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Expression of Cathepsin B by Oligodendrogliomas: An Immunohistochemical Study

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Abstract: **Degradation of extracellular matrix (ECM) is an essential prerequisite for invasive growth of brain tumours. There is evidence from investigations of CNS-neoplasmas that secreted proteolytic enzymes may facilitate tumour invasion by partial degradation of ECM. Among the enzymes which may be involved are cysteine proteinases, especially cathepsin B (CB).**
Cathepsin B is a lysosomal tissue proteine, which in cooperation with other enzymes is responsible for degradation of ECM and basement membrane (BM) components in gliomas. CB participates in the proteolysis and decomposition of laminin which is part of the basement membrane. Furthermore, CB probably can also degrade fibronectin and type IV-collagen, which are important components of ECM.
In the present investigations 78 oligodendrogliomas were examined immunohisto-chemically for CB-expression and increased levels of CB-expression were found in high grade oligodendroglial tumours.
Upregulation of CB-expression correlated in the present study with tumour dedifferentiation and in high-grade oligodendrogliomas the expression of CB correlated negatively with survival.

Keywords: **Oligodendrogliomas, Cathepsin B (CB), Basal Membrane (BM), Extracelluar Matrix (ECM), proteolysis.**

1. INTRODUCTION

The invasiveness and destructive features of malignant neoplasmas in the central nervous system (CNS) vary between different types of tumour (1-2, 8, 10, 12-16). Gliomas often show a diffuse growth, infiltration into the adjacent brain tissues, and spreading of great distances in the brain. Infiltration occurs along white-matter tracts, around nerve cells, along blood vessels, and beneath the pia mater (leptomeninx). Due to this growth pattern it is very difficult to accomplish a total resection (12-15, 17, 26, 27, 61, 63, 69, 77, 89, 90, 92) and local recurrence is frequently observed (58, 61).

Tumour cell migration may be facilitated by secretion of hydrolytic enzymes (especially proteinases) which partially degrade the extra-cellular matrix (ECM) and basement membrane (BM). This allows breaching of ECM and BM barriers, infiltration of the leptomeninx, remodelling of vasculature, ECM and BM, and destruction of normal brain tissue (42, 45, 47, 55-58, 62-64, 72,-73, 80, 82, 84, 91, 92).

The expression and secretion of proteolytic enzymes such as collagenases, cathepsins, plasminogen activators, and plasmin have been implicated in tumour invasion and metastasis formation (7, 12, 13, 23, 28, 29, 30, 31, 32, 35, 36, 40).

Cathepsin B (CB) is a lysosomal proteinase which is expressed in all cells. It is a cysteine endoproteinase which is structurally and functionally related to the papain family of proteinases. CB is sythesized as an inactive 43 kDa pro-enzyme which, by removal of a 62 amino acid propeptide, is activated to the single-chain form of 31 kDa or the two-chain

form (25 and 5 kDa subunits). Mature CB is normally localized in the lysosomes where it functions in protein turnover. It has also been shown to be capable of degrading extracellular matrix proteins at acidic and neutral pH (30, 32-33, 43, 44, 49, 52, 56, 65, 68, 74).

Endogenous inhibitors of cysteine proteinases, the cystatins and stefins, may play a major role in regulating their activity. In tumour cells and in cells exposed to mitogens, CB displays altered cellular trafficking resulting in the secretion of the 43 kDa precursor (66-67, 70).

Elevated levels of CB correlate with malignancy suggesting that this enzyme may be a useful prognostic marker for several types of human cancers. Cathepsin B has also been reported to be an important degradative enzyme in invasion and metastatic spreading (44, 84, 86). Intracellular activity and secretion of CB have been described in a number of human non-CNS tumours including malignant and non-malignant breast tumours and adenocarcinomas of the colon (6-9, 34, 35, 39, 66).

The ability of CB to activate other proteinases and to degrade ECM and BM components suggests that it may play a major role in brain tumour cell / ECM interactions (18, 29, 34, 42, 45). Human glioma cells were recently reported to secrete CB (47, 53, 55). However, the presence of cathepsin B in oligodendrogliomas has not been examined yet. In the present study, we demonstrate the expression of cathepsin B enzyme activity in oligodendroglial tumours. It was found that CB is expressed significantly more frequently in anaplastic tumours than in low grade oligodendrogliomas.

2. MATERIALS AND METHODS

Cases and Patients:

All cases were selected from the files of the Institute of Neuropathology, University of Hamburg. 78 oligodendroglial tumour cases were examined (male: 44, female: 34), which were reviewed by two neuropathologists for confirmation of the original diagnoses and tumour grading according to WHO.

The tumours were classified as grade II (n=46), grade III (anaplastic oligodendroglioma, n=18), and IV (glioblastoma with oligodendroglial growth pattern, n=14). The age varied between 17 and 70 years (mean age 47.5 years female, 43.7 **years male).**

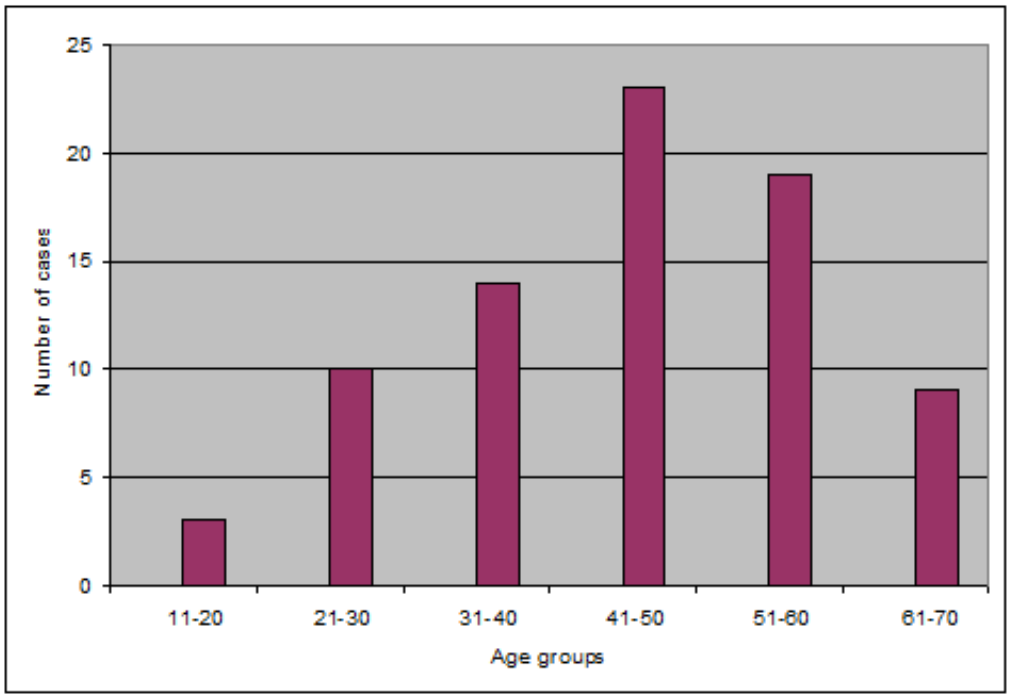

Figure 1: Age of oligodendroglioma patients

Immunohistochemical detection of CB:

Polyclonal rabbit anti-cathepsin B antibody was purchased from Oncogene Research Products (Cat # PC41, dilution 1:50) and applied according to the manufacturer´s specifications for 1 hr at room temperature after inactivation of endogeneous peroxidase by hydrogen peroxide and blocking of unspecific antobody binding by preincubation of the samples with 10% goat serum. Biotinylated secondary antibody (Sigma B7264, dilution 1:60) and Strept-Avidin-Biotin-Complex (Strept-ABC, Dako, HRP Duett Mouse/Rabitt, K 0492, dilution 1:50) were added in sequence. Diaminobenzidine (Sigma D 5637) was used as chromogen.

CB-expression was evaluated qualitatively as being present or absent in the tumour tissue. Negative stains were repeated at least once. A positive and negative control was implemented in every experiment by staining of a previously positive proven breast carcinoma specimen.

Statistical analysis:

Procedures of non-parametric statistical calculations according to Spearman-Rho were used (11, 59, 60). Tumour grading correlated with age (r = 0,327, p = 0,03) and with CB-expression (r = 0,3000, p = 0,017).

3. RESULTS

Immunohistochemical demonstration of CB accumulation:

In grade II oligodendrogliomas, CB was detected in significantly fewer cases (17/46) than in grade III oligodendrogliomas (9/18) and grade IV glioblastomas with oligodendroglial growth pattern (10/14) (p = 0,017) (Fig. 2, 3 and 4).

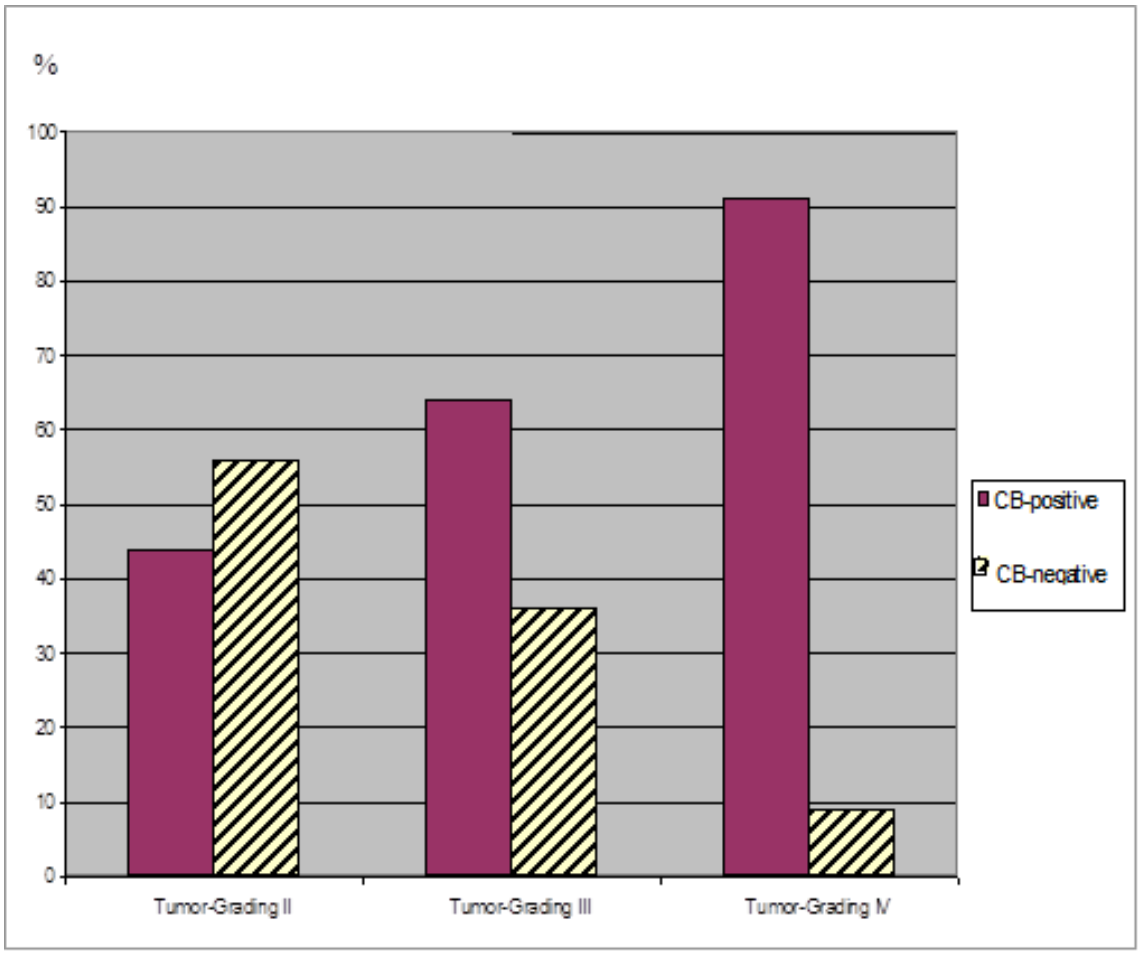

Figure 2: CB-expression in oligodendrogliomas

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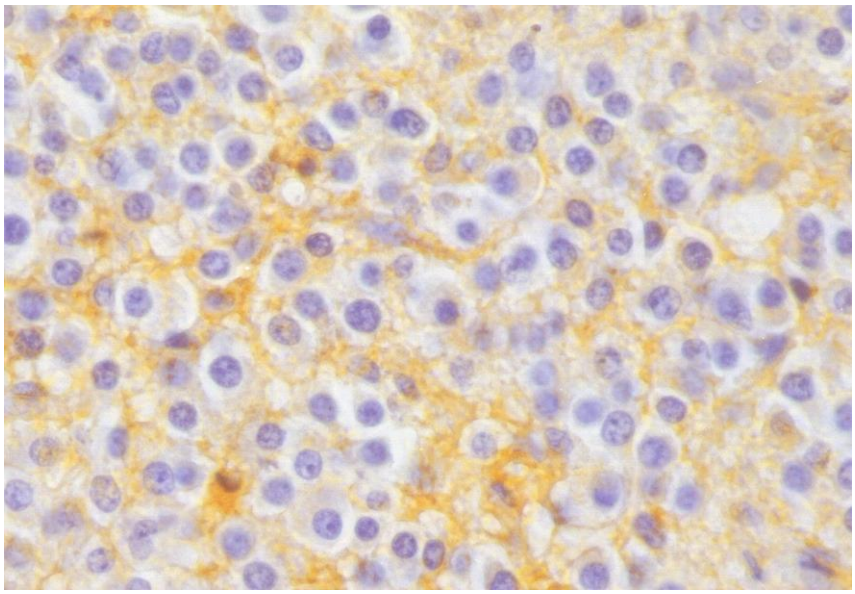

Figure 3: Immunhistochemical staining of CB-expression in a low grade ligodendroglioma. The tumour cells are mostly negative. Labelling of residual brain tissue between tumour cells (x600, counter-stain hemalaum).

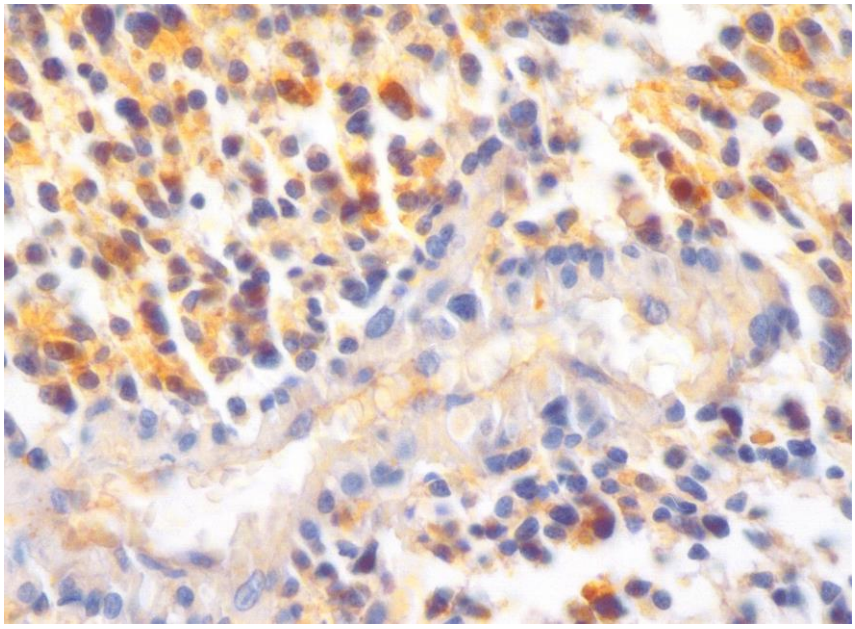

Figure 4: Immunhistochemical staining of CB-expression in an anaplastic oligogodendroglial tumour. Expression of CB in the majority of tumour cells (x 600, counter-stain hemalaum).

Correlation of age and grading:

In addition to correlation of grading and CB-expression, grading also correlated significantly with the age of the patients (r = 0,327, p = 0,03).

Correlation of CB-expression and survival:

Kaplan Meyer survival statistics of 52 cases, in whom catamnestic data was available, revealed a significantly shorter survival of patients with tumours expressing CB (log Rank statistics, p = 0.02, see Fig. 5). The mean survival time of patients with CB-positive oligodendrogliomas was 66 months (n = 31, 9 cases censored), whereas patients with CB-negative tumours had a mean survival time of 109 months (n = 21, 12 cases censored). However, in multivariate Cox regression computed for grading, gender, age and CB-expression only tumour grading was statistically significant (p = 0.002).

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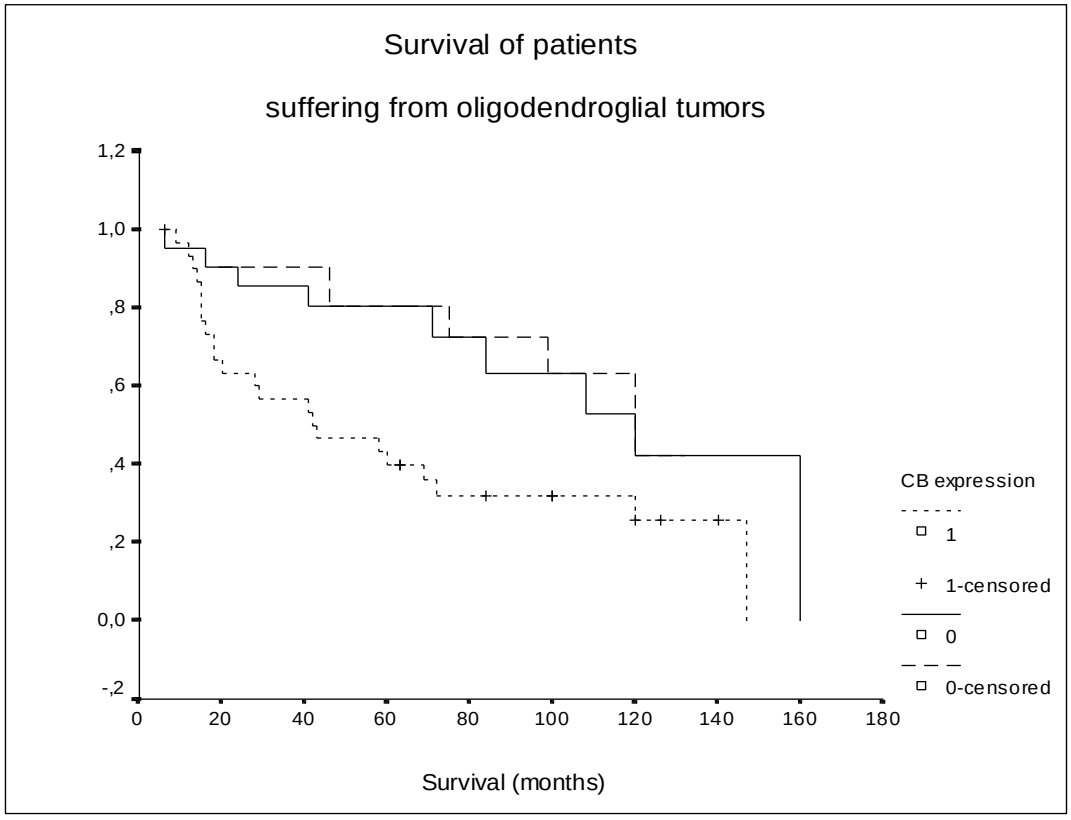

Figure 5: Survival of patients with oligodendrogliomas

4. DISCUSSION

Although gliomas rarely metastasize to distant sites, they show a marked propensity for fast and diffuse local invasion (62). The intrinsic neuronal and glial elements in the CNS are tightly packed as to preclude any significant extracellular space (71). Due to these facts, key elements in invasion of glioma cells include not only the tumour cell´s capacity for motility, but also its ability to manipulate the environment to facilitate cellular migration into the intact CNS (63).

The presence of cathepsin B in normal brain tissues and human brain tumours has been previously reported. The expression of proteases belonging to the cysteine protease superfamily, serine proteases and metalloproteases have been investigated. All proteases are thought to be involved in tumour invasion.

Several reports have indicated differences in the production of plasminogen activators in solid brain tumours and in cell lines derived from these tumours (39, 45, 64, 73). The synthesis of different metalloproteinases and tissue inhibitors of metalloproteinases by cultured fetal astrocytes and glioma cell lines has also been reported. Metalloproteases are capable of degrading fetal rat brain aggregates, and Caroni and Schwab described a metalloprotease activity that facilitates CNS invasion in an in vitro model (3). Sivaparathi et al. (79- 81) demonstrated higly elevated levels of 92 kDa type IV collagenase in glioblastoma samples in vivo.

Cathepsin B is normally localized in lysosomes in the perinuclear regions of cells as observed in the U251MGn cells. However, a redistribution of lysosomes toward the cell periphery appears to be common in cells that participate in degradative or invasive processes. These alterations in cathepsin B subcellular distribution predict an increasing mobilization of cathepsin B throughout glioma progression and suggest that altered trafficking of cathepsin B in glioblastomas contributes to the malignant invasive phenotype. (34-42, 55-62, 82).

In accordance with this assumption are findings that cathepsin B mRNA and protein is expressed in higher amounts in glioblastomas than in normal brain tissues and low-grade gliomas (46, 63, 64).

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5. CONCLUSION

The present study demonstrates that oligodendrogliomas of all grades express cathepsin B and that the CB-expression correlates with the tumour grading.

In cooperation with other enzymes cathepsin B may participates in the proteolysis and decomposition of extracellular matrix and basement membrane components such as laminin, fibronectin and type IV collagen. Furthermore it may be speculated that infiltration of the leptomeninx by oligodendroglial tumours among other factors depends on the expression of cathepsin B.

Concerning the survival statistics of patients with oligodendroglial tumours it could be shown, that patients with tumours expressing CB have a shorter mean survival time, which was 66 months, whereaspatients with CB-nagative tumours had a mean survival time of109 months.

However, in multivariate statistical calculations for grading, gender, age, and CB-expression only tumour grading was statistically significant, so that conventional histological grading of oligodendroglial tumours remains the undisputed standard.

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