Glutathione-*S*-transferases and Chemotherapy Resistance of Hodgkin's Lymphoma Cell Lines

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Abstract. *Background: Glutathione-S-transferases (GSTs) are associated with multidrug resistance of tumor cells and are involved in drug detoxification and control of apoptosis. We analyzed the impact of GSTs on apoptosis of Hodgkin's lymphoma (HL) cells. Materials and Methods: Expression of GST isoforms in HL cell lines was assessed by analysis of DNA microarray data and quantitative reverse transcription-polymerase chain reaction (qRT-PCR). The impact of the GST inhibitor ethacrynic acid (EA) on HL cell survival was analyzed in vitro. Results: DNA microarray analysis and qRT-PCR analysis demonstrated higher expression of GST isoforms in chemoresistant HL cells. Therefore, GSTs may contribute to chemoresistance of HL cells. Incubation of GST-expressing chemoresistant L-1236 HL cells with EA significantly enhanced the activity of cisplatin against these cells. Conclusion: Our data suggest that the combined treatment with chemotherapy and GST inhibitors such as EA might be an interesting option for patients with chemoresistant HL.*

Today, more than ninety percent of pediatric patients with Hodgkin's lymphoma (HL) are cured due to combined traditional chemo- and radiotherapy. However, currently established treatment options are associated with late adverse side-effects and patients with therapy-refractory HL have still a very poor prognosis (1, 2). New treatment strategies are needed to further improve the prognosis of patients with HL. Beside the identification of molecular structures for targeted therapy, the detection of markers associated with chemoresistance might be useful for the treatment of therapy-refractory HL.

By using DNA microarray analysis, we found a highly divergent gene-expression profile between chemoresistant and sensitive HL cell lines. For example, chemoresistant cell lines express preferentially antigen expressed in melanoma (PRAME) at a high level. In turn, we observed increased sensitivity to several cytostatic drugs after knocking down of *PRAME* using vector-based RNA interference (3, 4). Expression of histone deacetylase 6 (HDAC6) is also particularly high in HL. We showed that the HDAC inhibitor vorinostat inhibits cell proliferation and induces changes in the gene expression pattern of HL cells. Subsequently, vorinostat increased sensitivity of HL cell lines to chemotherapy, *i.e.* cisplatin (5). The cyclin-dependent kinase inhibitor roscovitine might be also a potential anticancer agent for treatment of HL. Roscovitine induces cell-cycle arrest and sensitizes HL cells to apoptosis (6). These and other data suggest that targeted agents which increase cytotoxic drug sensitivity of HL might be a treatment option for patients with chemoresistant HL.

Glutathione-*S*-transferases (GSTs) as detoxification enzymes are also potential targets for overcoming drug resistance. GSTs are expressed at high levels in several tumor cell lines and human malignant tumors. GSTs may contribute to the chemoresistant phenotype of malignancies due to their detoxification of different cytostatic drugs (*e.g.* cisplatin and doxorubicin) (7, 8). Ethacrynic acid (EA) and its derivatives inhibit the GST α, μ and π subclasses by binding to the substrate-binding site, as well as by depleting enzymatic cofactors (8-10). DNA microarray data have revealed distinct GST expression patterns between resistant and sensitive HL cell lines. Therefore, we assessed the effect of EA on cytotoxic drug sensitivity in regard to the GST status.

Materials and Methods

Cell lines, cell culture, and determination of drug sensitivity. HL cell lines L-1236, L-540, L-428, KM-H2 and HDLM-2 (11-15) were obtained from the German Collection of Microorganisms and Cell Cultures, Brunswick, Germany. All cells were cultured in RPMI-1640 medium (Invitrogen, Karlsruhe, Germany) supplemented with 10% fetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin at 37˚C in a humidified atmosphere with 5% CO_2.

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Cisplatin, roscovitine and ethacrynic acid were purchased from Sigma, Munich, Germany. For determination of sensitivity to chemotherapy, chemosensitive L-540 cells and chemoresistant L-1236 cells or L-428 cells were treated in 24-well plates with cisplatin (50 μg/ml) or roscovotine (60 μM) in the presence of increasing concentration of EA (0 μM, 12.5 μM, 25 μM, 50 μM, 100 μM) for 24 hours. The total volume per well was 2.0 ml (0.5 ml cell suspension of 500,000 cells, 1.0 ml EA solution, 0.5 ml cisplatin or roscovotine solution). To assess cell viability, cells were stained with propidium iodide after centrifugation and re-suspension in 500 μl phosphate-buffered saline. Cells were analyzed on a FACScan flow cytometer (Becton-Dickinson, Heidelberg, Germany) equipped with CellQest Pro software (Becton-Dickinson, Heidelberg, Germany).

Gene expression analysis. DNA microarray analysis of HL cell lines was performed by using HG_U133A microarrays (Affymetrix, Santa Clara, CA, USA) (3, 5). Additional data sets from HL cell lines (16, 17) and normal tissues (18) were downloaded from the Gene Expression Omnibus (GEO) database (19). All data sets were analyzed by using Expression console 1.3.1.187 (Affymetrix). Data visualization was performed with Genesis (20).

For validation of differentially expressed genes in HL cell lines, conventional reverse-transcription-polymerase chain reaction (RT-PCR) and quantitative RT-PCR (qRT-PCR) was applied. Therefore, RNA from cell lines was isolated by using TriFast™ peqGold reagent (PegLab, Erlangen, Germany) following the manufacturer's protocol. RNA from peripheral blood mononuclear cells (21), normal liver (purchased from Cyagen, Santa Clara, CA, USA), normal spleen and normal thyroid (Agilent, Santa Clara, CA, USA) was used as control. Reverse transcription of RNA was performed by using the following conditions: 4 μl 5x buffer, 1 μl Oligo-dT_{12-18} primer, 1 μl dNTP mix (10 mM), 1 μl RevertAid H Minus M-MuLV reverse transcriptase (Fermentas, St.Leon Rot, Germany) at 37˚C for 60 min, then 90˚C for 5 min. After reverse transcription, 2.0 μl cDNA were mixed with 2.5 μl 10x buffer, 1.5 μl $MgCl_2$ (25 mM), 0.2 μl *Taq* polymerase (Promega, Mannheim, Germany), 0.5 μl dNTP mix (10 mM; Fermentas), 0.25 μl of sequence-specific primers (Invitrogen, Karlsruhe, Germany; MWG-Biotech AG, Ebersberg, Germany) and 17.8 μl water. The following primer combinations (forward and reverse) and conditions were used: actin beta (*ACTB*): 5'-ggc atc gtg atg gac tcc g-3', 5'-gct gga agg tgg aca gcg a-3'; *GSTM1*: 5'-CCT GTC TGC GGA ATC CGC AC-3', 5'-GCT GAG TAT GGG CTC CTC AC-3'; *GSTM2* isoforms 1: 5'-CCT GTC TGC AGA ATC CAC AG-3', 5'-GCT GAG TAT GGG CTC CTC AC-3', *GSTM2* isoform 2: 5'-CCT GTC TGC AGA ATC CAC AG-3', 5'-TAG TGG CCA CAA GGA TCA CA-3'; *GSTM4* isoform 2: 5'-GAA TCG ACA CCA ACC AGC AT-3', 5'-CCC AGT CAA GTT GAT GCA GA-3', *GSTM4* isoforms 1 and 3: 5'-GAA TCG ACA CCA ACC AGC AT-3', 5'-GCT GAG TAT GGG CTC CTC AC-3'; *GSTT1*: 5'-TTC CTT ACT GGT CCT CAC ATC TC-3', 5'-TCA CCG GAT CAT GGC CAG CA-3'. The PCR conditions were: 94˚C for 30 s, 60˚C for 45 s, and 72˚C for 45 s (35 cycles). Each PCR program started with a denaturation step (95˚C for 5 min) and was finished with 72˚C for 5 min followed by cooling to 4˚C. Sequence-specific PCR primer pairs were designed by using web-based Primer3 software (22). The PCR products were subjected to agarose gel (1.5%) electrophoresis in the presence of ethidium bromide. qRT-PCR was performed using 2.0 μl cDNA, 10 μl Maxima SYRB Green qPCRMaster Mix (Fermentas, St. Leon Roth, Germany), 2.0 μl sequence-specific primer pair (25 mM) and 6.0 μl water with following conditions: 94˚C for 45 s, 62˚C for 45 s, and 72˚C for 60 s (40 cycles). Target genes and *ACTB* were amplified using a Rotor Gene RG-3000 (Corbett Research, Cambridgeshire, UK) and Rotor Gene 6 software. Determination of gene expression was performed using the $2^{-\Delta\Delta Ct}$ method (23).

Sequence analysis. For sequence analyses, RT-PCR products were eluted from agarose gels by using the MinElute Gel Extraction Kit (Qiagen, Hilden, Germany) as described in manufacturer's protocol. A 10 μl sequencing mix was used that contained 0.5 μl forward or reverse gene-specific sequencing primers (10 μM), 4 μl BigDyeTerminator Cycle Sequencing Kit 1.1 mix (Applied Biosystems, Foster City, CA, USA) and 10-30 ng DNA. Bi-directional sequence analysis was performed using ABI Prism™ 310 Genetic Analyzer (Applied Biosystems) under the following conditions: 96˚C for 10 s and 60˚C for 10 s (30 cycles). The following primers were used: *GSTM1*: 5'-CTG CTC GGT TTA GGC CTG T-3' and 5'-GCT GAG TAT GGG CTC CTC AC-3'; *GSTM4*: 5'-TCT GCA GAA TCG ACA CCA AC-3' and 5'-GCT GAG TAT GGG CTC CTC AC-3'; *GSTT1*: 5'-TTC CTT ACT GGT CCT CAC ATC TC-3' and 5'-AGG CTG AGC CCA GGT TTA TT-3'; *GSTT1* (additional sequencing of retained intron): 5'-TTC CTT ACT GGT CCT CAC ATC TC-3' and 5'-TCA CCG GAT CAT GGC CAG CA-3'. A BLAST search (24) was performed to align the obtained sequences with published sequences of the analyzed genes.

Detection of intracellular GSTM1 protein. Flow cytometry was performed essentially as described elsewhere (25). For detection of intracellular GSTM1 in L-1236 cells and L-428 cells, cells were fixed and permeabilized by using BD Cytofix/Cytoperm™ (Becton-Dickinson). A rabbit anti human GSTM1 polyclonal antibody (Proteintech Group, Inc., Chicago, IL, USA) was used as the primary antibody and phycoerythrin (PE)-labelled donkey anti rabbit IgG $F(ab')_2$ fragment (eBiosciences, San Diego, CA, USA) was the secondary antibody for flow cytometry. Cells were analyzed on a FACScan flow cytometer (Becton-Dickinson) equipped with CellQest Pro software (Becton-Dickinson).

Results

Gene-expression analysis. Differences between the gene-expression profile of chemotherapy-resistant and sensitive HL cell lines have been shown in high-density DNA microarrays analysis (3). In order to identify candidate genes that might be associated with different sensitivity to chemotherapy, we analyzed the gene expression of the six classes of cytosolic GST that are known to play a crucial role in development of drug resistance to chemotherapy agents. Only the genes of the μ class GST (*i.e. GSTM1, GSTM2* and *GSTM4*) were differentially expressed in the tested HL cell lines (Figure 1). The GST μ genes were up-regulated in the chemoresistant L-1236 cells in comparison to the other cell lines, including the highly chemosensitive L-540 cells. The genes of the other five analyzed GST classes were not differentially expressed between the four HL cell lines.

Following-up DNA-microarray data, we validated expression of selected differentially expressed GSTs (*i.e.* GST μ: *GSTM1, GSTM2* and *GSTM4*; GST θ: *GSTT1*) by

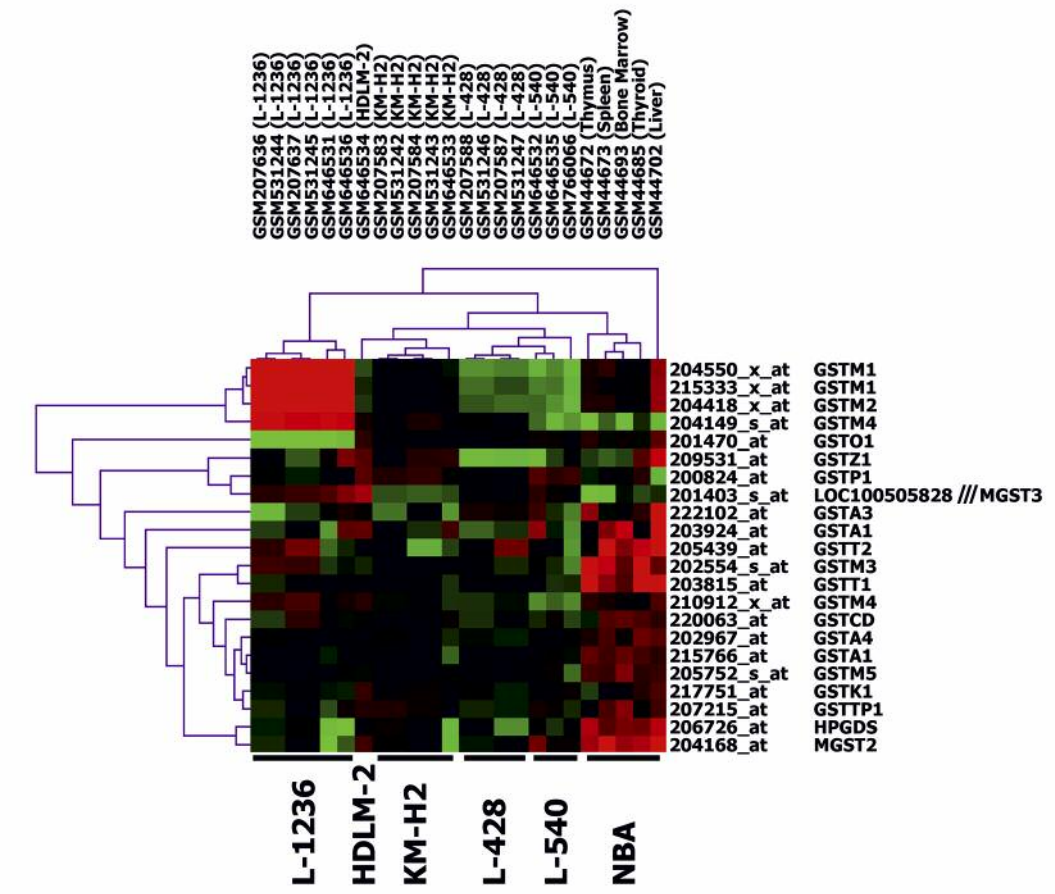

Figure 1. *Expression pattern of glutathione-S-transferases (GST) in Hodgkin lymphoma (HL) cell lines and normal tissues. DNA microarray data (3, 5, 16-18) from the GEO data base from the indicated cell lines and normal tissues (NBA) were filtered for probe sets with specificity for GST. Cluster analysis and data visualization was performed with Genesis (log2 transformed and median centered data, Manhattan distance, complete linkage clustering). Red color corresponds to high signal intensities. HPGDS: Hematopoietic prostaglandin D synthase=GST sigma 1; MGST2: microsomal GST2.*

conventional and quantitative RT-PCR. *GSTT1* was included as an example of a GST that was not overexpressed in L-1236 cells. The analyzed cell lines had distinct patterns of expressed classes of GST (Figure 2). The highest variability was observed for *GSTM1*, with high expression in the chemoresistant cell line L-1236, intermediate expression in the cell line KM-H2 and low or absent expression in the other cell lines. In subsequent flow cytometric analyses, expression of GSTM1 was tested by intracellular antigen detection in L-1236 cells and L-428 cells, which are known to be both resistant to cytotoxic drugs. Fluorescence-activated cell scanning (FACS) analyses confirmed the expression of GSTM1 in L-1236 cells, whereas in L-428 cells, no positive signals were detected (Figure 3).

Sequence analysis of GSTM1, GSTM4 and GSTT1 transcripts. In order to demonstrate the specificity of the PCR products, we performed sequencing analysis of the coding region of selected GST genes (namely *GSTM1, GSTM4* and *GSTT1*) in different HL cell lines. As shown before, *GSTM1* was highly expressed in L-1236 cells and KM-H2 cells. The sequence of cell line L-1236 showed no divergences from the reference sequence, whereas KM-H2 cells carried a known single nucleotide polymorphism (SNP) in *GSTM1* (rs1065411) that induces a non-synonymous amino acid change (AAG>AAC; Lys>Asp). According to the Ensembl database this variant has an allelic frequency of >40% in the Caucasian population (26). The variant might be associated with colon cancer risk (27). All analyzed HL cell lines expressed at least one isoform of *GSTM4*. In sequencing analyses, a known SNP (rs506008) was detected in all five HL cell lines. With a frequency of about 75%, this synonymous SNP (TTT>TTC; Phe=Phe) represents a common variant in the Caucasian population. *GSTT1* expression was low in most cell lines. By conventional PCR,

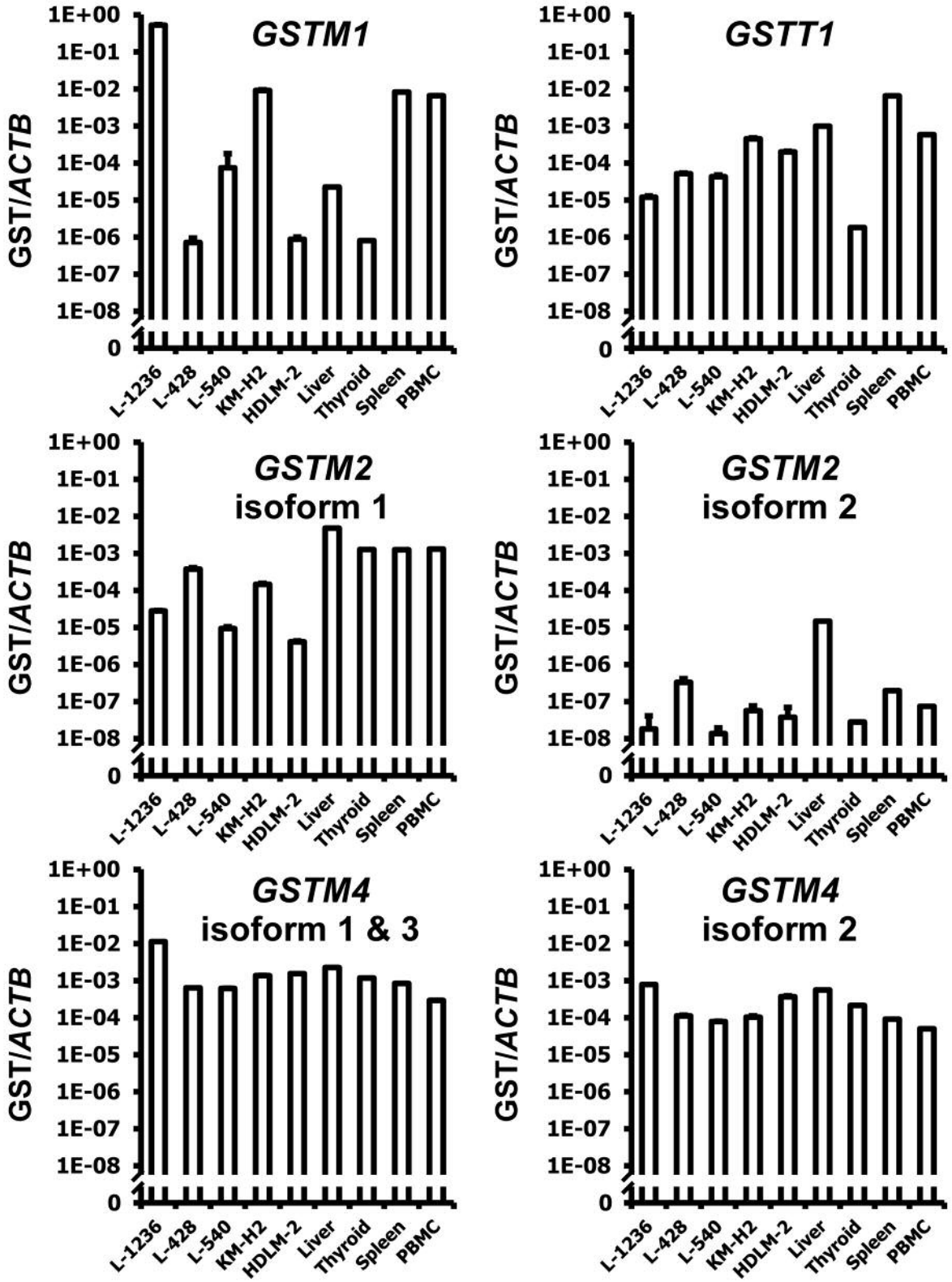

Figure 2. *Quantitative reverse transcription-polymearse chain recation (qRT-PCR) analysis of glutathione-S-transferase (GST) isoforms in Hodgkin lymphoma (HL) cell lines and normal tissues. qRT-PCR was used for quantification of GST expression in the indicated HL cell lines and normal samples. For normalization beta actin (ACTB) was used. Data are means and standard deviations from triplicates.*

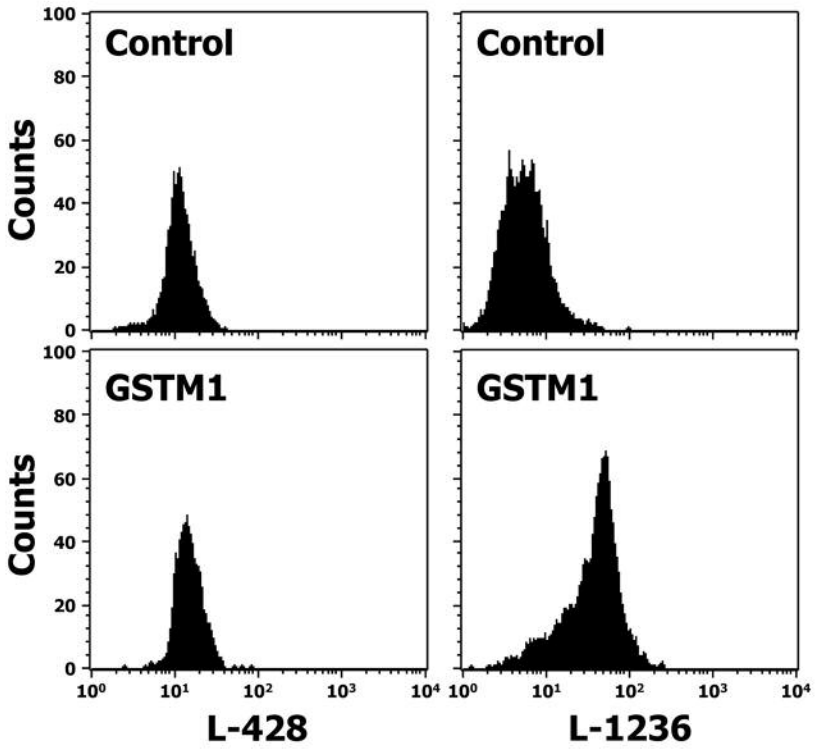

Figure 3. *Intracellular detection of glutathione-S-transferase M1 (GSTM1) in Hodgkin lymphoma (HL) cells. Presence of GSTM1 protein in the indicated cell lines was assessed by intracellular staining with GSTM1-specific antibodies. Cells after staining with secondary antibody alone served as control.*

we found signals only in KM-H2 and HDLM-2 cell lines. In the *GSTT1* sequence from KM-H2 cells and HDLM-2 cells, a known common variant (rs4630; C>T) in the 3' untranslated region was seen. This SNP was homozygous in KM-H2 cells, whereas it was heterozygous in HDLM-2 cells. This variant is also common in Caucasians and might be associated with thalidomide-induced neuropathy (28). Additionally, the PCR products of *GSTT1* had a size of 400-500 bp in both HL cell lines and were larger than expected from the reference sequence (expected size of PCR product: 255 bp). In an NCBI Blast search, we observed that the size difference was based on a retained intron sequence (Figure 4). These larger amplicons were also the prominent products in the qRT-PCR analysis of HL cell lines. In contrast, non-malignant control samples (spleen, blood cells, and liver) showed the expected 255 bp size of the PCR products and only a faint band between 400 and 500 base pairs (Figure 5).

Effect of EA in combination with cisplatin on HL cells. In an earlier work, we demonstrated that L-1236 cells were highly resistant to cytotoxic drugs, whereas the L-540 cell line showed a high sensitivity to cytotoxic drugs (3). Cells of both HL cell lines showed a decreased viability at higher concentrations of cisplatin. However, the 50% effective dose (ED_{50}) of cisplatin was lower for L-540 cells. These previous results suggested that GSTM1 might be a potential factor involved in drug resistance. Because the GST inhibitor EA enhances cytotoxicity of several anticancer drugs, we analyzed the effect of cisplatin combined with EA on the HL cell lines. Figure 6 demonstrates the sensitivity of different HL cell lines to EA. Increasing concentrations of EA reduced viability of L-540 cells in a dose-dependent manner. L-428 cells showed high resistance to EA. L-1236 cells showed an intermediate phenotype. Figure 7 demonstrates the sensitivity of L-428 cells and L-1236 cells for the combined treatment with EA and cisplatin. A comparison between the both HL cell lines L-428 (GSTM1-negative) and L-1236 (GSTM1-positive) highlights their differential sensitivity to chemotherapy. After treatment with cisplatin (50 μg/ml), EA (50 μM), or a combination of both for 24 h, the viability dropped in both cell lines. However, the viability of L-428 cells was comparable after treatment with cisplatin alone and after treatment with a combination of cisplatin and EA ($p>0.05$; Student's *t*-test). In contrast, viability of L-1236 cells significantly decreased in the simultaneous presence of cisplatin and EA compared to cisplatin ($p<0.05$; Student's

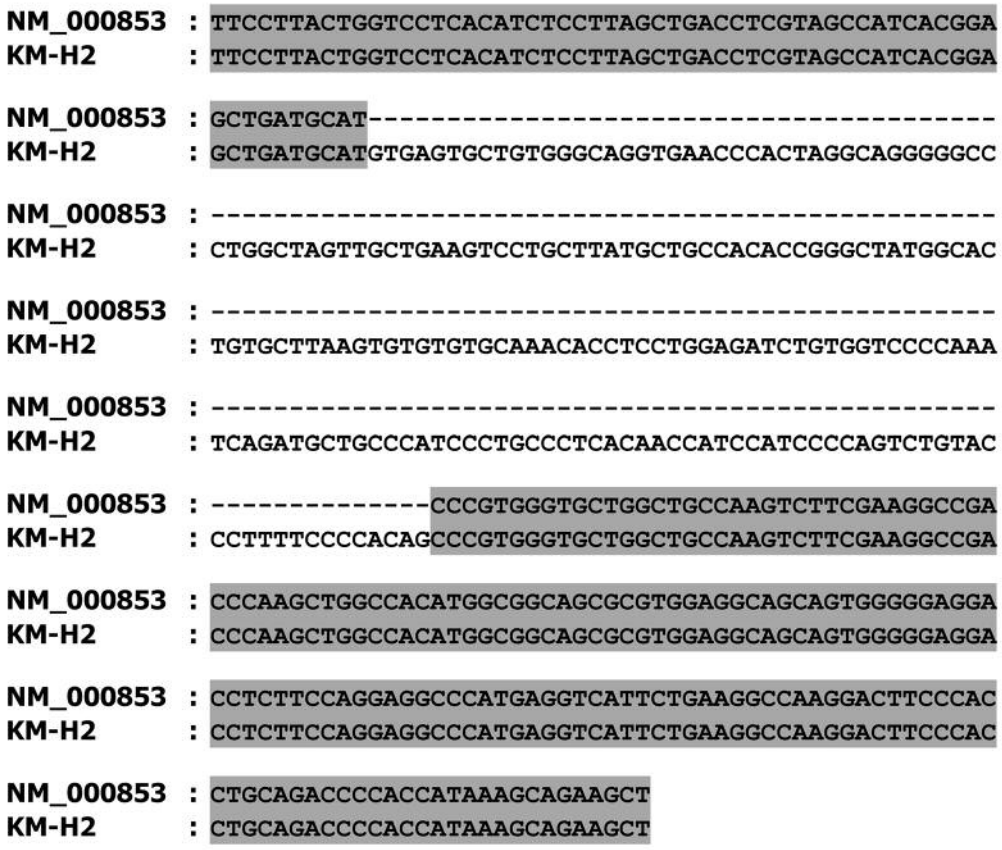

Figure 4. *Sequence alignment of glutathione-S-transferase T1 (GSTT1) from KH-H2 cells and the reference sequence (NM_000853). cDNA from cell line KM-H2 was subjected to conventional polymerase chain reaction (PCR) using GSTT1-specific primers and the PCR product was sequenced. Individual exons from the reference sequence are marked by shading.*

t-test) and to EA ($p<0.005$; Student's *t*-test) alone. Similar effects were seen for the combination of EA with roscovitine (Figure 8; EA plus roscovitine *versus* EA: $p<0.005$; EA plus roscovitine *versus* roscovitine: $p<0.00005$; Student's t-test). Due to the high sensitivity of L-540 cells to cisplatin and EA, no synergistic effects were observed in this cell line.

Discussion

In the present study, we analyzed the gene expression of six classes of cytosolic GSTs in different HL cell lines. GSTs affect the metabolism of alkylating agents and anthracyclines and play a crucial role in development of drug resistance (29). We show differential GST expression in the analyzed HL cell lines. The GST μ genes *GSTM1* and *GSTM4* were significantly up-regulated in the known chemoresistant L-1236 cells in comparison to the other cell lines (*GSTM1* and *GSTM4* isoform 1 and 3: $p<0.0005$; GSTM4 isoform 2: $p<0.001$ for all cell lines; Student's *t*-test), including chemosensitive L-540 cells. For the first time, our data revealed a different gene-expression profile of GST μ genes in chemoresistant and sensitive HL cells. The fact that *GSTM1* was not expressed in the chemoresistant L-428 cell line and subsequently EA did not increase drug sensitivity of these cells indicates that GST overexpression represents only one possible reason for failure of chemotherapy in patients with HL. The mechanisms for the chemoresistance of L-428 cells need to be identified.

Multidrug resistance in cancer therapy has been linked to overexpression of GSTs in different tumor entities and GSTs represent putative candidate genes that might be associated with different sensitivity to chemotherapy. Due to conjugation of glutathione (GSH) to cytotoxic drugs (carboplatin, cisplatin, doxorubicin, cyclophosphamide), the resulting more hydrophilic conjugates are rapidly eliminated from cells through transporter proteins located in the cell membrane (30, 31). In addition to this classic catalytic detoxification, certain GSTs (GSTP1 and GSTM1) affect cell survival pathways that are involved in stress regulation, apoptosis and cell proliferation. GSTM1 directly interacts

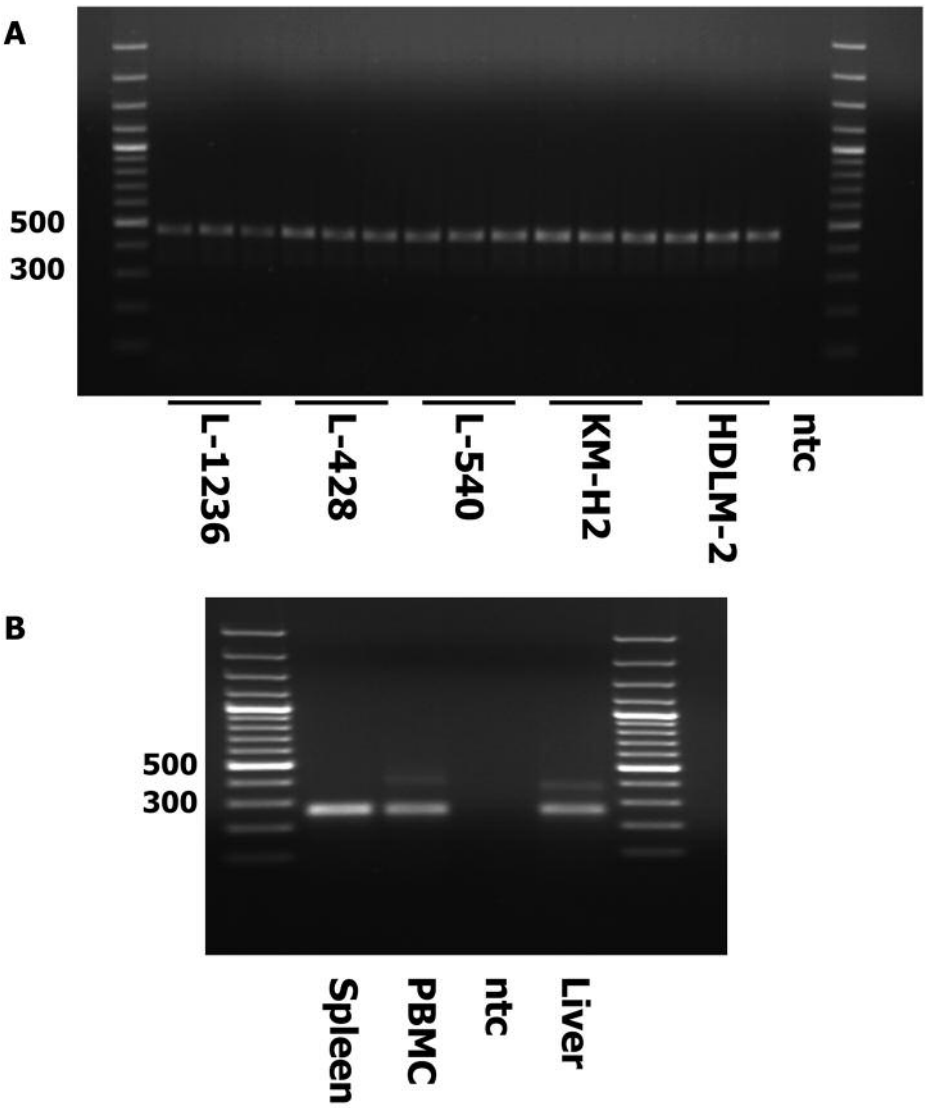

Figure 5. *Agarose gel electrophoresis of products from quantitative reverse transcription-polymerase chain reaction (RT-PCR) with glutathione-S-transferase T1 (GSTT1)-specific primers. A: cDNA from Hodgkin lymphoma cell lines was used as template for quantitative RT-PCR. Thereafter, PCR products were subjected to agarose gel electrophoresis in the presence of ethidium bromide. B: cDNA from the indicated normal tissues and cells was used as template for quantitative RT-PCR. Thereafter, PCR products were subjected to agarose gel electrophoresis in the presence of ethidium bromide. PBMC: Peripheral blood mononuclear cells; ntc: no template control.*

with apoptosis signal-regulating kinase 1 (ASK1), leading to down-regulation of the mitogen-activated protein kinase (p38-MAPK) pro-apoptotic pathway (32-34).

Although not as commonly reported as for GSTP1, increased expression of *GSTM1* as well as polymorphisms in GSTM1 have been associated with clinical outcome of various human malignancies (35-38). Children with acute lymphoblastic leukemia (ALL) who express GSTM1 seems to have a higher risk of relapse (39-41). The inhibition of dexamethasone-induced apoptosis due to down-regulation of p38-MAPK by GSTM1 has been proposed as one possible reason for treatment failure (42, 43). In different malignant cell lines, a correlation between anticancer drug resistance and overexpression of GSTM1 was demonstrated (44, 45). Variant alleles that are associated with increased GSTM1 expression also contribute to detoxification of platinating cytotoxic agents and lead to increased half maximal inhibitory concentration (IC_{50}) for lymphoblastoid cell lines, subsequently (45, 47). A genome-wide meta-analysis identified an association between variants of *GSTM1* and

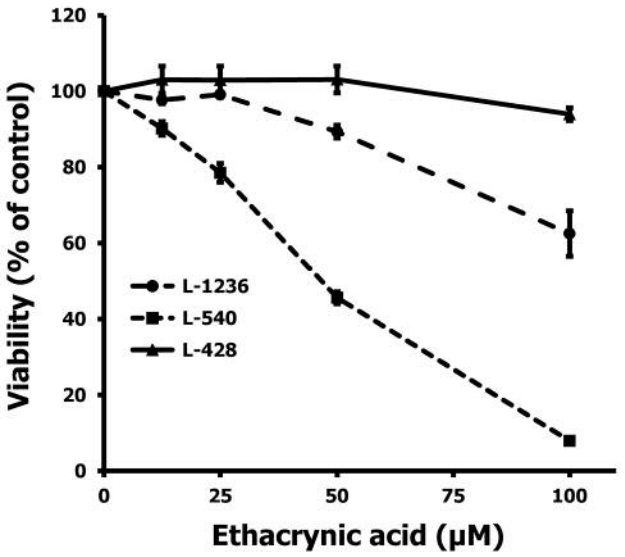

Figure 6. *Differential sensitivity of Hodgkin lymphoma (HL) cells to ethacrynic acid (EA). The indicated HL cell lines were treated with the indicated concentrations of EA. Viability was assessed by staining with propidium iodide. Data are means and standard deviations from two independent experiments.*

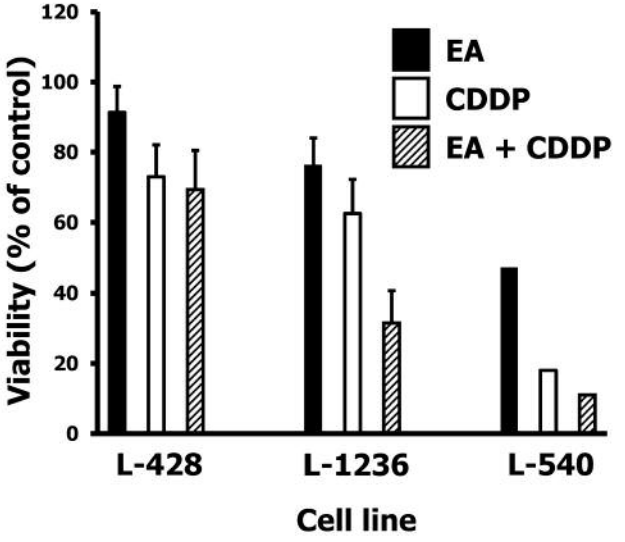

Figure 7. *Incubation of glutathione-S-transferase (GST)-positive Hodgkin lymphoma (HL) cells with ethacrynic acid (EA) increases drug sensitivity. The indicated cell lines were treated with EA, cisplatin (CDDP) or a combination of both drugs. Viability was assessed by staining with propidium iodide. Data are means and standard deviations from one (L-540 cells), three (L-428 cells) or four (L-1236 cells) independent experiments.*

susceptibility to both cisplatin and carboplatin (48). Recently, it was shown that inhibition of GSTM1 is able to overcome cisplatin resistance in lung cancer cells (49).

In our investigation, we demonstrated the possibility to overcome resistance to cytotoxic drugs in GSTM1-expressing L-1236 cells by adding EA. The higher sensitivity of L-1236 cells to cytotoxic drug in the presence of EA suggests that GSTM1 contributes to the resistant phenotype and could become a potential target for inhibition by EA. EA is one of best-characterized GST inhibitors that acts both as a non-competitive inhibitor of GSH for GST binding and by depleting the GSH cofactor by forming an EA–GSH conjugate (50). Because of the role of GST in drug detoxification and regulation of apoptosis, inhibitors of GST represent an interesting research target in order to modulate cancer cell resistance of anticancer drugs (51). EA has already been effectively utilized to enhance the cytotoxicity of alkylating drugs in cancer cells overexpressing GSTP1 (52, 53). Moreover, EA has been identified as one of the most effective agents among 1040 US Food and Drug Administration-approved compounds tested as putative inhibitors of GSTP1 (54).

In addition to the described role as an adjuvant, EA has probably its own antitumor effect by inhibition of wingless mouse mammary tumor virus integration site (WNT)/β-catenin signaling (55, 56). However, GST inhibition by EA as an adjuvant treatment modality has not been established in clinical practice because of substantial side-effects of the diuretic drug, especially in combination with other

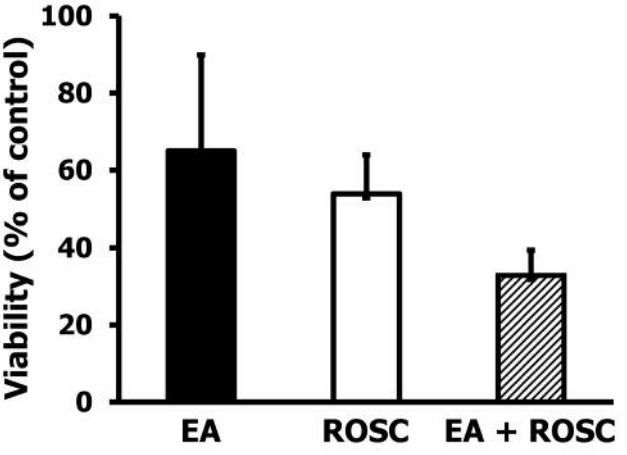

Figure 8. *Incubation of glutathione-S-transferase (GST)-positive L-1236 cells with ethacrynic acid (EA) increases sensitivity to roscovitine. L-1236 cells were treated with EA, roscovitine (ROSC) or a combination of both drugs. Viability was assessed by staining with propidium iodide. Data are means and standard deviations from three independent experiments.*

nephrotoxic chemotherapeutic agents (*e.g.* cisplatin) (62). Designing new drugs by synthetically combining a GST inhibitor such as EA with a cytotoxic drug could be a possible way to overcome drug resistance in the future. In

several cell lines, a higher sensitivity to the platinum–EA conjugate ethacraplatin compared to cisplatin has been observed. Moreover, a possible dose reduction of both components due to a higher therapeutic index of ethacraplatin could result in lower nephrotoxicity (57, 58).

The calculated ED_{50} of cisplatin highlights the synergistic effect of EA in L-1236 cells. The ED_{50} of cisplatin as single agent was 67.18 μg/ml, while it was only 45.9 μg/ml for combined treatment with EA (50 μM). Hence, the co-incubation of L-1236 cells with EA reduced by about a third the concentration of cisplatin required to devitalize half of the cells. Similar effects were seen for the combination of EA with roscovitine. Whereas the calculated ED_{50} for a single treatment with roscovotine was 116.36 μM, the concentration dropped to 72.73 μM when cells were treated in combination with EA (50 μM).

Co-expression of multiple GST isoforms in HL cells might result in functional redundancy. The presence of splice variants with putative functional differences can increase this redundancy. Whether the transcript variant of *GSTT1* that we observed in HL cells codes for a functional protein or not remains to be investigated. Multiple *GSTT1* splice variants have been described but the significance of these variants remains unclear (59).

Taken together, our results show that overexpression of *GSTM1* affect the sensitivity of HL cell lines to cytotoxic drugs. Therefore, *GSTM1* represents an interesting target to overcome drug resistance of HL cells. Further studies using both *in vitro* and *in vivo* models are required to investigate the potential role of GST inhibition in the multimodal treatment concept of HL.

Conflicts of Interest

The Authors declare that they have no competing interests. Funding sources had no involvement in the study.

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