RESEARCH ARTICLE

Expression of Antioxidant Enzymes in Astrocytic Brain Tumors

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We studied the expression of antioxidant enzymes (AOEs) and related proteins manganese superoxide dismutase (MnSOD), thioredoxin (Trx), thioredoxin reductase (TrxR), and the catalytic (GLCL-c) and regulatory (GLCL-r) subunits of glutamate cysteine ligase (γ-glutamylcysteinesynthetase) in 433 astrocytomas. Expression of MnSOD was found in 91%, Trx in 46%, TrxR in 66%, GLCL-c 73% and GLCL-r in 89% of the cases. Diffuse astrocytomas showed more intense staining for Trx (p = 0.002), TrxR (p = 0.004), GLCL-c (p = 0.001), GLCL-r (p = 0.04) and MnSOD (p = 0.01) than pilocytic astrocytomas. Within diffuse astrocytomas only Trx (p = 0.0001) and TrxR (p = 0.04) significantly associated with increased malignancy grade. Necrotic tumors were more often immunopositive for Trx (p = 0.001) and TrxR (p = 0.02) and AOE expression was generally higher in mitotically active tumors. Expression of Trx and lack of MnSOD expression was associated with a worse prognosis in diffuse astrocytomas. None of the AOEs had any prognostic value in pilocytic grade I astrocytomas. Familial astrocytomas, which included 23 of the cases studied, did not differ in their expression of MnSOD from sporadic ones. The results show that MnSOD and Trx may influence the biological behaviour of astrocytomas, possibly by modulating cell proliferation and necrosis in these tumors.

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Introduction

Reactive oxygen species (ROSes) are oxygen-derived reactive compounds which include both oxygen derived free radicals harbouring one unpaired electron on their outer orbital and other reactive oxygen derived metabolites (11). The most important ROSes are the superoxide radical, the hydroxyl radical and hydrogen peroxide (11). ROSes are formed in many different pathological situations such as chronic inflammations and ischemia and they may cause functional or structural disturbances by reacting with other constituents of the cell. Because of their noxious effects, cells have numerous antioxidant defence mechanisms that include small molecular weight vitamins, such as vitamin E, metal-binding proteins, such as ferritin and ceruloplasmin and antioxidant enzymes (AOEs) located in distinct cellular compartments and extracellularly. AOEs comprise multiple families of proteins the function of which is to protect cells from damage caused by free radicals, especially ROSes (11).

AOEs and related compounds include superoxide dismutases (SODs), catalase, glutathione-associated enzymes, such as glutathione peroxidase and glutamate cysteine ligase (γ-glutamylcysteine synthetase), the thioredoxin-thioredoxin reductase system and peroxiredoxins. Superoxide dismutases comprise three enzymes, manganese SOD (MnSOD), copperzincSOD (CuZnSOD) and extracellularSOD (ECSOD) (26, 30). MnSOD is an enzyme, which is located in the mitochondrial compartment of the cells (26). The gene encoding MnSOD is located at 6q25.3 (3), which is an area frequently lost in familial gliomas (32). CuZnSOD is a 32 kDa molecule mainly located in the cytosol, but is also found in the nucleus and peroxisomes (5). ECSOD, on the other hand, is excreted into the extracellular matrix (8). Especially MnSOD is induced by several factors, most important of them are hyperoxia, radiation, TNF-α, interleukin-1, lipopolysaccharides and interferon-γ (18). MnSOD knockout mice die at the age of 10 to 21 days with cardiovascular and neural manifestations (21). CuZnSOD and ECSOD knockout mice are viable but also they are sensitive to oxidative damage (1,36).

Thioredoxin is a 12 kDa protein, which takes part in the redox regulation of the cell. Its expression is induced by oxidative stress, etc (14, 15). By influencing the redox balance it can regulate the function of several transcription factors, such as p53, NK-κB and AP-1 (34). It has been shown that thioredoxin is able to induce proliferation in vitro, and it harbours the resistance of cells to cytostatic drugs (9, 34, 38, 47). Its function is regulated by thioredoxin reductase (TrxR). There are 3 subtypes of TrxR, named TrxR 1, 2 and 3(27, 43). They are located both in mitochondrial and cytosolic

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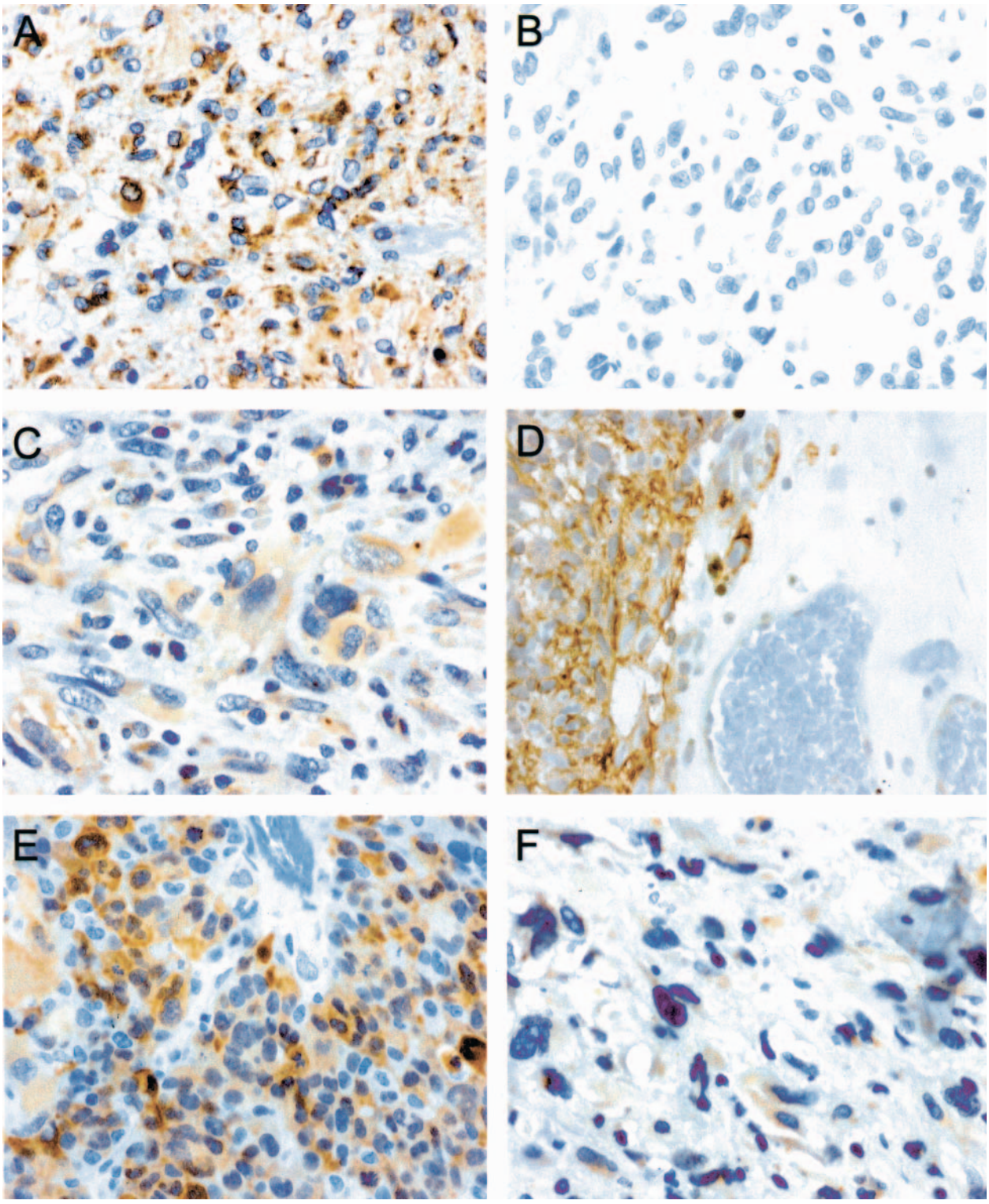
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compartments of the cell (27,43). The function of thioredoxin reductase is dependent on the selenium concentration of the cell (51).

Glutathione is a small molecule consisting of 3 amino acids. It participates in the scavenging of H_2O_2 in a reaction catalysed by glutathione peroxidase (44). It is also involved in the development of drug resistance by participating in various mechanisms that transport toxic metabolites through the cell membrane (13, 44, 48, 49). The synthesis of glutathione is regulated by 2 enzymes, glutathione synthase and glutamate cysteine ligase (GLCL), the latter being the rate-limiting enzyme in glutathione synthesis (44). GLCL consists of 2 molecules, heavy or catalytic and light or regulatory unit (40). The former is more important for the enzymatic function and GLCL-c knockout mice die soon after birth (6).

Given the putative role of endogenous mechanisms both in the development of astrocytic tumors and possibly also the resistance of astrocytomas to treatment we here wanted to study the expression of antioxidant enzymes MnSOD, Trx, TrxR and GLCL heavy and light chain in these tumors. Our basic hypothesis was that cells undergoing genetic changes and transformations to a malignant clone would lose some or all of its protective mechanisms towards oxidative damage. This would make them genetically more labile and susceptible to further damage. We also assessed the expression of these enzymes to some other parameters, such as proliferation, tumor grade, heredity and to the survival of the patients.

Materials and Methods

Study material. The brain tumor samples were obtained from patients who underwent surgery at the Tampere University Hospital, Tampere, Finland, during 1983-2000. The specimens had been fixed in 4% phosphate buffered formaldehyde, after which they had been processed into paraffin blocks. One neuropathologist (HH) evaluated all H&E-stained slides of the tumors, and the histopathological typing and grading were done according to the criteria presented by WHO (18). He pinpointed one histologically representative tumor region in each astrocytoma H&E-slide. From this tumor region a sample was included in multitissue microarray blocks representing 433 astrocytic tumors (20).The microarray blocks were constructed with a custom-built instrument (Beecher Instruments, Silver Spring, Md). The sample diameter of the tissue core in the microarray block was 600 μm. The astrocytomas of the microarray blocks comprised 354 primary and 79 recurrent astrocytomas (grade I: 33 primaries and 9 recurrences; grade II 50 and 11; grade III 35 and 16; grade IV 236 and 43, respectively).

A series of 23 familial gliomas (6 [gr I], 7 [gr II], one [gr III] and 9 [gr IV] astrocytomas) was included in the microarray analyses. Familial occurrence of 2 or more gliomas was identified among glioma patients treated at Tampere University Hospital during 1983-1994 (30). Three non-familial age- (±10 years), histology-, and grade-matched controls were selected for each familial case to compare MnSOD expression between familial and sporadic gliomas.

		Grade I	Grade II	Grade III	Grade IV	Total
TRX	0	83	74	63	45	54
	1	10	18	27	35	29
	2	5	9	10	14	12
	3	2	0	0	6	4
TRXR	0	55	31	42	29	34
	1	30	40	33	32	33
	2	13	26	24	31	28
	3	3	4	0	8	6
GLCLC	0	39	24	33	23	27
	1	27	31	22	26	26
	2	27	21	29	29	28
	3	7	24	16	22	20
GLCLR	0	8	3	2	13	11
	1	53	43	35	39	40
	2	38	41	52	38	40
	3	3	12	10	10	10
MNSOD	0	10	3	15	10	9
	1	53	36	27	29	32
	2	38	47	38	41	41
	3	0	14	21	20	17

Table 1. Distribution of AOE expression (0-3) in pilocytic (grade 1) and diffuse (grades II-IV) astrocytomas (in percents).

Figure 1. A. (Opposing page) Immunostaining of MnSOD in a diffuse astrocytoma. Granular cytoplasmic MnSOD immunostaining can be seen in the tumor cells. **B.** A rabbit isotype control is shown of the same case as in (**A**). **C.** Strong immunostaining for GLCL-r in a glioma. Pleomorphic tumor cells show positivity in the cytoplasm. **D.** Glioblastoma showing strong expression of GLCL-c in the cytoplasm of the tumor cells. Adjacent non-neoplastic tissue is negative. Faint positivity can be seen in endothelial cells of the blood vessels. **E.** Glioblastoma showing strong cytoplasmic positivity for Trx. Some nuclei are also positive. **F.** An astrocytoma showing positivity for TrxR in the cytoplasm of the tumor cells.

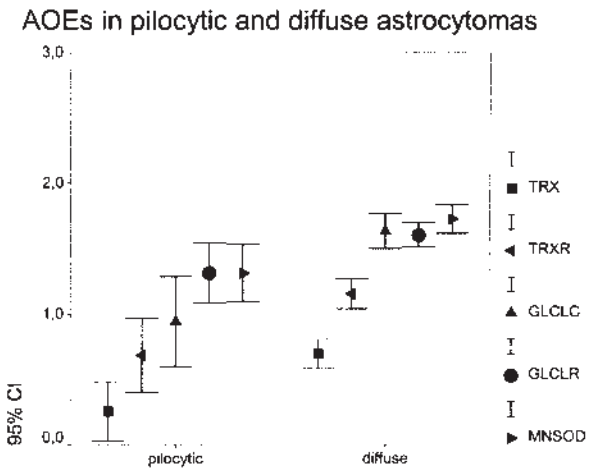

Figure 2. The distribution of AOE immunostaining in astrocytomas. Expression of the AOEs is stronger in diffuse than in pilocytic astrocytomas.

Immunohistochemistry/antibodies. The immunostaining procedure was as follows. Four-micron thick sections were cut from a representative array paraffin blocks. The sections were first deparaffinized in xylene and rehydrated in descending ethanol series. In order to enhance immunoreactivity, the sections were incubated in 10 mM citrate buffer (pH 6.0), boiled in a microwave oven for 2 minutes at 850 W, and after that 8 minutes in 350 W. Endogenous peroxidase activity was eliminated by incubation in 0.1% hydrogen peroxide in absolute methanol for 10 minutes. The antibodies used in the study were as follows. A polyclonal rabbit anti-human antibody to MnSOD (a gift from Professor J.D. Crapo, National Jewish Medical Center, Denver, Colo, dilution 1:1000), rabbit polyclonal anti-human antibodies to GLCL-c and GLCL-r (a gift from Dr Kavanagh, University of Washington, Seattle; dilution 1:1000 for both), an affinity purified goat polyclonal human Trx antibody (American Diagnostica, Greenwich, Conn; dilution 1:200), antibody to TrxR (generous gift from Professor Arne Holmgren, Karolinska Institutet, Stockholm, Sweden; dilution 1:1000) was the gammaglobulin fraction of a polyclonal rabbit anti-rat antibody directed against cytosolic TrxR in rat liver (27, 28).

The immunostainings for MnSOD, γGLCL, and TrxR were done using the Histostain-Plus Kit (Zymed Laboratories, South San Francisco, Calif) and the chromogen was aminoethyl carbazole (AEC) (Zymed Laboratories) In negative controls the primary antibodies were substituted with phosphate-buffered saline (PBS) or non-immune rabbit serum. For Trx, a biotinylated secondary anti-goat antibody was applied (dilution 1:400) followed by the avidin-biotin-peroxidase complex (all from Dakopatts, Glostrup, Denmark). The color was developed using 3,3′-diaminobenzidine, and the sections were lightly counterstained with hematoxylin and mounted with Eukitt (Kindler, Freiburg, Germany). Replacement of the primary antibody by phosphate buffered saline (PBS) at pH 7.2 and goat IgG immunoglobulin isotype (Zymed Laboratories, South San Francisco, Calif) were used as a negative controls.

Cell proliferation was analysed by a mouse monoclonal antibody MIB-1 (IgG, Immunotech, S.A. Marseille, France) recognizing the Ki-67 antigen. The antibody was used at a dilution 1: 40. The tissue sections were counterstained with ethyl green. The proliferation was reported as the percentage of immunopositive nuclei and evaluated by analysing all the tumor cells in the core tissue with an image analysis system (CAS-200 Software, Becton Dickinson, NJ) as described previously (37).

Additional tissue sections of grade II-IV astrocytomas (N = 61) were stained either with a MAb31G7 monoclonal antibody to human EGFR (0.5 μg/ml, Triton Diagnostics) or with a monoclonal antibody DO-7 to p53 (dilution 1:300, Novocastra Laboratories, Newcastle, UK) and analyzed as described previously (10).

Statistical analysis. SPSS for Windows (Chicago, Ill) was used for statistical analysis. The significance of associations were determined using Fisher's exact probability test, Mann-Whitney test, Kruskal-Wallis test, correlation analysis, 2-tailed t-test and Cox multivariate regression analysis. In survival analysis the Kaplan-Meier curve and the log rank test was used.

Results

The immunoreactivity pattern for AOEs in astrocytomas is shown in Figure 1. The distribution of immunostaining (negative (0) or weakly (1)/moderately (2)/intensively (3) positive) by AOEs in four malignancy grades of 433 astrocytomas is shown in Table 1. Immunoreactivity for MnSOD was granular and was seen in the cytoplasmic compartment of the positive cells. Ninety-one percent of the astrocytomas expressed positivity for MnSOD. Expression of Trx was seen both in the cytoplasmic and nuclear compartments of the cells and a similar distribution of positivity was seen for TrxR, but the nuclear positivity was far less pronounced. Trx and TrxR positivity were seen in 46% and 66% of the cases, respectively. GLCL-c and GLCL-r showed cytoplasmic expression in the tumor cells, and 73% and 89% of cases showing positivity.

	All tumors			Pilocytic tumors			Diffuse tumors		
	AOE-neg.	AOE-pos.	p-value	AOE-neg.	AOE-pos.	p-value	AOE-neg.	AOE-pos.	p-value
Trx	9.9 (10.9)	12.9 (11.9)	0.002	1.9 (2.8)	4.9 (8.3)	n.s.	11.3 (11.2)	13.2 (12.0)	n.s.
TrxR	10.6 (12.5)	11.2 (10.9)	n.s.	1.5 (1.3)	3.7 (6.1)	n.s.	12.5 (13.0)	11.8 ((11.0)	n.s.
Glcl-c	9.6 (11.0)	11.7 (11.7)	0.025	1.3 (1.5)	3.1 (5.2)	n.s.	11.1 (11.3)	12.6 (11.8)	n.s.
Glcl-r	11.1 (11.6)	11.1 (11.4)	n.s.	0 (0)	2.7 (4.4)	0.034	12.0 (11.7)	12.1 (11.5)	n.s.
MnSOD	8.4 (11.5)	11.3 (11.4)	0.025	0.4 (0.4.)	2.6 (4.5)	n.s.	9.3 (11.8)	12.2 (11.6)	0.042

Table 2. MIB indices (mean, SD in brackets) in different subsets of astrocytic tumors according to AOE expression.

AOE expression differed significantly between pilocytic (grade I) and diffuse (grade II-IV) astrocytomas (Figure 2). Diffuse astrocytomas had generally more intense staining by Trx ($p = 0.002$, Mann-Whitney test), TrxR ($p = 0.004$), GLCL-c ($p = 0.001$), GLCL-r ($p = 0.04$) and MnSOD ($p = 0.01$) (Figure 2). However, within diffuse astrocytomas only Trx ($p = 0.0001$) and TrxR ($p = 0.04$) showed significantly increasing expression with the malignancy grade (Kruskal-Wallis test). Interestingly, there were fewer MnSOD immunopositive (1+2+3) cases in recurrent gliomas ($p = 0.034$). Such association was not found with the other AOEs.

When compared with each other there was an association between MnSOD expression and TrxR ($p = 0.001$) and GLCL-r ($p = 0.039$) expression. Similarly, Trx associated with TrxR ($p = 0.001$), GLCL-r ($p = 0.039$) and GLCL-c ($p = 0.016$) and TrxR with GLCL-c ($p<0.001$).

Trx ($p = 0.001$) and TrxR ($p = 0.02$) correlated significantly also with the presence of necrosis in the diffuse astrocytomas, necrotic tumors being more often immunopositive (chi-square test). When mitotically active diffuse astrocytomas were compared with mitotically inactive tumors, Trx was the only AOE, which was significantly higher in proliferative tumors ($p = 0.005$, chi-square test). However, when AOE-negative and AOE-positive tumors were compared, proliferation by Ki67 (MIB1)-index differed significantly by GLCL-c, GLCL-r and MnSOD in addition to Trx (Mann-Whitney test, Table 2). Proliferation was generally higher in AOE-positive tumors. There was no association between the p53- or EGFR-immunohistochemistry and AOE-status in diffuse astrocytomas (Mann-Whitney test).

Protein expression of MnSOD was studied in 23 familial astrocytomas to find out whether the gene expression was similar in familial and sporadic gliomas. In the total series of 23 familial tumors,

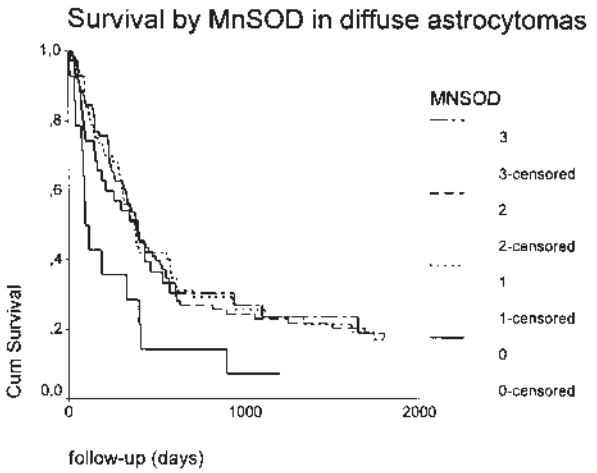

Figure 3. Kaplan-Meier curve showing patient survival in relation to MnSOD immunostaining in diffuse astrocytomas. Patients with no immunostaining have the worst prognosis ($p = 0.02$).

MnSOD expression was as frequent (19/23 cases, 83%) as in sporadic astrocytomas. Positive immunostaining pattern (1+2+3) was detected in 88% of familial diffuse astrocytomas, while the respective number in sporadic diffuse astrocytoma controls was 98% ($p=0.15$). Similarly, in pilocytic astrocytomas, the expression was less frequent in familial tumors (66% immunopositive) than in sporadic controls (78% immunopositive), but the trend was less prominent (p = n.s.).

Survival was known in 182 patients with primary grade I-IV astrocytomas (grade I: 14; grade II: 14 grade III: 20; grade IV: 134). In diffuse (grade II-IV) astrocytomas only MnSOD-expression (0, 1, 2 or 3) divided the tumors into 4 significantly differing prognostic subsets ($p = 0.02$, Figure 3). However, also Trx seemed to have prognostic value at a near-significant level ($p = 0.07$, log-rank test) (Figure 4).The survival difference by MnSOD was even more significant when immunoposi-

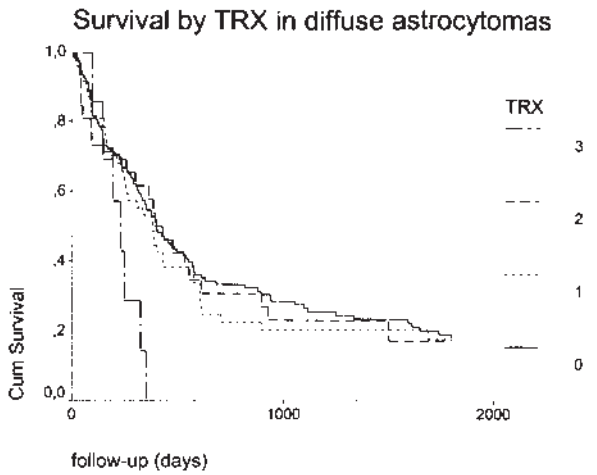

Figure 4. A Kaplan-Meier curve showing patient survival in relation to Trx immunostaining in astrocytomas. Patients with no immunopositivity have the best prognosis (p = 0.07).

	Exp(B)	95% CI for Exp(B)	Sig.
All tumors			
Grade	3.66	2.76-4.87	0.00
MIB-1	0.99	0.98-0.99	0.03
MNSOD	0.99	0.99-1.00	0.05
TRXR	1.00	1.00-1.01	0.03
TRX	1.00	0.99-1.00	0.09
Diffuse astrocytomas			
Grade	3.48	2.51-4.85	0.00
MIB-1	0.99	0.98-1.00	0.04
MNSOD	0.99	0.99-1.00	0.03
TRXR	1.00	1.00-1.01	0.01
TRX	1.00	0.99-1.00	0.09

Table 3. The independent molecular and histopathologic prognostic indicators of astrocytomas as evaluated by Cox's stepwise regression model (enter limit p<0.10).

tive (1+2+3) tumors were compared with immunonegative (0) neoplasms, the latter having worse prognosis (p = 0.008, log-rank test). In pilocytic astrocytomas none of the AOEs was of significant prognostic value. When all the AOEs (divided into immunonegative and positive tumors), histological grade and Ki-67 (MIB1) proliferation index were tested in the Cox multivariate analysis, MnSOD, TrxR and Trx were found to be of independent prognostic value in addition to histology and proliferation. The result was the same when analysed either in the total material (grades I-IV) or in the category diffuse astrocytomas(grades II-IV) (Table 3). However, only malignancy grade appeared to be an independent prognosticator in both categories if age-stratification was used in the Cox analysis. In spite of this, patient age was not significantly associated with the immunostatus of AOEs (Mann-Whitney test).

Discussion

In this investigation we studied the expression of AOEs MnSOD, Trx, TrxR, GLCL-c and GLCL-r in a large set of astrocytomas to see whether these enzymes would have a putative pathogenic significance and play a role in the behaviour of these tumors. Moreover, since some of the antioxidative enzymes modulate the chemoresistance of the tumors, their expression in astrocytomas could have also therapeutic importance. In our study we found that the majority of astrocytomas were positive for these proteins, the most significant expression being found for MnSOD and GLCL-r and the lowest expression for Trx. Generally, the expression of these enzymes was more pronounced in grade II-IV astrocytomas (diffuse astrocytomas) compared to grade I (pilocytic) astrocytomas, and within diffuse astrocytomas Trx and TrxR also showed an association with the grade. Within diffuse astrocytomas, Trx and TrxR expression was associated with a larger extent of necrosis and Trx also associated with a higher mitotic activity. Trx also associated with a worse survival of the patients while MnSOD had an opposite correlation; patients with tumors showing no MnSOD expression showed a significantly poorer prognosis than other cases.

In previous studies MnSOD expression has been shown in a wide variety of tumors, including breast, colon and thyroid carcinomas, and mesotheliomas (16, 29, 42, 45). In our previous study on breast carcinomas, 49% of invasive tumors showed MnSOD positivity, while in in situ lesions and benign hyperplasias 82% and 79% of the cases were positive (42). In thyroid, esophageal, gastric and colon carcinomas higher expression of MnSOD was found than in normal tissue (12, 29, 45). In agreement, MnSOD expression has been shown to be higher in brain tumors than in normal brain (4). In our material on astrocytomas, grade I pilocytic lesions did show less positivity than more malignant, grade II-IV diffuse astrocytomas, but there was not a significant difference between the grades within diffuse astrocytomas. On the other hand, tumors with a lack of MnSOD expression had a poor prognosis, a phenomenon that is in line with the suggested tumor suppressor nature of MnSOD (22). In previous in vitro studies and animal models overexpression of MnSOD has been shown to abrogate proliferation (23) and apoptosis (25).

MnSOD has also been shown to upregulate the expression of protease inhibitor maspin, which inhibits tumor cell motility and migration (24). On the other hand, its overexpression also leads to the synthesis of several matrix metalloproteinases (at least MMP 1, 2, 3 and 7), also suggesting the complex effect of this enzyme and cellular redox state in vivo (35). In cultured cells MnSOD overexpression appears to inhibit tumor promotion through redox regulation of transcirptional factors, such as c-jun and AP-1 (50). In cultured glioma cell lines, transfection and overexpression of the MnSOD gene lead to a less malignant phenotype with a slower rate of growth in nude mice (52). In our results there was, however, not an association between tumor grade or MnSOD expression within diffuse astrocytomas. MnSOD also appeared to be associated with increased proliferation. The lack of MnSOD expression, however, associated with a poorer prognosis in diffuse astrocytomas. A similar but marginal association has been previously found in mesothelioma (16). Similarly, recurrent glioma cases showed a diminished expression of MnSOD, which also is in line with its tumor suppressor nature. In fact, downregulation of MnSOD might be one late event in the progression of gliomas.

Putative tumor suppressor gene MnSOD is located at chromosome 6q25.3. In our earlier study by comparative genomic hybridization, loss at 6q was detected in 43% of familial gliomas, which was significantly more frequent than in sporadic controls (16%) (32). A tumor suppressor gene or a mismatch repair gene characteristic of tumorigenesis of familial gliomas is likely to locate in this region. Although familial diffuse astrocytomas expressed less frequently MnSOD (12% MnSOD immunonegative) than sporadic controls (2% immunonegative), the overall number of immunonegative familial astrocytomas was small (4/23). Thus, decreased MnSOD expression is not frequent in familial gliomas and the respective gene does not seem to play a central tumorigenic role in hereditary tumors. The slight excess of immunonegative tumors in the familial series probably reflects secondary genetic changes originating from other locations than 6q25.3 in this chromosome.

MnSOD expression may play a role in the development of drug resistance. Exposure of A549 lung adenocarcinoma cells with cytotoxic drugs such as paclitaxel, vinclastine or vincristine, lead to upregulation of MnSOD mRNA (7). Expression of Trx and TrxR has also been associated with development of drug resistance. Cisplatin-resistant HeLa cells express elevated levels and increased activity of Trx and TrxR but resistance is lost when the cells are transfected with TR antisense oligonucleotides (38,39). Also cisplatin-resistant human bladder and prostate cancer cells show a stronger expression of Trx than the more drug sensitive counterparts (47). According to this, tumors with a lower Trx or TrxR expression could have a better prognosis. Indeed, diffuse astrocytomas showing strong Trx expression seemed to have a poorer prognosis, but such association could not be observed with TrxR. In addition to drug resistance, the Trx-TrxR system also influences other cellular mechanisms, which may account for their effect on patient survival and tumor growth. Trx promotes cell proliferation and in transfection experiments it has been shown to increase the growth rate and colony formation of non-malignant and malignant cells (34). In line with this there was a significant association between tumor cell proliferation and Trx expression in astrocytomas.

There are no previous studies on Trx or TrxR expression in brain tumors or brain tumor cell lines. The level of their expression, however, suggests that the Trx-TrxR system probably plays an important part also in glial neoplasia. However, previous studies on other tumors, such as non-small cell lung carcinomas, show an even higher rate of Trx positivity with over 90% of tumors positive (41). Such a difference may be, in part, cell type specific, but may also be dependent on the more frequent exposure of lung tissue to exogenous carcinogens than the brain.

Similar to Trx and TrxR, expression of GLCL-c or GLCL-r has not been previously studied in brain tumors. In brain tissue, both subunits have been shown to be localised in astrocytes and in dopaminergic nigral neurons and their lowered expression in substantia nigra has been suggested to be associated with Parkinson's disease (46). Interestingly also, oxidized glutathione has been suggested to play a role in the regulation of sleep and in the pathogenesis of Alzheimer's disease, where lowered levels of glutathione has been found in lymphoblastic cell lines of familial Alzheimer patients even though the level of GLCLs have been on a normal level (2,17). Our results show a strong expression of these enzymes in astrocytomas the majority of the tumors being positive for both of them. In contrast to Trx and TrxR, the expression of GLCL is on a similar level in brain tumors as in lung carcinomas showing no evident cell type specific differences (41). Their expression has been shown to be stronger in low grade tumors and be inversely associated with apoptosis in lung tumors (41). In contrast to this, diffuse astrocytomas representing a higher grade, showed a stronger expression of

GLCL-c and GLCL-r, but no difference in the expression was found between the grades of diffuse astrocytomas. As with MnSOD and the Trx-TrxR system, also GLCL, through influencing the synthesis of glutathione, is involved in the development of drug resistance. Glutathione is involved in the metabolism of various cytotoxic drugs by cellular detoxification or scavenging free radicals released by these drugs and increased glutathione synthesis has been associated at least with resistance to alkylating agents such as cisplatin and certain anthracyclins (48). Thus , in addition to counteracting oxidative damage, the expression of GLCL in brain astrocytomas also suggest that a large subtype of them may develop drug resistance based on their expression of GLCL.

In conclusion, our results show a substantial expression of MnSOD, Trx, TrxR and GLCL catalytic and regulatory chain in brain astrocytomas suggesting that they play a role in ROS defense and in chemoresistance of these tumors. Their expression was higher in diffuse than in pilocytic astrocytomas. Generally, AOE expression was associated with increased proliferation, and with Trx and TrxR also with increased malignancy grade and necrosis. In diffuse astrocytomas, Trx seemed to be associated with a worse prognosis while MnSOD showed an opposite correlation. Interestingly, MnSOD was lower in recurrent tumors, suggesting some role in glioma progression. Even though the MnSOD gene is located in the long arm of chromosome 6, a frequent locus for loss of heterozygosity in gliomas, we did not find any differences in the immunohistochemical expression of MnSOD between sporadic and familial cases suggesting that MnSOD expression probably does not play any key role in tumorigenesis of familial gliomas.

Acknowledgments

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