Distribution of the extracellular matrix glycoproteins in ependymomas – an immunohistochemical study with follow-up analysis*

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The extracellular matrix (ECM) plays a critical role in influencing the biological behavior of brain tumors and the diagnostic detection of ECM components in ependymomas might be of prognostic value. In the present study we evaluated immunohistochemically the expression of a spectrum of ECM glycoproteins (tenascin, vitronectin, fibronectin, laminin, collagen types II, IV and VI) in a series of 36 pediatric intracranial ependymomas. The distribution of the ECM glycoproteins was evaluated both within the tumor tissue and at the tumor invasion front, and the prognostic value of the results was tested in a survival analysis. The expression of most of the ECM glycoproteins was associated only with blood vessels. Tenascin and vitronectin were found in a more diffuse pattern around the tumor cells and at the tumor invasion fronts of several cases. The progression-free survival was significantly decreased for patients with tenascin positive tumors (in any of the studied compartments) and for the tumors with vitronectin accumulation at their invasion fronts. In one ependymoma containing foci of cartilage with metaplastic ossification we demonstrated that collagen types II and VI and tenascin were present in ECM of both the cartilage and the ependymoma, and were accompanied by areas of necrosis and dystrophic calcifications. We suggest, that the rare simultaneous production of the specific ECM components might lead to the formation of chondroid areas in ependymomas.

An abundant production of some ECM glycoproteins (tenascin and vitronectin) is present in a proportion of ependymomas and its immunohistochemical detection is of prognostic relevance.

Key words: ependymoma, prognosis, extracellular matrix, tenascin, vitronectin, cartilage

The extracellular matrix (ECM) is an important component of the microenvironment of the nervous tissue and it is involved in both normal and pathological tissue remodeling processes such as embryonal development or tissue repair [24]. Because primary brain neoplasms have a marked propensity for an infiltrative growth, the matrix plays also a critical role in influencing their biological behavior. The tumor cell invasion within the CNS is a complex process including tumor cell receptor-ECM interaction, degradation of ECM by proteolytic enzymes and subsequent tumor cell movement [4, 9, 13]. Many ECM components such as fibronectin, tenascin, laminin, vitronectin, hyaluronic acid and collagen of different types were proved to be produced by glioma cells in tissue cultures and they were shown to be preferred substrates for the adhesion and migration of glioma cells (for review see ref. [4, 10, 13, 27]). It has also been suggested that the ECM distribution is modified at the brain/tumor confrontation zone [20, 35]. However, studies concerning the expression and role of the tumoral ECM were concentrated on the most frequent primary brain tumors, astrocytomas and glioblastomas, and mostly in studies on cultivated glioma cells. Considerably less attention has been paid to the characterization of the ECM composition of more slowly but also infiltratively growing tumors – ependymomas, which constitute up to 12% of all intracranial malignancies in childhood [39]. Although detection of individual ECM glycoproteins in tissue sections has been reported in single cases or limited series of ependymomas previously

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[22, 23, 25, 37], the distribution of these ECM components and the prognostic value of their detection have not been studied systematically in a larger ependymomas series so far.

Because the correlation between the histopathologic grade of ependymomas and the clinical outcome of the patients remains poor [2, 7, 30, 31, 40], there is a need to search for specific prognostic markers. A recent demonstration of an aggressive behavior of astrocytomas producing some of the ECM glycoproteins (mainly tenascin) [14, 15, 18, 26] gives us a clue that the ECM may have an impact on the tumor behavior also in ependymomas.

In the present study we evaluated the expression of a spectrum of ECM glycoproteins (tenascin, vitronectin, fibronectin, laminin, collagen types II, IV and VI) by the immunohistochemical method in tissue sections in a series of 36 pediatric intracranial ependymomas. The distribution of the ECM glycoproteins was evaluated both within the tumor tissue and at the tumor invasion front. The ECM expression was correlated with histological grade of the ependymomas and with markers, which were recently demonstrated to mirror the more aggressive properties of ependymomas, MIB-1 labeling index (LI) and expression of the oncoprotein p53 [6, 16, 29, 34, 40]. The prognostic value of immunohistochemical identification of ECM components was tested in univariate and multivariate survival analyses.

Material and methods

Patient characteristics. The tissue blocks of 36 intracranial ependymoma cases included in the study were retrieved from the Pediatric Tumor Registry of the Department of Pathology and Molecular Medicine, Charles University, 2nd Medical School, Prague, Czech Republic. Specimens were obtained only from children (age <18 years). The age of the patients at the time of diagnosis ranged from 1 to 14 years (mean 4.9 years, median 5 years). There were 21 boys and 15 girls. The tumor location was supratentorial in 17 patients and infratentorial in the remaining cases. The histological grade according to the WHO classification criteria [39] has been evaluated independently by three observers. We graded each sample as an anaplastic (grade III) or as a low-grade (grade II) ependymoma, when the consensus of at least two of the observers was achieved.

Progression-free survival (PFS) was defined as the time from the initial surgery to the date of evidence of the tumor progression confirmed radiologically or as the time to the last follow-up appointment of the patients without the tumor progression. The median PFS for the entire cohort of patients was 27.5 months. The up to date follow-up revealed that 15 (41.7%) patients were alive with no evidence of the disease within the period ranging from 73 to 176 months after the operation (mean, 132 months; median, 149 months). Local tumor recurrence developed in 21 (58.3%) cases with PFS time varying from 2 to 58 months (mean, 17.2 months; median 12 months). All of these patients died of tumor with the interval between the surgery and death ranging from 2 to 61 months (mean, 20.6 months; median 15 months).

Morphology and immunohistochemical analysis. All specimens were fixed in 10% neutral buffered formalin and paraffin-embedded. All paraffin blocks containing viable tumor tissue and the tumor borders were chosen for the study. 4 μm thick tissue sections were recut from the paraffin blocks for hematoxylin and eosin staining (HE) and for immunohistochemical studies. Tissue sections were deparaffinized in xylene and rehydrated through decreasing concentrations of ethanol to water. Following cooling for 20 min and blocking of endogenous peroxidase activity, sections were incubated overnight at 4 °C with antibodies directed against glycoproteins of the extracellular matrix (tenascin, vitronectin, laminin, fibronectin and collagen types II, IV, VI), antigen Ki-67 (MIB-1) and oncoprotein p53. In one case containing a cartilage in the tumor (see bellow) immunohistochemical reactions against glial fibrillary acidic protein (GFAP) were also performed. The antigen-antibody complexes were visualized using biotin-streptavidin detection systems (LSAB2 System, HRP, DakoCytomation, cat. no. K0675; ChemMate Detection kit, HRP, DakoCytomation, cat. no. K5001) and 3,3'-diaminobenzidine (DAB, Fluka Chemie). Details concerning the chemicals, dilutions and pretreatment methods used are indicated in Table 1. To avoid background staining, the optimal dilutions of the primary antibodies and tissue section pretreatments were determined prior to the study by test staining using checkerboard titrations on normal brain, kidney and skin. Positive and negative controls were provided with each assay.

The intensity of the immunohistochemical staining using antibodies directed against ECM glycoproteins was evaluated in the studied sections in three separate compartments: a) in association with the blood vessels, b) in the extracellular space surrounding the tumor cells and c) at the uninvolved tissue-tumor confrontation zone (when available). The results were expressed as absent or present accumulation of the ECM glycoprotein. The focal or diffuse distribution of immunopositivities was also noted within the tumor parenchyma. In the brain-tumor confrontation zone, the presence of glycoprotein was evaluated separately on the tumor-side and in the adjacent non-affected brain tissue.

The regions having the greatest number of immunoreactive cells were chosen for counting the MIB-1 labeling index (LI). At least 1000 cells in at least five HPFs (high-power field, x400, area comprising 0.017 mm^2) were counted. The MIB-1 LI was defined as the percentage of immunoreactive nuclei divided by the total number of the tumor cell nuclei in

Table 1. Antibodies and detection kits used for the immunohistochemical study

Antigen (antibody)	Source	Dilution	Pretreatment, detection kit
Tenascin (MM, clone BC-24)	*Sigma Aldrich, Co.*	1:500	MP*
Laminin (MM, clone LAM-89)	*Sigma Aldrich, Co.*	1:500	EP*
Vitronectin (MM, clone BV2)	*Chemicon, Co.*	1:100	MP**
Fibronectin (MM, clone IST-4)	*Sigma Aldrich, Co.*	1:50	EP,MP**
Type II collagen (RP, NCL-COLL-IIp)	*Novocastra, Co.*	1:20	EP*
Type IV collagen (MM, clone CIV 22)	*DakoCytomation*	1:50	MP*
Type VI collagen (MM, clone VI-26)	*Chemicon, Co.*	1:150	MP*
Glial Fibrillary Acidic Protein (MM, clone 6F2)	*DakoCytomation*	1:1000	MP*
Ki-67 (MM, clone MIB-1)	*DakoCytomation*	1:100	MP*
p53 (MM, clone DO-7)	*DakoCytomation*	1:100	MP*

MM – mouse monoclonal antibody, RP – rabbit polyclonal antibody, MP – microwave pretreatment, EP – enzyme predigestion, *LSAB2 System HRP detection kit; **ChemMate Detection kit

the evaluated areas. The results of p53 protein immunoreactions were expressed semiquantitatively (absent or present). To exclude evaluation of equivocal reactions the staining was considered p53 positive if more than 1% of the neoplastic cell nuclei were stained.

Statistical methods. Associations between categorical variables were assessed via the Pearson's chi-square test (χ^2) and associations between categorical and numeric variables were assessed using the Mann-Whitney test. The degree of dependence of nominal variables were assessed via computing the contingency coefficient (C). Classification and regression tree analysis was performed to identify the value of MIB-1 LI (cut-off point) dividing the cohort of patients into subgroups with most significant difference in the clinical outcome.

The PFS was the primary endpoint of the analysis of survival and prognostic relevance of the studied factors. The PFS curves were plotted using the Kaplan-Meier method. Univariate analysis was performed using a log-rank test to assess the strength of association between all subgroups of patients and the outcome. Relative risks (hazard ratios) were computed using univariate and multivariate Cox proportional hazards regression analysis. In the multivariate model, the variables were statistically selected by forward stepwise inclusion. All analytical work was performed using SPSS (version 10, SPSS Inc.) software. Probability (p) values <0.05 were considered significant. A confidence interval (CI) was taken 95%.

Results

All 36 tumors were characterized by perivascular pseudorosettes and presented histopathological features typical of "classic" cellular ependymoma. Based on the recommended histopathological criteria [39] 15 of them were evaluated as low-grade (grade II) and 21 as anaplastic (grade III) ependymomas. The problematic reproducibility of the current grading scheme was illustrated by the lack of interpersonal agreement in more than one third of our cases: in 22 cases (61.1%) the consensus concerning the grade was reached by all three observes, in the remaining 14 cases (38.9%) by two observers only.

In one case of anaplastic ependymoma of the fourth ventricle in one-year-old male, multiple foci of hyaline cartilage with a progressive calcification and with a formation of metaplastic bone in central parts of cartilage nests resembling that observed in enchondral ossification was found (Fig. 1) both in the first specimen of an incompletely removed tumor and at the autopsy 11 months later. The size of the foci ranged from 2 to 9 mm and they had a typical structure of a hyaline cartilage without any cellular atypia, and they were completely encapsulated by a variably thick layer of connective tissue. No other tissues suggesting a diagnosis of teratoma were identified. Specific features of this particular tumor were also areas of necrosis as well as calcifications. Focal deposits of collagen were identified in HE sections in this case and in further two posterior fossa ependymomas in our series.

All ependymomas exhibited nuclear MIB-1 accumulation (median MIB-1 LI, 8.7%). Anaplastic tumors revealed more prominent labeling indices of MIB-1 (medians, 13.2% vs. 1.9%; Mann-Whitney test; p<0.0005). The nuclear positivity of p53 protein was observed in 14 (38.9%) cases and it was significantly prevalent in anaplastic tumors (χ^2; p=0.02).

The presence and localization of ECM glycoproteins was investigated *in situ* by immunoperoxidase method. The ECM glycoproteins were distributed in the ependymomas in the following pattern:

A. ECM glycoproteins associated with blood vessel walls. The expression of laminin, fibronectin and type IV collagen was associated strictly with basement membranes of all vessels supplying both the tumor and the normal brain tissue. Collagen types II and VI were co-expressed in the adventitia of hyperplastic blood vessels of the tumor in 19 cases

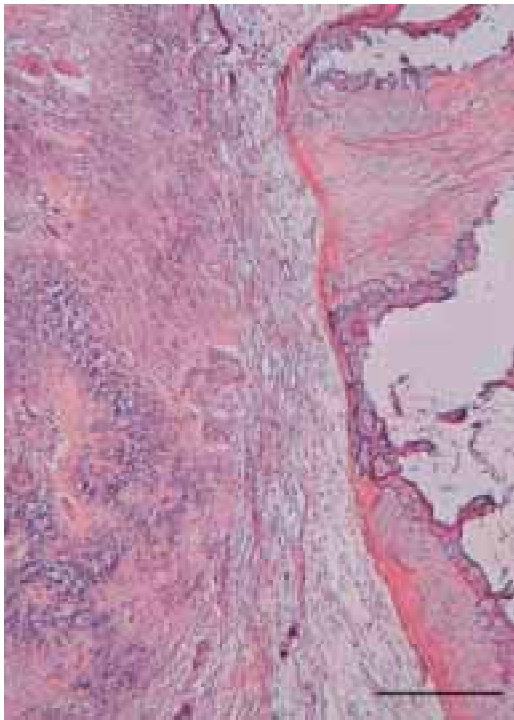

Figure 1. An anaplastic ependymoma of the fourth ventricle containing hyaline cartilage with a formation of metaplastic bone. Hematoxylin-eosin stain; scale bar = 1000 μm.

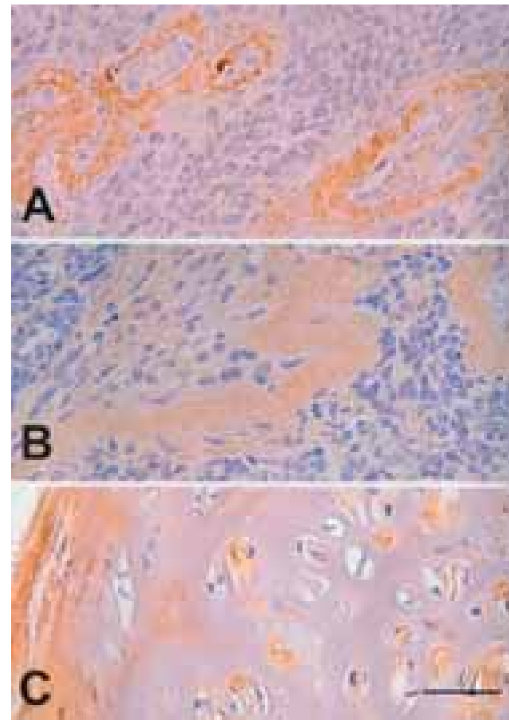

Figure 2. Ependymoma containing a cartilage: immunohistochemical demonstration of type II collagen in the walls of hyperplastic blood vessels (A), in the intersticial collagen bands (B) and in the cartilage (C). Scale bar = 100 μm.

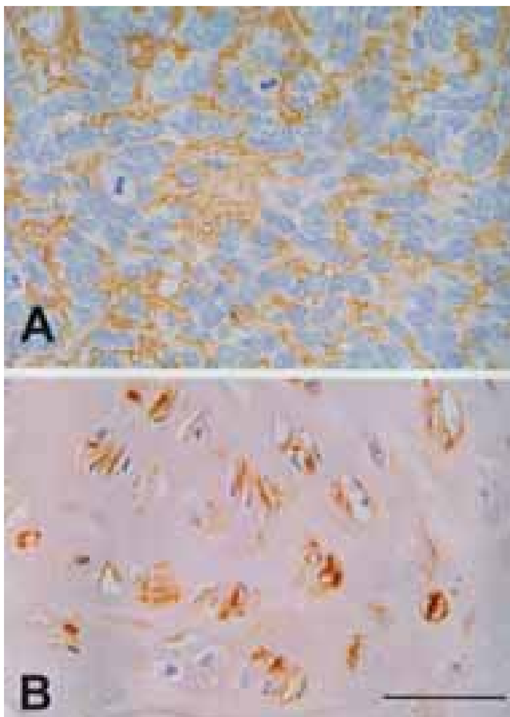

Figure 3. Ependymoma containing a cartilage: immunohistochemical demonstration of tenascin accumulation in both the extracellular space of the tumor (A) and in the cartilage (B). Scale bar = 75 μm.

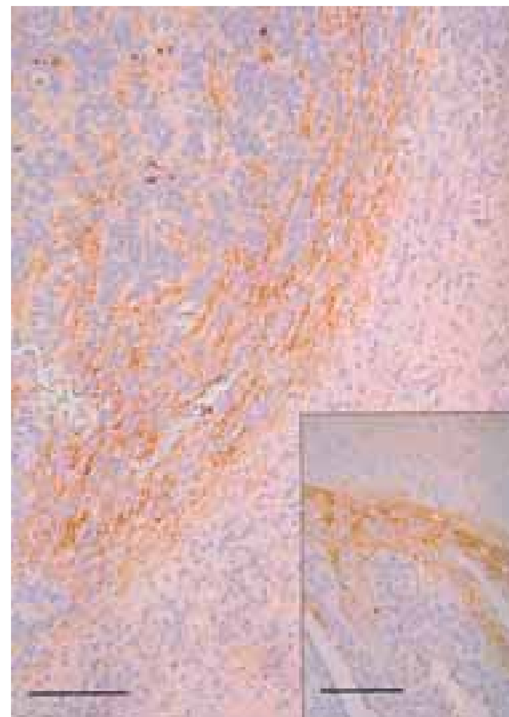

Figure 4. Immunohistochemical demonstration of enhanced vitronectin accumulation at the border zones between the ependymoma and the surrounding unaffected granular layer of cerebellum (scale bar = 100 μm). Inset: Enhanced tenascin accumulation at the tumor invasion front (scale bar = 100 μm).

Table 2. The degree of dependence of the expression of ECM components and factors displaying the aggressive biological behavior of ependymomas. Contingency coefficient (C).

	Grade 3	MIB-1 LI >7%	p53+
ECM associated with vasculature			
Tenascin	C=0.220 NS	C=0.470 p=0.002*	C=0.391 p=0.019*
Collagen types II and VI	C=0.082 NS	C=0.243 NS	C=0.084 NS
ECM in the intercellular spaces			
Tenascin	C=0.220 NS	C=0.470 p=0.002*	C=0.391 p=0.019*
Vitronectin	C=0.265 NS	C=0.142 NS	C=0.001 NS
Collagen types II and VI	C=0.083 NS	C=0.091 NS	C=0.092 NS
ECM at the tumor invasion front			
Tenascin	C=0.315 p=0.049*	C=0.412 p=0.003*	C=0.425 p=0.003*
Vitronectin	C=0.252 NS	C=0.453 p=0.002*	C=0.198 NS

ECM – extracellular matrix, LI – labeling index, NS – not significant, *statistically significant

Table 3. Univariate survival analysis of variables displaying the distribution of the ECM glycoproteins in ependymomas and of histological and immunohistochemical prognostic factors by Cox proportional hazards modeling

	Frequency (%)	PFS Risk ratio(CI)	p
Grade III	21(58.3%)	4.3(1.5–14.5)	0.025*
MIB-1 LI >7%	20(55.6%)	27.9(3.6–216.2)	0.001*
p53 positivity	14 (38.9%)	4.9(2.1–19.3)	0.002*
ECM associated with vasculature			
Collagen types II and VI	19(52.8%)	1.2(0.4–3.7)	NS
Hyaline vessels – collagen II	5(13.9%)	1.4(0.3–6.2)	NS
Tenascin	18(50%)	7.8(2.9–19.8)	0.002*
ECM in intercellular space			
Tenascin	18(50%)	7.8(2.9–19.8)	0.002*
Vitronectin	8(22.2%)	1.2(0.4–5.4)	NS
Collagen types II and VI	3(8.3%)	0.6(0.1–6.32)	NS
ECM in tumor invasion front			
Tenascin	13(43.3%)	5.0(1.5–16.7)	0.008*
Vitronectin	11(36.7%)	5.3(1.6–17.2)	0.005*

ECM – extracellular matrix, PFS – progression-free survival, CI – confidence interval, LI – labeling index, NS – not significant, *statistically significant

(52.8%). Collagen type II was also present in the wall of several hyalinized blood vessels of the tumors in 5 cases (13.9%). The walls of blood vessels in 18 ependymomas (50%) were diffusely positive for tenascin. No deposits of vitronectin were observed in association with blood vessels.

B. ECM glycoproteins of the tumor parenchyma. We did not identify any accumulation of laminin, fibronectin or type IV collagen in the intercellular space outside the blood vessel walls. Tenascin and vitronectin were the only ECM glycoproteins that were found in a more diffuse pattern in spaces around the tumor cells. In all 18 tenascin-positive cases (50%), the accumulation of the glycoprotein was observed also in the wall of tumor-supplying blood vessels. In six cases tenascin was observed only focally in the intercellular spaces, the remaining twelve tumors showed mostly diffuse fibrillary immunopositivities. Vitronectin was detected in extracellular spaces in 8 cases (22.2%) being only focally distributed within the tumor parenchyma. In 5 cases vitronectin was observed at the same time with tenascin deposits. Collagen types II and VI were immunohistochemically identified to be components of collagen fibrils or bands observed in HE sections in the intersticium of the three cases (8.3%) mentioned above. The hyperplastic blood vessels of all these tumors were also collagen types II and VI positive, but the extracellular deposits of collagens were obviously independent on the vasculature. In the ependymoma containing the cartilage, the collagen deposits were observed simultaneously with a massive tenascin accumulation in both the intercellular compartment of the tumor and in the walls of the blood vessels. Interestingly, the extracellular matrix of the cartilage in that case contained also collagen types II and VI and tenascin deposits (Fig. 2, 3). The fibrous tissue surrounding the cartilage nodules was weakly positive in the immunohistochemical reaction against these ECM glycoproteins. Although the anti-GFAP immunohistochemical reaction stained the cell processes of perivascular pseudorosettes of this ependymoma, the cells of the cartilage were GFAP negative.

C. ECM glycoproteins in the tumor invasion front. Of the 36 cases, border zones between the tumor and the surrounding unaffected tissue were present in 30 cases (83.3%). No obvious enhanced accumulation of collagen types II, IV and VI, laminin and fibronectin was noted at that site. Tenascin was found to be present in an increased amount at the front of the tumor in 13 (43.3%) cases, vitronectin in 11 (36.7%) cases. The co-expression of tenascin and vitronectin at the brain/tumor zone was observed in 4 cases (13.3%). All these ECM glycoproteins were present only at the tumor side of the junction with tumor mass (Fig. 4).

Correlative and survival analyses. The relationships between the presence of the ECM components and factors displaying the more aggressive biological behavior of the tumors (histological grade 3, increased MIB-1 LI and p53 protein positivity) were statistically tested. Because there was no statistically significant difference in survival between the subgroups of focally and diffusely tenascin-positive tumors (assessed via log rank test), the tumors entered the

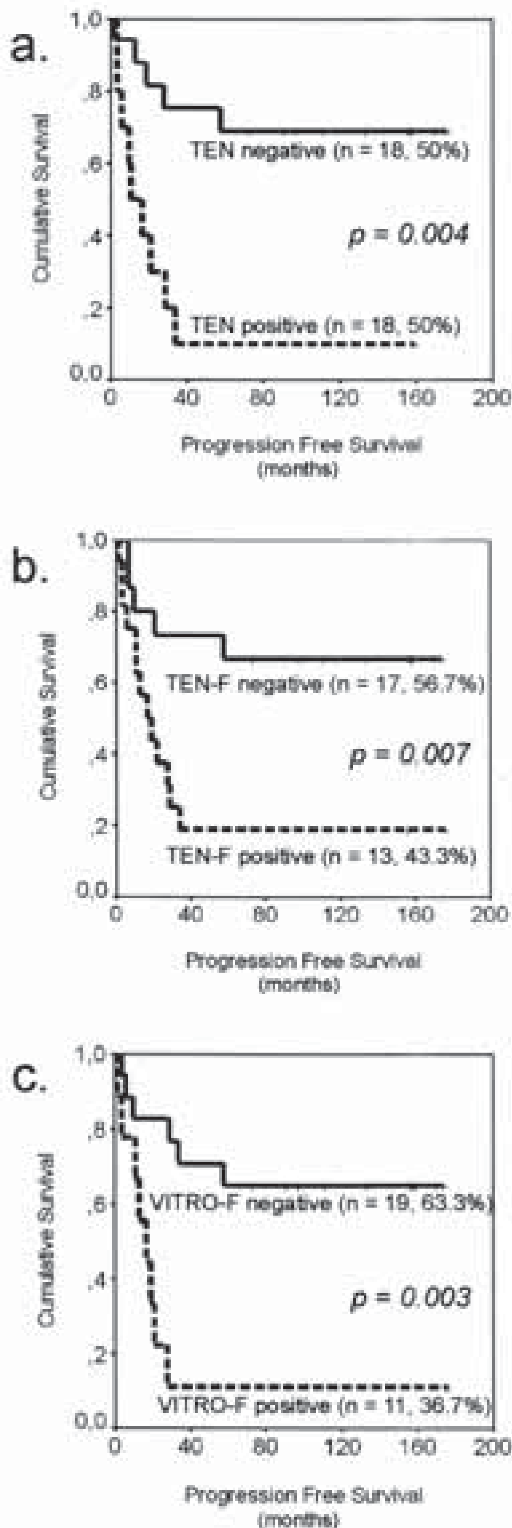

Figure 5. Graphs showing representative Kaplan-Meier survival curves of the patients with ependymomas grouped according to the accumulation of a) tenascin in the extracellular space (TEN); b) tenascin at the tumor invasion front (TEN-F); c) vitronectin at the tumor invasion front (VITRO-F). P values of log-rank testing.

statistical analyses as one subgroup. The MIB-1 LI of 7% was designed as a cut-off point. The results of the analysis are summarized in Table 2. The presence of tenascin in the extracellular space, in association with blood vessels and in the tumor invasion front of ependymomas was significantly associated with both the p53 protein positivity and MIB-1 LI >7%. The presence of tenascin in the brain/tumor confrontation zone was further statistically associated with grade 3 ependymomas, however, only at a low level of significance. The presence of vitronectin in the tumor invasion front was associated with MIB-1 LI >7%. No statistically significant associations were observed among all other possible pairs of tested variables.

The results of univariate survival analysis (Cox proportional hazard risk modeling) are given in Table 3. From all studied variables displaying the ECM glycoproteins distribution, the PFS was significantly decreased for the subgroup of patients with tenascin positive tumors (in any of the studied compartments) and for the tumors with vitronectin accumulation at their invasion fronts. The relevant survival curves plotted via Kaplan Meier method and assessed via the log-rank testing are given in Figure 5.

The multivariate analysis of the entire cohort revealed that decrease of PFS was significantly associated with MIB-1 LI >7% and with presence of tenascin in intercellular spaces and blood vessel walls. Risk ratios (CI) reached 10.3 (2.5–62.1) for MIB-1 LI >7% (p=0.002) and 3.8 (1.6–7.4) for the detection of tenascin (p=0.012).

Discussion

The ECM of glial tumors modulates a variety of cell functions, such as cell attachment, migration and proliferation [10]. Changes in glioma ECM composition have been intensively studied in tissue cultures of glioma cell lines. Although the capacity of cultured glioma cells to produce laminin, fibronectin and type IV collagen into the extracellular space was shown (reviewed in ref. [4, 10, 13, 27]), they were associated strictly with vasculature of both non-affected tissue and ependymomas in our *in situ* study, which is in accordance with some previous observations [1, 23, 37]. Because an extensive clonal selection and transdifferentiation to mesenchymal features with an extensive ECM production arise in the cultures [28], the results obtained by the tissue culture experiments do not necessarily reflect the situation *in vivo*. Fibronectin deposition in extravascular ECM, which was reported by OHNISHI et al [25] in a single case of ependymoma, was not detected in any of our cases. In contrast to HIGUCHI et al [15] and OZ et al [26], who found a significant decrease in fibronectin and laminin accumulation in the tumor blood vessels with increasing tumor grade of astrocytomas, the two glycoproteins were present in our series in vascular basement membranes in

all studied ependymomas irrespective of their biological behavior. Moreover, MAHESPARAN et al [20] showed recently in an experimental model that the production of laminin, fibronectin and type IV collagen is more likely derived from preexisting blood vessels than from the tumor. The detection of laminin in vascular basement membranes in all of our cases contradicts the finding of a complete absence of that ECM glycoprotein in ependymomas reported by FURNESS et al [8] and raises doubt on their recommendation of laminin-immunohistochemistry for distinguishing between ependymomas and choroid plexus neoplasms.

The association of type VI collagen with ependymoma's vasculature was reported previously in one case by McCOMB et al [23] and it is now confirmed by the results in our series. We have shown that this type of collagen is colocalized with deposits of type II collagen, presence of which has not been investigated in brain tumors so far. Rare cases of abundant interstitial production of collagen were reported previously [33, 36], particularly in spinal ependymomas. In three of our intracranial cases the interstitial bands of dense collagen were also observed and we demonstrated that both types II and VI collagens represent their structural components.

From the spectrum of investigated ECM glycoproteins, tenascin and vitronectin were observed to be accumulated in a more diffuse pattern in the extracellular space of 18 (50%) and 8 (22.2%) cases, respectively, and based on spatial relationships of their distribution, they seem to be produced by the neoplastic ependymal cells. A correlation between tenascin production and the malignancy or angiogenesis was clearly demonstrated in astrocytomas [14, 15, 18, 26]. To our best knowledge, it were only KORSHUNOV et al [19] who explored the expression of ECM (namely of tenascin) in a larger series of patients with ependymomas. They showed intercellular tenascin immunoreactivity in 52% of cases, which is in accordance with our data. In a good correlation with that study, tenascin-positive cases were more prevalent in anaplastic ependymomas in our series, and we demonstrated the usefulness of tenascin detection for prognostic evaluation of ependymomas. Moreover, we identified the accumulation of tenascin at tumor infiltration fronts in some cases and we showed, that the detection of tenascin-immunoreactive tumor borders was also strongly associated with reduced PFS in the survival analysis. We demonstrated a similar phenomenon also in detection of vitronectin at the brain/tumor confrontation zone. Although vitronectin deposits were detected in the extracellular space of some of the most malignant astrocytic tumors [11, 12], there was no evidence of correlation between the accumulation of this ECM glycoprotein within the parenchyma of ependymomas and the reduction of PFS in our series.

The presence of cartilage in neuroepithelial brain tumors, which was also a feature of one case in our series, was rarely reported in the literature and there are many controversies concerning its origin. KEPES et al [17] observed a smooth transition from glial to chondroid regions of three astrocytomas and one mixed ependymoma-astrocytoma with a distinct GFAP positivity of both the tumor cells and the chondrocyte-like cells of the cartilage, interpreting the cartilaginous islands as having their origin from neoplastic astrocytes. Similarly to our case, such a transition was not observed in several reported forth-ventricle ependymomas with inclusions of cartilage nodules [21, 32]. The cartilage was completely encapsulated by a fibrous tissue resembling the perichondrium, the cells of the cartilage had cytology features of typical chondrocytes and they were GFAP-negative. The origin of the cartilage was ascribed in these cases more likely to a metaplastic change of mesenchymal elements, as was once illustrated in the adventitia of a tumor blood vessel [17]. However, based on our immunohistochemical analysis of ECM distribution we can speculate, that the mesenchymal cells share the origin of cartilage with the tumor cells capable of manufacturing the ECM components. We have demonstrated that the type II collagen, which represents one of the major structural components of a cartilage [5, 38], was accumulated in the cartilage containing ependymoma in the wall of the tumor blood vessels, in their adventitia as well as in the extracellular space independently from the mesenchymal structures. A similar observation was made in case of the other ECM glycoproteins, tenascin and type VI collagen, which were also reported to be structural components of human cartilage [3]. The abundant ECM production was in this particular ependymoma accompanied by areas of necroses and dystrophic calcifications. This is in contrast to the other ECM glycoproteins producing tumors in our series, in which the chondroid metaplasia did not develop. In our opinion, a rare temporal-spatial coexistence of the specific conditions might lead to the formation of chondroid areas even with metaplastic ossification.

In conclusion, the distribution of major ECM glycoproteins in tissue sections of ependymomas was described in the present study. In contrast to tissue culture studies of glioma cell lines, most of the studied ECM glycoproteins were associated strictly with the vasculature of the ependymomas. However, we have demonstrated that an abundant production of some glycoproteins occurs in a proportion of cases and their detection within the tumor parenchyma (tenascin) or at the invasive fronts of the tumor (tenascin and vitronectin) are of prognostic relevance. Furthermore, a simultaneous abundant production of certain ECM components by both the neoplastic and mesenchymal cells may lead to the formation of cartilage, even with a metaplastic ossification.

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