

Expression of Invasion-Related Extracellular Matrix Molecules in Human Glioblastoma Versus Intracerebral Lung Adenocarcinoma Metastasis

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Key words

- glioblastoma
- metastasis
- lung cancer
- tumor invasion
- extracellular matrix

Abstract

Tumor cell invasion into the surrounding brain tissue is mainly responsible for the failure of radical surgical resection, with tumor recurrence in the form of microdisseminated disease. Extracellular matrix (ECM)-related molecules and their receptors predominantly participate in the invasion process, including cell adhesion to the surrounding microenvironment and cell migration. The extent of infiltration of the healthy brain by malignant tumors strongly depends on the tumor cell type. Malignant gliomas show much more intensive peritumoral invasion than do metastatic tumors. In this study, the mRNA expression of 30 invasion-related molecules (twenty-one ECM components, two related receptors, and seven ECM-related enzymes) was investigated by quantitative reverse transcriptase-polymer-

ase chain reaction. Fresh frozen human tissue samples from glioblastoma (GBM), intracerebral lung adenocarcinoma metastasis, and normal brain were evaluated. Significant differences were established for 24 of the 30 molecules. To confirm our results at the protein level, immunohistochemical analysis of seven molecules was performed (agrin, neurocan, syndecan, versican, matrix metalloproteinase 2 [MMP-2], MMP-9, and hyaluronan). Determining the differences in the levels of invasion-related molecules for tumors of different origins can help to identify the exact molecular mechanisms that facilitate peritumoral infiltration by glioblastoma cells. These results should allow the selection of target molecules for potential chemotherapeutic agents directed against highly invasive malignant gliomas.

Bibliography

DOI <http://dx.doi.org/10.1055/s-0030-1249698>
Published ahead of print:
15 April 2010
Cen Eur Neurosurg 2010;
71: 173–180
© Georg Thieme Verlag KG
Stuttgart · New York
ISSN 1868-4904

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Introduction

During malignant transformation, invasiveness is determined by complex functions of tumor cells of distinct histological types. A three-step model of invasion has been applied to a variety of malignant cell types. Tumor cells at the invasive site 1) detach from the growing primary tumor mass; 2) adhere to the extracellular matrix (ECM) via specific receptors; and 3) locally degrade the ECM components, forming a pathway for neoplastic cells to migrate into the surrounding tissue [51]. Malignant gliomas are the most common primary brain tumors. They are associated with the shortest survival time because of their infiltrative invasion of the normal brain, which makes complete removal of these tumors practically impossible. Invasive primary brain tumors are almost invariably fatal, recurring close to the resection margin in almost all cases. Primary brain tumors have a strong tendency to invade

and, with rare exceptions, do not metastasize outside the brain [5,24,26]. In contrast, carcinomas, which often metastasize to the brain, are more significantly and sharply delineated from the surrounding brain tissue, infiltrate only short distances, and invade as groups of cells rather than as single cells [4]. Consequently, successful total surgical resection of metastatic tumors of the brain can be achieved in many cases, whereas this is usually not possible for primary invasive brain tumors.

The ECM constitutes a considerable proportion of normal brain volume. There is much evidence that ECM components can modulate brain tumor growth, proliferation, and invasion by many different mechanisms [6]. The ECM is composed mainly of hyaluronan (HA), proteoglycans (PGs), and tenascin [12]. To allow cell adhesion and migration, the ECM components interact with specific receptors on the cell membrane, such as integrins, CD44, or CD168. Some proteases and

synthases also strongly influence invasiveness because of their capacity to alter the actual levels of the ECM molecules or to degrade the pericellular network [4].

To understand the evident differences between glioblastoma (GBM) and intracerebral metastasis, we compared the mRNA expression of 30 invasion-related molecules in GBM and intracerebral bronchial adenocarcinoma metastases. To analyze their relationship to the environment, the same expression patterns were also determined in normal brain tissue. For morphological information and to check whether or not differences in mRNA expression appear on a post-translational level, immunohistochemical analyses were performed for seven molecules (agrin, neurocan, syndecan, versican, matrix metalloproteinase 2 [MMP-2], MMP-9, and HA).

Material and Methods

Tissue samples

Thirty tissue samples were collected during neurosurgical operations. The samples were frozen promptly after removal on the surface of liquid nitrogen and stored at -80°C until processing. Each sample was collected from a different patient. Eleven pieces of infiltrated margins of GBMs, ten pieces of intracerebral lung adenocarcinoma metastases, and nine normal brain tissue samples taken from epilepsy surgery were carefully selected by an experienced neuropathologist for further investigation. Sections for histological analysis and immunohistochemistry were cut from the same samples used for the mRNA assays. The fresh frozen tissue samples were provided by the Neurosurgical Tissue and Tumor Bank of the Neurosurgical Department of our university. All procedures were approved by the Ethical Committee and every patient signed an informed consent form.

RNA analysis

The mRNA expression of 30 ECM-related molecules was determined by real-time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR): 21 PGs, three different MMPs, three hyaluronan synthases (HASs), chondroitinase ABC, and the two cell membrane receptors CD44 and CD168. Tumor markers (glial fibrillary acidic protein [GFAP], carcinoembryonic antigen [CEA], and cytokeratins 18 and 19) and the proliferation marker Ki-67, were also tested.

Fresh-frozen tissue samples were first pulverized with a manual CryoPress device (Microtec Co., Japan) precooled in liquid N_2 . The powdered still-frozen tissue was then scraped into the appropriate volume of TriReagent (Invitrogen, USA) and homogenized instantly with a rotor-stator homogenizer. Total RNA was isolated from the TriReagent lysates according to the manufacturer's instructions. RNA purity and quantity were assessed on a NanoDrop[®] ND-1000 spectrophotometer (NanoDrop Technologies, USA) and then stored at -80°C . RNA quality was checked on 1.2% agarose gel stained with ethidium bromide. Total RNA was converted to single-stranded cDNA with the High-Capacity cDNA Archive Kit with RNasin (Applied Biosystems, USA) using 600 ng of total RNA per sample in one reverse transcription reaction. The cDNA transcribed from 100 ng of total RNA was loaded per port of the microfluidic card.

TaqMan Low Density Array (TLDA) experiments were performed using the Applied Biosystems 7900HT real-time PCR system with the Micro Fluidic Card upgrade (Applied Biosystems, USA). The Micro Fluidic Card format allowed the analysis of 40 genes

per sample, and each sample was analyzed in duplicate. The Micro Fluidic Cards were analyzed with the SDS 2.1 software as relative quantification studies (maximum 10 cards per study) with automatic threshold settings, and the C_T values were exported for further analysis. Each 40-gene set contained three different assays for housekeeping genes: β -actin (ACTB), β_2 -microglobulin ($\beta_2\text{M}$), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). ACTB and GAPDH showed the least variation among the samples and were used as the reference genes to calculate the dC_t value for each gene.

Expression values were calculated using the comparative C_T method, as described previously [23]. Briefly, assuming that the PCR efficiency for any gene on the TLDA is close to 1, mRNA expression [15] for a given gene in the tumor or normal (calibrator) sample can be compared using the simplified equations [17]:

$$X_{\text{tumor}} = 2^{-dC_{T\text{tumor}}} \text{ and } X_{\text{normal}} = 2^{-dC_{T\text{normal}}}$$

2^{-dC_T} values are the input expression values during further analysis with GeneSpring 7.3 software (Silicon Genetics, Redwood City, CA, USA). Instead of calculating the expression values (2^{-dC_T}) relative to those of the normal samples, this allowed inspection of individual mRNA expression (X) differences within sample categories. To identify genes with significantly different expression levels between different sample groups Mann-Whitney U -test was performed. Significance was established at $p < 0.05$.

Immunohistochemistry

After evaluating the results of the mRNA determination seven molecules were selected and investigated at the protein level with immunohistochemistry: agrin, neurocan, syndecan, versican, MMP-2, -9, and hyaluronan.

Frozen and stored (-80°C) samples were fixed in Saint Marie's fixative [37,44] for 24 h at 4°C . After fixation and dehydration, the tissue samples were embedded in wax, and $5\mu\text{m}$ sections were cut. The sections were stained with hematoxylin-eosin and immunohistochemical reactions were carried out according to the following protocol. Slides were preincubated in ready-to-use (2.5%) normal horse serum (Vector, Burlingame, CA, USA) for 30 min at 37°C to prevent nonspecific binding of the primary antibodies. The sections were then incubated overnight at 4°C with the appropriately diluted antibodies. To promote the morphological evaluation a supplementary section for immunohistochemical and routine hemalaun staining was also performed. The immunohistochemical and HA reactions were visualized with the avidin-biotin-peroxidase complex, and the peroxidase was detected with a solution containing the H_2O_2 substrate and the diaminobenzidine (DAB) chromogen (ImmPress Reagent Kit, Vector; Peroxidase Substrate DAB Kit, Vector). Finally, the nuclei were labeled with hemalaun staining, and the sections were mounted in DePeX (BDH Laboratory Supplies, Poole, UK). To control for the specificity of the immunohistochemical reactions, sections were treated with the same protocol, excluding the primary antibodies, which were replaced with nonimmune IgG (Sigma, St Louis, MO, USA) solution at the optimal dilution for the different primary antibodies. HA was detected with a biotinylated HA-binding complex probe (bHABC), which contains the hyaluronan-binding G1 domain of the cartilage PG, aggrecan, and link protein, which have high affinity and specificity for HA [42]. The bHABC probe was kindly provided by

R. Tammi and M. Tammi (Institute for Biomedicine, Department of Anatomy, University of Kuopio, Kuopio, Finland). The probe was used at a concentration of 5 µg/mL, and the reaction was performed as described previously [9].

The reactions were evaluated by three different investigators experienced in histology on a score of 1–5 in three different locations: intracellular, cell-membrane, and extracellular regions, which were evaluated separately for morphological information. The scores of the regions were then summed for general comparison.

Results

Thirty tissue samples were investigated from three different homogeneous groups of tissues: eleven GBM samples originating from the infiltrated margins of the tumor, ten intracerebral lung adenocarcinoma metastases, and nine normal brain tissues. The results of the statistical analysis for differences in mRNA expression levels of 34 molecules between the investigated sample groups are summarized in **Table 1**.

mRNA expression level

The mRNA expression of the tumor markers and the Ki67 proliferation marker confirmed the histological diagnosis. The

mRNA expression of the investigated molecules is illustrated in **Fig. 1**.

GBM versus normal brain

The expression of mRNAs for agrin, fibronectin, laminin α -4, β -1 and β -2, perlecan, syndecan-1, tenascin-C, CD44, CD168, HAS-2, MMP-2 and -9 was significantly elevated in GBM relative to that in the normal brain, whereas the mRNA expression of syndecan-4, tenascin-R and HAS-1 was statistically lower in the GBM. The mRNA levels of aggrecan, brevican, laminin α -1, -2, laminin β -1, laminin γ -1, matrilin-1 and -2, neurocan, neuroglycan, syndecan-2, versican, chondroitinases, HAS-3, and MMP-8 did not differ significantly between normal brain and GBM.

Intracerebral lung adenocarcinoma metastases versus normal brain

Significantly reduced mRNA expression of brevican, laminin α -1, matrilin-2, neurocan, neuroglycan-C, syndecan-2, tenascin-R, and HAS-1 was detected in lung adenocarcinoma metastases compared with that in normal brain. Conversely, the expression of agrin, fibronectin, laminin β -1, β -2 and γ -1, perlecan, syndecan-1 and -4, CD168 and MMP-9 mRNAs was statistically elevated in the metastatic tumor relative to that in normal brain. There was no apparent difference in the expression of aggrecan, laminin α -2, -4, matrilin-1, tenascin-C, versican, CD44, chon-

Table 1 Significant differences (bold) in the mRNA expression levels of 30 invasion-related molecules and five tumor markers in glioblastoma (GBM), intracerebral lung adenocarcinoma metastasis (Met), and normal brain tissues (Norm).

	Norm – Met		Norm – GBM		GBM – Met	
	<i>p</i> -value	fold change	<i>p</i> -value	fold change	<i>p</i> -value	fold change
aggrecan	0.421	0.79	0.421	0.39	1.000	2.01
agrin	0.002	0.30	0.012	0.44	0.218	0.67
brevican	<0.001	172.46	0.820	1.27	<0.001	135.66
fibronectin	<0.001	0.08	<0.001	0.09	0.916	0.87
laminin alfa-1	0.032	4.55	0.548	2.98	0.421	1.53
laminin alfa-2	0.310	1.84	0.841	0.82	0.222	2.24
laminin alfa-4	0.713	0.85	0.023	0.47	0.098	1.79
laminin beta-1	0.020	0.36	0.001	0.27	0.379	1.33
laminin beta-2	0.020	0.44	0.005	0.34	0.130	1.29
laminin gamma-1	0.016	0.29	0.222	0.38	1.000	0.76
matrilin-1	0.548	1.69	0.310	2.34	1.000	0.72
matrilin-2	0.002	6.07	0.761	0.90	<0.001	6.72
neurocan	<0.001	44.76	0.095	2.29	0.001	19.50
neuroglycan-C	<0.001	46.94	0.058	3.15	<0.001	14.89
perlecan	<0.001	0.08	<0.001	0.12	0.342	0.70
syndecan-1	<0.001	0.01	0.008	0.23	<0.001	0.06
syndecan-2	0.008	3.27	1.000	1.44	0.151	2.27
syndecan-4	0.020	0.41	0.048	1.53	0.008	0.27
tenascin-C	0.348	0.40	0.006	0.06	0.007	6.91
tenascin-R	<0.001	136.10	<0.001	10.53	0.018	12.92
versican	0.596	1.27	0.289	0.56	0.307	2.29
CD44	0.094	0.39	0.003	0.15	0.012	2.70
CD168	<0.001	0.06	<0.001	0.12	0.053	0.46
chondroitinases (AC,ABC)	0.222	0.77	0.841	0.95	0.690	0.81
hyaluronan synthase-1	<0.001	26.98	<0.001	33.55	0.504	0.80
hyaluronan synthase-2	0.838	0.77	0.002	0.11	0.003	6.84
hyaluronan synthase-3	0.421	1.75	0.690	0.83	0.222	2.10
MMP-2	0.131	0.37	0.004	0.15	0.032	3.08
MMP-8	1.000	0.89	0.857	1.50	0.310	0.60
MMP-9	0.001	0.06	<0.001	0.05	0.805	1.11
cytokeratin 18	0.008	0.002	0.310	0.72	0.008	0.003
cytokeratin 19	0.008	0.003	0.151	4.49	0.008	0.001
GFAP	0.016	33.31	0.548	0.73	0.008	45.61
Ki67	0.008	0.01	0.008	0.01	0.548	0.82

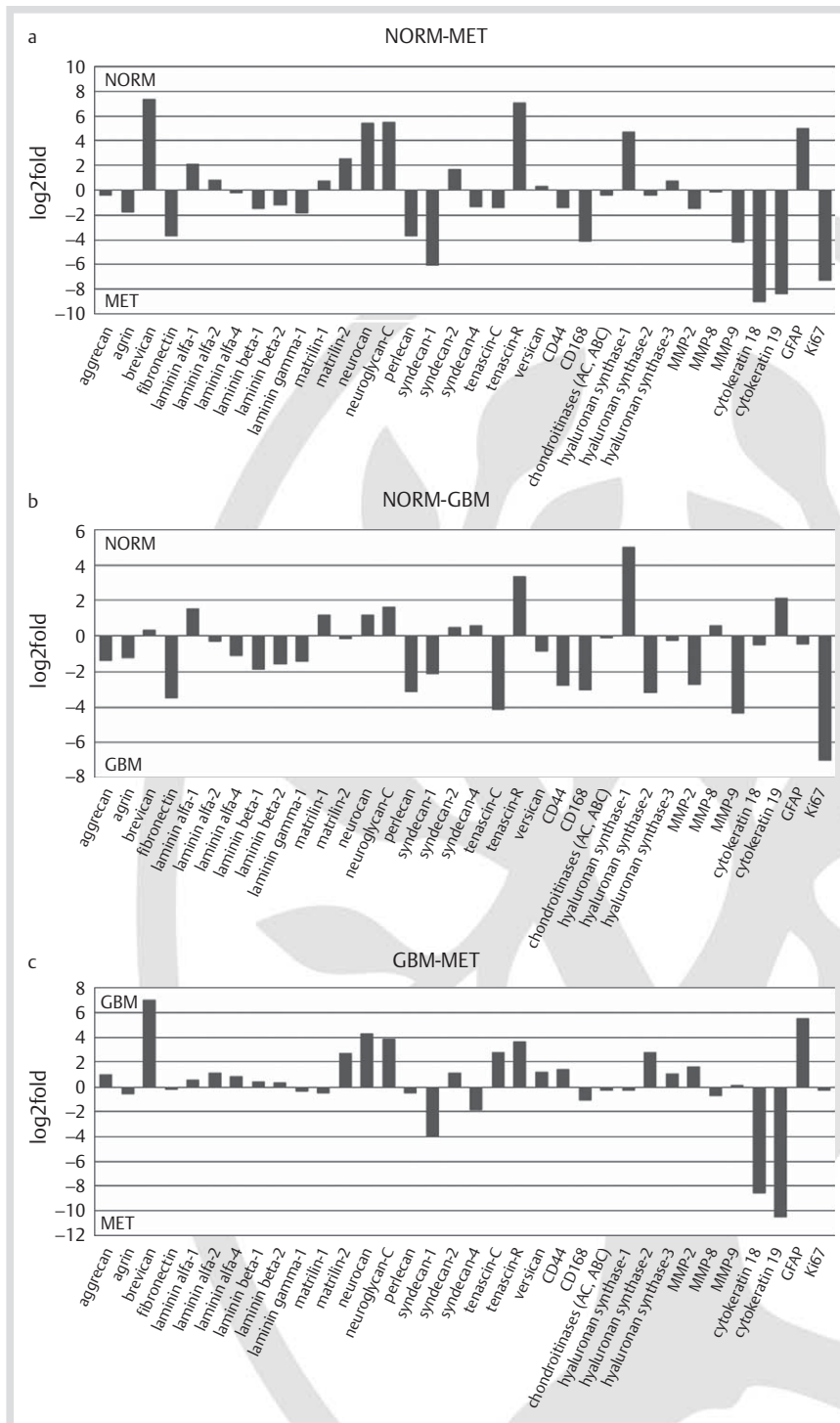


Fig. 1 mRNA expression of 30 invasion-related molecules, tumor markers and Ki67 proliferation marker in glioblastoma (GBM), intracerebral lung adenocarcinoma metastasis (Met), and normal brain (Norm). Relation between mRNA expression of each molecule (fold change) in normal brain and in metastasis (a); normal brain and GBM (b); GBM and metastasis (c).

droitinases, HAS-2, -3, MMP-2 and -8 transcripts between the two types of tissue.

GBM versus intracerebral lung adenocarcinoma metastases

In a comparison of the anaplastic tumors of different origins, the mRNA expression of brevican, matrilin-2, neurocan, neuroglycan-C, tenascin-C and R, CD44, HAS-2 and MMP-2 was significantly higher in GBM compared to lung adenocarcinoma metastases. The transcripts of syndecan-1 and syndecan-4 were significantly elevated in the intracerebral adenocarcinoma. We detected no statistically significant differences in agrin, aggrecan, fibronectin, laminin α -1,-2 or -4, laminin β -1, -2, laminin

γ -1, matrilin-1, perlecan, syndecan-2, versican, CD168, chondroitinase, HAS-1 and -3, or MMP-8, and -9 transcripts.

Immunohistochemistry

The immunohistochemical and HA histochemical reactions are summarized in **Fig. 2**. According to the morphological evaluation, immunoreactivity for agrin, neurocan, syndecan, and versican was most intense mainly on the cell membrane and in the extracellular space, whereas the MMPs showed strong immunoreactivity on the cell membrane and intracellularly. Most intense immunostaining of agrin, syndecan and MMP-9 was observed in the lung adenocarcinoma metastases, while MMP-2, neurocan and hyaluronan showed the highest values in GBM samples. The

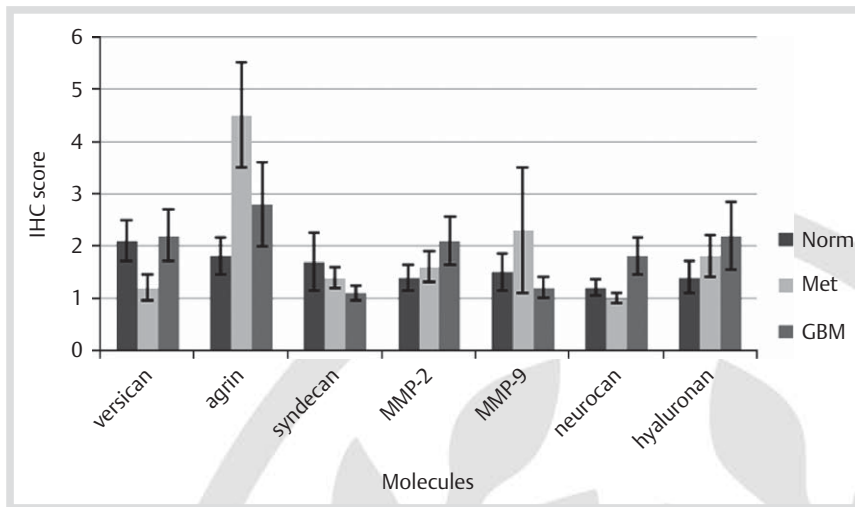


Fig. 2 Immunohistochemical (IHC) evaluation of four proteoglycans, two matrix metalloproteinases, and hyaluronic acid in glioblastoma, intracerebral lung adenocarcinoma metastasis (Met), and normal brain (Norm). The reactions were evaluated by three different investigators experienced in histology on a score of 1–5 in three different regions. The scores of the regions were then summed for general comparison.

staining intensity of tumor markers and Ki-67 was in accordance with the histology.

Discussion

Gliomas are the most common primary intracranial tumors. The incidence of brain metastasis is about double the number of primary brain tumors, and more than half of them originate from lung cancer [11]. Both tumor types are anaplastic, dedifferentiated neoplasms, but their biological behaviour and thus their therapeutic management differs considerably. The unambiguous ability of high grade gliomas to invade normal neural tissue makes them difficult to control and makes complete surgical resection almost impossible. This also explains the extremely high mortality associated with anaplastic gliomas. In contrast, the invasiveness of intracerebral metastases of anaplastic tumors is much more moderate, and radical surgical removal can be achieved in most cases.

The extracellular matrix plays a pivotal role in the tumorous infiltration of the surrounding tissue. The presence and functions of hyaluronic acid, PGs, synthetic and lytic enzymes, and specific adhesion receptors have already been intensively investigated to clarify the molecular mechanisms of invasion, and a positive correlation has been established many times [4, 17, 18, 22, 31, 39].

To identify the molecules that are probably responsible for the distinct invasiveness of high-grade gliomas and metastatic tumors, the mRNA expression of 30 invasion-related molecules was measured in fresh-frozen neurosurgical tissue samples of GBM, intracerebral lung adenocarcinoma metastases, and normal brain tissue. Based on data from the literature, 21 ECM components, seven proteases, the membrane receptor for hyaluronan (CD44), and CD168 were selected for quantitative RT-PCR. To verify the results of mRNA analysis, immunohistochemical analysis of seven molecules (agrin, neurocan, syndecan, versican, MMP-2, MMP-9, and hyaluronan) was performed. The mRNA expression patterns of the analyzed chosen molecules are illustrated in **Fig. 1**.

Previous studies have described a positive correlation between the invasion potential of gliomas and the expression of brevican [10, 46], fibronectin [25, 43], laminin [13, 25], syndecan [48], tenascin-C [16, 52], versican [32], MMP-9 [3, 40, 53], hyaluronan [7], and CD44 [30, 35, 49]. We also detected significant differ-

ences in the mRNA profiles of normal brain and GBM for fibronectin, laminin β -1, perlecan, syndecan-1, -4, tenascin-C, -R, CD44, CD168, HAS-1, -2, and MMP-2 and -9. There are no relevant data in the literature relating astrocytomas to changes in the levels of aggrecan, matrilin, perlecan, neuroglycan-C, neurocan, CD168, or chondroitinase. In this study the mRNA expression of perlecan and CD168 was significantly higher in glioblastomas compared to normal brain, while there were no evident differences between the others.

ECM components and invasion-related molecules have also been studied in some cases of non-small-cell lung carcinoma. In bronchial adenocarcinomas, elevated levels of hyaluronan [33], fibronectin [15, 19], laminin [28, 41], versican [34], MMP-9 [3], MMP-2 [8], and CD44 [21] have been reported, whereas increased levels of perlecan, tenascin-C, and syndecan do not correlate well with tumor progression [14, 27, 38]. There are no data supporting a definite correlation between agrin, brevican, matrilin, neuroglycan-C, neurocan, CD168, chondroitinase, or HAS and the tumor behavior of lung adenocarcinoma. Comparing the intracerebral lung adenocarcinoma metastases with normal brain, we detected a significant difference in the mRNA expression of 18 molecules out of the 30 tested (**Table 1**). There are some molecules that are specific for the tissue of origin (e.g. brevican, neurocan, neuroglycan-C in the brain samples), while others probably play an important role in peritumoral invasion (fibronectin, syndecan-1, -4, CD168 and MMP-9). But the explanation for the high expression of agrin, laminin β -1, β -2, γ -1 and perlecan in intracerebral lung adenocarcinoma metastasis needs further investigation.

The mRNA expression of 11 molecules differed significantly between GBM and lung adenocarcinoma metastases (**Table 1**). Since brevican, matrilin-2, neurocan, neuroglycan-C and tenascin-R had a higher mRNA expression both in normal brain and in GBM compared to the metastatic tumors, these molecules can be interpreted as specific molecules for glial tissues. On the other hand, the mRNA expression of tenascin-C, CD44, HAS-1 and MMP-2 was elevated only in the GBM compared to the metastatic tissue. Based on these observations the high level of the former molecules might help to understand the role of ECM in GBM in avoiding antitumoral reactions of the surrounding brain tissue. Furthermore, the role of the latter molecules in the difference in invasion effectiveness of GBM can be also hypothesized.

Lung adenocarcinoma metastases had high expression of syndecan-1 and -4, but this proved to be insufficient for invading the peritumoral brain.

BEHAB (brain enriched hyaluronan binding)/brevican, a brain-specific chondroitin sulfate PG, shows dramatic upregulation in gliomas and it is also upregulated during periods of increased glial cell motility during development and following brain injury [29]. Viapiano et al. established that brevican expression in gliomas have a restricted membrane localization, and its presence in high-grade gliomas suggests that it may play a significant role in glioma progression, making it an important new potential therapeutic target [45]. We detected no significant difference in mRNA expression of brevican between the normal brain and GBM, thus our data does not support the role of brevican in GBM progression.

Although the mRNA expression of fibronectin was found to be significantly higher in GBM than in normal brain, it was also definitely high in lung adenocarcinoma metastases as well, so that the role of fibronectin in the different invasion activity of the two different tumor types is not supported by our results.

Neurocan, a chondroitin-sulphate PG, is involved in the promotion of neurite outgrowth through interaction with heparan-sulphate PGs [1]. We found significantly lower mRNA expression of neurocan in the lung adenocarcinoma metastases than in GBM, but the highest value was detected in the normal brain, so the role of neurocan in the invasion process of GBM is not clear yet. Based on our results it could be hypothesized that neurocan plays a predominant part in cell differentiation and not in cell proliferation or migration. The reason might be that neurocan promotes normal neurite outgrowth and not tumoral membrane processes. Further investigations are needed for a more precise explanation.

Neuroglycan-C, another chondroitin-sulphate PG, is a ligand of epidermal growth factor receptor (EGFR) Erb-B3 and thus plays an active role in tumor growth [2,20]. The mRNA expression of neuroglycan-C was found to be significantly lower in lung adenocarcinoma metastases than in the other two groups, but the highest value was detected in normal brain, which does not support its putative role in glioma invasion.

Syndecans were previously found to play a part in glioma invasion [48]. We found the highest mRNA expression of syndecan-1 and 4 in lung adenocarcinoma metastases and syndecan-2 in normal brain. Based on these data we can not determine their specific role in the extremely high infiltrative activity of GBM.

Tenascin-C is a glycoprotein of the ECM that was reported to be positively correlated to glioma invasion [9,27] and have a negative correlation with tumor progression of bronchial adenocarcinomas [14,27,38]. Since we also detected a higher mRNA expression of tenascin-C in GBM than in lung adenocarcinoma metastases, and it was low again in normal brain, a specific role of tenascin-C in glioma invasion can be strongly hypothesized.

Versican is one of the most common ECM components in the brain and plays a role in neurite outgrowth, cell attachment and EGFR-mediated signal pathways [50]. Its elevated expression in gliomas has been described previously [32]. We detected elevated mRNA expression of versican in GBM compared to lung adenocarcinoma metastases and normal brain, but the difference was not confirmed statically.

The positive correlation between CD44 and glioma grading and the functional connection between HA and CD44 has been reported many times [30,35,49], and a similar association is also

known with regard to lung adenocarcinomas [22]. In our study we detected significantly elevated CD44 mRNA expression in GBM compared to lung adenocarcinoma metastases with a low CD44 mRNA expression in normal brain, indicating that CD44 may play an important role in the invasion process of malignant gliomas. HAS-2 also showed increased mRNA expression in GBM compared to lung adenocarcinoma metastases or normal brain, and immunostaining of HA was most intensive in GBM. These observations emphasize the common function of CD44 and HAS-2 in tumor infiltration of the surrounding brain.

The role of metalloproteinases in tumor invasion and their representative subtypes in gliomas and bronchial adenocarcinomas has already been described [3,8,40,53]. Our observation that mRNA expression of MMP-2 is significantly higher in GBM than in lung adenocarcinoma metastases and normal brain definitely underlines its role in the different invasiveness of the two different tumor types. The immunostaining profile of MMP-2 was in accordance with the results of the investigations into mRNA expression.

Comparison of mRNA expression and immunohistochemical findings

To investigate the appearance of the different mRNA expressions on a post-translational level and obtain morphological information, immunohistochemical analyses were performed for seven molecules (agrin, neurocan, syndecan, versican, matrix metalloproteinase 2 [MMP-2], MMP-9, and HA).

The changes in the intensity of immunostaining between the three tissue sample groups correlated well with the changes in mRNA expression for versican, agrin, syndecan and MMP-2 (● Fig. 2). In immunohistochemical analysis, agrin, syndecan and MMP-9 were predominant in bronchial adenocarcinoma, whereas MMP-2, neurocan and hyaluronan showed the greatest intensity of immunostaining in GBM.

Interestingly, the highest staining intensity and mRNA expression of agrin was detected in the metastasis group. Agrin is an important component of the blood-brain barrier [36,47], but its presence and role in intracerebral lung adenocarcinoma metastases has not yet been clarified.

MMP-9 showed the strongest immunostaining intensity in lung adenocarcinoma metastases, but its highest mRNA expression was measured in GBM. The highest mRNA expression of neurocan was detected in normal brain, but immunohistochemistry showed a slightly increased staining intensity in the tumors. These findings are probably attributable to post-transcriptional events, but further investigation is required.

HA was found to be mostly present in GBM. Since the mRNA expression of its receptor CD44 was also highest in GBM, their common role in the invasion process can be affirmed.

Conclusions

▼ By comparing the mRNA expression of 30 invasion-related molecules in GBM, normal brain, and intracerebral lung adenocarcinoma metastasis, some molecules that probably play a role in the extremely high invasive activity of GBM could be identified. Based on these results, tenascin-C, CD44, and MMP-2 seem to be predominantly involved in peritumoral infiltration by GBM, but a clear role of fibronectin and syndecans in the different infiltrative activity of these tumors could not be confirmed. Adding

new data to previous observations, the levels of brevican, neurocan, neuroglycan-C and matrilin-2 were also shown to differ significantly between lung adenocarcinoma metastasis and GBM. Because ECM components, proteases, and their receptors have been shown to play an important role in tumor cell migration and invasion, each identified component could be investigated further as a potential target for anticancer therapy.

Acknowledgements

This study was supported by the Hungarian Ministry of Education (OTKA, no. F-049050).

Conflict of Interest: None

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