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Glutathione S-transferase enzyme expression in hematopoietic cell lines implies a differential protective role for T1 and A1 isoenzymes in erythroid and for M1 in lymphoid lineages

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ABSTRACT

Background and Objectives. **Glutathione S-transferases (GSTs) are phase II metabolizing enzymes which catalyze the conjugation of glutathione (GSH) to electrophilic substrates and possess selenium-independent glutathione peroxidase activity. The GST enzyme family includes the cytosolic isoforms GST-α (GSTA), μ (GSTM), π (GSTP), θ (GSTT) and σ (GSTS). GSTT1, P1 and M1 are polymorphic and altered polymorphic frequency of genes encoding these proteins has been suggested as a potential risk factor for the development of hematopoietic malignancies. Overexpression of GSTs has also been implicated in chemotherapeutic drug resistance. This study was undertaken to elucidate the potential functional relevance of these genetic polymorphisms in hematopoiesis.**

Design and Methods. **GST genotype of 14 hematopoietic cell lines was determined by polymerase-chain-reaction (PCR). Gene expression of GSTs in cell lines was detected by real-time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) on TaqMan 7700 and by semi-quantitative RT-PCR. Cytosolic GST protein expression was detected by Western blot. GST conjugation activity was assayed using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate.**

Results. **GSTP1 expression was higher than other GSTs in 13/14 cell lines and paralleled CDNB conjugation activity. GSTP1 and GSTM1 predominated in lymphoid lines whilst T1 expression was relatively greatest in erythroid lines but was absent in 7/12 non-null lines. GSTT2 was expressed in only 3/14 lines. The 3 cell lines which expressed GSTA1 were all erythroid.**

Interpretation and Conclusions. **Glutathione S-transferases showed differential lineage expression in hematopoietic cell lines. This implies a greater cytoprotective role for GSTT1 and GSTA1 in erythroid cells and GSTM1 in lymphoid cells. We postulate that inherited gene deletion of GSTT1 and M1 may produce increased genotoxic susceptibility for erythroid and lymphoid cells respectively, following exposure to xenobiotics that are substrates for these enzymes.**

Key words: glutathione S-transferase, genotoxicity, antioxidant, hematopoietic cell lines

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Higher organisms have evolved complex mechanisms by which they can protect themselves from environmental challenge. The metabolism and detoxification of xenobiotic toxins provides the first line of intracellular defence and this is mediated by enzyme superfamilies, including the cytochrome p450, glutathione S-transferase (GST) and N-acetylTransferases. Glutathione S-transferase genes encode for five families of cytosolic enzymes: GSTs-α (GSTA), μ (GSTM), π (GSTP), θ (GSTT) and σ (GSTS). Each family consists of isoenzymes which homo or hetero-dimerize to catalyze enzymatic reactions using different substrates. GSTs are phase II metabolizing enzymes which catalyze the conjugation of glutathione (GSH) to electrophilic substrates, most of which are the products of phase I metabolism. This is a major pathway of protection against chemical toxins and carcinogens (xenobiotics). GSTs also have an important role in conjugating GSH to the products of endogenous lipid peroxidation and inactivating organic hydroperoxides via selenium-independent glutathione peroxidase activity, thus protecting the cell from the deleterious effects of oxidative stress. These enzymes therefore function to detoxify exogenous carcinogens (including cytotoxic drugs) and thus prevent cellular cytotoxicity/DNA mutation. They may also function as disease-modifying enzymes when oxidative stress is contributory. GSTs are constitutively expressed in a wide variety of tissues, each with different patterns of isoenzyme expression.[1,2] Only limited data concerning lineage specific expression of GSTs in hematopoiesis are available and a more detailed understanding will potentially clarify the role of GSTs in the etiology of human hematopoietic malignancy and in bone marrow chemotherapeutic drug resistance. This study describes lineage specific expression of 6 GST isoenzymes in 14 hematopoietic cell lines.

Design and Methods

Cell culture

Hematopoietic cell lines, source and culture conditions are listed in Table 1. Cells in logarithmic growth were collected for subsequent analysis.

DNA extraction and genotype analysis by polymerase chain reaction (PCR)

Genomic DNA was prepared from each cell line by a standard proteinase K-phenol-chloroform extraction method. DNA was amplified in a final volume of 25 µL containing 20 mM $(NH_4)_2SO_4$, 75 mM Tris-HCl, pH 8.8 at 25°C, 0.01% (v/v) Tween 20, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 0.5 unit of Red Hot DNA polymerase (Advanced Biotechnologies, Epsom, Surrey, UK) and 12.5 pmol of each primer were added in the 25 µL reaction. PCR conditions were denaturing at 95°C for 5 min, then 35 cycles of denaturing at 95°C for 30 sec, annealing at 60 °C for 30 sec and elongation at 72°C for 1 min, and finally incubation for 10 min at 72°C. Ten microliters of PCR product were run on 8% polyacrylamide (acrylamide: bisacrylamide=29:1) gel. The gel then was silver-stained.

Identification of GSTP1, GSTM1 and GSTT1 polymorphism

GSTM1 wild/null were checked by PCR performed with primers M1g2 and M1g3.[3] Fidelity of the DNA template in GSTM1*0 homozygotes was confirmed by co-amplification of a 90bp fragment of GAPDH gene. A modified PCR method which was first developed by Fryer *et al*.[4] was used to identify the GSTM1*A, GSTM1*B and GSTM1A/B. GSTP1 and GSTT1 polymorphisms were identified by PCR and PCR-RFLP assay.[5,6]

Table 1. Haematopoietic cell lines.

Cell lines	*Cell type*	*Source*
KG-1a	Undifferentiated	ECACC
F36p	Multipotential E/Me/My	RIKEN Cell Bank
MDS92	Multipotential: Me/My	Dr. K Tohyama, Kyoto, Japan
SKM-1	Myelomonoblast	Dr. T. Nakagawa, Hyogo, Japan
P39	Myelomonoblast	Dr. Yoshida, Kyoto, Japan
HL60	Myeloid/promyelocyte	ECACC
U937	Histiocytic/monocyte	ECACC
K562	Erythroid (Me)	ATCC
JK-1	Erythroid (Me)	Dr. T. Takahashi, Kobe, Japan
OCI-M1	Erythroid (Me/My)	Dr. Papayannopoulou, Seattle, USA
HEL	Erythroid (Me/My)	ECACC
MEG-01	Megakaryoblast	ATCC
Jurkat	T-lymphoblast	ECACC
Molt-4	T-lymphoblast	ECACC

Me: Megakaryocytic; My: Myeloid; E: Erythroid: ECACC: European Collection of Cell Cultures; ATCC: American Type Culture Collection;

Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from 10^6 cells using QIAGEN RNeasy Mini Kit (QIAGEN Ltd., Crawley, West Sussex, UK). DNase I (FPLCpure, Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK) was used to digest the genomic DNA in total RNA. cDNA was synthesized from the DNase-treated RNA using random hexamer (Promega, Southampton, UK) and SUPERSCRIPT™ II RNase H^- reverse transcriptase (GIBCOBRL BRL Life Technologies, Paisley, Renfrewshire, UK). For manual semi-quantitative RT-PCR, 10^6

Table 2. TaqMan real-time RT-PCR primers and probes and semi-quantitative RT-PCR primers

	Name	*Sequence*	*Amplicon (bp)*	*Position*
GSTP1 primers	pe3-4-89F	5'CTCACTCAAAGCCTCCTGCCTAT-3'	85	exon3-4
	pe3-4-173R	5'CAGGATGGTATTGGACTGGTACAG-3'		
GSTP1 probe	P1-121T	5'TCCCCAAGTTCCAGGACGGAGACCT-3'		
GSTM1 primers	M1340-6F	5'CTGGGCATGATCTGCTACAATC-3'	136	exon5-7
	M1340-141R	5'CAAAAGTGATCTTGTTTCCTGCAA-3'		
GSTM1 probe	M1-51T	5'AAGTACTTGGAGGAACTCCCTGAAAAGCTAAAGC-3'		
GSTT1 primers	t1e3-4-109F	5'CACGACTCTGCGGAGAAGCT-3'	126	exon3-4
	t1e3-4-234R	5'TGCAGGGTCACATCCAACTCT-3'		
GSTT1 probe	T1-140T	5'AACACAGGGAACATCACCTTAAGCCACAA-3'		
GSTA4 primers	a4-11F	5'GGATCTGCTGGAACTGCTTATCAT-3'	135	*
	a4-145R	5'TGTCCGTGACCCCTTAAAATCTT-3'		
GSTA4 probe	A4-39T	5'CCTTTCTTAAAACCAGATGATCAGCAAAAGGAAG-3'		
GSTT2 primers	t2e300-14F	5'TGCATCCGTGGCACCTTT-3'	109	exon3-4
	t2e300-122R	5'GGTCCATGGCAGTCCTGTTG-3'		
GSTA1 primers	ae5-6-128F	5'CCTGCCTTTGAAAAAGTCTTAAAG-3'	90	exon5-6
	ae5-6-217R	5'AAGTTCCACCAGGTGAATGTCA-3'		

Gene Bank Accession No. AF020918 nucleotide 370-504

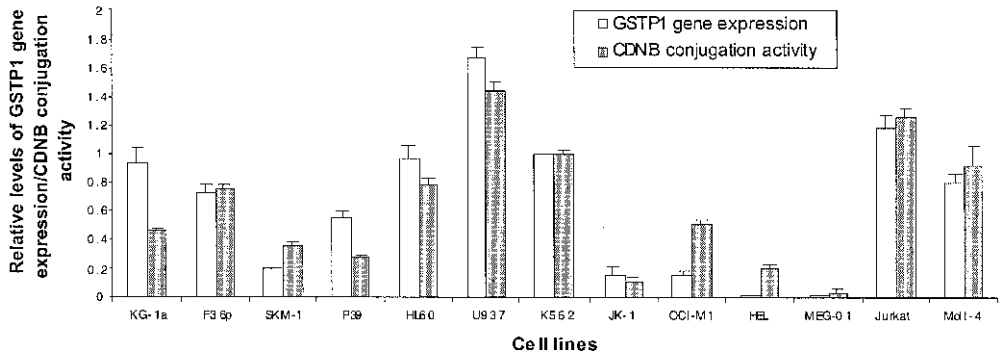

Fig 1. Correlation between GSTP1 gene expression and CDNB conjugation activity. K562 cell line was considered as an arbitrary figure of 1.0 for both GSTP1 gene expression and CDNB conjugation activity. All the other cell lines were compared to K562.

cells were used for RNA extraction and cDNA synthesis. The amplification of GAPDH gene or β-actin gene in separate tubes was used as internal semi-quantitative control. The primers used are listed as in Table 2. Every primer pair was checked by sequencing the PCR product to ensure the specificity of amplification.

Real-time quantitative RT-PCR

The primers and probes were designed by applying Primer Express™ 1.0 Software (PE Applied Biosystems, Warrington, UK). To ensure the specificity of each assay, PCR products were gel-purified and sequenced by an ABI Prism 377 Automated DNA Sequencer (PE-Applied Biosystems, Warrington, UK). The GSTP1, GSTM1, GSTT1 and GSTA4 primers and probes are listed in Table 2. Probes were 5' end FAM labeled and 3' end TAMRA labeled (PE Applied Biosystems, Warrington, UK). Primers were synthesized by MWG Biotech UK LTD (Milton Keynes, UK). cDNA of 18S rRNA was co-amplified with each of GSTP1, T1, M1 and A4 genes in the same well by using TaqMan ribosomal RNA internal control kit (PE-ABI). 18S rRNA gene primers were JOE labeled. Concentrations of primers and probes were optimized for each assay then real time PCR was performed in triplicate using the TaqMan PRISM®7700 Sequence Detection System (PE Applied Biosystems) in a 25 μL reaction volume. DNase treated RNA was also used as template to ensure no genomic DNA amplification for every gene. Relative gene expression was calculated as the ratio of signal for target gene (FAM) to signal for 18S rRNA (JOE). GSTP1, T1 and A4 gene expression of K562 cell line was considered as an arbitrary figure of 1.0. For GSTM1 gene expression, HEL cell line was normalized to 1.0 as K562 is null for GSTM1. To calculate relative levels of different isoenzymes in cell lines, the same threshold and baseline for the target gene FAM signal was set up. The internal control signal was normalized to the JOE signal of the same batch of K562 cDNA that was used as reference across the different plates. All values were normalized to an arbitrary value of 1.0 for GSTA4 expression in K562 cells.

Preparation of cytosol and microsomes

Cells in logarithmic growth were harvested and washed by cold phosphate-buffered saline (pH 7.4). Cell pellets were frozen at –70°C. Cytosol and microsomes were prepared by differential centrifugation.[7]

1-chloro-2,4-dinitrobenzene conjugation activity

GST conjugation activity was assayed as described elsewhere[8] using 1-chloro-2,4-dinitrobenzene CDNB (Sigma-Aldrich Company Ltd., Poole, Dorset, UK) as the substrate. Formation of the CDNB-GSH conjugate by cytosol was measured continuously by Cobas Fara II Chemistry System (Hoffman-La Roche Ltd, Basel, Switzerland) at 340 nm. The extinction coefficient is 38 nM^{-1} cm^{-1}. Results were expressed in nmol of CDNB conjugated per min per μg of cytosolic protein.

Western blot analysis

Cytosolic proteins from each cell line were freeze-dried and 10 μg separated by electrophoresis on sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE) as described by Laemmli[9] using a 12.5% separating gel. Resolved proteins were electrotransferred to a PVDF membrane (Sigma-Aldrich Company Ltd, Poole, Dorset, UK) using a continuous transfer buffer (39 mM glycine, 48 mM Tris, 0.0375% (w/v) SDS, and 20% methanol). Rabbit polyclonal antibody raised against human GSTT1, rat GSTA4 and rat GSTP1 (a gift from Prof. John Hayes, Biomedical Research Centre, University of Dundee) were used at dilutions of 1:1000, 1:5000 and 1:1000, respectively. The second antibody was goat anti-rabbit immunoglobulin-horseradish peroxidase conjugate (DAKO Ltd, Cambridge, UK) and was used at a dilution of 1:3000. An enhanced chemiluminescence system (Amersham Life Science, Little Chalfont, Bucks., UK) was used for protein detection.

Results

GST conjugation enzyme activity

CDNB conjugation activity paralleled GSTP1 expression in most cell lines (Figure 1 and Table 3).

Table 3. CDNB conjugation activity of cell lines.

Cell Lines	*CDNB conjugation activity (nmol/µg.min)*
KG-1a	21.7 ± 0.6
F36p	34.7 ± 1.1
SKM-1	16.5 ± 0.8
P39	12.6 ± 0.7
HL60	36.3 ± 1.2
U937	65.9 ± 2.8
K562	45.9 ± 1.5
JK-1	4.9 ± 1.6
OCI-M1	23.0 ± 2.0
HEL	9.5 ± 0.9
MEG-01	1.8 ± 0.9
Jurkat	57.9 ± 2.7
Molt-4	42.7 ± 5.6

GST subclass distribution (Table 4)

GSTP1. GSTP1 mRNA and protein were found in all cell lines and were well correlated (Figure 2, Table 4). GSTP1 RNA and protein were highly expressed in both lymphoid cell lines (Jurkat, Molt-4), and all three undifferentiated/pluripotential cell lines (KG-1a, F36p and MDS92). Three of four erythroid cell lines showed relatively low expression. Four of six AA genotype cell lines had lower expression, while all A/B or A/C cell lines had higher expression.

GSTT. Only JK-1 and MEG-01 cell lines were null for the GSTT1 gene. GSTT1 gene expression and protein were found predominantly in the erythroid cell lines. In 12 GSTT1 wild type cell lines, GSTT1 was highly expressed in K562, OCI-M1 and HEL cells (Table 4 and Figure 3). These cell lines express predominantly erythroid and megakaryocytic surface markers. GSTT1 gene expression was also detected in MDS92 and SKM-1 cells, both of which expressed a lower percentage of erythroid and/or megakaryocytic antigens. GSTT1 was not expressed in either lymphoid cell lines or in 3 myeloid cell lines. GSTT2 gene expression was found in only three cell lines: MDS92, HEL and MEG-01, with particularly high expression in the megakaryocytic cell line MEG-01.

GSTA. GSTA1 was expressed at low levels in 3 of 4 erythroid cell lines and not at all in undifferentiated, myeloid, megakaryocytic and lymphoid cell lines (Table 4 and Figure 3). GSTA4 was expressed in all cell lines.

GSTM1. Seven of 14 cell lines had the GSTM1*0 genotype. GSTM1 was highly expressed in two lymphoid (Jurkat and Molt-4) and one erythroid (HEL) cell lines. No GSTM1 mRNA was detectable in non-null genotype erythroid OCI-M1 cells or myeloid HL60 cells. Low levels of GSTM1 expression was found in MDS92 and P39 cell lines.

Relative lineage-specific expression of GST subfamilies

GSTP1 was the predominant GST enzyme in 13/14 hematopoietic cell lines (Table 5). Expression of all subfamilies studied was found at different levels in erythroid cell lines though GSTT1 was expressed predominantly in this lineage. GSTP1 expression was relatively lower than in non-erythroid cell lines. Lymphoid cell lines had relatively higher expression of GSTP1, GSTM1 and GSTA4 and did not express GSTT1, GSTT2 or GSTA1. In myeloid cell lines, the main GSTs expressed were GSTP1 and GSTA4 while neither GSTA1 nor GSTT2 was found. Finally, the megakaryoblast cell line MEG-01 expressed a relatively high level of GSTT2, median level of GSTA4, and relatively low level of GSTP1. No GSTA1 mRNA was found in MEG-01.

Table 4. Gluathione S-transferase genotype and gene expression in haematopoietic cell lines.

Cell lines	*GSTP1*		*GSTM1*		*GSTT1*		*GSTT2*	*GSTA1*	*GSTA4*
	Genotype	*Gene Expression*	*Genotype*	*Gene Expression*	*Genotype*	*Gene Expression*	*Gene Expression*	*Gene Expression*	*Gene Expression*
KG-1a	AB	0.94 ± 0.10	NULL	0	WT	0	-	-	0.08 ± 0.02
F36P	AB	0.72 ± 0.06	NULL	0	WT	0	-	-	0.92 ± 0.11
MDS92	BB	1.20 ± 0.01	B	0.16 ± 0.05	WT	0.17 ± 0.03	+	-	0.09 ± 0.08
SKM-1	AA	0.21 ± 0.00	NULL	0	WT	0.23 ± 0.06	-	-	0.31 ± 0.16
P39	AA	0.56 ± 0.03	B	0.19 ± 0.05	WT	0	-	-	0.07 ± 0.02
HL60	AA	0.97 ± 0.09	B	0	WT	0	-	-	0.08 ± 0.04
U937	AC	1.67 ± 0.08	NULL	0	WT	0	-	-	3.34 ± 0.63
K562	AB	1.00 ± 0.00	NULL	0	WT	0.98 ± 0.01	-	+	0.48 ± 0.05
JK-1	AA	0.15 ± 0.07	NULL	0	NULL	0	-	-	0.03 ± 0.02
OCI-M1	BB	0.16 ± 0.02	B	0	WT	0.85 ± 0.06	-	+	0.09 ± 0.04
HEL	AA	0.02 ± 0.00	A	1.00 ± 0.15	WT	0.67 ± 0.06	++	+	0.97 ± 0.08
MEG-01	AA	0.01 ± 0.00	NULL	0	NULL	0	+++	-	0.28 ± 0.08
Jurkat	AB	1.18 ± 0.09	A	0.66 ± 0.01	WT	0	-	-	1.17 ± 0.23
Molt-4	CC	0.80 ± 0.06	A/B	1.23 ± 0.17	WT	0	-	-	0.80 ± 0.14

After normalisation to internal control-18S rRNA signal, GSTP1, GSTT1 and GSTA4 gene expression of K562 cell line was considered ; as an arbitrary figure of 1.0. For GSTM1 gene expression HEL cell lines was normalised to 1.0. WT:wild type; NULL: null genotype; -: no expression; +/++/+++: semi-quantitative expression level relative to GAPDH signal.

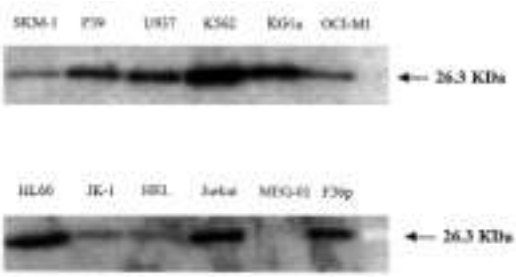

Figure 2. GSTP1 protein expression in hematopoietic cell lines. Ten µg cytosolic protein of each cell lines was analyzed by Western Blot with polyclonal rat GSTP1 antibody (1:1000 dilution).

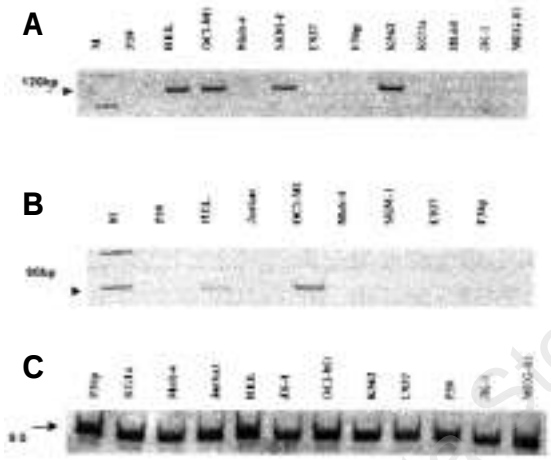

Figure 3. Semi-quantitative manual RT-PCR gene expression of A. GSTT1, B. GSTA1 C. GAPDH in haematopoietic cell lines. cDNA amplification fragments were resolved on 8% polyacrylamide gel and silver-stained. M: Marker-100bp DNA ladder.

Table 5. Relative gene expression of different GST subclasses within individual hematopoietic cell lines.

Cell lines	*GSTP1*	*GSTM1*	*GSTT1*	*GSTA4*
KG-1a	628.89	null	0.00	0.17
F36p	485.50	null	0.00	2.16
MDS92	712.46	30.48	8.94	0.18
SKM-1	117.51	null	12.47	0.64
P39	353.77	36.00	0.00	0.16
HL60	692.98	0.00	0.00	0.16
U937	1052.79	null	0.00	8.11
K562	680.29	null	54.44	1.00
JK-1	93.27	null	null	0.08
OCI-M1	99.04	0.00	46.85	0.18
HEL	12.27	218.27	38.05	2.35
MEG-01	7.82	null	null	0.58
Jurkat	761.83	129.79	0.00	2.53
Molt-4	532.51	236.11	0.00	1.79

GSTA4 gene expression level of K562 was considered as an arbitrary figure of 1.0. The same threshold and baseline was used for the target gene FAM signal (GSTP1, T1, M1, A4) on different plates. The internal control JOE signal (18s rRNA) of the same K562 cDNA was used as reference for different plates.

Discussion

We have demonstrated that expression of GST isoenzymes in hematopoietic cell lines shows distinct lineage differences. Hematopoietic cell lines harbor multiple genetic damage, which will differ substantially from one line to another. Whilst they are inherently imperfect models for the *in vitro* study of hematopoiesis, the use of more than one line with lineage-specific characteristics in our study goes some way towards overcoming these limitations. GST expression in humans is known to exhibit tissue specificity. GSTA class enzymes are expressed in the liver and kidney, GSTM in the brain, lymphocytes, muscle, and testes, and GSTP in the placenta, spleen, hepatic ducts, and kidney tubules.[2] This has potential significance for tissue-specific susceptibility to genotoxicity from endogenous and exogenous insult. High concentrations and activity of detoxification enzymes are expressed in the liver and this organ has the major role in carcinogen metabolism. Nevertheless, it is clear that many genotoxins are known to act directly *in vivo* on bone marrow cells and examples include benzene[10] and cytotoxic drugs.[11] Bone marrow cell protection from these insults thus depends upon intact detoxification pathways and DNA repair systems to prevent cellular mutagenesis/apoptosis. Differential expression of detoxification enzymes between cell lineages may lead to lineage-specific susceptibility to genotoxicity following a given insult. This insult could be an environmental toxin such as benzene/heavy metals implicated in the etiology of myelodysplastic syndromes,[12] cytotoxic drugs leading to differential chemosensitivity/chemoresistance[13] or other pharmacological agents producing idiosyncratic bone marrow cell toxicity such as agranulocytosis or aplasia.

In our study, GSTP class was the predominant GST in 13/14 cell lines analyzed, consistent with a previous report.[14] In most cell lines GSTP1 mRNA was at least twice as abundant as any other GST enzyme class and the major role of GSTP1 was emphasized by the fact that its expression paralleled CDNB conjugation activity. GSTP over-expression has been implicated in chemotherapeutic drug resistance for a number of hematopoietic malignancies,[14-16] though it has been little studied in the context of metabolism of other carcinogens by bone marrow cells.

From our report, and consistent with previous studies, GSTP1 and GSTM1 are the predominant GSTs in T-lymphoblast cell lines. GSTT1 and T2 were not expressed in these T-cell lines. GSTM expression (but not GSTTor GSTA) has been correlated with sur-

vival in acute lymphoblastic leukemia.[15]

The relatively high levels of GSTT1 and A1 and the relatively low GSTP1 in erythroid cell lines imply greater roles for GSTT1/A1 isoenzymes in the erythroid than in other lineages. GSTT1 is known to be highly expressed in erythrocytes.(17) An increased frequency of GSTT1 gene deletion in myelodysplastic syndrome (MDS) has been described[18] though this remains controversial.[19-20] Though MDS is a trilineage disorder, low risk MDS affects predominantly the erythroid lineage.

Finally, although platelets contain abundant GST,[21] our megakaryoblast cell line (in which no platelets were morphologically detectable) contained only a very low level of GSTP1. The functional significance of high expression of GSTT2 in the megakaryoblasts is unknown though this clearly differs from other lineages.

In conclusion, by analyzing 6 GST isoenzymes in hematopoietic cell lines, we found clear differences in lineage-specific expression. Our studies confirm the predominance of GSTP1 in hematopoietic cells but also imply a greater cyto-protective role for GSTT1 and GSTA1 in erythroid cells and GSTM1 in lymphoid cells.

Potential implications for clinical practice

1. This study will help to elucidate the cytoprotective role of GSTs against hematopoietic cellular toxicity from agents that are substrates for these enzymes. Such cytoprotection may be a pathway for prevention of
   - mutagenic events induced by leukemogenic xenobiotics
   - idiosyncratic drug-induced bone marrow cell dyscrasias
   - cytotoxic drug resistance in the therapy of hematopoietic malignancy
2. Our study suggests that the consequences of inherited allelic variants such as deletions of GSTT1 and M1 genes will differ for different hematopoietic lineages and this may influence the defence capability of cells from those lineages.

Contributions and acknowledgments

LW carried out most of the laboratory analysis, managed the data and wrote the paper. DTB designed the study, critically assessed the data, assisted with writing the paper and was responsible for the final version of the paper. MJG contributed to the genotyping of cell lines and both critically reviewed and assisted with writing the manuscript. MDH was involved in the cell culture of all cell lines and both critically reviewed and assisted with writing the manuscript.

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The order of authorship was decided as follows. LW was the major contributor both to the laboratory assays and manuscript preparation and was first author. DTB was a major contributor to the study design and the manuscript writing and therefore appears last. MJG and MGH contributed equally as above and therefore appear as second and third authors.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

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