ensure an accurate methylation profile, we sequenced a total of 18 to 27 clones from 3 independent PCR reactions. For each of the sequenced clones, the presence or absence of a methylated cytosine at the 15 CpG sites within the PCR fragment was scored. Table I shows the methylation patterns recorded for the different clones of the K562 and Raji cell lines. In K562 cells, which express *GSTP1*, 37% of the clones were almost completely demethylated (Table I). Raji cells, which do not express *GSTP1*, showed a very high level of methylation (90–95%), suggesting that promoter inactivation in these cells might result from methylation (Table I).

3.3. Inhibition of transcriptional activity by methylation of the minimal GSTP1 promoter

To study the possible cause and effect relationships between CpG methylation and transcriptional activity, we compared the ability of *in vitro* methylated and mock-methylated *GSTP1* minimal promoters to direct the reporter gene expression in transiently transfected leukemia cells expressing GSTP1-1 (K562, U937, and Jurkat) and the non-expressing Raji lymphoma cells. To accomplish complete methylation, we used *Sss*I methylase, which methylates all CpG dinucleotides in the methylation reactions. The comparison of results with methylated and unmethylated reporter plasmids indicates, in all tested cell lines, that methylation resulted in only residual reporter gene activity with respect to the control cells transfected with unmethylated pGST1128: 3.6% for K562, 0.8% for U937, 0.2% for Jurkat, and 0.7% for RAJI (Fig. 4).

To assess the role that methylation of the promoter relative to methylation of the plasmid backbone may have the gene expression results, we measured the reporter activity after exchanging the different plasmid components. The methylated *GSTP1* promoter fragment was religated into a mock-methylated plasmid backbone, and the methylated plasmid backbone was ligated to a mock-methylated promoter fragment. Transfection of the chimeric plasmids into K562 cells resulted in 96.3% reduction of luciferase activity when only the promoter was methylated and 66% reduction when only the plasmid backbone was methylated (Fig. 4). These results confirm that methylation significantly reduces the promoter activity of the *GSTP1* fragment, but also that other effects take place at CpG islands that may affect the expression of nearby genes.

4. Discussion

In this report, we have shown that transcription of the *GSTP1* gene is cell line-specific even among human leukemia cells in culture. We have also found that in cell lines

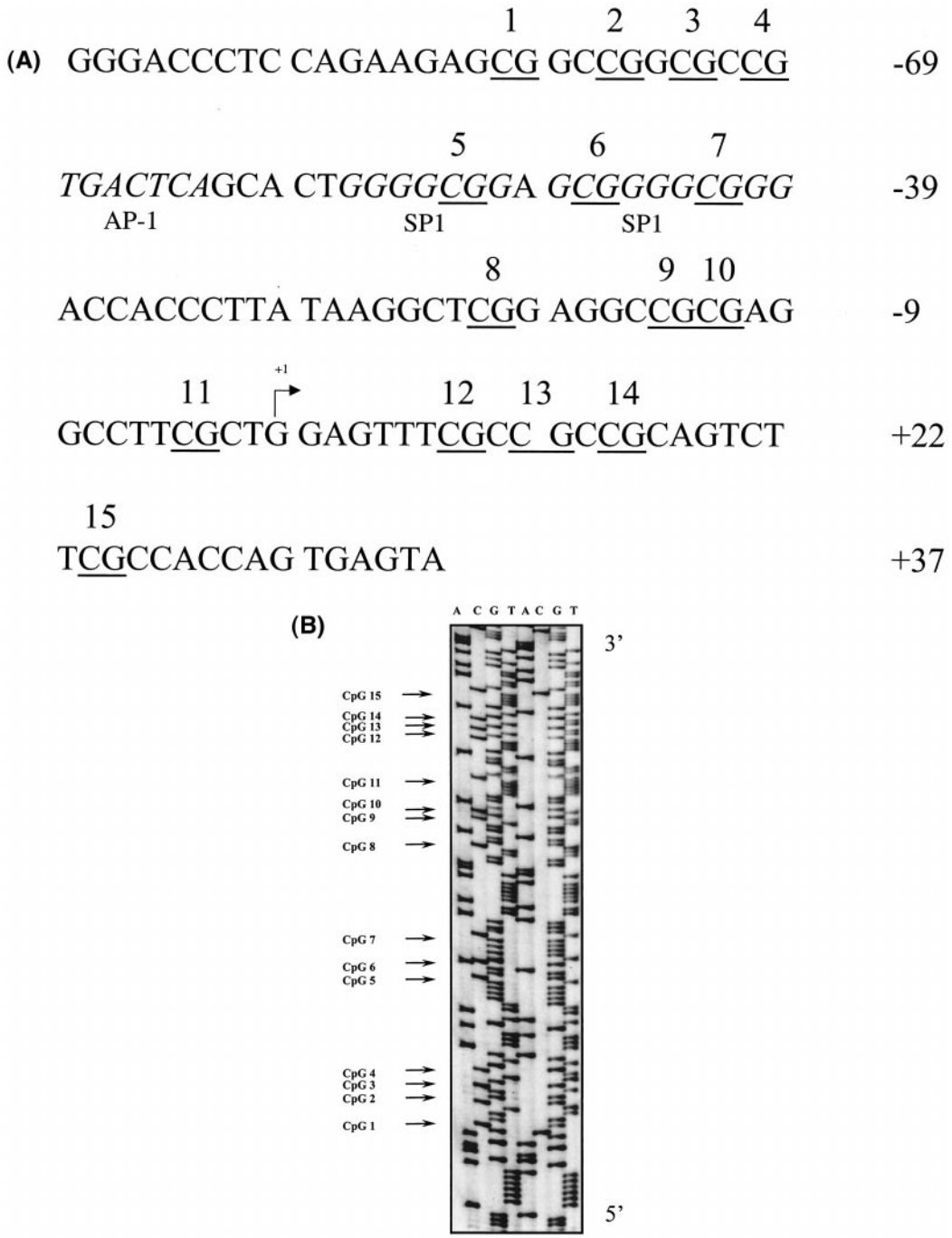

Fig. 3. (A) Alignment of the human *GSTP1* promoter sequences. CpG dinucleotides are numbered and underlined. Sequence is numbered relative to the transcriptional start site as indicated (+1); binding sites for SP1 and activator protein-1 are in italics. (B) Typical bisulfite sequence autoradiograph of two clones of the amplified region (complementary strand). All cytosines have been converted to thymines while 5-methylcytosines appear in the C track. Clones shown are methylated at all CpG sites (Raji cells, left) or unmethylated (K562 cells, right).

that express *GSTP1* mRNA, such as K562, a 136-bp fragment of the *GSTP1* minimal promoter was sufficient to direct the strong induction of a luciferase reporter gene. *In vitro* methylation of the promoter reduced its ability to direct the expression of the reporter gene. In Raji lymphoma cells, low levels of *GSTP1* mRNA could be correlated to a lower transcriptional activity and a stronger methylation of the promoter region. Those results correlate with those of Shea *et al.* [25], who measured very low anionic GST activity related or identical to GSTP1-1 (<1.0 milliunits/mg protein) in Raji cells.

Several proximal promoter elements have been shown to influence GSTP1-1 expression. A region spanning from position −80 to −8 is a prerequisite for reporter gene activity in transient transfection experiments [26] and for retinoic acid-dependent repression [27]. Of two GC boxes

Table 1
Methylation of individual clones of K562 and Raji cell lines

Cell line	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	No. of methylated sites
K562	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	1
	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	1
	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	1
	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	2
	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	2
	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	2
	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	2
	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	14
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Total %	84	63	63	63	58	63	63	63	63	63	63	63	63	63	100	
Raji	+	+	−	−	−	−	−	−	−	−	−	−	−	−	+	3
	+	+	+	+	+	+	−	−	−	−	−	−	−	−	+	7
	+	+	+	+	+	−	−	−	+	+	+	+	+	−	−	10
	+	+	+	+	+	−	−	+	+	+	+	+	+	−	−	11
	+	+	−	+	+	+	−	+	+	−	+	+	+	+	+	12
	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	13
	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	13
	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	13
	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	14
	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	14
	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	14
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Total %	96	96	93	96	96	81	81	96	93	96	93	93	93	85	93	

Each horizontal line represents a single sequenced clone; the sequencing results from three independent PCRs have been combined for each cell line. (+): methylated site and (−): unmethylated site; total %: average level of methylation of all clones sequenced for each CpG site.

located in this promoter region, the distal G/C box, but not the proximal, is essential for promoter activity, as shown by site-directed mutagenesis [16]. Other reports suggest that the activator protein-1 site (AP-1, nucleotides −69 to −63) is essential for promoter activity in a human breast carcinoma cell line [26,28]. Since the expression of various genes encoding drug-metabolizing enzymes, including the human NAD(P)H:quinone oxidoreductase1 (NQO_1) gene and the murine GST Ya and rat GST-P genes, is regulated through inducible AP-1 components, the involvement of the AP-1 response element in the transcriptional regulation of the human *GSTP1* gene might also be of importance. Recent studies have suggested that NF-E2-related factor 2 (Nrf2) is involved in regulating the basal expression of the rat

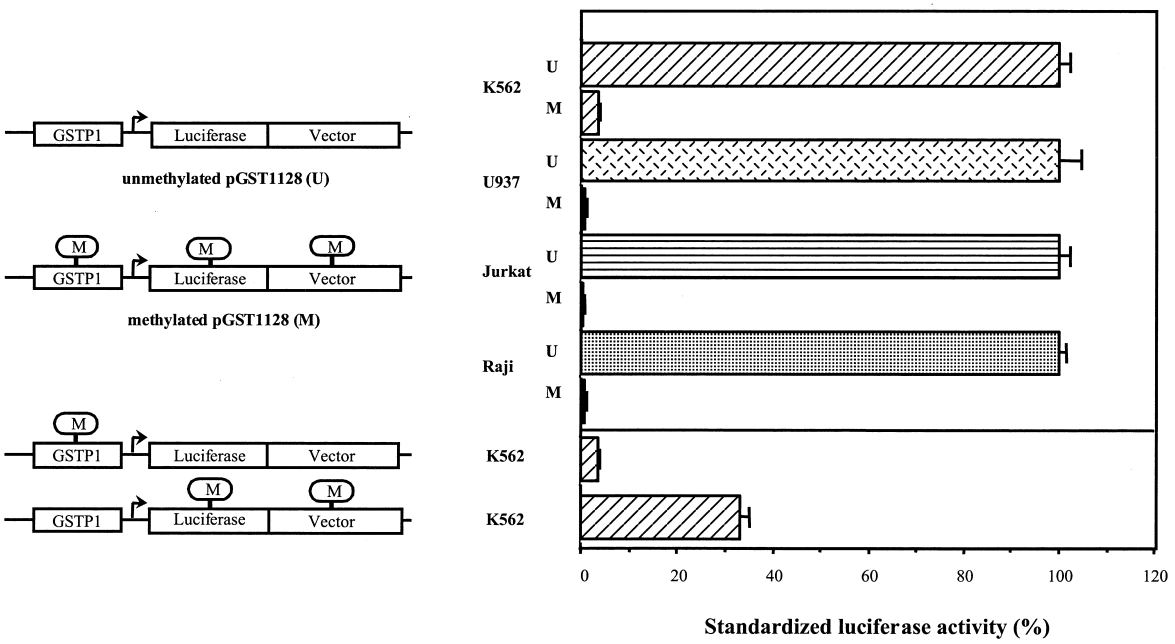

Fig. 4. Relative luciferase expression following *in vitro Sss*I methylation of plasmid construct pGST1128 containing the *GSTP1* promoter region upstream of a luciferase reporter gene and transfection into leukemia cells. Complete methylation of all CpG dinucleotides in a particular region of a given construct is represented by an attached M. Each bar represents the average ± SD. of three independent transfection experiments. Assays were repeated twice. U, unmethylated; M, methylated.

GSTA2 and NAD(P)H:quinone oxidoreductase1 genes [29]. Cell line-specific expression of these and related transcription factors might also regulate the expression of the *GSTP1* gene in human leukemia.

In K562, a chronic myelogenous leukemia (CML), methylation of the ABL1 promoter is limited to the allele nested within the Philadelphia fusion gene, whereas the allele on the normal chromosome 9 remains unmethylated [30]. The same authors previously showed that in chronic myelogenous leukemia, methylation is likely to be an allele-specific process. Moreover, for the ABL1 promoter, methylation appears to be closely correlated to CML progression and also correlates to a disease progression to a more aggressive form of the disease. As K562 cells form a homogenous culture and were established from a patient in the blastic phase of CML, further experiments will be needed to show whether or not every cell contains a methylated and an unmethylated promoter allele. *GSTP1* mRNA expression would then derive from the unmethylated allele, whereas the methylated allele would remain silent.

To assess the relationship between promoter methylation status and transcriptional activity, we analyzed the effect of *in vitro* methylation on the induction of the luciferase reporter gene transiently transfected in both expressing (K562) and non-expressing (Raji) cells. In both cell lines, *in vitro* methylation considerably reduced the expression level. Those results are in good agreement with a number of previous studies that correlate CpG methylation to transcriptional silencing of the minimal promoters of the $p16^{INKA}$ [3] and embryonic globin genes [31].

Hypermethylation of regulatory sequences at the *GSTP1* gene has been observed in human prostatic tissue specimens [32], where it was accompanied by a striking decrease in GSTP1-1 protein expression. Overexpression of *GSTP1* was observed during the development of multidrug resistance mechanisms in acute non-lymphoblastic leukemia and in acute myeloid leukemia [33], which led to the suggestion that expression of *GSTP1* might be a useful marker of clinical resistance to cytostatic drugs. This close association with multidrug resistance in human leukemia and in breast cancer makes the study of the molecular mechanisms regulating *GSTP1* expression of particular importance for the design of anticancer treatments. Conversely, we might expect that demethylation of otherwise CpG-rich promoter regions at that specific site will have an important role in relieving repression of *GSTP1* expression and play a key role in the switch from drug-sensitive cancer cells to the development of drug resistance in human leukemia cells.

In the present study, we have examined the relationship between methylation and the promoter activity of a 136-bp minimal *GSTP1* promoter in the human leukemia cell line K562. We find that *in vitro* methylation of this promoter with *Sss*I has a transcriptional inhibitory effect and that the methylation state of this *GSTP1* promoter fragment in expressing and non-expressing leukemia cell lines correlates with the degree of *GSTP1* RNA expression and transcriptional activity.

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