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Heterogeneity of Human Gliomas: Glutathione-S-Transferase Expression Profile During Disease Progression and Under Systemic Therapy

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Abstract. *Background/Aim: The glutathione S-transferase pi gene (GSTP1) is a polymorphic gene encoding functionally different Gstp1 isoenzyme proteins. These seem to contribute to xenobiotic metabolism and might also play a role in susceptibility to cancer. The aim of this study was to elucidate the potential role of GSTP1 as a biomarker for disease progression and predictor of chemotherapeutic effect in glioma. Materials and Methods: Using quantitative real time PCR and western blotting analysis, a total of 61 astrocytic tumor samples from WHO grade II-IV were investigated. Results: There were no differences in GSTP1 mRNA expression between diffuse astrocytomas, anaplastic astrocytomas, or GBM. No difference was seen between secondary GBM before and after radio-/chemotherapy. Conclusion: The expression of GSTP was highly heterogeneous within the surgical specimens. No significant differences in gene and protein expression were detected between the different types of gliomas, suggesting that glioma chemoresistance is probably multifactorial and GSTP1-independent.*

Gliomas are the most frequent primary brain tumors in humans (1). Despite the fact that these tumors share common features, their characteristics concerning infiltrative growth, growth speed and susceptibility to chemotherapeutical treatment are very heterogeneous. Combined radio- and chemo-therapy (most notably with the second-generation alkylating agent temozolomide) after surgical resection is the treatment of choice for high-grade glioma patients and has improved the patient's survival significantly (2). Unfortunately, anaplastic astrocytomas and especially glioblastomas are still two of the most lethal human cancers, with a dismal prognosis (3). However, about 3-5% of glioblastoma patients are so called long-term survivors, surviving at least 3 years after the initial diagnosis (4), presumably due to a combination of molecular and clinical factors. Resistance to chemotherapy, especially to alkylating agents like temozolomide, is one of the most important factors of chemotherapy failure in glioma patients. Causes of drug resistance include increased DNA repair and increased intrinsic detoxification of glioma cells. These chemoresistance mechanisms in gliomas are only vaguely understood and only few proteins have been investigated concerning their role in drug resistance. O-6-methylguanine-DNA methyltransferase (Mgmt) is the most studied DNA repair protein involved in the resistance mechanisms to alkylating drugs in glioma patients and is the only one routinely tested in clinical practice. In this case gene silencing due to methylation of the *MGMT* promoter leads to a better response to temozolomide (2) and to longer survival (4). DNA-repair mechanisms by *MGMT* are rapid, but become saturated at a point in time, which is dependent on the cell type (5). Recently, a study showed that glioblastoma cells showing overexpression of the DNA repair protein AlkB homolog 2 (*ALKBH2*), a protein regulated by the p53 pathway, had an increased resistance to methylating agents like temozolomide (6). Another protein potentially involved in resistance to alkylating agents is the phase-II-enzyme Glutathione-S-Transferase-π (GSTP1), and presumably other GST-isoforms may be involved to a lesser

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extent. Glutathione-S-Transferases (GST) belong to a family of detoxifying enzymes which are suspected to play a major role in drug resistance in a variety of human cancers such as breast cancer (7, 8), prostate cancer (9), lung cancer (10, 11) osteosarcoma (12) and may also play a role in resistance to treatment in epileptic patients (13). Nine isoforms of soluble GST have been identified in mammalian cells, but only three (GST-α, μ, π) are found in the brain (13, 14). The placental isoform of GST - GST-π (GSTP1) - is already expressed after 12 weeks of gestation (15) and shows expression in many different brain cells (including astrocytes) and brain regions (even in the choroid plexus), in contrast to other isoforms of GST which are only expressed locally (15). This suggests that GSTP1 is the most important GST isoform in the human brain. Earlier studies found that 49% of the investigated glioma samples showed overexpression of GSTP1 and 67% showed overexpression of the methylguanine DNA-methyltransferase (MGMT) biomarker (16). These findings are suggesting that an increased activity of GST plays a role in malignant transformation and progression in gliomas and also possibly in drug resistance. A study published in 1997 found a correlation between tumor grade and GSTP1 expression and concluded that a high GSTP1 expression in glioma cells is associated with shorter survival (17). In glioblastoma tissue samples investigated by Juillerat-Jeanneret *et al.* (18-20) all samples showed expression of GSTP1, but the pattern of expression was very heterogeneous. Clinical implications concerning the status of GSTP1 expression in gliomas possibly include increasing prognostic accuracy and prediction of patient's response to alkylating chemotherapeutical drugs, and therefore may help customize the individual patient's drug regimen (16). A recent study published by Kogias *et al.* showed that nitric oxide (NO), produced by PABA/NO in glioma cells after activation by GSTP1, reduced glioma cell proliferation (21). In melanoma, *in vitro* and *in vivo* experiments have shown that adding the GSTP1-1 inhibitor NBDHEX synergistically improved the anti-proliferative effects of temozolomide without increasing its myelotoxicity (22). However, many studies conducted earlier, concerning GSTP1 and gliomas, used unselected populations and only a small number of patients (n=1-3) and also did not include anaplastic astrocytomas, WHO-grade-II astrocytomas or did not distinguish between different grades. Moreover, the results are heterogeneous or even contradicting. Due to the abovementioned methodological restrictions of most other studies (small sample numbers, only semiquantitative methods, *etc.*), a new approach was applied. In order to explore the pattern of GSTP1 expression in gliomas of different grades and after (different) treatment conditions, peritumoral 'control' tissue was compared with WHO-grade-II diffuse astrocytomas, WHO-grade-III anaplastic astrocytomas and glioblastomas by using quantitative RT-PCR on the transcription level, and, by western blotting and immunohistochemistry on the protein level. Within the glioblastoma group, primary and secondary glioblastomas were distinguished, both before and after chemotherapeutical treatment in the recurrent situation. Due to the conflicting publications in the literature with studies using mainly small patient numbers and using only semiquantitative methods, we aimed to clarify the question whether GSTP1 plays a role in human gliomas or not. The hypothesis was that with increasing glioma grades, the number of samples showing GSTP1 overexpression would raise proportionally. It was also assumed that a higher percentage of glioblastomas after chemotherapy would show GSTP1 overexpression as compared to glioblastomas before chemotherapy, as higher detoxifying capabilities are needed after exposure to chemotherapeutical agents. So far, it is not known to what extend the selected gene and its corresponding protein are implicated in the metabolism of anti-cancer drugs. Using the abovementioned techniques, GSTP1 was studied at the gene and protein level in an effort to identify part of the metabolic profile of the different brain tumors, and the possible correlation between expression levels and different types of neoplasias.

Materials and Methods

Patients. Brain tumor specimens were obtained from 35 patients treated in the Department of Neurosurgery of the University of Cologne from 1991 to 2003. A total of 61 tumor-tissue samples altogether were analyzed, because many patients were followed during disease progression in the longitudinal course. Histological interpretation of tumor type and grade was updated by an experienced neuropathologist, based on the 2007 WHO classification criteria (23) and divided into six different groups based on the grade of malignancy and prior treatment: astrocytoma WHO grade II, astrocytoma WHO grade III, glioblastoma WHO grade IV before and after chemotherapeutical treatment (Table I). Furthermore, primary and secondary glioblastomas were distinguished according to history of former low-grade glioma. Perilesional brain tissue from resections of deep-seated benign lesions without histological detectable tumor cells served as control. Informed consent of the patients was obtained according to the Helsinki declaration of ethical requirements and the local ethical committee (Application No. 03-170).

Tissue sampling and isolation of nucleic acids. Tissue samples were snap-frozen in liquid nitrogen immediately after excision and stored in our tumor bank at –80˚C until further treatment. Ten μm cryostat sections were taken from each sample and stained with hematoxylin/eosin for histological examination to assure that tumor-representative tissue was used for the molecular and biochemical analysis.

Total RNA was isolated from frozen tissue using the RNeasy Mini Kit (Qiagen, Hilden, Germany). RNA concentration and purity were assessed spectrophotometrically at 260 and 280 nm. After elimination of genomic DNA, reverse transcription was performed using the QuantiTect Reverse Transcription Kit (Qiagen).

Table I. *The groups analyzed and the numbers used in the experiments (n) are shown.*

Group:	Abbreviation:	Number in qPCR	Number in western blot
Peritumoral control tissue	Con		5
Diffuse astrocytomas (WHO°II)	A	10	6
Anaplastic astrocytomas (WHO°III)	AA	10	6
Secondary glioblastomas (WHO°IV)	sGBM w/o Cx	10	7
Primary glioblastomas (WHO°IV)	pGBM w/o Cx	10	
Recurrent secondary GBM (WHO°IV)	sGBM with Cx	10	6
Recurrent primary GBM (WHO°IV)	pGBM with Cx	10	

GBM: Glioblastoma; Cx: chemotherapy, Cx; qPCR: quantitative RT-PCR, qPCR; without, w/o:. without.

Realtime PCR. PCR was carried out in a final volume of 20 μl in the presence of 1x iQ *SYBR* Green Supermix (BioRad, Munich, Germany; containing dNTPs, iTaq™ DNA polymerase, $MgCl_2$, SYBR® Green I), 1x GSTP1 primers (QuantiTect Primer Assay, Qiagen; Accession No. NM 000852, amplicon length 83 bp) and 5 μl cDNA (diluted 1:200). Amplification was performed on a Rotor-Gene Q cycler (Qiagen) using a two-step cycling programme according to the following conditions: initial denaturation for 3 min at 95˚C, followed by 45 cycles of 95˚C for 5 sec and 60˚C for 10 sec. After each run, a melting curve was added to confirm the specificity of the amplification. The mRNA level of the samples was normalized to the RNA concentration of the reference gene *SDHA* (succinate dehydrogenase complex, subunit A).

Immunohistochemistry. For immunohistochemistry both cryo-conserved glioma and control tissue samples, were fixed using ice cold acetone and were then subjected to chloroform. In detail, the sections were incubated in acetone for 10 min, stored at –20˚C until usage, then again incubated in ice-cold acetone, shortly washed with PBS, and further incubated for 7 min in chloroform. After washing in PBS, sections were blocked in a mixture of 5% BSA, 0.25% Triton-X-100 and PBS for 60 min. Sections were then incubated overnight, in a dark, in a humidified environment, with the primary anti-GSTP1 polyclonal antibody (ABclonal, Manhattan Beach, CA, USA) at a dilution of 1:200 in 1% goat-serum. Negative control glioma sections were exposed only to 1% goat serum and treated equally afterwards. After washing the sections in PBS, the secondary anti-rabbit antibody (Alexa 488, Life Technologies, Carlsbad, CA, USA) was applied at room temperature in a 1:200 dilution for 30 min. Slides were also incubated with DAPI for 5 min (dilution 1:1,000) at room temperature, washed in PBS and stored at 4˚C before imaging using a fluorescence microscope.

Immunocytochemistry. In addition to the commercially available U87 cell line, primary BT16 cells were obtained during surgery from a patient with glioblastoma multiforme. Both, U87 and BT16 cells were cultured at 37˚C with a carbon dioxide concentration of 5% in Dulbecco's Modified Eagle's Medium (DMEM) enriched with 10% fetal calf serum (FCS). Penicillin and streptomycin (Gibco, Waltham, MA, USA) were added to reduce the risk of bacterial contamination. After reaching at least 80% confluence the medium was removed, cells were washed using PBS (Gibco), then fixed using 4% PFA (paraformaldehyde) for 15 min and incubated for 1 h in blocking solution containing 3% BSA with 0.1% Triton-x-100 in PBS. Then cells were incubated with the primary Gstp1 antibody (dilution 1:200) overnight at 4˚C. After washing with PBS, cells were incubated with a secondary anti-rabbit antibody (Alexa 488, Life Technologies) at a dilution of 1:4,000 for 30 min and DAPI at a dilution of 1:2,000 for 5 min (both at room temperature). Cells were washed in PBS and then stored in the dark at 4˚C before imaging using a fluorescence microscope.

Western Blot analysis. Thirty different samples of brain tissue were analyzed by western blotting which included five to seven samples for each of the groups. For each sample, 50 μg of protein was used for gel electrophoresis and then transferred to a nitrocellulose membrane (Porablot NCP). Precast gels (NuPage 4%-12% Bis-Tris 1mm gel, Life Technologies) were chosen to ensure maximum reproducibility. Protein concentration was determined using Bradford assay (Biorad). For blocking, 5% dry non-fat milk (Cell Signaling, Cambridge, UK) dissolved in tris-buffered saline at pH=7.5 with 0.05% Tween 20 as detergent (TBST) was used. Overnight-incubation with the primary anti-GSTP1 polyclonal antibody (ABclonal) was performed at a dilution of 1:2,000 in 3% milk and TBST at 4˚C, followed by incubation with the secondary horseradish peroxidase-conjugated anti-rabbit antibody (JacksonImmunoResearch, West Grove, PA, USA); dilution 1:10,000 in TBST at room temperature (RT)). Detection of horseradish signals by chemiluminescence was followed by densitometric analysis (Imagelab, Biorad).

The same membranes were incubated overnight at 4˚C with a primary antibody to β-actin (Cell Signaling; dilution 1:10,000) and then incubated with the secondary horseradish peroxidase-conjugated goat anti-mouse antibody (dilution 1:10,000 in TBST) at RT in order to verify the protein load for each sample and to normalize GSTP1 levels.

Normalization. In order to correct the results gathered by western blotting and quantitative real-time PCR tests for possible variations in the amount of protein or DNA, the concentrations of each sample were divided by the concentrations of housekeeping proteins or DNA. The concentrations of housekeeping DNA and proteins were determined for each sample separately.

Statistical analysis. To assess differences in the means of protein and gene expression between the defined groups, an analysis of variance (ANOVA) was applied. Bonferroni correction was as posthoc test. All statistical analyses were conducted using the commercially available program IBM SPSS Statistics (Version 20). The level of significance was set at 0.05%. All results are depicted with the S.E.M. (standard error of the mean).

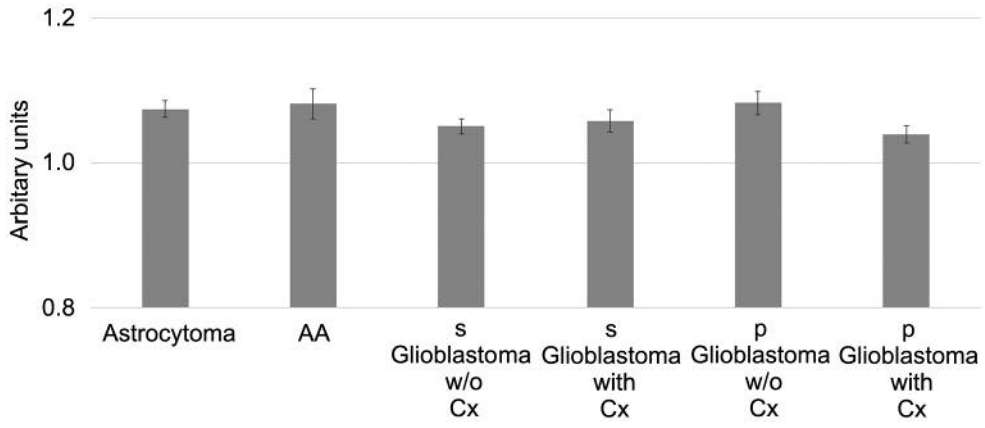

Figure 1. *Mean GSTP1 expression values analyzed by quantitative RT-PCR. Results are normalized to reference gene and depicted plus/minus SEM. aa: Anaplastic astrocytoma; s: secondary; p: primary; w/o: without; cx: chemotherapy.*

Results

Quantitative real time (qRT)-PCR revealed a homogenous distribution between different groups. Using qRT-PCR, a total of 61 tumors, consisting of 11 WHO-grade II astrocytomas, 10 WHO-grade III astrocytomas, 20 glioblastomas (10 primary and 10 secondary GBMs) before and 20 WHO-IV glioblastomas after chemotherapeutical treatment (10 primary and 10 secondary GBMs), were tested for *GSTP1* gene expression. No significant differences in *GSTP1* expression were noticed between tumors of different WHO-grades (Figure 1). To evaluate whether chemotherapeutical treatment has an influence on *GSTP1* expression, secondary and primary glioblastomas before and after chemotherapeutical treatment were compared. In the latter ones, the greatest difference between all groups analyzed was found. The highest value of Δ *GSTP1* (GSTP expression normalized to the housekeeper gene) was found for primary WHO-grade IV glioblastomas before chemotherapeutical treatment (1.083; SEM: 0.02) and the lowest for primary WHO-grade IV glioblastomas after chemotherapeutical treatment (recurrent disease) (1.040; SEM: 0.01). The slightly higher *GSTP1* gene expression before chemotherapeutical treatment was not significant (p=0.291) (Figure 1). In secondary glioblastomas just the opposite was found, as secondary glioblastomas before chemotherapy showed a slightly lower *GSTP1* expression than secondary glioblastomas after chemotherapy (1.051±0.01 to 1.058±0.02, p>0.05). In summary, a difference in *GSTP1* gene expression was found neither between various grades of malignancy, nor between primary and recurrent tumors. Furthermore, no significant differences were identified when examining individual patients during the longitudinal course of their progressive disease (data not shown). In some (most) patients, GSTP1 increased during disease progression, in others it decreased.

No alteration of GSTP1 expression in different cell lines. In order to compare the GSTP1 expression in established GBM cell lines and primary GBM cells, immunocytochemistry (ICC) was used. The levels of GSTP1 in the established U87 cell line and our primary BT16 cells were compared. The latter ones were primary GBM cells derived from an operation specimen. Both cell types showed an equal level of GSTP1 expression (Figure 2).

Heterogeneous distribution of GSTP1 between groups, as shown by immunohistochemistry. To validate the PCR data on a protein level, the levels of GSTP1 expression in peritumoral control brain tissue and gliomas were assayed by immunohistochemistry (IHC). No qualitative difference was detectable between control and tumorous brain tissue. Moreover, no difference in GSTP1 expression was observed between tumors of different grades. All grades of malignancy showed the same level, distribution and localization of GSTP1. Furthermore, primary and recurrent disease had also no influence on GSTP1 IHC. Taken together, no significant differences in GSTP1 expression between the various tissues were identified (Figure 3).

Western blotting showed a slight increase in GSTP1 expression from low- to high-grade gliomas. The expression of GSTP1 was determined in a total of 30 different tumors (Figure 4). Comparing control and tumor tissue, a small but non-significant GSTP1 elevation in the tumors (0.43 vs. 0.68, data not shown) was found. In detail, western blotting results confirmed our quantitative real-time PCR data as no significant differences were detected. Protein analysis revealed some non-significant changes: (i) GSTP1 protein expression increased from low grade gliomas (WHO˚II) to high grade gliomas (WHO˚III) from 0.5±0.08 to 0.9±0.20, and (ii) GSTP1 protein levels decreased in GBM after

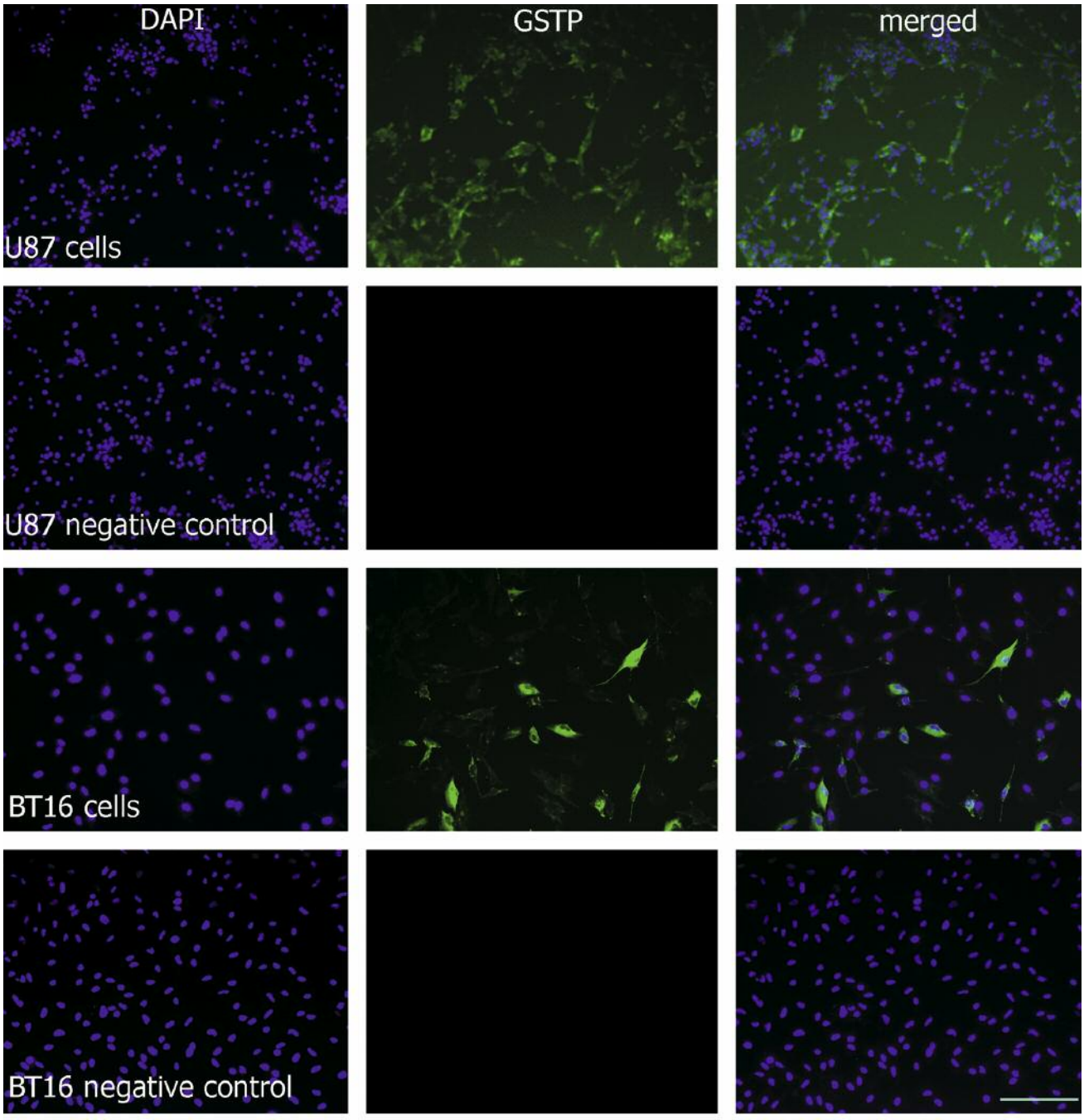

Figure 2. *Representative immunocytochemistry images showing staining in 2 different glioma cell lines (U87 and primary BT16) and their negative controls. First column shows DAPI nuclear staining, second column shows GSTP1 staining and the third column is an overlay of both images. Scale bar represents 200 μm.*

chemotherapy from 0.82±0.15 (primary disease) to 0.47±0.05 (recurrent disease) (Figure 4). The highest expression of GSTP1 was found in WHO-grade III astrocytomas (0.90; SEM 0.20) and the lowest expression of GSTP1 in control tissue (0.43; SEM: 0.07).

Discussion

Predicting the tumor's sensitivity to chemotherapeutic agents would be another step towards a more personalized treatment, tailored to fit the molecular profile of the tumor. Besides the

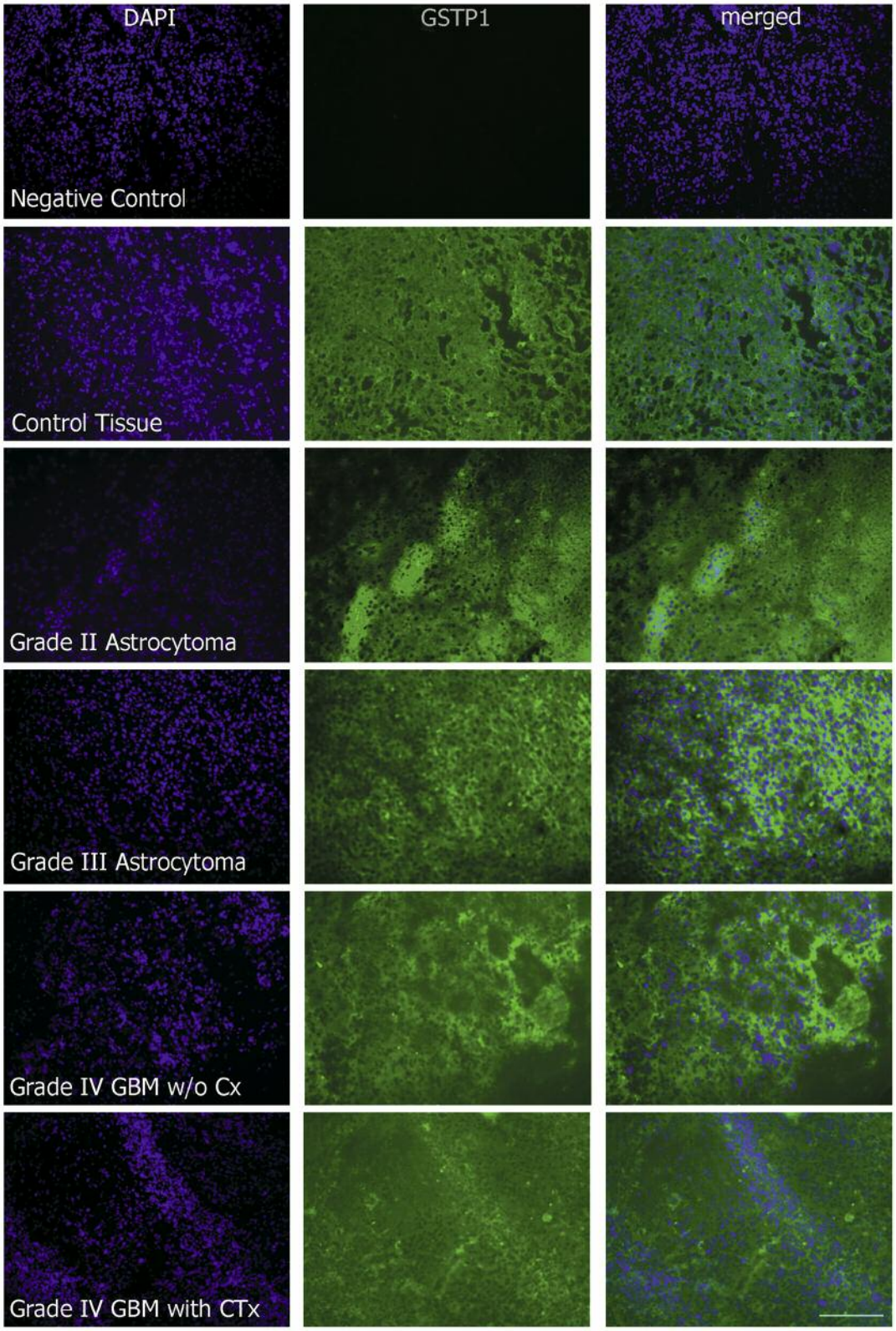

Figure 3. *Representative immunohistochemistry images taken from glioma tissue of different grades, control tissue and negative control, respectively. First column shows DAPI nuclear staining, second column shows GSTP1 staining and the third column is an overlay of both images. Scale bar represents 200 μm. CTx: Chemotherapy.*

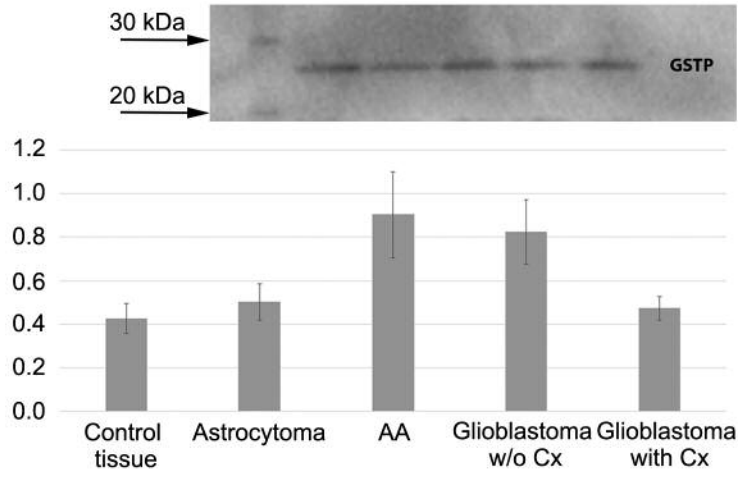

Figure 4. *Western blot showing GSTP1 protein levels in different tumor grades. From left to right: marker, control tissue, WHO-grade II astrocytoma, WHO-grade III astrocytoma, WHO-grade IV glioblastoma before chemotherapy, and WHO-grade IV glioblastoma after chemotherapy. GSTP1protein at approx. 23 kDa. Bottom: Quantification of Western blot results: GSTP1expression in respect to reference protein (arbitrary units). Error bars represent standard error of the mean (SEM). aa: Anaplastic astrocytoma; s: secondary; p: primary; w/o: without; cx: chemotherapy.*

well-established screening for the O-6-methylguanine-DNA methyltransferase (MGMT) methylation status there are multiple other genes and proteins that could potentially be responsible in restricting chemotherapeutical efficiency on tumor cells. *GSTP1* was chosen to be investigated based on previous studies that showed that GSTP1 is the most prominent protein in brain tumors while GST-α and GST-μ, are only present in trace amounts in astrocytoma cell lines (24).

The aim of this study was to correlate *GSTP1* gene and GSTP1 protein expression with tumor grades and exposure to chemotherapy, in an effort to identify GSTP1 as a potential biomarker for disease progression or as an indicator of therapy success in glioma patients. Previous studies addressing similar questions have shown very heterogeneous results and were only using semi-quantitative analyses, *in vitro* tests, or were performed before more accurate and sensitive tests were widely available (Table II). Semi-quantitative analysis by Kogias *et al*. (21) showed a higher expression of GSTP1 in the U87 glioma cell line and in four primary glioblastoma cell lines compared to fibroblasts. A study published in 1997 by Ali-Osman *et al*. (17) found a positive correlation between tumor grade and GSTP1 expression when comparing 33 grade IV, 13 grade III astrocytomas and 15 other gliomas using immuno-cytochemistry. Calatozzolo *et al*. (25) found no differences in GSTP1 expression between primary and recurrent gliomas. In 2006, Fruehauf *et al*. (16) published an immunohistochemistry study testing *in vitro* drug resistance and the expression of molecular markers in 148 high-grade gliomas and 35 low-grade gliomas. They found a slight, non-significant, trend towards higher GSTP1 expression in high-grade gliomas. Although these studies do not necessarily contradict each other, the influence of *GSTP1* on chemoresistance in gliomas remains uncertain. Many studies over the past decades analyzed this interesting question, however, about half of the studies found a (mostly not significant) correlation between tumor grade and GSTP1 expression, whereas the other half did not (overview in Table II). A more recent study, found high GSTP1 expression in pituitary adenomas, while the least GSTP1 expression was detected in glioblastoma (26).

In this study, quantitative real-time PCR and western blotting were used, as both methods are well established and qRT-PCR is known for its high sensitivity and high level of reproducibility (27).

Overall, no significant difference in GSTP1 expression was found between the different tumor grades or between GBM before or after chemotherapy treatment. In western blot analysis a non-significant trend towards higher GSTP1 expression in high-grade gliomas compared to low-grade gliomas was noted.

Our results suggest that chemoresistance against alkylating agents in gliomas is either intrinsic, multifactorial or even GSTP-1 independent.

Conclusion

In conclusion, our results showed, in a greater patient cohort and with quantitative methods both, on gene and protein levels, that GSTP1 levels were not different between (i) various degrees of malignancy in gliomas and (ii) before and after chemotherapy in gliomas. These findings suggest that

Table II. *Review of the literature*.

Publication	Method(s) used	Specimen	Number	Result	Significance	Remarks
Kogias *et al*., Int. J. of Cancer, 2012 (21)	IHC, ICC, WB	U87 Human primary glioma cells Animal tissue Fibroblasts	1 cell line 4 30 (four groups) 1	WB: Stronger GSTP1 expression in U87 and primary glioma cell lines than in fibroblasts. ICC: Slightly stronger GSTP1 expression in U87 than in fibroblasts.	Qualitative only or significance not stated	
Juillerat-Jeanneret *et al*., Cancer Investigation, 2008 (18)	IHC, ICC, WB, PCR, Methylation-Sensitive Dot Blot Assay	LN18, LN229, LNZ308 Human primary glioma cells	3 cell lines 14	Highly heterogeneous expression of GSTP1 in primary glioma cells.	Qualitative only or significance not stated	
Calatozzolo *et al*., J. of Neuro-Oncol., 2005 (25)	IHC, qRT-PCR, Flow cytometry (FC)	Human primary glioma cells	WHO˚II: 18 — qRT-PCR: 7, FC: 18, IHC: 12 WHO˚III: 6 — qRT-PCR: 3, FC: 6, IHC: 2 WHO˚IV: 24 — qRT-PCR: 16, FC: 24, IHC: 11	No significant differences in GSTP1 expression between primary or recurrent gliomas.	Not significant/ not stated	Flow cytometry: GSTP1 more expressed in recurrent high-grade gliomas compared to primary high grade gliomas (not sig.); PCR: higher levels of GSTP1 in tumor samples compared to control tissue (not sig.), no significant differences in mRNA expression between high grade and low grade tumors.
		Human endothelial cells	3			
Ali-Osman *et al*., Clinical Cancer Research, 1997 (17)	ICC	Human primary glioma cells	61	Not significant: higher GSTP1 expression with degree of malignancy.	p=0.16	Trend towards higher expression percentage in high-grade gliomas.
Kural *et al*., Med.Princ. Pract., 2018 (26)	IHC	Human tissue	9 glioblastoma, 5 WHO˚III glioma, 2 Oligodendroglioma	Lower GSTP1 expression in GBM compared to other brain tumors (*e.g*. pituitary adenoma)	n.s.	
Fruehauf *et al*.; Clinical Cancer Research, 2006 (16)	EDR assay, IHC	Human primary glioma cells	478	No significant differences in biomarker expression noted between high-grade and low-grade gliomas.	p=0.46	Trend towards higher expression percentage in high-grade gliomas.
Strange *et al*., Biochimica et biophysicaacta, 1992 (29)	Immuno-blotting	Human primary glioma cells 38	Control tissue: 21 WHO˚ II Astrocytoma: 1 WHO˚ III Oligo-astrocytoma: 1 WHO˚ III or IV Astrocytoma: 15	Significantly higher GSTP1 expression in tumor samples compared to control samples.	p<0.05	
Singh *et al*.; Biochemical Pharmacology, 2010 (30)	Quantitative RT-PCR, WB	MGR1 (anaplastic astrocytoma) and MGR3 (glioblastoma multiforme) cell lines	2 Cell lines	Higher GSTP1 expression in anaplastic astrocytoma cell line compared to glioblastoma cell line	Significance not stated	

Table II. *Continued*

Table II. *Continued*

Publication	Method(s) used	Specimen	Number	Result	Significance	Remarks
Von Bossanyi *et al*., Acta Neuropathologica, 1997 (31)	IHC	Primary glioma tissue 53	WHO° I astrocytoma: 8 WHO° II astrocytoma: 11 WHO° III astrocytoma: 9 WHO° IV glioblastoma: 25	No significant difference in number of cells showing GSTP1 expression between different levels of anaplasia	Significance not stated	Highest rate of tumors with GST-positive in WHO-grade II astrocytomas (significance not stated)
Winter *et al*., Journal of the Neurological Sciences, 2000 (32)	IHC, PCR, Immunoblotting	Primary human glioma cell lines 22	Radiochemotherapy: 12 Radiotherapy only: 10	No significant differences in GSTP1 expression between primary and recurrent GBMs	p=0.084	
Nagane *et al*., Japanese Journal of Clinical Oncology, 1999 (33)	Immunoblotting	Primary gliomatissue 28	WHO° II astrocytoma: 1 WHO° III astrocytoma: 4 WHO° IV glioblastoma: 4 Other (including: oligodendrogliomas, gangliogliomas, ependymoma, brain metastases *etc*.): 19	Higher levels of GSTP1 expression in tumor tissue compared to non-neoplastic control tissue	Qualitative only or significance not stated	
		Non-neoplastic control tissue	3 Benign astrocytoma: 4	Intensity of immunoreactivity	Qualitative only	
Hara *et al*., Journal of Cancer Research and Clinical Oncology, 1993 (34)	Immunoblotting	Primary glioma cells 13	WHO° III astrocytoma: 5 WHO° IV glioblastoma: 4	increases with level of malignancy	or significance not stated	
Grant *et al*., Journal of Neuro-Oncology, 1995 (35)	IHC	Primary glioma tissue	30	GSTP1 staining increased from less malignant tumors to glioblastoma.	Qualitative only or significance not stated	Staining was most intense in gemistocytic and giant tumour cells.
Hara *et al*., Journal of Cancer Research and Clinical Oncology, 1993 (36)	IHC	Primary glioma tissue 31	WHO° II astrocytoma: 5 WHO° III astrocytoma: 10 WHO° IV glioblastoma: 16	Higher level of GSTP1 staining in more malignant tumors	Qualitative only or significance not stated	
Weyerbrock *et al*., Neurosurgery. 2012 (37)	ICC, PCR, WB	U87	1	ICC: Higher GSTP1 expression in U87 cells, and four primary GBM cell lines compared to astrocytes and fibroblasts	Qualitative only or significance not stated	
			Primary glioblastoma cell lines	4		
			Fibroblasts, astrocytes (non-neoplastic control tissue)	1,1		

GSTP1 alone is not suitable as a biomarker for disease progression or as a predictive biomarker of chemotherapy efficacy. These findings are supported by a meta-analysis, which suggested that no association between GST variants and the risk of glioma (except a single variant A114V) exists (28). Future studies concerning GSTP1 and gliomas should therefore investigate GSTP1 together with other detoxifying enzymes and drug resistance genes as it is probable that the tumor's chemoresistance is determined by multiple molecules working simultaneously.

Conflicts of Interest

None of the Authors have any conflicts of interest to disclose regarding this study.

Authors' Contributions

RT: Performed most of the experiments; PS: made critical comments on the manuscript and helped with study design; GR: qPCR; BK: made comments on the manuscript; AK: pathological evaluation of tissue; RG: made comments on the manuscript; MT: study design, statistics, wrote manuscript.

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