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*Cellular and Molecular Biology*

# Expression of glutathione S-transferase T1 (GSTT1) in human brain tumours

**A. Diedrich[1], H.C. Bock[2], F. König[3], T.G. Schulz[4,5], H.C. Ludwig[2], R. Herken[1*] and F. Quondamatteo[1]**

[1]Department of Histology, [2]Clinic for Neurosurgery, [3]Department of Neuropathology and

[4]Department of Occupational Health, University of Göttingen, Germany

[5]Present address: Federal Institute for Risk Assessment, Berlin, Germany

**Summary.** Glutathione S-transferases (GSTs) play a central role in a number of metabolic processes. Glutathione S-transferase T1 (GSTT1) is a polymorphic cytosolic enzyme and a member of the theta class of GSTs. Typical substrates for GSTT1 are industrial compounds, such as dichloromethane and ethylene oxide. It has been shown that also chemotherapeutic drugs such as BCNU [i.e. 1,3-bis(2-chloroethyl)-1-nitrosourea] are efficiently inactivated by GSTT1. BCNU is a drug which is increasingly used locally in the chemotherapy of glioblastoma multiforme WHO grade IV. Therefore, if GSTT1 were expressed in neoplastic cells of brain tumours it could be a factor for chemoresistance. In order to clarify a possible role of GSTT1 in chemoresistance, as a first step, we localized this enzyme in malignant gliomas such as glioblastoma multiforme WHO grade IV and oligodendroglioma WHO grade II. Because of its polymorphism we first genotyped the samples for GSTT1 by PCR. Using in situ hybridization, we then demonstrated that GSTT1 transcripts are expressed in neoplastic cells of both tumour types. Immunohistochemistry revealed then that whereas neoplastic cells in glioblastoma multiforme WHO grade IV contain GSTT1, it was not localized in oligodendroglioma cells. Given the polymorphism of GSTT1 and its potential activity towards BCNU, the localization of GSTT1 in glioblastoma cells can be considered as a possible factor of non-homogeneous chemotherapy response among patients with different GSTT1 genotypes.

**Key words:** Malignant gliomas, *In situ* hybridization, Metabolism

*Offprint requests to:* Fabio Quondamatteo, Department of Histology, University of Göttingen, Kreuzbergring 36, D-37075, Göttingen Germany. e-mail: fquonda@gwdg.de

*deceased 3rd of November 2005

## Introduction

Glutathione S-transferases (GSTs) are able to conjugate electrophilic compounds to glutathione and play a central role in a number of metabolic processes (Hayes and Pulford, 1995). Glutathione S- transferase T1 (GSTT1) is a member of the theta class of cytosolic GSTs (Hayes and Pulford, 1995; Hayes et al., 2005) and has been characterized in human and animal tissues (Meyer et al., 1991; Pemble et al., 1994; Mainwaring et al., 1996a,b, 1998; Quondamatteo et al., 1998; Schulz et al., 2000; Sherratt et al., 1997, 2002). For the human (h)GSTT1 a deletion polymorphism is known and about 20% of all Caucasians are homozygous carriers of this deletion. Such individuals lack functional GSTT1 and are unable to conjugate glutathione to GSTT1 substrates, therefore, they are termed "non-conjugators" (Pemble et al., 1994; Hayes and Strange, 2000). In addition to products used in industry, such as halogenated organic compounds or ethylene oxide (Hallier et al., 1993; Thier et al., 1993, 1996; Hayes and Strange, 2000; Landi, 2000; Hayes et al., 2005), GSTT1 can also metabolize BCNU [i.e. 1,3-bis(2-chloroethyl)-1-nitrosourea] with great efficiency (Lien et al., 2002). BCNU is a cytostatic drug of the nitrosourea group. These are drugs used in the chemotherapy of malignant gliomas (Burton and Prados, 2000; Engelhard, 2000; Nieder et al., 2000; Selker et al., 2002; Giese et al., 2004). Among glial tumours, glioblastoma multiforme accounts for about 50% of the gliomas. Glioblastoma are astrocytic tumours which display extremely high local invasiveness. The surgical removal of glioblastoma tissue is mainly carried out to reduce the intracranial pressure (Giese et al., 2003; LeFranc et al., 2005). On account of the high invasiveness of this tumour, the possibility of addition of antimigratory agents to the conventional radio- and chemotherapy has been the object of discussion (Giese et al., 2003; LeFranc et al., 2005). Also, to directly target neoplastic cells, the use of intracranial BCNU implants has been proven to increase post-operative survival in

glioblastoma patients (Giese et al., 2004). For the systemic chemotherapy of glioblastomas, in addition to nitrosoureas, another alkylating agent, i.e. temozolomide, is becoming increasingly used (Chang et al., 2004; Hegi et al., 2005; Stupp et al., 2005), also at the Neurosurgical Clinic of the University of Göttingen. However, one of the major problems in chemotherapy of glioblastomas is chemoresistance. At the molecular level, it has been shown in vitro, that the sensitivity of astrocytes to alkylating agents is significantly increased by blocking the DNA-repair enzyme $O_6$-methylguanine-DNA-methyltransferase (MGMT) and by blocking glutathione synthesis (Nutt et al., 2000). The role of MGMT in the induction of chemoresistance to temozolomide in glioblastoma patients has recently been highlighted by the study of Hegi et al. (2005). With respect to the glutathione-dependent pathway in chemoresistance of glioblastomas, the role and expression of glutathione transferases of classes alpha, mu and pi (Awasthi et al., 1994) has been largely investigated in glial tumours (Strange et al., 1992; Grant and Ironside, 1995; Ali-Osman et al., 1997; von Bossanyi et al., 1997). In contrast, nothing is known on the expression of theta class glutathione transferases in malignant gliomas. The fact that GSTT1 is able to metabolize BCNU with great efficiency (Lien et al., 2002) and that one of the modern therapeutic approches in glioblastomas is the local use of BCNU (Giese et al., 2004) led us to the idea that GSTT1 might have a negative influence on the chemotherapy in conjugators. This could be possible if GSTT1 was active in tumour tissue. However, this question has still not been answered. In the present study, as a first step towards clarifying the biological relevance of GSTT1 in chemoresistance, we aimed to answer the question as to whether neoplastic cells of human malignant gliomas express GSTT1. To this end, we investigated expression of hGSTT1 in an astrocytic tumour type (i.e. glioblastoma multiforme WHO grade IV) and in an oligodendrocytic tumour type (i.e. oligodendroglioma WHO grade II), as well as in human control brain tissue.

## *Materials and methods*

### *Tissue studied*

Paraffin-embedded archival brain tissue of patients operated on in the Neurosurgery Clinic of the University of Göttingen was studied. This investigation was approved by the local Ethics Committee. All samples had previously been histologically examined by the Department of Neuropathology. The archival samples with the various diagnoses (n=36) were first genotyped for GSTT1. Among the samples with a GSTT1 positive genotype we chose for the localization of gene expression the samples of glioblastoma multiforme WHO grade IV (n=6) and of oligodendroglioma WHO grade II (n=2) as well as two samples obtained by resection following epilepsy (control brain tissue). Data related to the tissue in which GSTT1 expression was studied are summarized in Table 1. A histological overview of the tissue prior to in situ hybridization experiments was achieved by staining a few 5$\mu$m sections from each block using routine staining.

### *Genotyping of the tissue*

Given the known deletion polymorphism (Pemble et al., 1994), the tissues samples were first examined by PCR analysis in order to exactly define the ”conjugators” which were then suitable for the study of GSTT1 gene expression. From each sample, 10$\mu$m paraffin sections were cut and the genomic DNA was extracted from the tissue by means of proteinase K digestion and by processing in a BioRobot® EZ1 (Qiagen, Hilden, Germany) using the tissue extraction

**Table 1.** Tissue samples with GSTT1-positive genotype in which GSTT1 expression was studied.

| Sample # | Gender | Age | Diagnosis | Chemotherapy/Radiotherapy after surgery |
|---|---|---|---|---|
| 1340 | f | 60 | GBM WHO IV ri. parieto-occipital | R,ACNU |
| 1567 | f | 63 | GBM WHO IV le. parieto-temporal | R,ACNU |
| 1471 | m | 37 | GBM WHO IV ri. parietal | R,Br,ACNU,PCV |
| 1451 | m | 70 | GBM WHO IV ri. temporal | R |
| 1569 | m | 71 | GBM WHO IV ri. parieto-temporal | R,ACNU |
| 1490 | f | 70 | GBM WHO IV ri. temporal | R, |
| 1379 | f | 41 | OG WHO II ri. frontal | R,PCV |
| 1487 | m | 27 | OG WHO II le. frontal | none |
| 1482 | f | 27 | Focal epilepsy temporal ri. | none |
| 1498 | f | 43 | Focal epilepsy temporal ri. | none |

The age corresponds to that of the first diagnosis. All tumor patients underwent surgical resection of the tumoral mass. Patients with focal epilepsy (samples # 1482 and 1498) underwent resection of temporal lobe. Tumoral tissue samples derived from first surgical resection. No tissue was included from patients who received chemotherapy prior to first surgery. f: female, m: male, ri.: right, le.: left, GBM: glioblastoma multiforme, OG: oligodendroglioma, R: radiotherapy, Br: brachytherapy, PCV: combined therapy with procarbazine/CCNU [1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea]/vincristine, ACNU: (1-4-amino-2-methyl-5-pyrimidinyl)-methyl-3-(2-chloroethyl)-3- nitrosourea.

programme. Finally, the genomic DNA was collected and processed for multiplex PCR (Haase et al., 2002) which simultaneously amplified bands for GSTT1 (Primers: forward 5' TTCCTTACTGGTCCTCACATC TC 3', reverse 5' TCACCGGATCATGGCCAGCA 3', product: 480 bp), GSTM1 (Primers: forward 5' GAACTCCCTGAAAAGCTAAAGC 3', reverse: 5' GTTGGGCTCAAATATACGGTGG 3', product: 215 bp) and ß-globin (Primers forward: 5' CAACTTCAT CCACGTTCACC 3', reverse: 5' GAAGAGCCAAG GACAGGTAC 3', product: 260 bp). An example of this step is depicted in Fig. 1a. Since the product of GSTT1 was relatively long and there was a chance of DNA degradation in archival specimens, genotyping of the samples was further confirmed by using a second PCR assay with a primer pair which amplified a shorter fragment of 214 bp of the hGSTT1 gene (forward: 5' CATCCCTGCCCTCACAACCA 3', reverse: 5' CTTCTGCTTTATGGTGGGGTCTG 3') (Ambrosone et al., 2001). An example of this assay is depicted in Fig. 1b. Only DNA preparations in which a clear band for beta-globin was visible in the triplex PCR assay were considered. Among these, only the samples in which a specific band for GSTT1 was visible in both PCR assays were classified as "conjugators" and were suitable for the localization of GSTT1 expression. From the pool of samples with a GSTT1 positive genotype, we then took those of glioblastoma multiforme WHO grade IV (n=6, as an example of astrocytic tumour) and of oligodendroglioma WHO grade II (n=2, as an example of oligodendroglial tumour), as well as two GSTT1-positive samples obtained by resection following epilepsy (control brain tissue).

### *RNA probes*

A sequence of 307 bp specifically coding for the C-terminal exon of hGSTT1 (bp 638-944) of the hGSTT1 mRNA (Accession Nr. NM_000853) was amplified from human genomic DNA using specific primers flanked by the T7 and T3 promoter sequences for the forward (5' TGTAATACGCACTATAGGGTCATTCTGAAGGCCA AGGACT 3') and reverse primer (5' GCAATTAACCC TCACTAAAGGGTTAAAGGACACAAGGCCTCA 3'), respectively. These amplified a product of 351 bp bridging the 307 bp of the GSTT1 exonic sequence. The 351 bp product amplified by these primers had been previously sequenced and shown to correspond to the GSTT1 sequence. After PCR and loading of the product onto an agarose gel, a band of approximately 351 bp was cut from the gel and the DNA was purified using the phenol-chloroform method and washed in ethanol. An aliquot of the purified DNA used for in vitro transcription was again mounted on a 1.5% agarose gel and a specific 351 bp band was seen. Digoxigenin-labelled RNA probes were yielded by in vitro transcription using the RNA-polymerases T7 (sense) and T3 (antisense).

### *In situ hybridization*

In situ hybridization was carried out according to our standard protocol (Krengel et al., 1996) with slight modifications. Briefly, after deparaffinization,

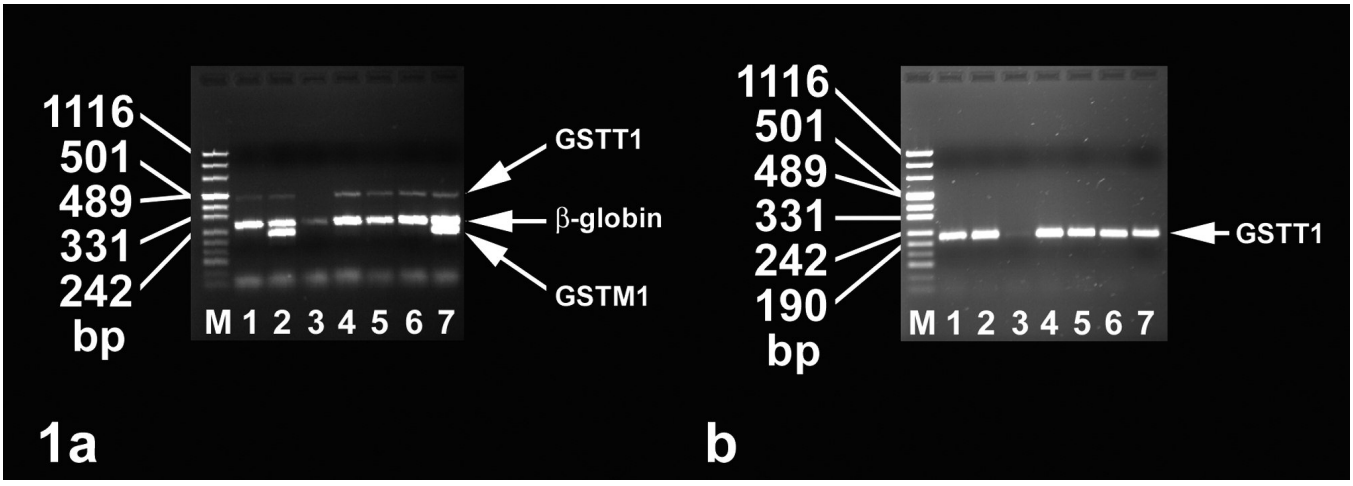



**Fig. 1.** Example of PCR analysis for the determination of the GSTT1-positive genotype in the paraffin embedded tissue samples. **a**. Primers for GSTT1, GSTM1 and ß-globin were applied to genomic DNA extracts obtained from three different cases of anaplastic astrocytoma WHO grade III (lanes 1, 2 and 3), glioblastoma multiforme WHO grade IV (lane 4), oligodendroglioma WHO grade II (lane 5), control brain tissue (lane 6) and another specimen of glioblastoma multiforme (lane 7). Only samples in which a clear positive band for ß-globin was obtained, were considered (for example, in this gel, the samples in lanes 1, 2, 4, 5, 6 and 7). A clear specific band of 480 bp corresponding to the amplification of the hGSTT1 gene is seen in lanes 1, 2, 4, 5, 6 and 7. M: marker. **b.** primers which amplified the shorter fragment of the hGSTT1 gene were applied to the same templates as depicted in 1a. A clear specific band of 214 bp is seen in lanes 1, 2, 4, 5, 6 and 7. M: marker.

rehydration, proteinase K treatment, postfixation with 4% paraformaldehyde and blocking of the background using acetic anhydride, RNAse-free paraffin sections of 5$\mu$m were hybridized using digoxigenin-labelled RNA probes. Sections were then washed in an SSC series and treated with RNAse A. Subsequently, after a rinse, the sections were processed for the immunohistochemical detection of digoxigenin using an anti-digoxigenin antibody conjugated to alkaline phosphatase and the reactions were developed using NBT/BCIP. In some sections, the nuclei were lightly counterstained with nuclear fast red. The sections were then dehydrated in a graded ethanol series, cleared in xylene and cover slipped. Negative controls were performed by incubating sections with a digoxigenin-labelled RNA sense probe substituted for the antisense probe simultaneously to the antisense incubation in each experiment. Additional negative controls were carried out by omitting the RNA probes.

### *Immunohistochemistry*

Paraffin sections were deparaffinized, rehydrated and the endogenous peroxidase activity was blocked by $H_2O_2$: methanol (1:100). Sections were pretreated with protex I (Quartett, Berlin, Germany, 20 min at 60°C) followed by protease XXIV (Sigma, Taufkirchen, Germany, 5 min at room temperature) and then incubated with the primary antibody anti-human GSTT1 raised in rabbit (kindly provided by Prof. John D. Hayes, University of Dundee, Scotland; Sherratt et al., 2002) diluted 1:50, applied overnight at room temperature. Immunoreactions were then visualized by the peroxidase-anti-peroxidase-diaminobenzidine method (Quondamatteo et al., 1998). Nuclei were counterstained with haematoxylin and the sections were then dehydrated in a graded ethanol series, cleared in xylene and cover slipped. In negative controls, sections were incubated with TBS/BSA substituted for the primary antibody.

## Results

### *Glioblastoma multiforme WHO grade IV*

#### Morphology

Most of the glioblastomas WHO grade IV analyzed were characterized by a high cell density with typical pleomorphic features of the glial tumour cells. In addition to regions in which a high amount of small roundish cells were found, there were bundles of spindle shaped cells intermingled with larger round cells. Many mitotic figures were found. Some of the cells had duplex nucleoli. Atypical vascular proliferation with thrombosis as well as regions of necrosis containing degenerating and pyknotic cells were also present. Such necrotic regions were surrounded by pseudo-palisades of tumour cells. In one case, a less cellular and less pleomorphic tissue was seen which, however, had histological features of glioblastoma multiforme WHO grade IV.

#### GSTT1-expression

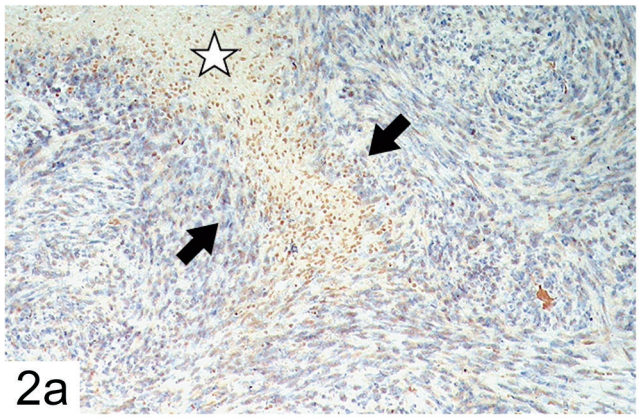
After treatment with the antisense RNA probe (Fig. 2a,b), neoplastic cells showed a clear specific signal towards GSTT1 transcripts. The signal was ubiquitously recognizable in the tumour tissue with the exception of the necrotic regions which showed no expression. GSTT1 gene expression was, however, found in the pseudo-palisades which surrounded the necrotic regions. In sections incubated with the sense RNA probe (Fig. 2c) or after omission of the RNA probe, no intracellular signal was seen. At the protein level (Fig. 3a-c), neoplastic cells of the specimens of glioblastoma multiforme WHO grade IV showed specific localization of GSTT1. The enzyme was irregularly distributed within the tumour areas and was present in the majority of the neoplastic cells. Also, in cells of pseudo-palisades (Fig. 3b,c) adjacent to the necrotic regions staining for GSTT1 was found. Control sections did not show any

**Fig. 2.** Glioblastoma multiforme WHO grade IV. In situ hybridization for hGSTT1. **a.** Antisense probe. Intracellular staining is visible throughout the neoplastic tissue. No signal for GSTT1 is present in necrotic areas (star). Black arrows: pseudo-palisades of neoplastic cells surrounding the necrotic areas. x 70. **b.** Antisense probe, same sample as in Fig. 2a, higher magnification. Staining of neoplastic cells is visible in spindle-like cells (black arrows) and round cells (black/white arrows). x 700. **c.** Sense probe, same sample as in Figs. 2a and b (adjacent section). No staining is visible. Black arrows: spindle-like cells. Black/white arrow: round cells. x 300

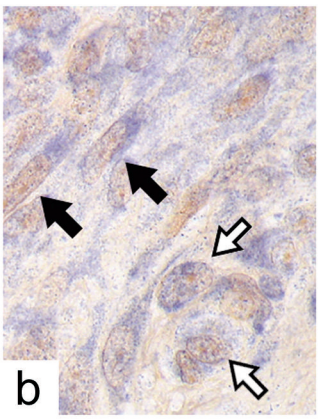
**Fig. 3.** Glioblastoma multiforme WHO grade IV. Immunohistochemistry for hGSTT1. **a.** Staining of neoplastic cells is clearly recognizable (black/white arrows). x 400. **b.** Region adjacent to necrotic areas (star) with pseudo-palisade (black arrow). x 100. **c.** Higher magnification of the pseudo-palisade depicted in Fig. 3b. Neoplastic cells in the pseudo-palisade show staining for GSTT1 (black/white arrows). x 600

**Fig. 4.** Oligodendroglioma WHO grade II, specimen with higher cellularity. In situ hybridization for GSTT1. **a.** Antisense probe. Intracellular staining is visible throughout the neoplastic tissue. x 200. **b.** Antisense probe, higher magnification of the specimen depicted in Fig. 4a. Cellular staining is visible in most of the cells (black arrow). Some cells are not stained (black/white arrow). x 800. **c.** Sense probe, same specimen as the one depicted in Figs. 4a and 4b, adjacent section. No staining is visible. x 200

**Fig. 5.** Control brain tissue. *In situ* hybridization for hGSTT1. **a.** Antisense probe. A positive signal for GSTT1 (black arrows) is seen in nerve cells. No signal is visible in the capillary (black/white arrow). x 400. **b.** Sense probe, same specimen as the one depicted in Fig. 5a, adjacent section. No staining is visible. Black arrow: neuron. x 400. **c.** Control brain tissue. Antisense probe. No staining is visible in the white matter. x 800



2a



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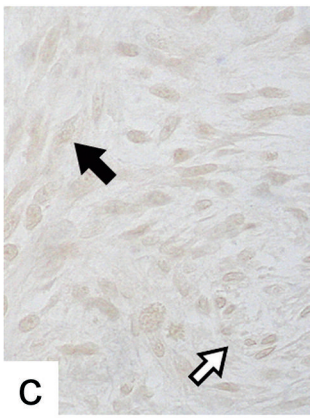

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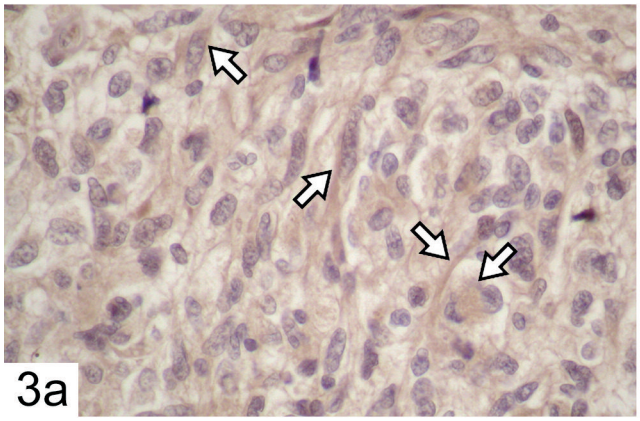

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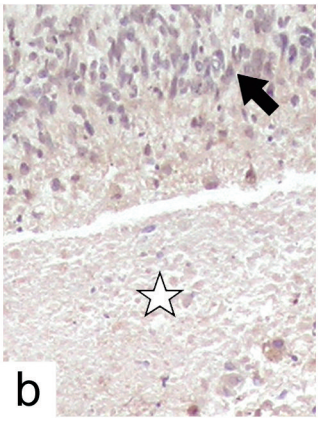

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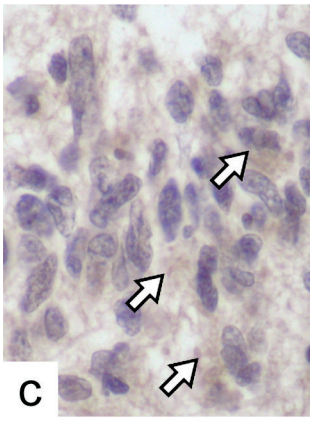

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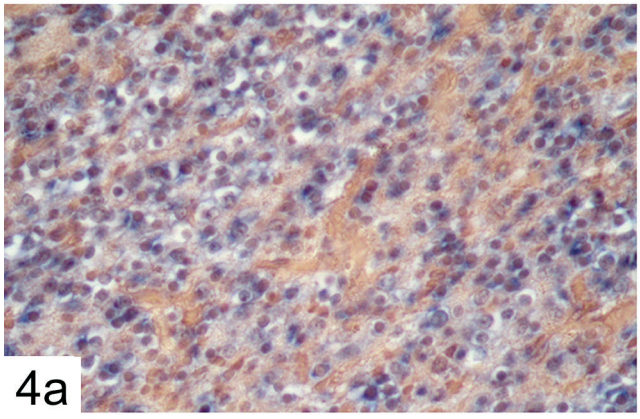
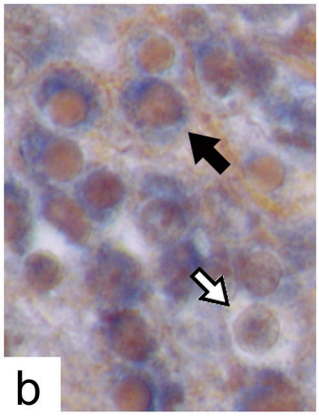


4a



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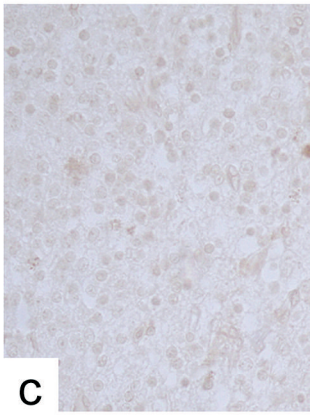

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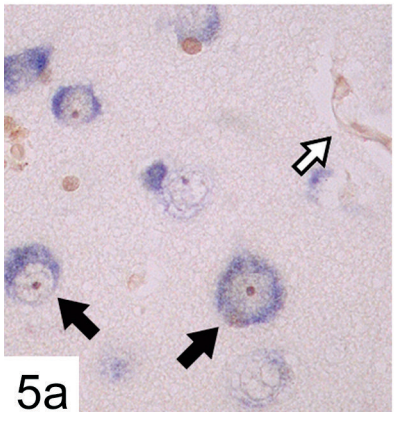

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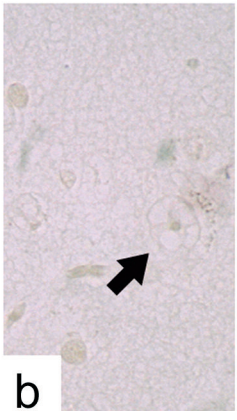

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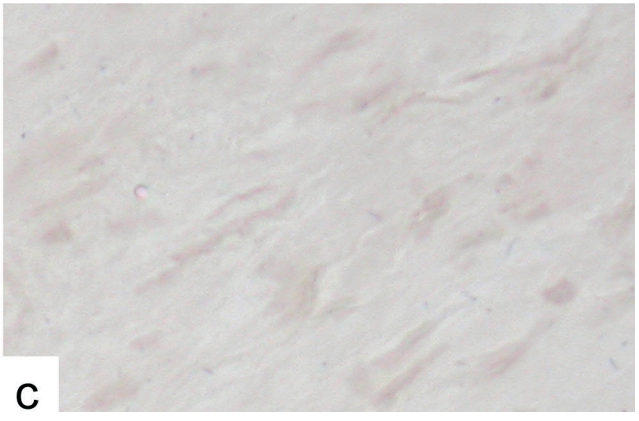

c

staining of glioblastoma neoplastic cells.

*Oligodendroglioma WHO grade II*

Morphology

One case was characterized by lower cell density and typical fried-egg-like cells could be clearly recognized. Minigemistocytes could also be seen. The other case showed higher cell density and a honeycomb aspect. Signs of mineralization, pigment containing cells and minigemistocytes were found. Both cases had a high vascularization and haemorrhages but no signs of higher malignancy.

GSTT1-expression

In the tumour with lower cell density, after incubation with the antisense RNA probe, numerous neoplastic cells showed clear GSTT1 expression. In the sample with higher cell density, after incubation with the antisense RNA probe (Fig. 4a,b), GSTT1 transcripts were seen and expression was localized in nearly every cell of the tumour. In sections incubated with the sense RNA probe (Fig. 4c) or after omission of the RNA probe no signal was observed. At the protein level, in contrast to the specimens of glioblastoma multiforme WHO grade IV, no clear localization of the GSTT1 protein in neoplastic oligodendroglial cells was observed.

*Control brain tissue*

Morphology

One specimen was lined by a regular leptomeningeal sheath with loose connective tissue without cell proliferation, lymphocytic or granulocytic infiltrates or pathologic depositions. No signs of thrombosis were present in the leptomeningeal vessels. In the other specimen, the leptomeningeal sheath was not present. The grey matter showed a layered organisation which corresponded to the cortical structure, and larger neurons as well as some pyramidal cells were also well recognizable. Among these, some uniform roundish cells were also present. In the neuropil, a regular structure was seen. Cortical regions showed lower cell density than the other tumorous tissues and they were characterized by a regular distribution of the cell bodies. In one specimen, fragments of white matter showing an ordered aspect of myelin sheaths were found. The tissue showed a regular distribution of capillaries and no presence of necrosis, mineralisation, inflammation or degenerative alterations.

GSTT1-expression

After incubation with the antisense RNA probe against GSTT1 (Fig. 5a), a clear positive signal was well identifiable in larger neurons. Staining was also found in some other smaller cells of the cortical regions. In contrast, in the white matter (Fig. 5c), no signal was seen. In sections incubated with the sense RNA probe (Fig. 5b) or after omission of the digoxigenin-labelled RNA probe, no signal was observed. Immunohistochemically, in sections of control brain tissue samples incubated with the anti-GSTT1 antibody, staining for GSTT1 was specifically recognized in neurons as well as in some capillaries. In negative controls no such staining was seen.

## Discussion

In the present work, we show localization of GSTT1 in neoplastic cells of glioblastoma multiforme WHO grade IV. This enzyme is able to conjugate electrophiles to glutathione and thus it plays a role in detoxification processes (Hayes et al., 2005). The glutathione-dependent pathway of detoxification has been implicated in chemoresistance in brain tumours. It was shown that astrocytic cells in vitro display a low sensitivity to alkylating agents which can be increased by blocking the synthesis of glutathione (Nutt et al., 2000). Among GSTs, alpha, mu and pi class enzymes have generally been well studied for several years (Awasthi et al., 1994) and their significance in brain tumours has largely been investigated as well (Strange et al., 1992; Grant and Ironside, 1995; Ali-Osman et al., 1997; von Bossanyi et al., 1997). Grant and Ironside (1995) found a correlation between the expression of such GST enzymes with the malignancy grade of the tumours, class pi being the predominant form. Strange et al. (1992) showed that brain pi class is increased in tumours. Also, Ali-Osman et al. (1997) showed that a higher expression of GST-pi correlates to a poorer prognosis of glioblastoma patients thus pointing to a possible role of this enzyme as a determinant of chemoresistance (Ali-Osman et al., 1997). In a mouse model, however, it has recently been shown that experimental oligodenroglial tumours are much more sensitive than corresponding astrocytic tumours to alkylating agents and that such oligodendroglial tumours possess a higher content of pi class enzymes than experimental astrocytic gliomas (Branle et al., 2002).

In the present work, we focus on the member of the theta class GSTT1. The significance of this enzyme in chemoresistance of brain tumours is not clear. A first study which specifically considered GSTT1-polymorphism as a determinant of chemoresistance in brain tumours has already been published (Okcu et al., 2004). In this study, polymorphisms for GSTT1, GSTM1 and GSTP1 were examined retrospectively in a heterogeneous group of patients after surgery who then received chemotherapy for different types of malignant gliomas (Okcu et al., 2004). The authors did not report any influence of the GSTT1 genotype on the outcome of the chemotherapy. However, as the authors of the study also stated, one main limit of their investigation was a non-uniform utilization of multiple chemotherapy

agents, most of them probably not being substrates for the GSTs studied (Okcu et al., 2004). Therefore, the significance of GSTT1 in chemotherapy resistance in malignant gliomas still remains unclear. To address this issue, the first question to be answered is whether GSTT1 is expressed in malignant gliomas of conjugators.

In the present work, we provide, for the first time, clear evidence of expression of hGSTT1 in neoplastic cells of gliomas of conjugator patients. Both tumour types studied, i.e. glioblastoma multiforme WHO grade IV and oligodendroglioma WHO grade II, express mRNA, however, localization of the ezyme at the protein level in neoplastic cells was obvious in glioblastoma cells though not in oligodendroglioma cells. In control brain tissue of conjugators, GSTT1 expression seems, in contrast, to be restricted to neurons. In this context, the question arises as to which biological function GSTT1 in glioblastoma multiforme WHO grade IV may have.

Possibly, glioblastomas may use GSTT1, perhaps together with other metabolizing enzymes (e.g. GST pi-class enzymes, Ali-Osman et al., 1997), for the inactivation of chemotherapy drugs.

There is, generally, hardly any information available on the metabolic potential of GSTT1 towards drugs used in antitumour therapy. However, the fact that a possible metabolic activity of GSTT1 towards chemotherapy drugs may represent a determinant for variations in the efficacy of the chemotherapy, has in recent years been a subject of discussion for other tumours. In fact, although some contradictory results exist (Stanulla et al., 2000; Davies et al., 2002; Haase et al., 2002; Krajinovic et al., 2002; Naoe et al., 2002; Chacko et al., 2005; Yang et al., 2005), a null genotype for GSTT1, sometimes associated with a null genotype of GSTM1, has been correlated with increased toxicity of the chemotherapy or with a reduced risk of cancer relapse, e.g. in breast cancer or leukemias (Stanulla et al., 2000; Haase et al., 2002; Naoe et al., 2002; Chacko et al., 2005).

GSTT1 is capable of inactivating BCNU in vitro by means of a denitrosating mechanism (Lien et al., 2002). Nitrosoureas can, in general, be denitrosated by cytosolic GSTs and their denitrosation inactivates these drugs (Talcott and Levin, 1983; Smith et al., 1989; Tuvesson et al., 1993). However, the study of Lien et al. (2002) clearly shows that among 12 different cytosolic GSTs tested (among which are also GSTM1, GSTP1*A and *B), only three enzymes, namely GSTM2, GSTM3 and GSTT1 showed activity towards BCNU. Furthermore, the activity of GSTT1 was shown to be about 20-fold higher than that of the other two GSTs (Lien et al., 2002). This could mean that the major part of the metabolism of cytosolic GSTs towards nitrosoureas might be dependent on GSTT1.

This could be of particular importance for glioblastomas, in fact, BCNU is currently used for local implantation in this tumour type (Giese et al., 2004). In conjugators, i.e. in those individuals who have a functional GSTT1 gene, presence of GSTT1 in neoplastic cells of glioblastoma multiforme WHO grade IV, as shown in the present work, if also supported by enzyme activity, could negatively influence the efficacy of the pharmacological effects of chemotherapy drugs in comparison to non-conjugators.

Further, it is not known whether in cells nitrosoureas are inactivated by GSTT1 as has been shown in enzyme assays (Lien et al., 2002). The fact that inhibition of glutathione in astrocytes may sensitize cells towards BCNU (Nutt et al., 2000), speaks, however, in favour of this.

In contrast, a possible implication of the GSTT1 genotype as a chemoresistance factor could be less significant in the case of oligodenrogliomas. On the one hand, in oligodendrogliomas the problem of chemoresistance is less marked than in astrocytic tumours (Soffietti, 2004). On the other hand, oligodendroglial cells as well as experimental oligodendrogliomas are more sensitive to BCNU than their astrocytic counterparts (Nutt et al., 2000; Branle et al., 2002). This is also in line with our finding that the enzyme was not localized in neoplastic oligodendroglial cells and would further speak for a possible involvement of GSTT1 in the inactivation of BCNU in vivo.

In conclusion, in the present work we show for the first time that neoplastic cells of glioblastoma multiforme WHO grade IV express and contain the enzyme GSTT1. In the light of the in vitro activity of GSTT1 towards BCNU (Lien et al., 2002), of the GSTT1 polymorphism (Pemble et al., 1994; Hayes and Strange, 2000) and of the use of BCNU in glioblastoma multiforme (Giese et al., 2004), our results suggest that not only GST enzymes of the classes alpha, mu and pi (Ali-Osman et al., 1997; von Bossanyi et al., 1997) but also the theta class member GSTT1 may be considered as a factor of relevance for chemotherapy of glioblastomas. A further clarification of the significance of the GSTT1 genotype in glioblastoma patients treated with BCNU will help in the understanding of the role of GSTT1 in chemotherapy resistance.

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