ORIGINAL ARTICLE

UDC 616.831-006.328-091.8

IMMUNOHISTOCHEMICAL EXPRESSION OF MMP-9 AND VEGF IN BENIGN AND MALIGNANT BRAIN MENINGIOMAS

Vanda Voteva,
e-mail: vanda.voteva@yandex.ua

W.Voteva, S. Tertishniy

Zaporozhye State Medical University

Key words: *meningioma, neoangiogenesis, MMP-9, VEGF.*

Introduction. Meningiomas of the brain are generally benign slow growing tumors with favorable outcome, but in some cases they can recur and demonstrate unpredictable course, despite their benign histological structure. According to the classification of CNS tumors given by WHO 2007 edition, histological malignancy grade and proliferation index are one of the most important predictors of recurrence rate [1,2]. On the other hand, the extent of surgical resection is also an important factor, influencing on the risk of recurrence, which in turn depend upon the tumor localization, its relation to the adjacent anatomical structures, extent of invasion and peritumoral brain edema [2,3].

Little is known about mechanisms of meningiomas invasion into the surrounding structures to date. It is believed that expression of matrix metalloproteinases (MMPs) can be related with this process, especially the matrix metalloproteinase of 9 type (MMP-9) [4,5]. Matrix metalloproteinases are able to degrade proteins of extracellular matrix and basement membranes, playing the defining role in invasion and metastasis. The role of MMPs in invasion and metastasis of prostate, breast and lung cancer is reported in recent studies [6,7]. The tendency of increased expression of MMP-9 with increasing grade was revealed in meningiomas [8, 9].

Except the determinant influence on the invasive abilities of meningiomas, MMP-9 also has important relation to the neoangiogenesis. The growth of the tumor and its progression depends upon the formation new capillary network to the great extent, which happens because of secretion of different angiogenic substances by the tumor tissue and underlies the so called "angiogenic switch" [6,10]. MMP-9 is one of the crucial proangiogenic molecules, which induces this event in many neoplasms, especially in meningiomas [5,9,10]. MMP-9 causes not only the degradation of the collagen IV in basement membranes, which promotes invasion and migration of endothelial cells, but also has an equally important indirect effects on the induction of new microvessel formation from preexisting capillaries. One of the mechanisms underlying the "angiogenic switch" is mobilization and activation of the angiogenic mitogens from matrix stores, this process is facilitated by MMP-9, which acts as an exceptionally potent nanomolar angiogenic factor, releasing vascular endothelial growth factor (VEGF) from matrices [6, 9].

The results of researches concerning the expression of MMP-9 and VEGF in benign and malignant meningiomas are controversial. Many authors directed their search towards detection of the relation between expression of MMP-9 and invasion or tumor grade or between expression of MMP-9 and microvessel density [5,9]. By the way, special attention should be devoted to the positive induction of neoangiogenesis caused by MMP-9 in case of growth factors recruitment, such as VEGF.

Goal: determination of the expression level of MMP-9 and VEGF in different subtypes of benign meningiomas and in anaplastic brain meningiomas, to establish the correlation between these markers.

Materials and methods. All specimens involved in this study were collected from 36 patients treated by neurosurgical resection of meningiomas. The age range was 25 to 75 years. All benign meningiomas revealed location on the convexity of the cerebral hemisphere. Among anaplastic meningiomas 60% had convexity location, 20% – supratentorial and 20% – parasagittal. In the samples we studied, 26 cases were benign meningiomas (grade I), among them 8 meningiomas were represented by meningothelial subtypes (n=8), 8 meningiomas – by fibroblastic (n=8),

transitional meningiomas comprised 10 cases (n=10). The histological type and grade of the specimens were classified according to the WHO 2007 standard [1]. Group of malignant meningiomas (grade III) consisted of 10 specimens (n=10) with 6 recurrent cases (n=6). Routine histological sections were cut from formalin fixed, paraffin embedded tissue and stained with hematoxylin and eosin (H&E).

The expression levels of MMP-9 and VEGF were measured by immunohistochemical staining and En Vision. Tissues were prepared as paraffin sections. Prior to immunohistochemistry, the sections were deparaffinized with xylene and rehydrated in graded ethanol. The sections were then prepared in a pressure cooker to 121°C for 2 minutes in citrate buffer solution (pH=6,0) to restore the antigen immunoreactivity. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide, serum was then applied to prevent unspecific adherence of serum proteins. After the incubation with primary antibodies corresponding antigens were revealed using the DAB visualization. The expression level of VEGF was detected with the help of monoclonal antibodies *Mo a-Hu Vascular Endothelial Growth Factor (VEGF), Clone VG1 ("DAKO" – Denmark).* The expression level of MMP-9 was determined using policlonal antibodies *Rb a-Hu MMP-9 (92 kDa Collagenase IV) ("Thermo Fisher Scientific Inc." – USA).* The result of every immunohistochemical reaction was assessed semiquantitatively by calculating percentage of positively stained cells in the standardized microscopic field of vision of the Axioplan 2 microscope ("Carl Zeiss" – Germany) under 200x field, in each case 200 cells were analyzed in 5 fields of vision.

The expression strength was analyzed and graded based on the positive ratio and intensity of immunoreactivity. The immunohistochemical score (IHS) for the MMP-9 and VEGF was evaluated semiquantitatively using the immunoreactive scale from 0 to 3 scores, according which 0 was equal to the absence of expression, 1 – weak immunoreactivity less than in 30% of cells, 2 – moderate diffuse expression in 31-60% of cells, 3 – strong diffuse expression more than in 60% of cells. High expression considered when IHS was 2 and more, low expression – when IHS was less than 2.

All statistical analyses were carried out using SPSS 17.0 software for Windows. The expression of MMP-9 and VEGF were expressed as the mean ± standard deviation (mean ± SD). Spearman's rank correlation coefficient test was applied for examining the correlations among the expressions of MMP-9 and VEGF. The association between the clinicopathological parameters and immunohistochemical results was analyzed with the Chi-square or Fisher's exact test (if N < 5). MMP-9 and VEGF expression were compared between benign meningiomas subtypes by using the Kruskal-Wallis H test and Mann-Whitney U test. Between tumor grades these markers were compared by using the Mann-Whitney U test. P-values < 0.05 were regarded to be statistically significant.

Results and discussion. Most of the benign (grade I) meningiomas revealed positive immunoreactivity of MMP-9. Positive expression was mainly observed in the cytoplasm of tumor cells and in the majority of microvascular endothelial cells as brownish granules. The positively stained cells had flaky, spotty or scattered appearance. Meningiomas with the low expression level of MMP-9 comprised 53,85% of cases (14/26), medium and high expression level was detected in 46,15% of cases (12/26).

The highest expression level of MMP-9 was observed in the transitional subtypes of grade I meningiomas, the score was 2,32±0,14. It was higher than the expression level of this marker in meningothelial subtypes, which in turn was 1,95±0,21, but the difference was not statistically significant (p=0,191). Fibroblastic subtypes were characterized by the less expression level of MMP-9 (1,75±0,18), than meningothelial, but the results did not reach the statistical significance (p=0,423). Only transitional subtypes revealed the statistically higher expression level of MMP-9 comparing to the fibroblastic tumors (p=0,016). Other researches also noted presence of the differences between the immunoreactivity of this marker in benign meningiomas. Some authors reply that the highest expression level of MMP-9 is peculiar to the fibroblastic subtypes, as well as for the transitional ones [9,12], others suggest that the highest expression levels belong to the psammomatous and meningothelial subtypes, but they did not obtain the statistically significant results [13]. In our research any considerable differences were absent between the expression levels of MMP-9 in grade I meningiomas (p=0,062, Kruskal-Wallis H test), table 1.

What concerns VEGF expression, the majority of benign meningiomas showed low expression level of this marker in 69,23% of cases (18/26). Positive staining was noted both in the microvascular endothelial cell cytoplasm and in cytoplasm of tumor cells. The high expression level was present in 30,76% of grade I meningiomas (8/26). The highest expression level of VEGF was observed in the transitional subtypes of grade I meningiomas, the score was 1,64±0,16, which is not controversial to the data obtained by other scientists [13,14]. It was considerably higher than the extent of expression in meningothelial subtypes (the score was 0,85±0,08), p=0,001 and significantly higher than the expression level of VEGF in fibroblastic meningiomas (0,95±0,14), p=0,003. There were no statistically significant differences between immunohistochemical score of VEGF among meningothelial and fibroblastic subtypes (p=0,530). With the help of Kruskal-Wallis H test the expression level of VEGF was compared between different subtypes of benign

Table 1.

The comparative analysis of the VEGF and MMP-9 expression level in different subtypes of benign meningiomas according to the Kruskal-Wallis H test (mean ± SD)

Immunohistochemical marker	Meningothelial subtype (mean ± SD)	Fibroblastic subtype (mean ± SD)	Transitional subtype (mean ± SD)	P
VEGF	0,85±0,08	0,95±0,14	1,64±0,16	0,001
MMP-9	1,95±0,21	1,75±0,18	2,32±0,14	0,062

meningiomas, the statistically significant data were established (p=0,001), (table 1).

Anaplastic meningiomas represented high expression level of both antigens, the score for the MMP-9 was 2,8±0,8 and 2,08±0,16 for the VEGF. What is more, all specimens obtained from recurrent tumors revealed high expression level of MMP-9, which comprised 60% of cases (6/10). The positive staining was present as the brownish granules within the cytoplasm of tumor cells as well as in the cytoplasm of the endothelial cells lining microvessels. The positive immunoreactivity of VEGF in the endothelial cells of anaplastic meningiomas was noted in all cases, while cytoplasmic expression in tumor cells was absent in 40% of specimens (4/10). Comparative analysis of the MMP-9 expression between the grade I and grade III meningiomas established that malignant tumors were characterized by statistically higher expression levels of this marker than the benign (2,03±0,1 балів), p=0,001. The IHS of VEGF was also higher in the anaplastic meningiomas than in benign tumors (1,18±0,09), the result reached statistical significance (p=0,001). The comparative analysis of the VEGF and MMP-9 expression between different grades is shown in the table 2.

The formation of new blood vessels during tumor growth is a highly orchestrated process that depends upon the balance between stimulating and inhibiting angiogenic factors and involves matrix remodelling, cell migration, and regulated adhesive interactions between vascular cells and with the extracellular matrix. It is known that MMP-9 can promote the induction of neoangiogenesis in an indirect way through the VEGF activation [6,9,10]. So MMP-9 is able to modulate the endothelial cells behavior, which acquires the migration and invasive capabilities. Except this, MMP-9 is also connected with the vascular mimicry – the process when the tumor cells obtain the properties of the endothelial cells and create the tubular network from new microvessels with the help of the VEGF and corresponding receptors for this growth factor [11].

Taking into account the mechanisms of the neoangiogenesis mentioned above we decided to establish the presence of the correlation between the expression level of the MMP-9 and VEGF in benign and anaplastic meningiomas. The researches conducted in recent years contain sparse data about the relationship of these markers. The investigation provided by Barresi V. et al. [9] revealed the trend towards the correlation between the MMP-9 and VEGF, but results did not reach the statistical significance. Other authors found out that the considerable expression of both markers favored the incidence of the peritumoral brain edema due to the appearance of new pial blood vessels [13]. In our research the statistically significant relations were established between the expression levels of MMP-9 and VEGF in different histological subtypes of benign meningiomas as well as among the anaplastic meningiomas (table 3).

As the result of examination of the relationship between the clinicopathological variables and MMP9 expression it was found that the expression level of MMP-9 was associated with gender and recurrence. The association between the strong immunoreactivity of MMP-9 and the presence of recurrence is proved with the data obtained by other researchers [5, 8, 9]. In case of VEGF no statistically significant correlations were present except age (table 4).

Conclusions. 1. In benign meningiomas the low expression of MMP-9 was observed in 53,85% of cases, medium and high expression of MMP-9 was noted in 46,15% of meningiomas. At the same time statistical differences in the expression level of this marker among various subtypes were not found. The VEGF expression, conversely was significantly distinct between different histological subtypes of grade I meningiomas, with the highest immunoreactivity in the transitional tumors.

2. Anaplastic meningiomas were characterized by significantly higher expression levels of MMP-9 and VEGF than benign; besides, the expression of MMP-9 was associated with the tumor recurrence, which reflects the important role of this marker in the acquirement of invasive abilities by the tumor cells.

3. Using the correlation analysis it was detected that the statistically significant correlation exists between the MMP-9 and VEGF expression in meningiomas, which point to the association of this markers during the neoangiogenesis in brain meningiomas.

Prospects for further research in this area include exploring new interactions between potential angiogenic factors and inhibitors of neoangiogenesis in meningiomas. The data obtained in such researches can be used for the development of molecular targeted therapy of these tumors.

Table 2.

The expression level of the VEGF and MMP-9 in benign and malignant meningiomas according to the Mann-Whitney U test (mean ± SD)

Histological grade	VEGF (mean ± SD)	P	Histological grade	MMP-9 (mean ± SD)	P
grade I	1,18±0,09	0,001	grade I	2,03±0,1	0,001
grade III	2,08±0,16		grade III	2,8±0,08	

Table 3.

Association between expressions of MMP-9 and VEGF in benign and malignant meningiomas using Spearman's correlation analysis

Histological grade and subtype	VEGF (mean ± SD)	MMP-9 (mean ± SD)	Spearman's correlation coefficient	P value
Meningothelial subtype (grade I)	0,85±0,08	1,95±0,21	+0,46	0,042
Fibroblastic subtype (grade I)	0,95±0,14	1,75±0,18	+0,73	0,001
Transitional subtype (grade I)	1,64±0,16	2,32±0,14	+0,59	0,002
Anaplastic meningiomas (grade III)	2,08±0,16	2,8±0,08	+0,43	0,033

Table 4.

Correlation among VEGF, MMP-9 expression and clinicopathological parameters of patients with meningioma. investigated through Fisher's exact and Chi-squared tests

Variable	MMP-9 expression		P	VEGF expression		P
	Low (0–2 score)	High (≥2 score)		Low (0–2 score)	High (≥2 score)	
Gender						
Male (n=6)	0	6		4	2	
Female (n=30)	16	14	0,024	20	10	1,000
Age						
<45 years (n=10)	6	4		10	0	
≥45 years (n=26)	10	16	0,285	14	12	0,015
Grade						
Grade I (n=26)	14	12		18	8	
Grade III (n=10)	2	8	0,133	6	4	0,700
Invasion						
Present (n=6)	2	4		4	2	
Absent (n=30)	14	16	0,672	20	10	1,000
Recurrence						
Present (n=6)	0	6		4	2	
Absent (n=30)	16	14	0,024	20	10	1,000

REFERENCES

1. *WHO Classification of Tumors of the Central Nervous System / Eds D.N. Louis [et al.]. – Geneva, 2007. – 309 p.*
2. *Prognostic significance of Ki-67/MIB-1 proliferation index in meningiomas / S.H. Torp, C.F. Lindboe, B.H. Gronberg [et al.] // Clin. Neuropathol. – 2005. – Vol.24, № 4. – P. 170-174.*
3. *Meningioma grading: an analysis of histologic parameters / A. Perry, S.L. Stafford, B.W. Scheithauer [et al.] // Am. J. Surg. Pathol. – 1997. – Vol.21, № 12. – P.1455–1465.*
4. *Von Randow A.J. Expression of extracellular matrix degrading proteins in classic, atypical and anaplastic meningiomas / A.J. Von Randow, S. Schindler, D.S.Tews // Pathol. Res. Pract. – 2006. – Vol.202, № 5. – P. 365–372.*
5. *Increased co-expression of macrophage migration inhibitory factor and matrix metalloproteinase 9 is associated with tumor recurrence of meningioma / H. Quan, Zh. Song-Li, T. Xiao-Ying [et al.] // Int. J. Med. Sci. – 2013. – Vol.10, №3. – P. 276–285.*
6. *Farina A.R. Gelatinase B/MMP-9 in tumour pathogenesis and progression / A.R. Farina, A.R. Mackay // Cancers. – 2014, Vol.6, №1. – P. 240-296.*
7. *Krstic J. Transforming growth factor-beta and matrix metalloproteinases functional interplay in cancer; implications in epithelial to mesenchymal transition / J. Krstic, J.F. Santibanez // Cell Biol: Res. Ther. – 2014. – doi:10.4172/2324-9293.S1-004.*
8. *Expression of cell adhesion proteins and proteins related to angiogenesis and fatty acid metabolism in benign, atypical, and anaplastic meningiomas / A.T. Panagopoulos, C.L. Lancellotti, J.C. Veiga [et al.] // J. Neurooncol. – 2008. – Vol.89, №1. – P. 73–87.*
9. *MMP-9 expression in meningiomas: a prognostic marker for recurrence risk? / V. Barresi, E. Vitarelli, G. Tuccari [et al.] // J. Neurooncol. – 2011. – Vol.102, №2. – P. 189-196.*
10. *Barresi V. Angiogenesis in meningiomas / V. Barresi // Brain Tumor Pathol. – 2011. – Vol.28, №2. – P. 99-106.*
11. *Tubular network formation by adriamycin-resistant MCF-7 breast cancer cells is closely linked to MMP-9 and VEGFR-2/VEGFR-3 over-expression / A. Karoum, P. Mirshahi, A.-M. Faussat [et al.] // Mol. Cell. Pharmacol. – 2012. – № 685. – P. 1–7.*
12. *Expression of MMP-2 and -9 in short-term cultures of meningioma: influence of histological subtype / H.K. Rooprai, T.E. van Meter, S.D. Robinson [et al.] // Int. J. Mol. Med. – 2003. – № 12. – P. 977–981.*
13. *Role of VEGF and matrix metalloproteinase-9 in peritumoral brain edema associated with supratentorial benign meningiomas / E. Iwado, T. Ichikawa, H. Kosaka [et al.] // Neuropathology. – 2012. – Vol.32, №6. – P. 638-646.*
14. *Dharmalingam P. Vascular endothelial growth factor expression and angiogenesis in various grades and subtypes of meningioma / P.Dharmalingam, VR Roopesh Kumar, S.K. Verma // Indian J. Pathol. Microbiol. – 2013. – V.56, №4. – P.349-354.*

ІМУНОГІСТОХІМІЧНА ХАРАКТЕРИСТИКА ЕКСПРЕСІЇ MMP-9 ТА VEGF В ДОБРОЯКІСНИХ ТА ЗЛОЯКІСНИХ МЕНІНГІОМАХ ГОЛОВНОГО МОЗКУ

Вотєва В.Є., Тертишний С.І.

Запорізький державний медичний університет

Резюме. За допомогою імуногістохімічного методу дослідження нами була проаналізована експресія MMP-9 та VEGF в зразках доброякісних та злоякісних менінгіом, видалених під час нейрохірургічних операцій у 36 хворих. В результаті дослідження було встановлено, що анапластичні менінгіоми характеризуються достовірно вищими рівнями експресії MMP-9 та VEGF, ніж доброякісні, при цьому, рівень експресії MMP-9 пов'язаний з рецидивом пухлин. За допомогою кореляційного аналізу було виявлено, що між MMP-9 та VEGF існують достовірні прямі зв'язки.

Ключові слова: менінгіома, неоангіогенез, MMP-9, VEGF.

ИММУНОГИСТОХИМИЧЕСКАЯ ХАРАКТЕРИСТИКА ЭКСПРЕССИИ MMP-9 И VEGF В ДОБРОКАЧЕСТВЕННЫХ И ЗЛОКАЧЕСТВЕННЫХ МЕНИНГИОМАХ ГОЛОВНОГО МОЗГА

Вотева В.Е., Тертышный С.И.

Запорожский государственный медицинский университет

Резюме. С помощью иммуногистохимического метода исследования нами была проанализирована экспрессия MMP-9 та VEGF в образцах доброкачественных и злокачественных менингиом, удаленных во время нейрохирургических операций у 36 больных. В результате исследования было установлено, что анапластические менингиомы характеризуются достоверно большими уровнями экспрессии MMP-9 и VEGF, чем доброкачественные, при этом, уровень экспрессии MMP-9 связан с рецидивом опухолей. С помощью корреляционного анализа было выявлено, что между MMP-9 и VEGF существуют достоверные прямые связи.

Ключевые слова: менингиома, неоангиогенез, MMP-9, VEGF.