Expression of Matrix Metalloproteinases and Their Inhibitors in Medulloblastomas and Their Prognostic Relevance

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ABSTRACT

Purpose and Experimental Design: **The cellular mechanisms leading to metastatic disease in medulloblastoma (MB), the most common malignant brain tumor in childhood, are mainly unknown. Recently, however, the involvement of matrix metalloproteinases (MMPs) has been suggested. We examined the expression and localization of four MMPs—MMP-2 and -9, membrane-type 1 and 2 MMP (MT1- and MT2-MMP)—and correlated the data with those for their main inhibitors, tissue inhibitors of metalloproteinases (TIMP-1, -2, and -3), in 83 classical and 18 desmoplastic MBs.**

Results: **Independent of the histological subtype, MMP-2 expression was found in a small percentage of tumors, whereas MMP-9 and MT1- or MT2-MMP were expressed in >75% of tumor samples. The expression of TIMP-1, -2, and -3, on the other hand, was found to depend on the histological subtype: TIMP-3 was often found in classical MB, whereas TIMP-2 was often expressed in desmoplastic MB ($P = 0.007–0.001$). In addition, both TIMP-3 and -2 correlated significantly with the expression of all studied metalloproteinases except MMP-2. TIMP-1, detected only in classical MB in a low percentage, was the only TIMP that correlated with the expression of MMP-2. Kaplan–Meier estimation revealed significantly reduced long-term survival of patients with strong MMP expression in tumor samples. In multivariate logistic regression analysis, however, the prognosis was significantly determined only by clinical parameters.**

Conclusions: **TIMP-3 and -2 expression is highly correlated with histological subtypes of MBs and strongly associated with the expression of certain MMPs. The expression of TIMPs and MMPs, however, does not determine prognosis independently of clinical parameters.**

INTRODUCTION

Medulloblastoma (MB), the most common malignant brain tumor in childhood, comprises up to 25% of all intracranial neoplasms and is defined as a small blue-cell tumor arising in the cerebellum. Despite recent improvements in the survival of patients with MB, secondary tumor growth is observed in up to 40% of patients, resulting in a poor prognosis. Dissemination to the leptomeninges of the brain or seeding of tumor cells in the neuroaxis are the most common forms of metastasis (1–5). The underlying molecular mechanisms of brain tumor invasiveness are complex and have been studied mainly in malignant glial tumors and are closely related to proteolytic degradation of the extracellular matrix (ECM; Ref. 6). The ECM accounts for up to 20% of the total volume of the central nervous system and is composed of glycoproteins and proteoglycans that are secreted into a network to which cells adhere. This network consists predominantly of the proteins fibronectin, laminin, vitronectin, thrombospondin, tenascin, heparin sulfate proteoglycan, and collagen IV (7). Several proteases are thought to be involved in the degradation of ECM components, including matrix metalloproteinases (MMPs), serine proteinases (urokinase and tissue plasminogen activators), cysteine proteinases (cathepsin B and S), aspartic proteinases (cathepsin D), and glycosidases (8). Among these, MMPs are thought to play a major role in tumor invasion and metastasis (9–14).

The MMPs constitute a multigene family of >25 secreted and cell surface enzymes that process or degrade various pericellular substrates (15). Their targets include other proteinases, proteinase inhibitors, clotting factors, chemotactic molecules, latent growth factors, growth factor-binding proteins, cell surface receptors, cell–cell adhesion molecules, and almost all structural ECM proteins (15). Collagenases; gelatinases; membrane-type MMPs (MT-MMPs); matrilysin; stromelysin-1, -2, and -3; and metalloelastase are members of the MMP family (7). All MMPs contain at least three protein domains: a "pre" domain that is cleaved after it directs synthesis of the MMP to the endoplasmic reticulum; a "pro" domain that maintains enzyme latency until it is removed or inhibited; and a catalytic domain that contains a conserved zinc-binding region. MMPs are often classified according to these structural domains (15).

Under normal conditions, MMPs are tightly regulated at the levels of gene transcription, activation of inactive zymogens, and inhibition by tissue inhibitors of metalloproteinases (TIMPs; Refs. 7, 8, 15–17). TIMPs are a family of secreted glycoproteins that includes at least four members, TIMP-1, -2, -3, and -4. All of the TIMPs form high-affinity, noncovalent, and essentially irreversible complexes with the active forms of MMPs with a 1:1 stoichiometry (7, 8, 15, 16). TIMP-1 and -2

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are soluble inhibitors, whereas TIMP-3 is insoluble and bound to the ECM (15). After proteolytic injury, ECM remodeling normally occurs, accompanied by elevations in TIMPs, probably to interfere with further action by MMPs. An imbalance between production of MMPs and their inhibition by TIMPs can underlie many pathologies, particularly the invasive potential of brain tumors (11, 12, 18–20). Therefore, maintenance of an appropriate balance among these glycoproteins is essential.

Several MMPs have been implicated as key promoters of tumor invasion, metastasis, and angiogenesis in glial brain tumors. Among them, MMP-2 and -9 and MT1-MMP are in the focus of attention because of their link with the invasive behavior of malignant gliomas (21–24). Nevertheless, there is growing evidence for involvement of several other members of the MT-MMP family, including MT2-MMP (25). Expression of MMP-2 and -9 *in vitro* in MB cell lines has already been analyzed by several authors (22, 26–29), whereas *in vivo*, only very small numbers of MBs have been examined. In addition, little is known about the expression patterns of different MT-MMPs in a sufficient number of tumor samples and their relationship with tissue inhibitors, including TIMP-3. Moreover, the authors of several studies even suppose a possible prognostic relevance of the expression of metalloproteinases in MBs, which to date has not been reported.

In the present study, we used immunohistochemical methods to examine the expression of MMP-2, MMP-9, MT1-MMP, and MT2-MMP, which are considered to be particularly important for the malignant behavior of brain tumors, and their correlation with three tissue inhibitors in 83 classical MBs and 18 desmoplastic MBs.

MATERIALS AND METHODS

Tissue Samples. Tumor tissue used in this study included 101 formaldehyde-fixed, paraffin-embedded MB specimens obtained from 101 patients who underwent surgery between 1974 and 2000 at the university hospitals of Göttingen, München, Münster, Hannover, and Frankfurt. Histopathological diagnosis of MB was established by standard light-microscopic evaluation of the sections stained with H&E and confirmed in each sample based on the criteria of the latest WHO brain tumor classification by at least two neuropathologists (30).

The mean age at diagnosis was 12.3 years (age range, 4 months to 55 years). The male/female ratio was 1.8:1, which is in accordance with the literature (31). Clinical data concerning the extent of resection, determined by surgical reports, and postoperative therapy were available for 92 cases. Postoperative therapy before the late 1980s consisted of "empirical" non-trial-based treatment. From then onward, trial-based therapy was adopted, including a combination of craniospinal radiation and chemotherapy according to standard protocols (32, 33). Radiation therapy of MB consisted of doses of 50–55 Gy and 35 Gy to the posterior fossa and craniospinal axis, respectively. Further clinical information on follow-up time and outcome could be obtained retrospectively from the tumor registry, surgical reports, and clinical reports for 89 cases. For 12 patients, the outcome was unknown. Follow-up time ranged from 4 to 173 months with a mean of 54 months. Seventy-two patients could be followed for more than 24 months.

Immunohistochemical Analysis. Formaldehyde-fixed, paraffin-embedded tumor tissues were sectioned and immunostained after deparaffinization and rehydration. We used monoclonal antibodies to MMP-2 (Ab-3; monoclonal mouse; clone 42-5D11; Oncogene), MMP-9 (Ab-3; monoclonal mouse; clone 56-2A4; Oncogene), MT1-MMP (Ab-4; monoclonal mouse; clone: 113-5B7; Oncogene), MT2-MMP (Ab-1; monoclonal mouse; clone 162-22G5; Oncogene), TIMP-1 (Ab-2; monoclonal mouse; clone 147-6D11; Oncogene), TIMP-2 (Ab-2; monoclonal mouse; clone: 67-4H11; Oncogene), TIMP-3 (Ab-1; monoclonal mouse; clone 136-13H4; Oncogene).

Immunohistochemical procedures were carried out with a DAKO Envision Peroxidase System (DAKO Diagnostica, Hamburg, Germany) according to the following protocol:

For MMP-9 and TIMP-3 staining, antigen retrieval was performed in a microwave oven in 10 mM citrate buffer (pH 6.0) at 700 W for 15 min. Endogenous peroxidase activity was then blocked with 0.3% H_2O_2 for 15 min. After incubation for 10 min with 5% BSA in Tris-buffered saline [50 mM Tris-HCl, 150 mM NaCl (pH 7.4)] for blocking of nonspecific binding, sections were incubated with primary antibodies diluted 1:50 in Tris-buffered saline for 2 h in a humidified chamber at room temperature (for TIMP-1 and TIMP-2, overnight at 4°C). Sections were then incubated with peroxidase-labeled polymer for 30 min, followed by prepared diaminobenzidine substrate-chromogen solution. They were then counterstained with hematoxylin and mounted. Between steps, the slides were washed twice in Tris-buffered saline.

For negative controls, the primary antibodies were omitted, and nonimmune serum was used instead. For positive controls, sections of suitable tissues (as indicated in manufacturer protocols) were stained.

The extent and intensity of expression were evaluated semiquantitatively. Tumors were classified into the following four groups: 0, no expression; 1, low expression; 2, moderate expression; and 3, high expression (examples are shown in Fig. 1). For analysis of the prognostic significance of the expression strength of the three MMPs found most often in classical MB, we summed the expression strength score values. A tumor with a sum >3 was taken as a tumor with "high" expression, a tumor with a sum ≤ 3 was classified as having "low" expression.

Statistical Analysis. The results were entered into a database and analyzed with the statistics software packages Systat 5 and Survival 1.00 (both from Systat Inc.) and StatView (SAS Institute, Cary, NC). Overall survival was determined by Kaplan–Meier analysis (34). To examine the significance, we used the χ^2 test or Fisher's exact test for univariate analysis with discrete parameters and the Mantel–Cox test to compare the equality of survival distributions (all time points weighted equally). To relate the risk of death from disease to clinical and immunohistochemical data, we performed multivariate logistic regression analysis on patients with classical MB. The following candidate variables were included: age at diagnosis, sex, therapy, metastatic stage at diagnosis according to Chang *et al.* (35), extent of surgical resection, and MMP-9, MT1-MMP, MT2-MMP, TIMP-1, TIMP-2, and TIMP-3 expression. $P < 0.05$ was considered significant.

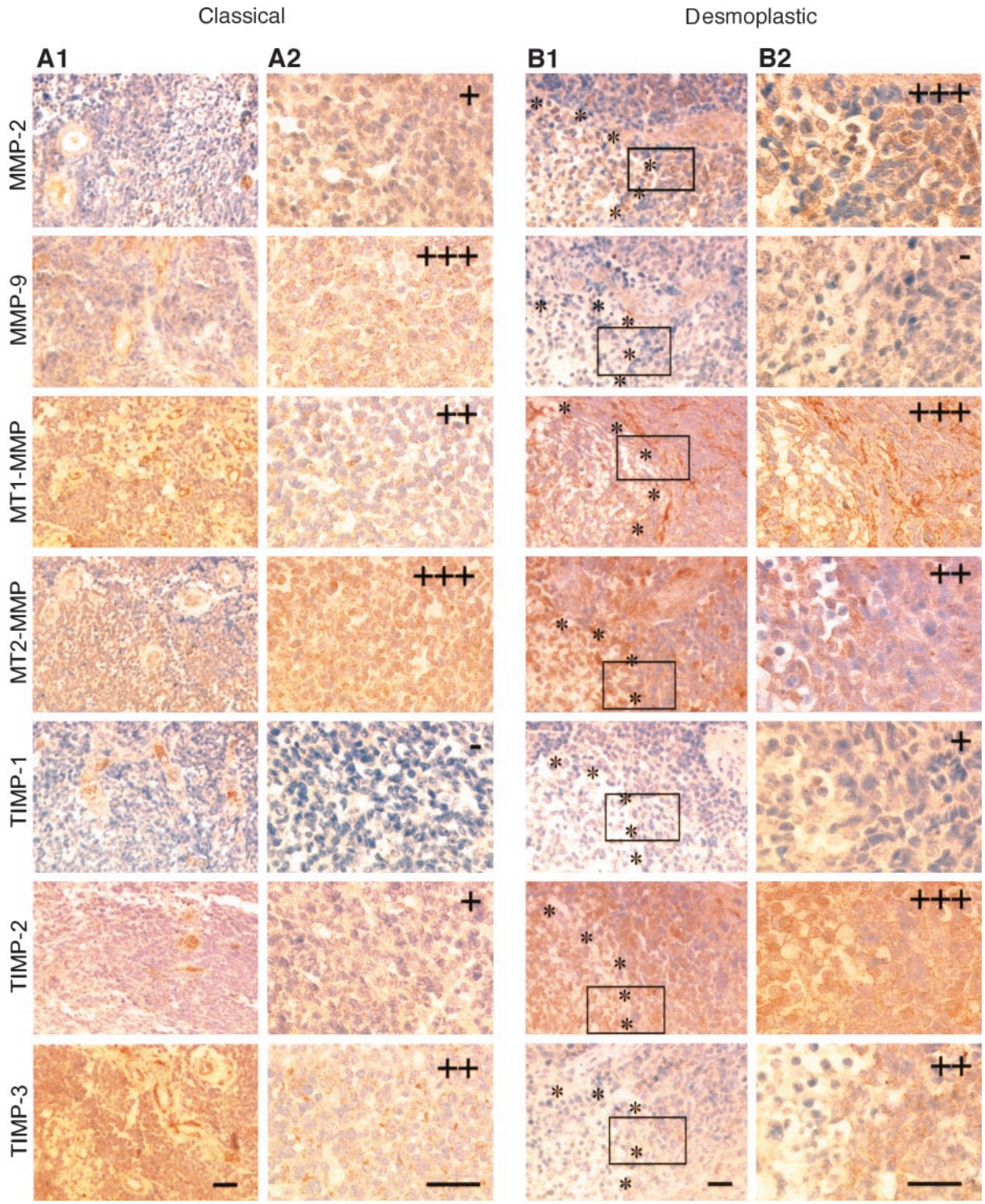

Fig. 1 Expression of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) in classical and desmoplastic medulloblastoma. MMP-2, MMP-9, membrane-type 1 (MT1)-MMP, MT2-MMP, TIMP-1, TIMP-2, and TIMP-3 expression in representative sample of a classical (*A*) and a desmoplastic (*B*) medulloblastoma. *A1* and *B1* show low magnifications, *A2* and *B2* show high magnifications. *A*, whereas endothelial cells of vessels within the tumor tissue express all studied MMPs and TIMPS and could serve as positive controls, the tumor cells express only MMP-9, MT1-MMP, MT2-MMP, and TIMP-3 to a moderate (++) or high extent (+++) and MMP-2 to a low extent (+). *B*, the tumor cells in the cell-rich desmoplastic areas of the desmoplastic medulloblastoma show moderate to high expression of MMP-2, MT1-MMP, MT2-MMP, TIMP-2, and TIMP-3. Note that the tumor cells within the areas of highly differentiated cells ("pale islands," on the *left* on *B1*, indicated by ∗) are negative for TIMP-3. *Boxed areas* in *B1* are shown in *B2*. *Bars*, original magnification, 50 μm.

Table 1 Expression of matrix metalloproteinase (MMP)-2 and -9, membrane-type 1 (MT1)- and type 2 (MT2)-MMP, and their tissue inhibitors (TIMP-1, -2, and -3) in 83 patients with classical medullablastoma and 18 with desmoplastic medulloblastoma

	Total (*n*)	MMP-2, *n* (%)	MMP-9, *n* (%)	MT1-MMP, *n* (%)	MT2-MMP, *n* (%)	TIMP-1, *n* (%)	TIMP-2, *n* (%)	TIMP-3, *n* (%)
All	101	34 (34)	87 (87)	77 (77)	81 (81)	23 (23)	49 (49)	62 (62)
Histology								
Classical	83	31 (37)	62 (75)	62 (75)	63 (76)	23 (28)	34 (41)	56 (67)
Desmoplastic	18	3 (17)	15 (83)	15 (83)	18 (100)	0	15 (83)	6 (33)
P[a]		0.09	NS[b]	NS	0.02	0.008	0.001	0.007

[a] Pearson's χ^2 test was used to assess the significance.
[b] NS, not significant.

RESULTS

Expression of MMPs and TIMP-1, -2, and -3 in MB. We observed a highly significant difference in the expression of MT2-MMP and TIMP-1, -2, and -3 in classical *versus* desmoplastic MB ($P = 0.02$, 0.008, 0.001, and 0.007, respectively; Table 1). We therefore analyzed the expression and the correlation of the different proteins separately in both histological subtypes (Table 2).

Expression of MMP-2 and -9, MT1- and MT2-MMP, and TIMP-1, -2, and -3 in Classical MB. Immunohistochemical analysis revealed that MMP-2 was expressed in only

Table 2 Association of the expression of matrix metalloproteinase (MMP)-2 and -9, membrane-type 1 (MT1)- and -type 2 (MT2)-MMP, and their tissue inhibitors (TIMP-1, -2, and -3) with each other in 83 patients with classical and 18 with desmoplastic medulloblastoma (MB)

	Total (*n*)	MMP-2, *n* (%)	MMP-9, *n* (%)	MT1-MMP, *n* (%)	MT2-MMP, *n* (%)	TIMP-1, *n* (%)	TIMP-2, *n* (%)	TIMP-3, *n* (%)
Classical MB ($n = 83$)								
MMP-2								
+	31		25 (81)	28 (90)	26 (84)	14 (45)	14 (45)	24 (77)
−	52		37 (71)	34 (65)[a]	37 (71)	9 (17)[b]	20 (38)	32 (62)
MMP-9								
+	62			50 (81)	47 (76)	19 (31)	25 (40)	49 (79)
−	21			12 (57)	16 (76)	4 (19)	9 (43)	7 (33)[b]
MT1-MMP								
+	62				50 (81)	21 (34)	29 (47)	47 (76)
−	21				13 (62)	2 (10)	5 (24)	9 (43)[b]
MT2-MMP								
+	63					20 (32)	28 (44)	46 (73)
−	20					3 (15)	6 (30)	10 (50)[a]
TIMP-1								
+	23						12 (52)	20 (87)
−	60						22 (37)	36 (60)
TIMP-2								
+	34							27 (79)
−	49							29 (59)
Desmoplastic MB ($n = 18$)								
MMP-2								
+	3		3 (100)	3 (100)	3 (100)	0	3 (100)	2 (67)
−	15		12 (80)	12 (80)	15 (100)	0	12 (80)	4 (27)
MMP-9								
+	15			13 (87)	15 (100)	0	12 (80)	6 (40)
−	3			2 (67)	3 (100)	0	3 (100)	0
MT1-MMP								
+	15				15 (100)	0	13 (87)	6 (40)
−	3				3 (100)	0	2 (67)	0
MT2-MMP								
+	18					0	15 (83)	6 (33)
−	0					0	0	0
TIMP-1								
+	0						0	0
−	18						15 (83)	6 (0)
TIMP-2								
+	15							6 (40)
−	3							0

[a,b] Pearson's χ^2 test was used to assess the significance of the association of the expression of the different proteins in classical medulloblastoma: [a] $P < 0.05$; [b] $P < 0.005$.

Table 3 Survival significance of clinical variables and matrix metalloproteinase (MMP) and tissue inhibitor of metalloproteinase (TIMP) expression in 83 patients with classical medulloblastoma (cMB) and clinical variables in 18 patients with desmoplastic MB (dMB)

Variable (n = cMB/dMB)	Classical MB, n (%)	Survival (P)[a]	Desmoplastic MB, n (%)
Sex (83/18)			
Female	32 (39)		5 (28)
Male	51 (61)	NS[b]	13 (72)
Age (83/18)			
<3 years	15 (18)		6 (33)
≥3 years	68 (82)	<0.05	12 (67)
M-stage (50/10)[c]			
M0	40 (80)		9 (91)
M1–M3	10 (20)	<0.05	1 (9)
Surgical resection (71/16)[c]			
Gross total	45 (63)		13 (81)
Near or subtotal	26 (37)	NS	3 (19)
Therapy (66/15)[c]			
Radio- or chemotherapy	12 (18)		6 (40)
Radio- and chemotherapy	54 (82)	<0.001	9 (60)
MMP-2 (83/18)			
−	52 (63)		3 (17)
+	31 (37)	NS	15 (83)
MMP-9 (83/18)			
−	21 (25)		3 (17)
+	62 (75)	NS	15 (83)
MT1-MMP (83/18)			
−	21 (25)		3 (17)
+	62 (75)	NS	15 (83)
MT2-MMP (83/18)			
−	20 (24)		0
+	63 (76)	NS	18 (100)
Sum MMP-9 + MT1-MMP + MT2-MMP			
≤3	45 (54)		8 (44)
>3	38 (46)	<0.05	10 (56)
TIMP-1 (83/18)			
−	60 (72)		18 (100)
+	23 (28)	NS	0
TIMP-2 (83/18)			
−	49 (59)		3 (17)
+	34 (41)	NS	15 (83)
TIMP-3 (83/18)			
−	44 (33)		12 (67)
+	56 (67)	NS	6 (33)

[a] Survival analysis (log-rank test) for patients with classical medulloblastoma. Note: This analysis could not be performed on desmoplastic MB because of the low number of cases.
[b] NS, not significant.
[c] These variables were not known for all patients.

31 of 83 MB cases (37%), whereas MMP-9 immunoreactivity was seen in 62 MBs (75%; Table 1). The staining was localized in the cytoplasm of tumor cells for both antibodies (Fig. 1*A*). Activated endothelial cells of blood vessels were occasionally immunostained, and the expression pattern was more striking for MMP-9 than for MMP-2 (Fig. 1*A*). In contrast to MMP-2 expression, which indicated a faint immunoreactivity in the ECM adjacent to blood vessels, MMP-9 expression was limited to tumor and endothelial cells. In 16 cases, neither MMP-2 nor MMP-9 was detected (Table 2). A significant association between MMP-2 and MMP-9 expression was not observed. On the other hand, in 62 cases of MB, tumor cells exhibited positive immunoreactivity for MT1-MMP (75%). MT1-MMP was also immunostained predominantly in tumor cells and endothelial cells (Fig. 1*A*). All tumor specimens expressing high levels of MT1-MMP showed an intense staining pattern distributed throughout the tumor, including the ECM surrounding the tumor cells and blood vessels. Because of the abundant cytoplasmic staining, discriminating between positive membrane immunoreactivity and/or cytoplasmic staining was very difficult.

Marked immunostaining was also seen for MT2-MMP in 63 of 83 cases (76%; Table 1). The staining pattern was similar to that of MT1-MMP (Fig. 1*A*). We observed no significant correlation between all studied metalloproteinases except for a correlation between expression of MMP-2 and MT1-MMP (P = 0.013; Table 2). However, the expression of all metalloproteinases that where found in the majority of MBs (MMP-9 and MT1- and MT2-MMP) was highly significantly correlated with the expression of TIMP-3. TIMP-3 expression was found in 56 cases of MB (67%), and the staining pattern observed with this antibody was similar to that seen for MMP-9, MT1-MMP, and MT2-MMP (Fig. 1*A*). Tumor cells and endothelial cells expressed medium to high levels of TIMP-3. In addition, TIMP-1 and -2 expression was seen in only 23 (28%) and 34 (41%) of

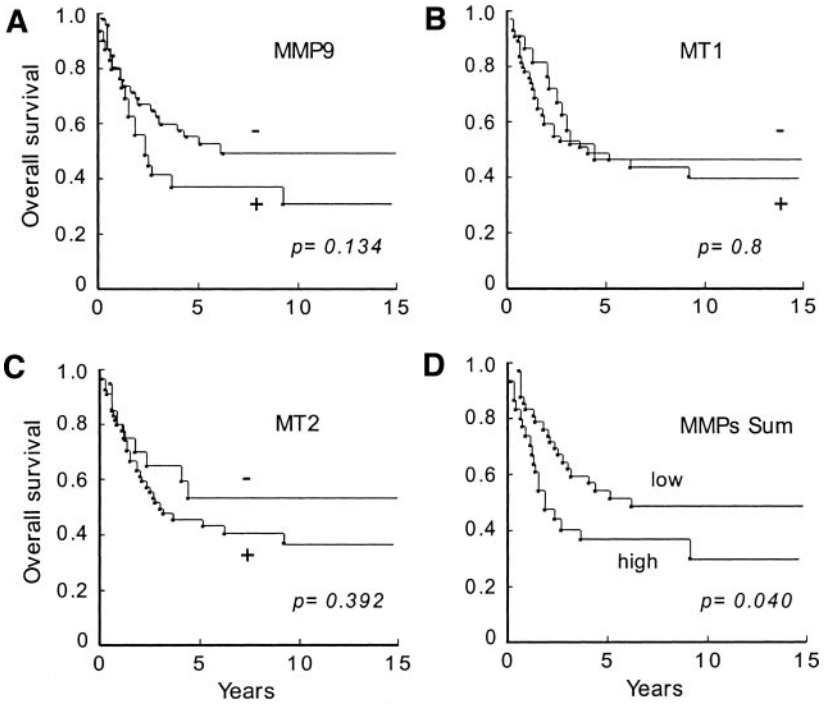

Fig. 2 Prognostic relevance of matrix metalloproteinase-9 (MMP-9), membrane-type 1 (MT1)-MMP, and MT2-MMP expression in classical medulloblastoma tumor specimens. Shown are curves for overall survival of 72 patients with classical medulloblastomas with (+; independent of expression strength) or without (−) expression of MMP-9 (*A*), MT1-MMP (*B*), and MT2-MMP (*C*). *Panel D* shows results of analysis of the sum of MMP-9, MT1-MMP, and MT2-MMP expression. *High* indicates the curve for the group of patients for whom the sum of expression of all three proteins was >3; *low* indicates patients with a lower expression.

the 83 MB specimens, respectively. Furthermore, in most cases, only faint immunoreactivity for TIMP-1 and 2 was detected, which was restricted to the cytoplasm of the tumor cells, in contrast to the intense staining seen in the tumor cells and surrounding ECM for MMPs. On the other hand, endothelial staining was much stronger.

The only significant correlation of TIMP-1 with MMPs was a negative correlation with MMP-2 ($P = 0.0052$). There were no significant correlations between TIMP-2 and all studied MMPs; however, the TIMP expressed most often, TIMP-3, was positively correlated with all studied MMPs with the exception of MMP-2. The strongest correlation was for MMP-9 ($P = 0.0005$), and the lowest was for MT2-MMP ($P = 0.05$; Table 2).

Expression of MMP-2 and -9, MT1- and MT2-MMP, and TIMP-1, -2, and -3 in Desmoplastic MB. Eighteen cases of desmoplastic MB were investigated with the antibodies specific for MMP-2, MMP-9, MT1-MMP, MT2-MMP, and TIMP1, TIMP-2, and TIMP-3 (Table 1; Fig. 1*B*). MMP-2 expression was seen in only 3 of a total of 18 cases (17%), whereas MMP-9 and MT1-MMP-9 were expressed in 15 cases (84%). In addition, all desmoplastic MBs studied expressed MT2-MMP at high levels. Interestingly, cellular and reticulin-free islands showed uniform staining for all MMPs studied. The staining was immunolocalized in the cytoplasm of the tumor cells as well as in activated endothelial cells of blood vessels and the extracellular space. Although TIMP-2 expression was seen in 15 of the 18 desmoplastic MB specimens (84%), only 6 cases were immunostained for TIMP-3 (33%). This expression pattern was found to differ significantly from the expression of TIMP-3 in classical MB (Table 1). Immunoreactivity of TIMP-2 and -3 was localized mainly in the cytoplasm of the tumor cells. Furthermore, a variable degree of immunostaining was detected in the ECM surrounding the tumor cells and blood vessels.

Prognosis of Patients with Classical MB with Regard to Clinical Features at Diagnosis and Immunohistochemical Results. To determine whether MMP or TIMP expression affects the survival of patients with MB, we prepared Kaplan–Meier survival curves and analyzed them statistically (35). Because several clinical parameters are known to affect survival, we also analyzed clinical parameters including sex, age at diagnosis, M-stage, extent of surgical resection, and postoperative therapy. Because the expression of MMPs and TIMPs was significantly different in classical MB compared with desmoplastic MB, we excluded patients with desmoplastic MB from the life-table analysis. The key survival results are summarized in Table 3. Patients receiving a combination of standard chemotherapy and radiotherapy (32, 33) showed a significantly improved survival (log-rank test, $P < 0.001$). The survival was found to be reduced in patients younger than 3 years of age at diagnosis and in patients with evidence of metastatic disease at diagnosis. However, as shown in Table 3, none of the analyzed MMPs or TIMPs showed a significant correlation with survival. Life-table analysis showed that MMP-9 expression was linked to a poor outcome but did not reach a significant level in the log-rank test (Fig. 2*A*). The expression of MT1- and MT2-MMP was not found to have a significant influence on prognosis. When the absolute expression of all three metalloproteinases was pooled, however, patients with strong metalloproteinase expression were found to have a significantly worse outcome than those with low expression (Fig. 2*D*; log-rank test, $P = 0.04$). The biological explanation for the reduced survival in patients with high MMP expression might be related to involve-

ment of the MMPs in tumor cell invasion and cerebrospinal fluid dissemination. Indeed, we observed a significant correlation between the stage of metastatic disease at diagnosis and expression intensity of the three MMPs ($r = 0.22$; $P = 0.03$, Kendall's τ). The correlation was strongest for the expression of MT2-MMP ($r = 0.33$; $P = 0.002$, Kendall's τ). The expression of the three TIMPs, however, was not found to be associated with prognosis or with the M-stage at diagnosis.

To evaluate the association of strong MMP expression within the tumor tissue with clinical parameters that affect prognosis, such as sex, age, therapy, M-stage, and the degree of surgical resection, we performed a multivariate logistic regression analysis. The analysis of 44 patients included in the logistic model identified only therapy ($P = 0.043$) as an independent predictive factor of death from disease.

DISCUSSION

MBs are invasive, malignant embryonic tumors of the cerebellum, mostly occurring in childhood. They are known to exhibit predominantly neuronal differentiation and a tendency to metastasize via cerebrospinal fluid pathways. Although significant advances have been made in the treatment of MBs, the 5-year survival rate of 50% still represents an unfavorable course (1). Because the initial step of intraspinal spread is characterized by both seeding of tumor cells on the arachnoid or pia mater as well as the lysis of collagenous barriers to enhance angiogenesis and local invasion, attention has been focused on the ECM degradation. Together with cysteine and serine proteases, MMPs have been suggested to play an important role in the proteolytic degradation of ECM (7, 8, 15). Within this group, MMP-2 and -9 are thought to have a pivotal role in tumor progression, angiogenesis, and invasion (8, 13, 14, 21, 22, 24, 28, 36, 37).

The present investigation has demonstrated that, among the different MMPs studied, MMP-9, MT1-MMP, and MT2-MMP are often and strongly expressed in classical and desmoplastic MBs. Strong expression of MMP-9, MT1-MMP, and MT2-MMP was found to correlate with prognosis in classical MB. Moreover, the strength of expression of these MMPs was found to correlate with the M-stage at diagnosis. The expression of the different TIMPs that we studied, which are known to inhibit the enzymatic activity of MMPs, was not, however, found to be linked with prognosis or M-stage. Additionally, using multivariate regression analysis, we discovered that the prognosis of patients with classical MB depends primarily on therapy and to a lesser degree on the extent of surgery, but not on expression of the MMPs and TIMPs studied. Obviously, MMP expression does not affect survival independent of clinical factors. Therefore, it is unlikely that expression of certain MMPs such as MMP-9 will be useful in clinical decision-making, as suggested for the expression of c-myc (38, 39), TrkC (40, 41), or ErbB2 (41, 42) in MBs. Moreover, the complex and, in some cases, obviously synergistic action of different MMPs in MBs and their subtypes observed in the present study make it clear that the role of MMP expression for prognosis cannot be estimated by simply analyzing the expression of a few MMPs.

The expression patterns of the MMPs and TIMPs observed in the present study give new insights with regard to the biology and pathogenesis of MBs. Similar to malignant gliomas (24), we observed that MMP-2-positive MBs often showed TIMP-1 expression (45%), whereas it was very uncommon in MMP-2-negative cases ($P = 0.005$). TIMP-2 and -3 were also found to be highly significantly correlated to MMP-9 and MT1- and MT2-MMP in desmoplastic MB and classical MB, respectively. Therefore, the expression of all of the three TIMPs in this study significantly correlated with the expression of different MMPs in MBs, indicating a significant functional relationship of these proteins in the two different histological entities of MB. The combination of certain MMPs found in brain tumors evidently differs substantially from one tumor entity to another, as well as within a single entity (17, 24, 25, 43). Nakada *et al.* (25) examined the expression and tissue localization of MT-MMPs in human malignant brain tumors and showed a strong correlation between the expression of MT1-MMP plus MT2-MMP and the activation of pro-MMP-2. More recently, Choe *et al.* (24) observed a differential association between MMP-9 activation and the different glioblastoma multiforme subtypes, *i.e.*, primary and secondary glioblastoma multiforme.

In conclusion, our study adds to the evidence for a major interplay between different metalloproteinases and TIMPs in MB, as documented previously in human malignant gliomas (17, 25, 43). With regard to our aim of identifying a prognostic marker in MB, the expression of the four MMPs and three TIMPs studied did not appear to be useful. However, the result showing a significant association of the expression strength of three MMPs (MMP-9, MT1-MMP, and MT2-MMP) with survival and M-stage indicates that MMPs may modulate survival of patients with MB in a complex and synergistic manner. Additional studies may help to elucidate whether a subset of patients with MB, particularly patients with metastatic disease at diagnosis, my benefit from the anti-MMP therapy proposed at present for the treatment of malignant glioma (44).

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