SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSPOHY (PhD)

Comparison of peritumoral invasiveness of lung cancer cerebral metastases and primary brain tumors by determining the expression profile of invasion related extracellular matrix components

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The Examination takes place at the library of the Department of Anatomy, Histology and Embriology, Faculty of Medicine, University of Debrecen at 11 a.m. 29th May, 2014.

Head of the Defense Committee:	Prof. Miklós Antal MD, DSc
Reviewers:	Eszter Csánky MD, PhD
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Members of the Defense Committee:	Prof. Ilona Kovalszky MD, DSc
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The PhD Defense takes place at the Lecture Hall of the Department of Anatomy, Histology and Embriology, Faculty of Medicine, University of Debrecen at 1:00 p.m. 29th May, 2014.

1. Introduction

“Plague of our era", this term has already belonged to many diseases, but perhaps it fits in principally to the malignant tumors. Among them prominent is the lung cancer, because it is common (with increasing incidence), its screening is difficult and its mortality parameters and therapeutic benefits remain under other malignant diseases.

The reason for therapeutic failure is in general the late detection and impossibility of surgical treatment – that would be the only hopeful procedure aiming definitive healing. The most common reason of inoperability is the presence of distant metastases. Among the many histological types of non small cell lung cancer, adenocarcinoma causes the most of brain metastases.

On the other hand metastases are the most common tumor in the brain responsive for about 20% of all tumor deaths. The lung cancer metastases are most common metastases in the brain.

The early detection of brain metastasis very impotant, because it can determine the further prognosis and curability of disease. Unfortunately, in many cases the first symptoms are due to metastases in central nervous system. At the begin these symptoms are very similar to symptoms appearing in case of primary brain tumors.

It is a well known neurosurgical dilemma: the ablastic surgical removal of malignant gliomas is impossible due to infiltrative propagation in most of cases, but the removal of lung cancer metastases is a routine neurosurgical intervention. These declarations are true of course only in case of solitary and easily accessible nodules, the multiple metastases cannot be removed surgically.

Primary brain tumors and metastases can be differentiated by good probability using a simple imaging technique, CT or MRI. The differentiation is based on different appearance: primary brain tumors infiltrate deeply the environment, they are irregular shaped surrounded by some edematic area, while the metastasis differs sharply from normal brain, appears as a regular spheroid nodule with typical perifocal edema.

It’s very important to detect brain metastases of lung cancer even in symptomless cases, because the surgical treatment of pulmonary disease and the prognosis basicly depends on it. The primary tumor alone with a solitary brain metastasis can be curable by an adequate treatment, thanks to the minimal or no invasiveness of metastasis in the brain. Accordingly to international literature, the peritumoral invasion can be related definitely to extracellular matrix (ECM) components. The reason of different resecability observed between the glioma

and lung cancer metastasis is based mainly on the differences of ECM structure of both tumor types and the different properties of macromolecular ingredients of ECM.

The multicellular organisms need an intercellular matrix providing connection, communication, cooperation, migration, defense and development of cells. This matrix is named extracellular matrix (ECM). The ECM components are produced intracellular, and transported via exocitosis into the environment, or a part of these molecules will be built into the cell membrane. Thus, if we search ECM-components, we can found them intra- or extracellularly or even in the cell membrane.

In this trial we investigated the different molecular background of invasiveness in both tumors using mRNA-and protein analysis techniques.

2. Arising questions and aim of the study

a. To collect ECM components playing pivotal role in peritumoral invasion reported in the literature, and create a so-called „invasion panel" of them.

b. To find ECM molecules responsible for the different activity in peritumoral invasion in case of gliomas and lung cancer brain metastases by mRNA and protein analysis.

c. To identify the molecules playing role in invasion of astrocytoma grade II, a tumor with benign properties but with ability to infiltrate.

d. To develop a simple microscopic routine test for identification of ECM components, promoting the further clinical application.

3. Tissue samples and methods

Tissue samples

Tissue samples were collected during neurosurgical operations. The samples were frozen promptly after removal on the surface of liquid nitrogen and were stored at –80°C until processing. Each sample was collected from a different patient. Sections for histological

analysis and immunohistochemistry were cut from the same samples used for mRNA analysis. All procedures were approved by the National Ethical Committee, and every patient signed an informed consent form.

RNA analysis

The mRNA expression of the ECM-related molecules was determined by real-time quantitative reverse transcriptase–polymerase chain reaction (RT–PCR). The expression of tumor markers (glial fibrillary acidic protein [GFAP] and cytokeratins 18 and 19), the proliferation marker Ki-67 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was also tested. RNA analysis was performed as described in our articles. Briefly, total RNA was isolated from TriReagent lysates according to the manufacturer's instructions. Total RNA was converted to single-stranded cDNA using the High Capacity cDNA Archive Kit with RNasin (Applied Biosystems, USA) and 600 ng of total RNA per sample in one reverse transcription reaction. cDNA transcribed from 100 ng of total RNA was loaded into each 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 Cards were analyzed with the SDS 2.1 software as relative quantification studies with automatic threshold settings, and the C_T values were exported for further analysis. GAPDH showed the least variation among the samples and was used as the reference gene to calculate the dCt value for each gene. Expression values were calculated using the comparative C_T method.

Histochemistry

Frozen samples were fixed in Saint Marie's fixative for 24 h at 4°C. After fixation and dehydration, the tissue samples were embedded in wax, and 5-μm sections were cut. The sections were stained with hematoxylin–eosin, and immunohistochemical reactions were carried out according to the protocol detailed in our articles. Briefly, slides were pre-incubated in ready-to-use 2.5% normal horse serum (Vector, Burlingame, CA, USA) for 30 min at 37°C to prevent nonspecific binding of primary antibodies. The sections were then incubated overnight at 4°C with the appropriately diluted antibodies. The immunohistochemical 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). Immunohistochemistry results were evaluated by three different investigators experienced in histology and scored from 1 to 4. Morphological evaluations were conducted separately.

We performed our investigations in four phases.

In the first phase we assembled the list of molecules reported significant role in peritumoral invasion. The mRNA expression of the genes was determined in five glioblastoma, five peritumoral normal brain tissue and five lung adenocarcinoma intracerebral metastasis samples. By comparing the mRNA expression of different molecules the group of molecules for following examinations was selected. This list of molecules, assembled by preliminary examinations was named „invasion panel".

In second phase we investigated the invasion panel molecules in more glioblastoma and pulmonary adenocarcinoma metastasis samples. The mRNA expression level of 23 molecules of peritumoral invasion was evaluated, and in five of them immunohistochemical examination (IHC) were also performed.

In the third phase we analyzed 30 molecules in 30 samples: eleven glioblastomas, ten adenocarcinomas of pulmonary origin and nine peritumoral normal brain tissues. Immunohistochemical staining was made for seven molecules.

In phase four we investigated 36 samples altogether: nine astrocytoma grade II, ten pulmonary adenocarcinoma metastases, nine schwannomas and nine tumor-free peritumoral brain samples were analyzed. In this phase the invasion panel contained 26 molecules and four molecules were stained immunohistochemically.

Each sample designed for mRNA analysis was first sent for histological investigation to confirm the histological diagnosis. The histological diagnosis was established in all cases by an experimented neuropathologist. The samples were coded and used independently from further clinical procedures.

Statistical evaluations

The Mann-Whitney U test was used to identify genes with significantly different expression levels between different sample group, and $p < 0.05$ was considered statistically significant. For testing the specificity of mRNA expression patterns of distinct histological tissue groups, hierarchical clustering was performed using complete linkage analysis with Pearson correlation.

4. Results

a. mRNA expression

Phase 1.

In the 1st phase we determined the expression level of 96 molecules in glioblastoma, pulmonary adenocarcinoma brain metastasis and peritumoral brain tissue samples. Based on our evaluations we established a group of molecules, found to be useful for further examinations. This group of target molecules was named „invasion panel".

Phase 2.

The mRNA expression of the invasion panel was determined on a larger number of tissue samples. By comparison of mRNA expression values in tumors of different origin the brevican, neurocan, neuroglycan-C, syndecan-2, tenascin-C, versican and MMP-2 showed significantly higher values in glioblastomas. The mRNA expression of syndecan-1 and 4 was higher in metastatic tumor. Regarding other molecules there was no significant changes between glioblastoma and the metastatic tumor.

The tumor markers showed specific expressions in each tumor type. Their mRNA level was typically increased, clearly concerning the histological type of sample. The expression of Ki-67 was increased similar to each tumor cases, concerning the high proliferation activity and the plenty of cell (and not necrosis) of samples.

Phase 3.

In the third phase thirty samples were investigated in three different homogenic histological groups: eleven GBM samples, originating from the marginal zone, ten samples from intracerebral metastasis of adenocarcinoma, and nine from normal brain tissue. Tumor markers and the Ki-67 proliferation marker concerned the diagnosis.

<u>A) GBM versus normal brain</u>

The agrin, fibronectin, laminin α-4, β-1 and ß-2, perlecan, syndecan-1, tenascin-C, CD44, CD168, HAS-2, MMP-2 ands-9 mRNS expression was significantly higher in GBM, compared with normal brain tissue, while the mRNA expression of syndecan-4, tenascin-R and HAS-1 were statistically lower in GBM. The mRNA levels of other investigated molecules were not significantly different in the normal brain tissue and GBM.

B) Intracerebral adenocarcinoma metastasis and normal brain tissue

We have found significantly decreased mRNA expression in case of brevican, laminin α-1, matrilin-2, neurocan, neuroglycan-C, syndecan-2, tenascin-R, and HAS-1 in pulmonary adenocarcinoma versus normal brain tissue. In contrary, mRNA levels of agrin, fibronectin, laminin ß-1, ß-2 and γ-1, perlecan, syndecan-1 and -4, CD168 and MMP-9 mRNS were statistically higher in metastasis. There was no significant difference in mRNA levels of aggrecan, laminin α-2, -4, matrilin-1, tenascin-C, versican, CD44, chondroitinases, HAS-2, -3, MMP-2 between the two tumor types..

C) GBM and pulmonary adenocarcinoma metastasis

Comparing the tumors of different origin, the mRNA expression of brevican, matrilin-2, neurocan, neuroglycan-C, tenascin-C and R, CD44, HAS-2 and MMP-2 were found significantly higher in GBM, than in adenocarcinoma metastasis. Syndecan-1 and -4 was higher in adenocarcinoma metastasis. There were no statistically significant differences in cases of other molecules.

Phase 4.

In this phase of the study the results of 3 tumors of different origin and peritumoral brain tissue were analyzed. Comparing the gene expression in adenocarcinoma metastases and in grade II astrocytoma, cadherin-3, collagen type I, III and IV, fibronectin, perlecan, syndecan-1 and -4 and MMP-9 expression were significantly higher in metastasis. On the contrary, brevican, cadherin-2, laminin alpha-4 and beta-2, matrillin-2, neurocan, syndecan-3, tenascin-C and -R, versican, HAS-2, and MMP-2 mRNA levels were higher in astrocytoma.

The mRNA expression of cadherin-3, neurocan, syndecan-1 and MMP-9 was found to be higher in metastatic tumor in comparison to schwannoma, while cadherin-2, collagen IV and VIII, laminin alpha-4, beta-1 and beta-2, matrillin-2, neuroglycan-C, perlecan, syndecan-3, HAS-2 and MMP-2 levels were found to be higher in schwannoma.

In comparison of schwannoma to astrocytoma samples, collagen alpha -1, collagen III, IV and VIII, fibronectin, perlecan, matrillin-2, syndecan-4, laminin alpha-4, beta-1 and beta-2 were found to be higher in schwannoma, while brevican, neuroglycan-C, tenascin-C and -R, neurocan and versican was dominantly expressed in astrocytoma samples.

By comparing astrocytoma to normal brain tissue we detected higher mRNA expression of agrin, brevican, cadherin-2, collagen I, III and IV, fibronectin-1, laminin alpha-4 and beta-

2, matrillin-2, neurocan, perlecan, syndecan-3, tenascin-C, versican, HAS-2, and MMP-2 and -9 in astrocytoma samples. On the contrary, syndecan-4 and HAS-1 mRNA levels were found to be higher in normal brain tissue.

In schwannoma samples mRNA level of agrin, collagen I, III IV and VIII types, fibronectin, laminin alpha-4, beta-1 and beta-2, matrillin-2, perlecan, syndecan-3 and -4, HAS-2 and MMP-2 were higher in comparison to normal brain while in normal brain level of mRNA of brevican, neurocan, neuroglycan-C and tenascin-R showed higher mRNA expression.

The mRNA level of agrin, cadherin-3, a collagen I, III, IV and VIII types, fibronectin, perlecan, syndecan-1 and -4 was significantly higher in brain metastases compared to brain tissue. On the contrary, mRNA expression of brevican, cadherin-2, neurocan, neuroglycan-C, matrillin-2, syndecan-3, tenascin-R and HAS-1 were found lower in metastases than in the normal brain tissue.

b. Immunohistochemical results

Phase 1.

There were no IHC examinations in this phase.

Phase 2.

We examined the intensity of immunohistochemical reaction of five molecules. The staining intensity of neurocan, versican and MMP-2 was higher in glioblastoma compared to metastasis. The syndecan and MMP-9 were most intensive in adenocarcinoma.

Phase 3.

In the third phase we performed immunohistochemical staining in glioblastoma, adenocarcinoma and peritumoral normal brain tissue samples by seven molecules: agrin, hyaluroronan, neurocan, syndecan, versican, MMP-2 and 9. Accordingly to morphological evaluation the immunoreactivity was most expressed in case of agrin, neurocan, syndecan and versican, both in extracellular space and in the cell membrane, while the MMP-s showed strong immunoreactivity in the membrane and intracellular. The strongest immune staining was in case of agrin, syndecan and MMP-9 in pulmonary adenocarcinoma, while MMP-2, neurocan and hyaluronan had highest values in GBM samples. The tumor marker levels and the Ki-67 level were in accordance to the histology.

Phase 4.

In the last phase of our trial we investigated four histological types (astrocytoma grade II, brain metastasis of adenocarcinoma, schwannoma and peritumoral ("normal" brain tissue) IHC staining was performed to detect four molecules: brevican, neurocan, tenascin – C and versican). The immunoreactivity of neurocan, tenascin-C and versican were most intensive on cell membrane and in the extracellular space. The most intensive staining of every molecule was observed in astrocytoma grade II.

The immunostaining was less intensive in normal brain for neurocan, tenascin-C and versican; schwannoma samples were less intensive in immunostaining for brevican and neurocan. It was found less intensive staining in normal brain in case of neurocan, tenascin–C and versican, in the schwannoma samples a less intensive immune staining was found for neurocan and brevican. The less immunostaining reaction for versican was detected in metastatic tissue. The tenascin–C staining was less expressed both in schwannoma and adenocarcinoma. The tumor markers and the Ki-67–intensity were in accordance to the histological diagnosis.

5. Discussion

a. The role of ECM in peritumoral invasion of primary and metastatic brain tumors

There is no evident conclusion in the literature about molecules that are responsible for invasiveness of different tumor types in the brain.

The two types of malignant tumors investigated in this study represent two endpoints: the astrocytoma produces no metastases despite of its highly active invasiveness; the pulmonary adenocarcinoma metastasizes easily and fast but does not infiltrate the peritumoral brain parenchyma. Brain is not the only target site for lung cancers, but the most frequented one. The reason for all these phenomena can be found in the structure and function of ECM.

The efficacy of oncotherapy is determined by the extent of tumor resection. It is true especially in brain tumors, where the peritumoral invasion makes the radical resection impossible. The peritumoral infiltration is observed not only in malignant gliomas, but also in low grade tumors like grade II. astrocytomas. On the contrary, the metastases of pulmonary

adenocarcinoma are much less invasive, so their radical surgical removal is a routine neurosurgical intervention.

The aim of this study was to define and compare the expression pattern of molecules involved in the peritumoral invasion process of brain tumors of different origin.

Furthermore, it was tried to identify molecules that are mainly responsible for the peritumoral invasiveness of grade II astrocytoma. While the invasion is mainly dependent on interactions between tumor cells and numerous ECM components, the first step was to select target molecules participating in peritumoral infiltration. 96 molecules were tested based on the literature. By targeted examinations, we assembled from these 96 components the group of molecules typical to intracranial tumors. This selected group of molecules was named „invasion panel". In our further examinations and mRNA expression evaluations we investigated this panel in different intracranial neoplasms.

b. The examination of invasion panel in adenocarcinoma brain metastases and glioblastoma

In comparison of lung cancer brain metastases and glioblastoma we determined the gene expression of 30 ECM components, to clear the molecular background of different invasivity. The mRNA expression of 21 ECM components, seven proteases, hyaluronic acid membrane receptor (CD44) and CD168 was determined by quantitative RT-PCR (reverse transcriptase-polymerase chain reaction). Based on results of mRNA analysis seven molecules (agrin, neurocan, syndecan, versican, MMP-2 and 9, and hyaluronic acid) were also investigated by immunohistochemical staining.

Our evaluations partially concerned, partially completed the results published previously in the literature. We detected significant differences between the normal brain and glioblastoma samples regarding mRNA profiles of fibronectin, laminin β-1, perlecan, syndecan-1, -4, tenascin-C, -R, CD44, CD168, HAS-1, -2, and MMP-2.

There are no sufficient data in case of astrocytomas about aggrecan, matrilin, perlecan, neuroglycan-C, neurocan, CD 168 or chondroitinases. According to our observation the mRNA expression of CD168 and perlecan is significantly higher in glioblastoma (versus normal brain tissue) and there are no similar differences between these tumors regarding other ECM molecules.

We compared intracerebral adenocarcinoma metastases to normal brain tissue, and among the 30 investigated molecules significant differences were observed in 18 molecules.

Some molecules are typical to the original tissue, as well as brevican, neurocan, neuroglycan-C in brain tissue samples, and others are playing probably role in peritumoral invasion (fibronectin, syndecan-1, 4, CD-168 and MMP-9). The explanation of increased expression of agrin, laminin, ß-1, ß-2, γ- and perlecan in intracerebral adenocarcinoma metastasis requires further investigations.

According to our results mRNA expressions of 11 molecules were different between GBM and adenocarcinoma metastases. While the mRNA expression of brevican, matrilin-2, neurocan, neuroglycan-C and tenascin R was higher both in normal brain and glioblastoma compared to metastases, these molecules seem to be specific for glial tissue. On the other hand, the mRNA expression of tenascin-C, CD44, HAS-1 and MMP-2 was higher in glioblastoma compared to metastases.

It can be concluded that these molecules, found to be present in higher levels in both glioblastoma and normal brain suggest the similarity of primary brain tumor and peritumoral brain tissue, so that the tumor cells are familiar with the surrounding brain parenchyma and are able to infiltrate it. The molecules, expressed higher in glioblastoma compared to other tissues, are probable necessary for the invasion process.

Finally, higher levels of syndecan 1 and 4 were detected in adenocarcinoma, but it seems to be not sufficient for invasion in this strange environment.

These results suggest that syndecan 1 and 4 have probably no important role in intracerebral peritumoral invasion. To determine the posttranslational manifestation of mRNA- results and to gain morphological information, we performed immunohistochemical analysis in case of seven molecules (agrin, neurocan, syndecan, versican, MMP-2, MMP-9, hyaluronic acid). The differences in staining intensity of three histological groups correlated well with mRNA expression levels of these molecules. During immunohistochemical analysis agrin, syndecan and MMP-9 was concerned to be predominant in pulmonary adenocarcinoma, while MMP-2, neurocan and hyaluronan showed the strongest immunostaining in glioblastoma.

Interestingly the most intensive staining and mRNA expression agrin was found in metastasis. Agrin is a very important component at blood-brain barrier, but its role in intracerebral adenocarcinoma metastases are not yet clarified.

The most intensive immunostaining of MMP-9 was found in adenocarcinoma metastases, but the highest mRNA expression was evaluated in glioblastoma group. Although the highest mRNA expression of neurocan was found in normal brain tissue,

immunohistochemistry showed higher intensity in tumors. These changes are possibly related to post-translational events, and further investigations are needed to clarify them.

The hyaluronic acid was present mainly in glioblastoma. While its receptor CD44 had higher mRNA expression too, their general role in process of invasion can be concerned.

c. Conclusions

By comparison of mRNA expression of 30 invasion related molecules in glioblastoma, normal brain and intracerebral adenocarcinoma metastasis we identified some molecules possibly participating in the extremely high invasiveness of glioblastoma. Tenascin-C, CD44, and MMP-2 seem to be mostly involved in the peritumoral infiltration of glioblastoma, but we cannot concern the positive role fibronectin and syndecans in invasiveness of gliomas. Further investigations suggest the possible role of brevican, neurocan, neuroglycan-C and matrilin-2 in the invasiveness of glioblastomas.

In phase 4 we investigated the gene expression of invasion panel on samples of astrocytoma grade II, schwannoma and pulmonary adenocarcinoma. By evaluation of mRNA expression of 26 invasion-related molecules an expression pattern specific for each histological types was generated. The specificity of changes of expression pattern was tested by cluster analysis. This statistical test clarified that each histological tumor type has an own characteristic molecular pattern („invasive spectrum"). These results suggest that the pattern of mRNA expression of 26 molecules is very typical to the investigated tumors.

Brevican, neurocan, neuroglycan, tenascin, versican and MMP-2 were typical in normal brain tissue and grade II astrocytoma, while schwannoma and adenocarcinoma pattern contained mainly collagens, fibronectin, syndecans, laminins and cadherin. This typical invasive spectrum suggests high amount of connective tissue in schwannoma and metastasis, while in gliomas appear some specific glucose-aminoglycans and proteoglycans. The surprisingly high similarity between the grade II astrocytoma and normal brain tissue can explain the highly active invasion of astrocytoma into the surrounding brain tissue. The great difference between the invasive spectrum of adenocarcinoma and normal brain helps us to understand the decreased peritumoral infiltration of metastasis.

The IHC results supported that brevican, neurocan, tenascin-C and versican are present in high level in astrocytoma. These proteins are also present in normal brain tissue, but appear in small amount in schwannoma and metastasis as well.

The efficacy of traditional oncotherapy in inoperable cases of metastasizing solid tumors – radiotherapy and chemotherapy – is limited. These procedures are toxic not only on tumor cells, but normal tissues too. Their selectivity can be a little bit increased, but their doses is limited, not enough for curative action in case of lung cancer.

In the future probably more effective chemotherapeutic agents will be achievable or new devices in radiotherapy providing e.g. the better planning of target volume. Despite of new opportunities in treatment of lung cancer increasing efficacy of oncotherapy, there is not yet significant improvement in international statistics about survival data in case of lung cancer. One of the reasons is surely the extremely high metastasizing potential of lung cancer. The chances for curative surgery are reducing due to the early appearance of metastases, and there is not else curative standard procedure available. Every new result about possible target for anticancer treatment or developing drugs to inhibit tumor invasion and development of metastases is very valuable for the future.

6. Summary and new results

Effectiveness of therapy in case of malignant intracerebral tumors depends mainly on the invasive behavior of the tumor. In recent work we tried to find new perspectives in inhibition of tumor metastases development and invasion.

We compared the molecular composition of extracellular matrix of different tissues like normal brain, glioblastoma, astrocytoma grade II and intracerebral pulmonary adenocarcinoma metastasis. For these purposes fresh frozen tissue samples were used collected by routine clinical neurosurgical procedures at the Department of Neurosurgery, University of Debrecen.

For determination of the mRNA expression of the invasion-related extracellular matrix proteins quantitative reverse transcriptase polymerase chain reaction (QRT-PCR) was used. Then immunohistochemical staining was performed for ECM molecules found to be in connection with invasion processes in the peritumoral brain.

The trial was made in four phases. At the first phase 96 molecules reported in the literature as possibly active agents in tumor growth and metastatization were investigated in glioblastoma and lung adenocarcinoma brain metastases. The mostly differ 30 molecules were selected and named „invasion panel”. In the second phase we checked the invasion panel in further glioblastoma and intracerebral metastases samples of pulmonary adenocarcinoma. In

the third phase our examinations were extended to normal brain tissue and in the fourth phase we analyzed four different histological tissue types, as well as normal brain, schwannoma, astrocytoma grade II, and intracerebral adenocarcinoma metastasis.

The mRNA-levels of invasion panel molecules were analyzed by cluster analysis that proved tumor specific expression pattern of the selected molecules. We named this „invasion spectrum".

Our observations partially concerned, partially completed the results reported preliminarily in the literature. New results:

1.) An „ invasion panel" was identified, consisting ECM components probably responsible for peritumoral invasion.

2.) Definite differences could be established regarding the expression pattern of the invasion panel in cases of distinct tumor types. This specific expression panel is named as „invasion spectrum" characterizing tissues of different histological types.

3.) Four molecules (brevican, neurocan, tenascin C and versican) were identified which are probably responsible for the extremely high invasion feature of gliomas.

In summary, identification of exact molecules playing indispensable role in peritumoral invasion can serve as new targets for anticancer therapy in the future.

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List of publications related to the dissertation

1. **Varga, I.**, Hutóczki, G., Szemcsák, C.D., Zahuczky, G., Tóth, J., Adamecz, Z., Kenyeres, A., Bognár, L., Hanzély, Z., Klekner, Á.: Brevican, Neurocan, Tenascin-C and Versican are Mainly Responsible for the Invasiveness of Low-Grade Astrocytoma.
Pathol. Oncol. Res. 18 (2), 413-420, 2011.
DOI: http://dx.doi.org/10.1007/s12253-011-9461-0
IF:1.366

2. **Varga, I.**, Hutóczki, G., Petrás, M., Scholtz, B., Mikó, E., Kenyeres, A., Tóth, J., Zahuczky, G., Bognár, L., Hanzély, Z., Klekner, Á.: Expression of Invasion-Related Extracellular Matrix Molecules in Human Glioblastoma Versus Intracerebral Lung Adenocarcinoma Metastasis.
Cent. Eur. Neurosurg. 71 (4), 173-180, 2010.
DOI: http://dx.doi.org/10.1055/s-0030-1249698
IF:0.472

List of other publications

3. Klekner Á., **Varga I.**, Bognár L., Hutóczki G., Kenyeres A., Tóth J., Hanzély Z., Scholtz B.: Különböző invazivitású agydaganatok extracelluláris mátrixának expressziója.
Ideggyógy. Szle. 63 (1-2), 38-43, 2010.
IF:0.236

4. Bágyi, K., Haczku, A., Márton, I., Szabó, J., Gáspár, A., Andrási, M., **Varga, I.**, Tóth, J., Klekner, Á.: Role of pathogenic oral flora in postoperative pneumonia following brain surgery.
BMC Infect. Dis. 9, 104-113, 2009.
DOI: http://dx.doi.org/10.1186/1471-2334-9-104
IF:2.55

5. Petrás M., Hutóczki G., **Varga I.**, Jr. Vereb G., Szöllősi J., Bognár L., Ruszthi P., Kenyeres A., Tóth J., Hanzély Z., Scholtz B., Klekner Á.: Különböző eredetű malignus agydaganatok invazivitásának panelszerű vizsgálata.
Magyar Onkol. 53 (3), 253-258, 2009.
DOI: http://dx.doi.org/10.1556/MOnkol.53.2009.3.3

6. Bágyi, K., Márton, I., Szabó, J., Andrási, M., Gáspár, A., **Varga, I.**, Bognár, L., Klekner, Á.: Efficacy of pre-operative cephalosporin prophylaxis in controlling pathogenic oral bacterial growth in comatose patients.
J. Med. Microbiol. 57 (Pt1), 128-129, 2008.
DOI: http://dx.doi.org/10.1099/jmm.0.47381-0
IF:2.19

7. Jenei M., Veres I., Schmidt E., **Varga I.**, Remenyik É.: Az erythrodermáról és rühatkafertőzésről scabies norvegica két esete kapcsán.
Orv. Hetil. 149 (47), 2229-2235, 2008.
DOI: http://dx.doi.org/10.1556/OH.2008.28479

8. **Varga I.**, Dezső B., Horváth Á., Kiss S.S.: A tüdő, a mellhártya és a mediastinum daganatai.
In: Klinikai onkológia a gyakorlatban. Szerk.: Szántó János, Medicina Könyvkiadó Rt., Budapest, 129-157, 2005.

9. **Varga I.**, Szűcs M.Z., Moldvay J., Jáger M., Szűcs G., Bordás M., Molnár L., Szilasi M., Strausz J.: A bronchoszkópia szerepe a nyelőcsődaganatok kivizsgálásában és kezelésében.
Orv. Hetil. 145 (45), 2285-2288, 2004.

10. **Varga I.**, Brugós L., Farkas M., Szilasi M.: Bronchoszkópia az intenzív osztályokon.
Orv. Hetil. 145 (18), 957-961, 2004.

11. Czirják, L., Koncz, A., **Varga, I.**, Dévényi, K., Kumánovics, G., Szűcs, G.: Investigation of the alveolar macrophages and T lymphocytes in 15 patients with systemic sclerosis.
Clin. Rheumatol. 18 (5), 357-363, 1999.
DOI: http://dx.doi.org/10.1007/s100670050119
IF:0.615

12. **Varga I.**, Koncz A., Brugós L., Szilasi M., Szakács É.: Első tapasztalataink a légúti stent-tel.
Med. Thorac. 51 (4), 154-157, 1998.

13. Koncz A., **Varga I.**, Kollár J., Winkler I.: Többszörös kerekárnyék radiologiai képével jelentkező pilmonális tuberkulózis esete.
Med. Thorac. 46 (7), 241-246, 1993.

DEBRECENI EGYETEM KENÉZY ÉLETTUDOMÁNYI KÖNYVTÁRA ÉS EGYETEMI LEVÉLTÁR

14. Koncz A., Herman K., **Varga I.**, Szabó Z., Szilasi M.: Sürgős bronchológiai vizsgálatok indikációja és kivitelezése szívműtétek perioperatív időszakában.
Med. Thorac. 46 (12), 467-474, 1993.

15. Dobrán I., Mórocz I., Vezendi S., **Varga I.**: Endobronchialis szemcsés-sejtes tumor: Abrikosoff myoblastoma.
Med. Thorac. 44 (11), 487-491, 1991.

16. Faragó E., Szilasi M., Csontos Z., **Varga I.**, Mihóczy L.: Antibiotikum szint mérések hörgőkarcinomás betegek köpetében.
Med. Thorac. 43 (6), 249-258, 1990.

17. Szilágyi J., Bene J., **Varga I.**: Új módszer az orrlégzés vizsgálatára.
Pneumonol. Hung. 41, 57-63, 1988.

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