Pathol. Oncol. Res. (2018) 24:35–43
DOI 10.1007/s12253-017-0194-6

ORIGINAL ARTICLE

Tumor Grade versus Expression of Invasion-Related Molecules in Astrocytoma

József Virga[1] · László Bognár[1] · Tibor Hortobágyi[2] · Gábor Zahuczky[3] · Éva Csősz[4] · Gergő Kalló[4] · Judit Tóth[5] · Gábor Hutóczki[1] · Judit Reményi-Puskár[1] · László Steiner[3] · Almos Klekner[1]

Received: 15 January 2016 / Accepted: 9 January 2017 / Published online: 4 February 2017

Abstract Peritumoral infiltration is characteristic of astrocytomas even in low-grade tumors. Tumor cells migrate to neighbouring tissue and cause recurrence. The extracellular matrix (ECM) plays a role in tumor invasion; expression levels of its components' have been linked to tumor invasion. This study determines the mRNA and protein expression of 20 invasion-related ECM components by examining non-tumor brain; grade I-II-III astrocytoma and glioblastoma samples. Expression levels were measured by QRT-PCR and mass-spectroscopy. The connection between the expression pattern and tumor grade is statistically analyzed. During the analysis of data, key molecules (brevican, cadherin-12, fibronectin and integrin-β1) correlating the most with tumor grade were selected. While the mRNA level of brevican, ErbB2, fibronectin, integrin-β1 and versican discriminates low-grade from high-grade gliomas, of proteins RHAMM, integrin-α1 and MMP2 seems important. The expression pattern was found to be distinctive for tumor grade, as statistical classifiers are capable of identifying an unknown sample's grade using them. Furthermore, normal brain and glioma expression patterns, along with low-grade astrocytoma and glioblastoma samples, differ the most. Determining the invasion-related molecules' expression profile provides extra information regarding the tumor's clinical behavior. Additionally, identifying molecules playing a key role in glioma invasion could uncover potential therapeutic targets in the future.

Keywords Gliomas · Tumor grade · Extracellular matrix · Invasion · Expression

József Virga and László Bognár contributed equally to this work.

✉ Almos Klekner
neurosurgery.debrecen@freemail.hu

[1] Department of Neurosurgery, University of Debrecen Clinical Center, Nagyerdei krt. 98, Debrecen 4032, Hungary

[2] Department of Pathology, University of Debrecen Clinical Center, Nagyerdei krt. 98, Debrecen 4032, Hungary

[3] UD-GenoMed Medical Genomic Technologies Research & Development Services Ltd., Nagyerdei krt. 98, Debrecen 4032, Hungary

[4] Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Nagyerdei krt. 98, Debrecen 4032, Hungary

[5] Department of Oncology, University of Debrecen Clinical Center, Nagyerdei krt. 98, Debrecen 4032, Hungary

Introduction

Gliomas are the most common primary malignant brain tumors and originate from glial cells. They comprise approximately 80% of all malignant brain tumors [1]. Of all gliomas, 75% are astrocytomas, making them the most common type [2]. Current glioma classification, which defines treatment, is based on the level of malignancy and the cytological features seen on routinely stained hematoxylin and eosin histological slides [3]. An astrocytoma's clinical behavior and evaluation, however, is not necessarily identical to its histopathological classification used for the WHO classification [3]. Even though WHO grade II astrocytomas are histologically benign, their clinical behavior may show some malignant traits. This is mainly due to the invasion of tumor cells into the brain's neighboring parenchyma, a trait of both low- and high-grade astrocytomas, making radical resection of high grade gliomas practically impossible [4, 5]. These infiltrative tumor cells almost inevitably originate local recurrence; hence, complex oncotherapy is required after surgical treatment [4].

The interaction of tumor cells and neighboring extracellular matrix (ECM) is necessary for tumor invasion. Previous

research shows that peritumoral ECM in gliomas differs from ECM present in healthy brain tissue and that ECM has a direct role in the development of high-grade gliomas' invasiveness [6–8]. Also, interconnectedness among ECM macromolecules enables the migration of tumor cells to healthy brain parenchyma [9–11]. These explain the extensive research toward the development of novel anti-invasive agents which can be used for glioblastoma treatment.

One of the goals of this research is to study the connection between the expression levels of invasion-related ECM components and the grade of astrocytomas. The other goal is to identify the key molecules that are responsible for invasive behavior. Furthermore, the relation of the expression spectrum of ECM components in astrocytomas to the grade of tumor was also tested. Examinations are not only performed on tumor samples; the difference of low-grade glioma and normal brain tissue is also analyzed, a rarity in the literature.

Methods

Tissue Samples

Samples were taken from patients who were routinely operated on due to neurosurgical illnesses. An informed consent form was signed by each patient and the research was permitted by the National Research Ethics Committee. Normal samples were brain tissues that was removed during functional neurosurgical procedures. Samples were frozen intraoperatively on the surface of liquid nitrogen, and then stored at −80 °C until further use. Each sample was evaluated and classified by an experienced neuropathologist into one of the following categories: normal brain; astrocytoma grade I, grade II, or grade III; and glioblastoma based upon the WHO classification criteria. (See Table 1)

The mRNA and protein expression levels were measured to ascertain the presence of 20 invasion-related molecules which were selected after extensive literature review and based upon previous findings of our research group. The measured ECM components include cell-ECM and cell-cell adhesion molecules expressed on the surface of the cells, ECM ligands of these receptors and enzymes (See Table 2) [9, 12–15]. To confirm glial origin of tumors, GFAP expression was measured, and to prove that the sample used for the analysis consists malignant cells, Ki-67 expression was tested.

Table 1 Number of investigated samples for mRNA and protein expression analysis

Histological diagnosis	No. of samples for mRNA analyis	No. of samples for protein analyis
non-tumor brain	18	36
astrocytoma, grade I	14	12
astrocytoma, grade II	14	12
astrocytoma, grade III	14	-
glioblastoma	16	12

mRNA Expression Measurements

The mRNA expression level of these molecules was determined through real-time quantitative reverse transcriptase–polymerase chain reaction (QRT–PCR) in the samples. Freshly frozen tissue samples were first pulverized, and then homogenized using TriReagent® (Invitrogen, USA). Total RNA was isolated from TriReagent lysates according to the manufacturer's instructions. A NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, USA) was used to measure the quantity and purity of RNA. In the next step, reverse transcription was performed converting total RNA to single-stranded cDNA with the help of a High-Capacity cDNA Archive Kit with RNasin (Applied Biosystems, USA). The cDNA was then loaded onto a microfluidic card (cDNA from 100 ng of total RNA per port). An Applied Biosystems' 7900HT real-time PCR system with Micro Fluidic Card upgrade (Applied Biosystems, USA) was used to perform TaqMan Low Density Array (TLDA) experiments. The Micro Fluidic Cards were analyzed with SDS 2.1 software as relative quantification studies, and the Ct (Cycle threshold) values were exported for further analysis. β-actin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) housekeeping genes exhibited the least variation among the samples. GAPDH was chosen as reference genes to calculate the dCt (deltaCt) value for each gene. Expression values were calculated using the comparative CT method, as described previously [16].

Table 2 Molecules selected to the invasion panel (that are in connection to ECM components and peritumoral invasion in astrocytoma)

Molecule	
brevican	laminin α4
cadherin-N2	laminin β1
EGFR (ErbB1)	matrix metalloproteinase 2 (MMP2)
ErbB2 (HER2/neu)	matrix metalloproteinase 9 (MMP9)
fibronectin	neurocan
integrin α1	RHAMM (CD168, HMMR)
integrin α3	syndecan-1
integrin α7	tenascin-C
integrin β1	tenascin-R
collagen III, α1 chain	versican

Protein Expression Measurements

After determining mRNA levels, concentrations of the transcribed proteins were measured with a mass spectrometer to uncover changes in the protein fingerprint of the samples. Tissue homogenization for protein analysis was performed as described in the case of RNA purification; however, a lysis buffer containing 50 mM Tris, 1 mM EDTA, 17 mM beta-mercaptoethanol and 0.5% Triton-X100™ was used in this case for tissue lysis. The protein content was measured using the Bradford method, and equal amounts of proteins were used for in-solution trypsin digestion [17]. The selected reaction monitoring (SRM)-based targeted proteomic method was developed for relative protein amount determination, using bovine serum albumin (BSA) as reference protein [18–21]. For protein concentration estimation, the area under the curve of the acquired spectra was calculated; SRM spectra were used for AUC calculations where the intensity of the signal exceeded 500 cps. Data integration was done with the help of Analyst 1.4.2 software based on the curve shape determined from pilot analyses.

Statistical Analysis

Linear discriminant analysis (LDA) was used to identify the key molecules playing a crucial role in the invasion of astrocytomas and separate grades from one another. Furthermore, with LDA of the typical expression pattern of each histopathological group, the invasion spectrum could be also established. To confirm the connection between the invasion spectrum and the grade, the grade of the unknown samples was identified using a closest neighbor search and LDA.

Results

LDA of mRNA Expression Data Separates Various Grades of Glioma and Selects Key Molecules of Glioma Invasion

Using LDA allowed the separation of grades based upon the expression level of the measured molecules in the samples while certain key molecules in the differentiation of grades were also identified. The expression levels of these molecules distinguished the samples' grades from each another. Fig. 1 shows the results of LDA performed with all groups (i.e., normal brain, grade I-II-III astrocytoma and glioblastoma). The graph shows how various grades break down based upon their expression profile. LDA found the following key molecules during the joint analysis of the groups: brevican, cadherin 12, fibronectin and integrin β1.

LDA performed with two groups only showed a better separation of the groups. Figures 2a-d show the graphic results of the analysis, Table 3 contains key molecules identified with these LDAs. Table 3 contains the key molecules identified by LDA of low-grade and high-grade gliomas.

LDA of Protein Expression Data Confirms Separation of Grades Based upon the Levels of Invasion Related Molecule in Tumor Samples

LDA performed with the protein expression data identified the key molecules that can be found in Table 4. Figure 3 shows the graphic results of the LDAs of protein expression data. The graph shows the separation of normal brain from grade I astrocytoma, together with the distinct expression profiles of grade I and grade II astrocytomas. (Fig. 3a-b) Low-grade astrocytoma and glioblastoma samples have been compared as well. The expression profile of low-grade astrocytomas differs from that of glioblastoma (Fig. 3c). Three proteins were selected by the analysis as key molecules: RHAMM (CD168), integrin α1 and MMP2. (Table 4)

The Expression Profile of Invasion-Related Molecules Creates the Invasion Spectrum which Is Characteristic of each Grade

The connection between tumor grade and the expression pattern was also tested by determining the grade of an unknown sample using the expression values of invasion-related molecules. Table 5 shows the percentage of correctly identified instances from LDA and nearest neighbor search (IB1) following cross-validation. The results of the classifiers using protein expression data showed better results than the ones of mRNA expression. Statistical classifiers managed to identify the grade of unknown samples by using the mRNA and protein expression profile of normal brain tissue and various grades of astrocytoma, therefore, it can be stated that each grade can be characterized by a special expression pattern of invasion molecules. This expression pattern can serve as a characteristic invasion spectrum for different grades of astrocytomas. Identifying a clear distinction in the invasion spectrum of non-tumor brain tissue and grade I astrocytoma, as well as the clear distinction between low-grade and high-grade astrocytomas is of upmost importance (also see Fig. 3C and Fig. 4). The set of key molecules selected by LDA gives extra credence to this finding, indicating that certain ECM components influence the invasiveness and tumor grade more than others (see Tables 3, 4 and 5).

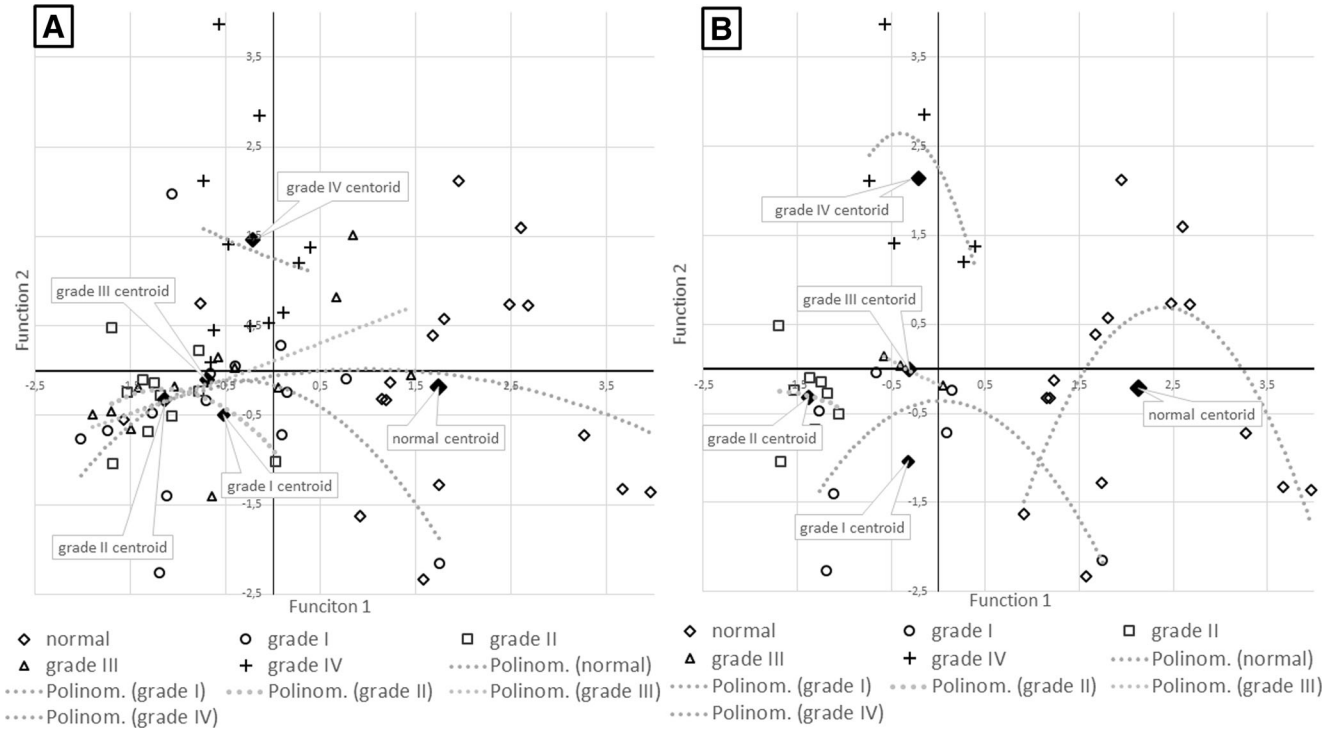

Fig. 1 The scattered plot graphs show how various grades of astrocytoma and normal brain tissue are separated in the graphic results of linear discriminant analysis (LDA) of the expression levels of invasion-related molecule mRNAs in each group. Dots represent the canonical discriminant function value of each sample which is calculated from the mRNA expression of the key molecules identified by the LDA of the five groups. Figure 1a shows all the cases while 1B shows correctly identified cases only. Misclassified samples came from patients whose prognosis did not follow the expected prognosis of the WHO grade, suggesting the importance of expressional analysis. Centroids show the average of each group, dotted trend lines represent the polynomial trend lines of the expression levels in each grade

Discussion

Astrocytoma is the most common malignant intracranial neoplasm [1]. The WHO classification separates subgroups of astrocytoma based upon histopathological classification into four grades, glioblastoma being grade IV [1]. Clinical diagnosis and treatment protocols depend definitely of this classification system therefore accurate pathological diagnosis is extremely important. Histopathological diagnosis and WHO classification are mainly based upon morphological features of the tissue sample and known molecular alterations typical in astrocytomas are not automatically considered in this classification – despite their importance in choosing targeted and effective anti-invasive therapy [1, 3].

Previous research confirms the different composition of extracellular matrix in normal brain tissue and astrocytoma [7, 8, 11]. It is known that many compounds of ECM play an important role in extensive peritumoral infiltration and that this process is important to tumor recurrence [5, 6, 13]. Our results not only confirm but also supplement data available in the literature. Similar findings are hardly available in literature, especially considering data from non-tumor brain samples. Linear discriminant analysis of the expression data of invasion-related ECM components has selected a set of molecules that play a key role in glioma invasion and show a strong relationship to the grade of astrocytoma, however the grade is, of course, not solely dependent on the level of these molecules (see Tables 2 and 3). It can be easily understood why a single molecule alone does not affect tumor grade to a significant degree, however, with joint assessment of the expression of these molecules using various statistical methods (nearest neighbor search or LDA) it is possible to identify the grade of samples using the invasion spectrum with great accuracy (Table 5, Fig. 1, Fig. 3C and Fig. 4).

Key ECM Components Selected by LDA and their Role in Glioma Invasion

Integrins These cell connecting αβ heterodimer ECM components bind to a wide range of substrates including laminin, fibronectin, tenascin and collagens [22]. It was found that glioma cell lines overexpressing β1 integrin are more infiltrative compared to glioma cell lines without increased expression [7, 11, 22]. Our research identifies α3 and β1 integrins as key molecules of tumor invasion. The two types of integrin are often simultaneously overexpressed, and the α3β1 heterodimer plays a central role in the migration of glioma cells [8, 11, 23]. Protein expression of β1 integrin was much higher in glioma samples than non-tumor samples. Intergin α1 protein's expression is higher in gliomas than in non-tumor samples.

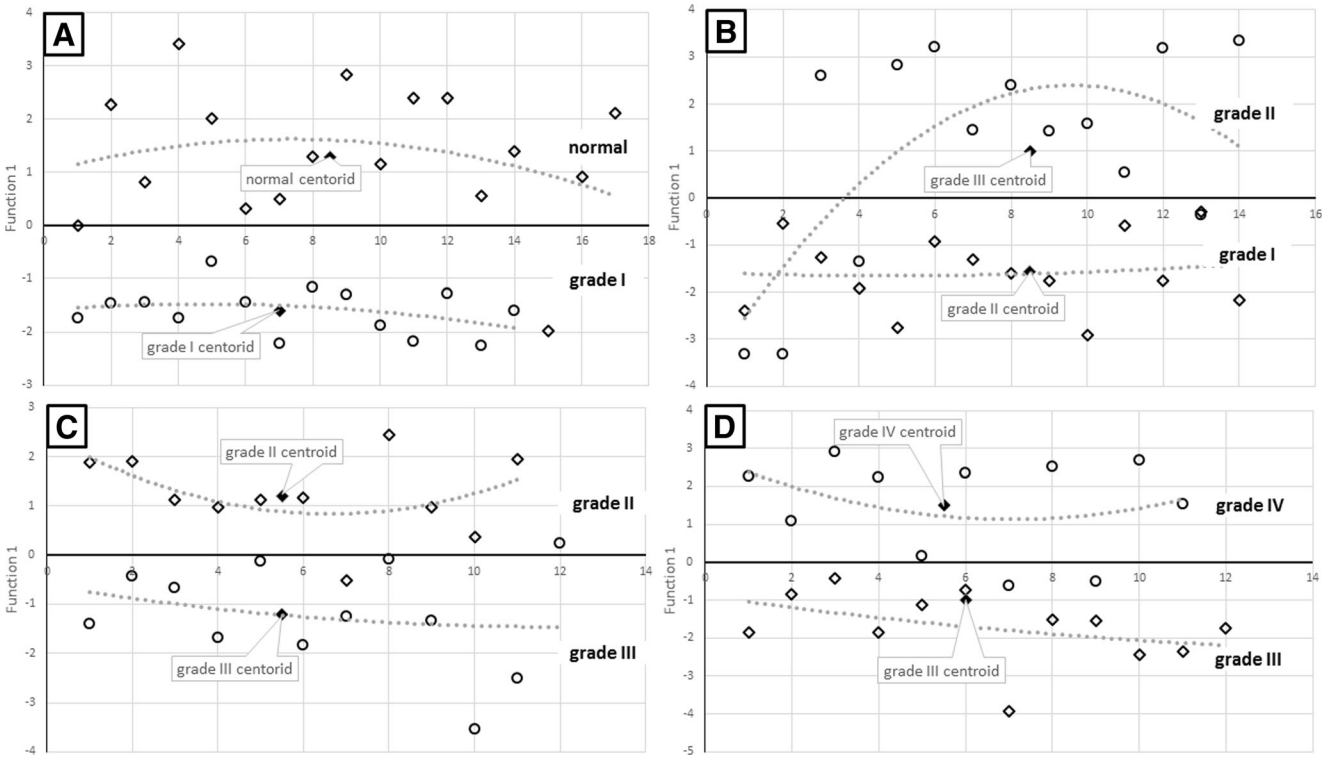

Fig 2 **a-d** Separation of two grades after LDA of mRNA expression data. Dots represent the canonical discriminant function value of each sample that is calculated from the mRNA expression of each sample. The LDA separates samples based upon their canonical discriminant function values. Dotted lines represent the polynomial trend lines of the values. (Fig. 2a: normal brain vs. grade I astrocytoma, 2B: grade I vs. grade II astrocytomas, 2C: grade II vs. grade III astrocytomas, 2D: grade III astrocytoma vs. glioblastoma)

Fibronectin This important integrin substrate for glioma migration was also identified as a key molecule in our research. Levels of fibronectin mRNA were found to be inversely proportional to tumor grade in our samples, which seems logical as fibronectin plays a role in cellular adhesion. However, it is also known that the expression of fibronectin is increased in the perivascular stoma of tumors and in the migrating edge of the tumor mass (gliomesenchymal junction); specific inhibition of fibronectin-binding integrins diminishes migration of tumor cells [6, 24, 25].

Table 3 Key molecules identified by linear discriminant analysis (LDA) of the expression levels of invasion-related molecule mRNAs in normal brain tissue and various grades of astrocytoma

non-tumor vs. A I	A I vs. A II	A II vs. A III	A III vs. GBM	low grade vs. high grade
integrin β1	integrin α3	cadherin 12	brevican	brevican
laminin α4	integrin α7	ErbB2	cadherin 12	ErbB2
tenascin C	integrin β1	neurocan	fibronektin	fibronektin
tenascin R	laminin α4	syndecan 1	laminin β1	ingerin β1
	MMP2	versican	neurocan	versican
	tenascin R		tenascin	
	versican			

non-tumor normal brain tissue, *A I* astrocytoma grade I, *A II* astrocytoma grade II, *A III* astrocytoma grade III, *GBM* glioblastoma

Laminins These giant molecules contribute to cellular adhesion and motility; they are typically expressed around blood vessels. It is postulated that the overexpression of α4 laminin is a sign of neovascularisation in cancer. Furthermore, it may play a role in tumor invasion and the development of recurrence [26, 27]. Levels of laminin α4 mRNA increased in our samples from high-grade gliomas compared to low-grade gliomas and the expression of laminin α4 was strikingly high in GBM samples, suggesting its role in the development of invasive potential. As GBM is known to be an extensively vascularized type of tumor, we also confirm the role of

Table 4 Key proteins identified with linear discriminant analysis (LDA) of the expression levels of invasion panel molecules in normal brain tissue and various grades of astrocytoma. Laminin α4 and intergrin β1 were selected as key molecule during the analysis of mRNA data in the respective groups

non-tumor vs. A I	A I vs. A II	low grade vs. GBM
RHAMM (CD168)	RHAMM (CD168)	RHAMM (CD168)
integrin α1	collagen 3 α1	integrin α1
laminin α4	*integrin β1*	MMP2
laminin β1	laminin β1	
MMP2		

non-tumor normal brain tissue, *A I* astrocytoma grade I, *A II* astrocytoma grade II, *GBM* glioblastoma

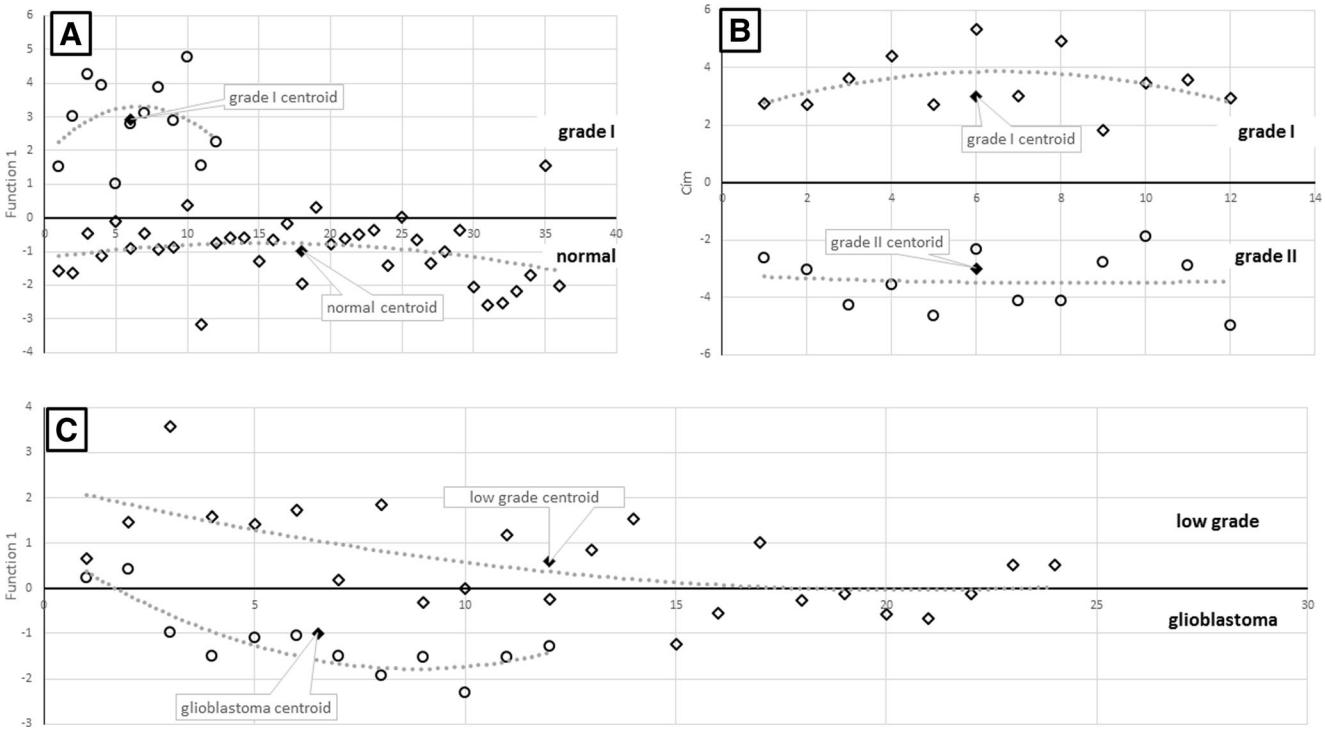

Fig 3 Separation of grades after LDA of protein expression data. Dots represent the canonical discriminant function value of each sample that is based upon the protein expression. Dotted lines represent the polynomial trend lines of the values. (Fig. 3a: normal brain vs. astrocytoma grade I, 3B: astrocytoma grade I vs. astrocytoma grade II, 3C: low grade astrocytomas vs. glioblastoma)

laminin α4 in the formation of new blood vessels in tumors. As a novel finding, non-tumor samples expressed lower levels of laminin β1 protein compared to astrocytoma samples.

Tenascin-C and -R In adults, tenascins, out of which tenascin-R isoform is CNS specific, are present during pathological processes in which tissue remodeling is of great importance, such as wound healing and tumor stroma; where they play a role in increased motility and cancer progression [28, 29]. The level of tenascin-R increased with grade, was especially high in GBM. Tenascin-C did not show a gradual change in expression, but the difference was noticable between non-tumor and grade I, as well as grade III and GBM samples.

Syndecans The transmembrane receptor syndecans, with 3–5 heparan-sulfate arms, participate in many processes like cell-cell and cell-ECM connections, cell division, cellular adhesion, and cellular migration [30]. Syndecan-1 was selected as a key molecule in the separation of grade II and grade III

Table 5 Percentage of correctly identified instances of statistical classifiers using the invasion spectrum (mRNA and protein expression profile of invasion-related molecules) following cross validation. Cross validation was done by rebuilding the classifier model without using the expression data of the sample in question

Level of differentiation	RNA expression data			Protein expression data		
	Correctly identified instances (%)		No. of key molecules	Correctly identified instances (%)		No. of key proteins
	IB 1	LDA		IB 1	LDA	
non-tumor vs. AI	90.62	96.0	4 genes	97.92	95.8	5 proteins
AI vs. AII	78.57	89.3	7 genes	100.00	100.00	4 proteins
AII vs. AIII	85.71	82.6	5 genes	NA	NA	NA
AIII vs. GBM	80.00	82.6	6 genes	NA	NA	NA
low grade vs. high grade	77.58	78.9	5 genes	94.45	86.10	3 proteins
of all grade and normal	51.30	50.8	4 genes	87.50	79.20	10 proteins

non-tumor normal brain tissue, *A I* astrocytoma grade I, *A II* astrocytoma grade II, *A III* astrocytoma grade III, *GBM* glioblastoma, *IB1* nearest neighbor search, *LDA* linear discriminant analysis, *NA* not applicable

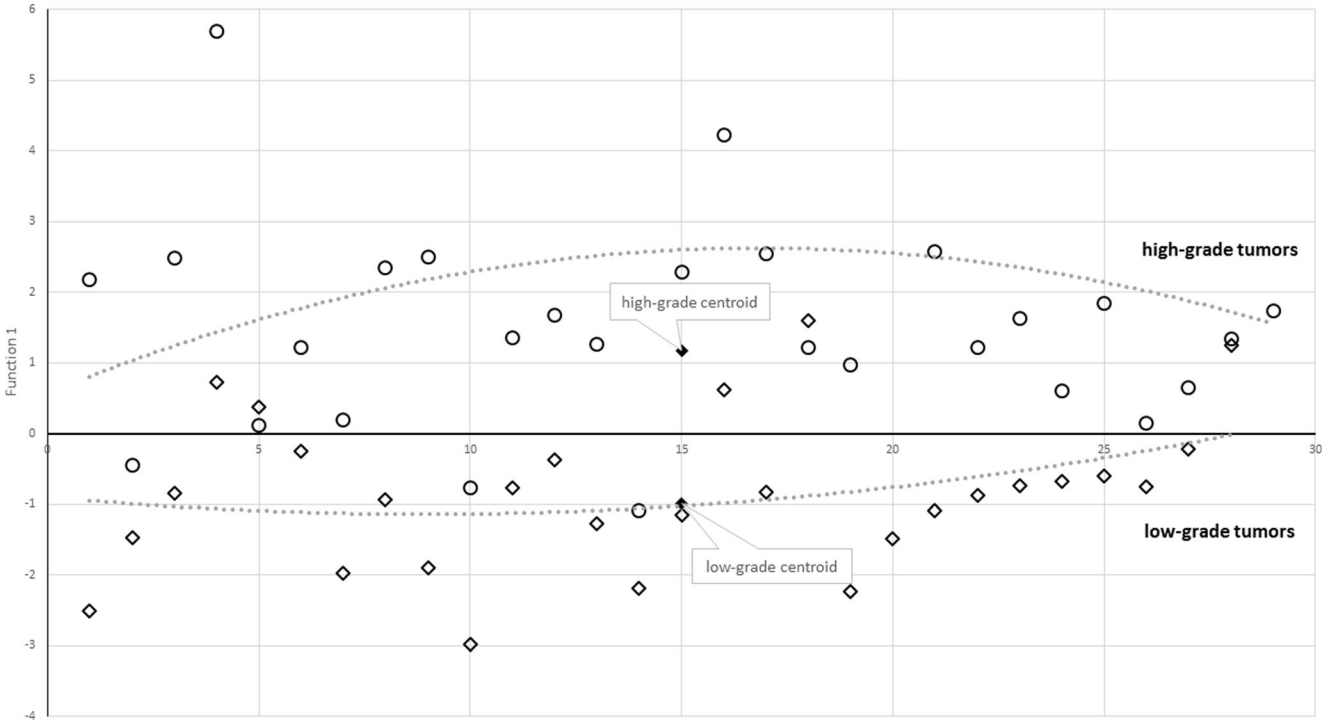

Fig. 4 Separation of low-grade and high-grade tumors based on protein expression data. Dots represent the canonical discriminant function value of each sample that is based upon the protein expression. Dotted lines represent the polynomial trend lines of the values

tumors, when the invasiveness of gliomas change expressly, further confirming the role of syndecan-1 in tumor invasiveness and glioma progression [31].

Brevican, Neurocan, and Versican These proteoglycans of the lectican family bind to the main ECM component hyaluronan. CNS specific brevican should be highlighted from the group; while expression of brevican in a healthy brain is barely detectable, it is remarkably overexpressed in glial tumors [32]. These chondroitin-sulfate proteoglycans promote tumor motility and progression via a yet unknown mechanism [33, 34]. The expression of brevican increased with tumor grade in our samples. High-grade gliomas expressed noticably more neurocan and versican than did low-grade gliomas and normal brain tissue.

Matrix Metalloproteinase 2 (MMP) Members of the MMP protease family participate in the remodeling and degrading of the ECM, many isoforms have been described to be overexpressed in gliomas [35, 36]. We could further confirm the role of MMP 2 in glioma invasion, as we also found that glioma cells synthetize high amount of MMP2 themselves.

Cadherin 12 (N-Cadherin 2) The cellular adhesion glycoprotein family of cadherins has a role in tumor invasion, as previously confirmed [37, 38]. Cadherin-12 was found to be a key molecule in the development of glioma invasion since its expression abruptly increases in GBM samples compared to grade III ones, but a bigger increase is also seen in grade I astrocytoma compared to non-tumor samples. The role of cadherin 12 was described in other cancers but not yet in gliomas [39].

ErbB2 A well-known oncogene member of the human epidermal growth factor receptor family (Her2/neu), the role of ErbB2 in tumor biology is common knowledge. Its increased expression in gliomas has been described already, and ErbB2 possibly contributes to radiosensitivity and peritumoral infiltration of gliomas as its signal transduction pathways promote MMP2 expression [40, 41]. The expression of ErbB2 protein in tumor samples was found to be increased compared to non-tumor samples.

RHAMM (HMMR, Cd 168) As its name suggests, the receptor for hyaluronan-modulated motility (RHAMM) is an extracellular receptor that interacts with the widespread ECM component hyaluronan. The complicated signaling pathways of RHAMM stimulate the expression of factors that promote cellular motility [42]. RHAMM expression is increased in gliomas compared to normal brain tissue; its quantity increases with tumor grade [43]. The results from our research match with literature data suggesting that the expression of CD168 protein in gliomas is increased compared to normal brain tissue, the highest in GBM.

Conclusions

In this study the expression pattern of invasion-related ECM molecules (invasion spectrum) in astrocytoma samples was determined and a set of ECM molecules have been identified as key molecules in glioma invasion. Furthermore, our research verifies the relation between invasion spectrum and the tumor grade of astrocytomas. It is possible to identify the grade of unknown astrocytoma samples at a high level of confidence by testing the invasion spectrum.

The aim of performing molecular analysis and determining the invasion spectrum is not to replace pathohistological grading but to access extra information that can be used to aid the neuropathologists in the grading process. The invasion spectrum can be also used to gain knowledge of the molecular changes (e.g. overexpression of specific molecules) in the tumor in order to correctly assess clinical prognosis and choose the right targeted treatment regimen (e.g. specific inhibitor of the overexpressed molecule). It was previously proven that using appropriately chosen novel molecular chemotherapy can contribute to better surgical outcome and can also increase resecability [5]. Determining the invasion spectrum, therefore, could be helpful when selecting the right anti-invasive regimen to improve surgical effectiveness in the future.

Acknowledgements This study was supported by Hungarian Brain Research Program - Grant No. KTIA_13_NAP-A-V/3, II/7. and A. Klekner by the János Bolyai Scholarship of the Hungarian Academy of Sciences. Supported by the ÚNKP-16- 3-I New National Excellence Program of the Ministry of Human Capacities.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Goodenberger ML, Jenkins RB (2012) Genetics of adult glioma. Cancer Genet 205:613–621. doi:10.1016/j.cancergen.2012.10.009
2. Ostrom QT, Gittleman H, Liao P et al (2014) CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2007–2011. Neuro-Oncology 16:iv1–iv63. doi:10.1093/neuonc/nou223
3. Louis DN, Ohgaki H, Wiestler OD et al (2007) The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol 114:97–109. doi:10.1007/s00401-007-0243-4
4. Tysnes BB, Mahesparan R (2001) Biological mechanisms of glioma invasion and potential therapeutic targets. J Neuro-Oncol 53: 129–147
5. Agnihotri S, Burrell KE, Wolf A et al (2013) Glioblastoma, a brief review of history, molecular genetics, animal models and novel therapeutic strategies. Arch Immunol Ther Exp 61:25–41. doi:10.1007/s00005-012-0203-0
6. Chintala SK, Rao JK (1996) Invasion of human glioma: role of extracellular matrix proteins. Front Biosci 1:d324–d339
7. Gingras M, Roussel E, Bruner JM et al (1995) Comparison of cell adhesion molecule expression between glioblastoma multiforme and autologous normal brain tissue. J Neuroimmunol 57:143–153. doi:10.1016/0165-5728(94)00178-Q
8. Mahesparan R, Read T-A, Lund-Johansen M et al (2003) Expression of extracellular matrix components in a highly infiltrative in vivo glioma model. Acta Neuropathol 105:49–57
9. Klekner A, Virga J, Toth J et al (2013) The role of extracellular matrix components in the invasion of intracranial malignancies. Magy Onkol 57:222–231
10. Coniglio SJ, Segall JE (2013) Review: molecular mechanism of microglia stimulated glioblastoma invasion. Matrix Biol 32:372–380. doi:10.1016/j.matbio.2013.07.008
11. Knott JCA, Mahesparan R, Garcia-Cabrera I et al (1998) Stimulation of extracellular matrix components in the normal brain by invading glioma cells. Int J Cancer 75:864–872
12. Varga I, Hutóczki G, Szemcsák CD et al (2012) Brevican, neurocan, tenascin-C and versican are mainly responsible for the invasiveness of low-grade astrocytoma. Pathol Oncol Res 18:413–420
13. Varga I, Hutóczki G, Petrás M et al (2010) Expression of invasion-related extracellular matrix molecules in human glioblastoma versus intracerebral lung adenocarcinoma metastasis. Zentralbl Neurochir 71:173–180
14. Klekner A, Varga I, Bognár L et al (2010) Extracellular matrix of cerebral tumors with different invasiveness. Ideggyogy Sz 63:38–43
15. Bellail AC, Hunter SB, Brat DJ et al (2004) Microregional extracellular matrix heterogeneity in brain modulates glioma cell invasion. Int J Biochem Cell Biol 36:1046–1069. doi:10.1016/j.biocel.2004.01.013
16. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) method. Methods 25:402–408
17. Bradford M (1976) Rapid and sensitive method for quantification of microgram quantities of protein utilizing principle of protein-dye-binding. Anal Biochem 72:248–254
18. James A, Jorgensen C (2010) Basic design of MRM assays for peptide quantification. Methods Mol Biol 658:167–185
19. Iuga C, Seicean A, Iancu C et al (2014) Proteomic identification of potential prognostic biomarkers in resectable pancreatic ductal adenocarcinoma. Proteomics 14:945–955
20. Qian W-J, Jacobs JM, Liu T et al (2006) Advances and challenges in liquid chromatography-mass spectrometry-based proteomics profiling for clinical applications. Mol Cell Proteomics 5:1727–1744
21. Lange V, Picotti P, Domon B, Aebersold R (2008) Selected reaction monitoring for quantitative proteomics: a tutorial. Mol Syst Biol 4:222
22. D'Abaco GM, Kaye AH (2007) Integrins: molecular determinants of glioma invasion. J Clin Neurosci 14:1041–1048. doi:10.1016/j.jocn.2007.06.019
23. Fukushima Y, Ohnishi T, Arita N et al (1998) Integrin alpha3beta1-mediated interaction with laminin-5 stimulates adhesion, migration and invasion of malignant glioma cells. Int J Cancer 76:63–72. doi:10.1002/(SICI)1097-0215(19980330)76:1<63::AID-IJC11>3.0.CO;2-H
24. Yeh W-L, Lu D-Y, Liou H-C, Fu W-M (2012) A forward loop between glioma and microglia: glioma-derived extracellular matrix-activated microglia secrete IL-18 to enhance the migration of glioma cells. J Cell Physiol 227:558–568. doi:10.1002/jcp.22746
25. Färber K, Synowitz M, Zahn G et al (2008) An alpha5beta1 integrin inhibitor attenuates glioma growth. Mol Cell Neurosci 39:579–585. doi:10.1016/j.mcn.2008.08.005

26. Ljubimova JY, Lakhter AJ, Loksh A et al (2001) Overexpression of α4 chain-containing laminins in human glial tumors identified by gene microarray analysis. Cancer Res 61:5601–5610
27. Nagato S, Nakagawa K, Harada H et al (2005) Downregulation of laminin alpha4 chain expression inhibits glioma invasion in vitro and in vivo. Int J Cancer 117:41–50. doi:10.1002/ijc.21102
28. Tanaka K, Hiraiwa N, Hashimoto H et al (2004) Tenascin-C regulates angiogenesis in tumor through the regulation of vascular endothelial growth factor expression. Int J Cancer 108:31–40. doi:10.1002/ijc.11509
29. Herold-Mende C, Mueller MM, Bonsanto MM et al (2002) Clinical impact and functional aspects of tenascin-C expression during glioma progression. Int J Cancer 98:362–369. doi:10.1002/ijc.10233
30. Rousseau C, Ruellan AL, Bernardeau K et al (2011) Syndecan-1 antigen, a promising new target for triple-negative breast cancer immuno-PET and radioimmunotherapy. A preclinical study on MDA-MB-468 xenograft tumors. EJNMMI Res 1:20. doi:10.1186/2191-219X-1-20
31. Xu Y, Yuan J, Zhang Z et al (2012) Syndecan-1 expression in human glioma is correlated with advanced tumor progression and poor prognosis. Mol Biol Rep 39:8979–8985
32. Gary SC, Kelly GM, Hockfield S (1998) BEHAB/brevican: a brain-specific lectican implicated in gliomas and glial cell motility. Curr Opin Neurobiol 8:576–581. doi:10.1016/S0959-4388(98)80083-4
33. Wegrowski Y, Maquart F-X (2006) Chondroitin sulfate proteoglycans in tumor progression. Adv Pharmacol 53:297–321. doi:10.1016/S1054-3589(05)53014-X
34. Silver DJ, Silver J (2014) Contributions of chondroitin sulfate proteoglycans to neurodevelopment, injury, and cancer. Curr Opin Neurobiol 27:171–178. doi:10.1016/j.conb.2014.03.016
35. Egeblad M, Werb Z (2002) New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2:161–174
36. Lampert K, Machein U, Machein MR et al (1998) Expression of matrix metalloproteinases and their tissue inhibitors in human brain tumors. Am J Pathol 153:429–437. doi:10.1016/S0002-9440(10)65586-1
37. Takeichi M (1993) Cadherins in cancer: implications for invasion and metastasis. Curr Opin Cell Biol 5:806–811. doi:10.1016/0955-0674(93)90029-P
38. Asano K, Kubo O, Tajika Y et al (1997) Expression and role of cadherins in astrocytic tumors. Brain Tumor Pathol 14:27–33
39. Zhao J, Li P, Feng H et al (2013) Cadherin-12 contributes to tumorigenicity in colorectal cancer by promoting migration, invasion, adhersion and angiogenesis. J Transl Med 11:288
40. Xu G-F, Xie W-F (2013) Effect of ERBB2 expression on invasiveness of glioma TJ905 cells. Asian Pac J Trop Med 6:964–967. doi:10.1016/S1995-7645(13)60172-8
41. Gao L, Li F, Dong B et al (2010) Inhibition of STAT3 and ErbB2 suppresses tumor growth, enhances radiosensitivity, and induces mitochondria-dependent apoptosis in glioma cells. Int J Radiat Oncol Biol Phys 77:1223–1231. doi:10.1016/j.ijrobp.2009.12.036
42. Turley EA, Hossain MZ, Sorokan T et al (1994) Astrocyte and microglial motility in vitro is functionally dependent on the hyaluronan receptor RHAMM. Glia 12:68–80. doi:10.1002/glia.440120109
43. Akiyama Y, Jung S, Salhia B et al (2001) Hyaluronate receptors mediating glioma cell migration and proliferation. J Neuro-Oncol 53:115–127