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VEGF-A, VEGFR-2 and MVD in brain tumor tissue

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Abstract

Neoangiogenesis plays a crucial role in tumor development. The method that is most commonly recommended for assessing this process is determining the so-called microvessel density (MVD) in the tissue sample. In relation to tumors of the central nervous system, it seems that VEGF-A along with its receptor VEGFR-2 are the most significant factors, and the important role of both of them is connected with their increased expression in tumor tissue. The study concerned 48 adult patients of both genders, treated surgically for newly diagnosed solid brain tumors and consisted of histological assessment of the material gained from the surgically removed brain tumor. The highest level of VEGF in tumor tissue was observed in gliomas – mostly HGG - 12.61 (N = 23, p = 0.000059). As for VEGFR-2 in tumor tissue, the highest average level was characteristic for metastatic tumors - 13.67 (N = 6, p = 0.037) and it was slightly lower in HGG - 12.2 (N = 23, p = 0.037). The average MVD in tumor tissue was the highest in HGG - 20.13 (N = 23, p = 0.047) and other tumors - 18.33 (N = 3, p = 0.047). The remaining types of tumors in the studied group had similar microvessel density – from 13.67 to 14.71.

Key words: VEGF-A; VEGFR-2; MVD; brain tumors; angiogenesis.

Abbreviations

MVD microvessel density
VEGF vascular endothelial growth factor
VEGF-A vascular endothelial growth factor A
VEGFR-1 vascular endothelial growth factor receptor 1
Flt-1 fms related tyrosine kinase 1
VEGFR-2 vascular endothelial growth factor receptor 1
KDR/Flk-1 kinase insert domain receptor
CD105 endoglin antigen
CD34 hematopoietic progenitor cell antigen
SD standard deviation
HGG high grade glioma

LGG low grade glioma

Introduction

Neoangiogenesis plays a crucial role in the development of cancerous tumor [1]. Its development and metastasis are determined by blood vessels [2]. One may thus state that a greater number of microvessels in the tumor correlates with its level of metastasis (microvessel density – MVD – is considered to be a prognostic factor in many types of tumor). In the process of angiogenesis, the vascular endothelial growth factor (VEGF) plays the most significant role [3], along with the receptors of high affinity – VEGFR-1 (Flt-1 - fms related tyrosine kinase 1) and VEGFR-2 (KDR/Flk-1 - kinase insert domain receptor) – that are found in the blood vessels' endothelium cells [4]. Based on the previous research, VEGF-A along with its VEGFR-2 receptor play the most important role in tumors of the central nervous system [5,6,7]. The significant role of vasculotropin in the process of neoangiogenesis in solid tumors is connected with its increased expression in tumor tissues.

In the case of histopathological examinations, the most recommended method of assessing neoangiogenesis is determining the so-called microvessel density (MVD) in the tissue sample [8-9], in the areas of their highest density (hot spots), using the expression of endothelium's CD105 (endoglin) antigen, CD34 (hematopoietic progenitor cell antigen), von Willebrand Factor [10] or fascin (CD31), using the light microscopy method. In this paper, in order to measure MVD in tumor tissues, immunohistochemical staining was used for CD34, using Rabbit Polyclonal CD34 Antibodies, ABCAM. Steiner et al. [11] in their study of 63 autochthonic gliobliastoma tissues showed the co-expression of VEGF and Flt-1 (46 cases) and KDR (45 cases). The aforementioned paper and many other publications that confirm this correlation induced broadening the histopathological examination and additionally determining the presence of VEGF-A in the tissue samples, using ABCAM's rabbit polyclonal to VEGF Antibodies, as well as VEGFR-2 using ABCAM's rabbit polyclonal VEGF Receptor 2 Antibodies.

Materials and methods

In order to conduct the research, the approval of the Bioethics Committee of Nicolaus Copernicus University Collegium Medicum in Bydgoszcz was obtained, its number being: KB-665/2009. The study involved 48 adult patients (M_b=60.16) of both genders (21 women and 27 men), treated surgically for brain tumor in the Department of Neurosurgery, Nicolaus Copernicus University Collegium Medicum in Bydgoszcz. Qualification of patients was based on interview, physical as well as neuroimaging examinations, and in the later stage it was confirmed with the post-surgical histopathological examination of the removed tumor. All people who were qualified for the study gave their written consent to take part in it. Within the studied group, the youngest person was 26 years old, and the oldest one was 79 years old. The diversity of age was equal to over a decade (SD=10.79). The majority of patients had grade III (27.08%) or IV (33.33%) tumors, according to WHO. Grade I and II was characteristic for 20.83% and 18.75% of patients, respectively.

The frequency of the occurrence of the particular tumor types was assessed by the division into the 5 general groups of intracranial tumors: high grade gliomas (HGG), low-grade gliomas (LGG), meningiomas, metastatic tumors and others (Antoni B type schwannoma, Antoni A type schwannoma and adenoma hypophysis). The majority of people of the studied group had glioma-type tumors – in total, they constituted 62.5% of the studied group. The type that prevailed the most

often were HGG; they were found in almost half of the studied group (47.92%). 14.58% of the studied group had low-grade gliomas. The next type – meningiomas, was characteristic in every 6th studied patient (18.75%). Metastatic tumors were characteristic for 12.5% of patients. The tumors from the last group were found the least often – in slightly more than 6% of the studied group.

The study in the researched group was conducted according to the following scheme:

1. Gaining written, informed patient's consent to take part in the study.
2. Performing the planned surgery consisting in removing the brain tumor.
3. Histological assessment of the material gained from the surgically removed brain tumor.

The brain tumor samples gained during the surgery underwent histopathological examination in the Department of Clinical Pathomorphology, Nicolaus Copernicus University Collegium Medicum in Bydgoszcz. The specimens made with paraffin blocks were assessed as follows:

1. Determining the histological type of the tumor and its grade of malignancy.
2. Assessing VEGF-A in tumor tissue, using the following reagents: Rabbit Polyclonal to VEGF by ABCAM and EnVision® + Kits, HRP Rabbit (AEC+) by Dako.
3. Assessing VEGFR-2, Flk-1 in tumor tissue, using the following reagents: Rabbit Polyclonal to VEGF Receptor 2 by ABCAM and EnVision® + Kits, HRP Rabbit (AEC+), by Dako.
4. Assessing MVD in tumor tissue, using immunohistochemical staining for CD34, using the following reagents: Rabbit Polyclonal to CD34, by ABCAM and EnVision® + Kits, HRP Rabbit (AEC+), by Dako.

The histological type of tumors and their malignancy grade were determined according to the WHO classification of tumors of the central nervous system. A detailed analysis was carried out on one slice of each tumor sample, that contained at least three angiogenic "hot-spots" (areas with the greatest density of microvessels on a tissue section), that were representative for the whole lesion. Then, the selected specimens were stained and checked in three "hot-spots" for the presence of VEGF-A, VEGFR-2 and MVD. The intensity of the immunohistochemical reaction was assessed by using light microscope, using the IRS scale according to Remmele, taking into account the intensity of staining and the proportion of the cells in which the reaction was observed.

The statistical analysis of the collected data was carried out using the Statistica 9.0 kit. Descriptive statistics and descriptive characteristics were used to describe the variables. For most of the tested variables, a deviation from the normal distribution was found, tested by Lillefors test ($p < 0.01$), that's why nonparametric statistics were used. Spearman's correlation coefficient matrix was used to examine relations among variables. The Man-Whitney U test was used examine the differences between the variables in the comparison of divalent variables, and to compare multivariant variables - Anova Kruskal-Wallis test. Comparison of the same variable between the two measurements was made using the Wilcoxon pair test. All results with the condition <0.05, were considered statistically significant.

Results

Ranges of expressions of VEGF-A, VEGFR-2 and MVD measured in all collected tumors (N=48) in the studied group were respectively: VEGF-A: 3-21 (median value: 9.00), VEGFR-2: 3-27 (median value: 9.00), and MVD: 7-27 (median value: 18.50).

At the first stage, the results were checked for the expression of VEGF-A in tumor tissue, depending on the type of tumor. The greatest amount of VEGF-A was characteristic of gliomas, however this observation mostly concerned HGG - 12.61 (N = 23, p = 0.000059). The remaining types of tumors were not distinguished by the amount of VEGF-A in their tissues – it oscillated between 6.22 and 7.00 (p = 0.000059).

The next stage consisted in checking the results for the expression of VEGFR-2 in tumor tissue, depending on the type of tumor. The highest average level of VEGFR-2 was characteristic of metastatic tumors - 13.67 (N = 6, p = 0.037); it was only slightly lower in HGG - 12.22 (N = 23, p = 0.037). The lowest level was characteristic of LGG - 5.43 (N = 7, p = 0.037), which was equal to less than half of the respective indicator of HGG. Meningiomas and other types of tumor had this indicator at average level, from 8.56 (N = 9, p = 0.037) to 9.00 (N = 3, p = 0.037). Interestingly, in HGG and metastatic tumors the standard deviation was relatively low and oscillated between 5.26 and 5.68, whilst meningiomas had an exceptionally high level of variability, almost 100% of the mean value (8.17).

At the third and last stage the results were checked for MDV in tumor tissue, depending on the type of tumor. The average density of microvessels in tissue was the highest in HGG - 20.13 (N = 23, p = 0.047) and other tumors - 18.33 (N = 3, p = 0.047). The remaining types of tumors had similar density of microvessels (from 13.67 to 14.71).

Discussion

There have been many reports on the relations between the expression of VEGF, VEGFR or MVD in tumor tissue and neoangiogenesis and the grade of malignancy of tumor.

Clara et al. [12] analysed 208 tissue samples of glioblastoma multiforme, checking for the expression of VEGF in tumor tissue and endothelium cells. The presence of VEGF was observed in 131 tumors (63%). The expression of VEGF in cancerous cells was correlated with its expression in blood vessels ($p < 0.0001$). Nuclear staining of VEGF in cancerous cells ($p = 0.002$), as well as VEGF staining for endothelium cells ($p = 0.005$) correlated with surviving.

Wenje et al. [13] carried out metanalysis, the aim of which was to assess the relation between overexpression of VEGF and the survival time of the patients suffering from gliomas, and the influence of VEGF overexpression over the malignancy grade of the tumor. 32 articles have been analysed – 2307 cases in total, 31 of which reported general surviving, and 5 – surviving without cancer progression. As it was noticed, overexpression of VEGF influenced general survival in a statistically significant negative way (HR = 1.647, 95% CI: 1.324 ~ 2.048, $p < 0.001$, Z = 4.48), but not survival without cancer progression (HR = 1.021, 95% CI: 0.974 ~ 1.070, $p = 0.393$). Furthermore, a statistically significant correlation between VEGF expression and tumor malignancy grade was indicated ($r = 0.307$, $p < 0.001$). It led to the conclusion that the level of VEGF has a high correlation level with glioma progression and may be a valuable prognostic factor for general survival of patients suffering from this kind of tumor.

Huang et al. [14] checked various tumors of the central nervous system for the presence of VEGF and its receptors. VEGF protein had the highest expression in gliomas and metastatic tumors of kidneys. The level of expression of VEGF receptors did not necessarily correlate with the level of VEGF – they both were very explicitly evident in gliomas, in meningiomas VEGF was low and VEGFR low, whilst in metastatic tumors it was just the opposite - VEGF was high and VEGFR was low. With a few exceptions (especially in the case of oligodendrogliomas), VEGFR-1 expression was equal to the expression of VEGFR-2. It was also noticed that in gliomas the expression of

VEGFR correlated in a positive way with the malignancy grade, to an even greater extent than the expression of VEGF. The results showed that the expressions of VEGF and VEGFR differ in various types of tumors of the central nervous system and are not necessarily parallel.

Hlobilkova [15] examined immunohistochemical specimens of 66 patients suffering from brain tumors (29 astrocytomas WHO II and 37 astrocytomas WHO III and IV) for, among others, the expression of VEGF, Flt-1, Flk-1 and their correlation with the malignancy grade of the tumor. The expression of VEGF receptors did not indicate any significant changes between tumors of low and high malignancy levels, whereas the expression of VEGF was higher in those of higher malignancy levels.

Yoo [16], Karayan-Tapon [17] and Schmidt [18] reached similar conclusions. Yoo studied the expression of VEGF in astrocytomas (14 cases of WHO II, 30 – WHO III and 34 – WHO IV) and its relation to the grade of histological malignancy and survival time. The expression of vasculotropin showed a significant correlation with the malignancy grade of the tumour ($p = 0.02$) and a relation to general survival time ($p = 0.1$). Karayan-Tapon examined 38 gliomas for the expression of, among others, vasculotropin. The level of VEGF correlated with the malignancy grade of the tumor and worse survival. Schmidt examined the level of expression of 3 factors of vascular growth factors, including VEGF, in 71 gliomas using the ELISA immunoenzymatic method. The average level of VEGF was 11 times greater in high-grade gliomas, correlated positively with the density of microvessels in tumor ($p < 0.001$). Thus, he found out that after the induction of angiogenesis in high-grade tumor, the level of VEGF in the tumor tissue still remains high.

In their independent studies, Samoto [19] and Ding [20] assessed the expression of VEGF mRNA in intracranial tumors' samples. Ding examined the expression of VEGF and VEGF mRNA in 40 patients suffering from meningiomas. He determined its levels in the tumor mass, in the area close to the tumor and in healthy brain tissues. Only 28 cases of meningiomas showed the expression of VEGF. In the case of the area close to the tumor, only 21 had detectible vasculotropin. It was also noticed, that the greater distance from the tumor is, the more decreased the gradient of expression of VEGF becomes. According to the expression of VEGF, in 28 cases of meningiomas, VEGF mRNA was found. However, in the area close to the tumor and in the healthy brain tissue it was almost undetectable. Samoto examined 17 gliomas and 16 meningiomas, and then correlated the level of expression of VEGF mRNA with the number of microvessels in the tumor. For gliomas, the correlation was 0.499 ($p < 0.05$) and for meningiomas 0.799 ($p < 0.001$). Based on this, the author arrived at the conclusion, that VEGF induces angiogenesis in both tumor types.

A similar relation was examined by Wang [21]. He determined the level of expression of VEGF in 40 hemangioblastoma tumors and correlated it with MVD in tumor tissue (using CD34 immunohistochemical staining). The results he achieved showed the same kind of relation as in the following paper – MVD correlated positively with the level of expression of VEGF in tumor tissue ($p < 0.001$).

Jianguo [22] examined the expression of p53 and VEGF in gliomas, using immunohistochemical staining. In the studied group, the prevalence of p53 was 73.3%, and VEGF – 76.7%, which was significantly more than in the adjacent healthy tissues and in the control group ($p < 0.05$). The expression of p53 was correlated positively with the expression of VEGF ($p < 0.05$). The levels of expression of p53 and VEGF correlated with the pathological type of glioma, WHO grade, distant metastasis and survival time ($p <0.05$), but not with age or general survival time ($p > 0.05$). In the studied group, high levels of p53 and VEGF were observed in both

serum and tumor tissue. Based on the research, Jianguo made an assumption that both those factors might play a crucial role in the development of gliomas.

A significantly higher level of VEGF in tumor tissue in gliomas was also noticed by Takano [23]. The concentration of vasculotropin in those tumors was the highest (6281.6 ± 3317.2 pg/mg), in comparison with other studied tumor types (anaplastic astrocytoma 493.6 ± 916.0 pg/ml, low-grade astrocytoma 600.4 ± 944.0 pg/ml, meningioma 1096.6 pg/ml, malignant lymphoma 48.8 pg/ml, metastatic tumor 332 pg/ml), as well as with normal brain tissue (25.8 ± 15.0 pg/ml, $p < 0.01$).

Deb et al. [24] correlated MVD with the WHO histological malignancy grade in various types of primary tumors of the central nervous system. Thirty tumors of the central nervous system that were surgically removed were then stained with CD34. MVD was raised in all the tumors except for meningiomas, and its level was significantly increased in gliomas ($p < 0.0062$). A positive correlation between malignancy grade and MVD level was indicated ($r = 0.547$).

In this paper, the results of the examination for the expression of VEGF-A, VEGFR-2 and MVD in tissue samples showed a positive correlation among them. A higher expression of both VEGF-A and KDR, to a lesser extent, was related to a greater number of microvessels in the tumor. The highest level of VEGF in tumor tissue ($p = 0.000059$) was observed in gliomas – mostly HGG (12.61). The expression of vasculotropin in other types of tumor was not distinctive – it oscillated between 6.22 and 7.00. In the case of VEGFR-2 in tumor tissue ($p = 0.037$), the highest average level was characteristic of metastatic tumors (13.67); for HGG it was slightly lower (12.22). The lowest level was characteristic of LGG (5.43), which was less than half of the same indicator for HGG. Meningiomas or other types of tumors gave the aforementioned indicator at the average levels of 8.56 - 9.00. In metastatic tumors and high-grade gliomas the standard deviation was relatively low and oscillated between 5.26 and 5.68. In the case of meningiomas, the variation was exceptionally high and was equal to almost 100% of the average value (8.17). The average density of microvessels (MVD) in tumor tissue ($p = 0.047$) was the highest in HGG (20.13) and other tumors (18.33). The remaining types of tumors in the studied group had similar microvessel density – from 13.67 to 14.71.

Conclusions

Based on the obtained results and performed statistical analyses, following conclusions were drawn:

1. In patients suffering from central nervous system tumors, the highest expression of both VEGF-A, and less VEGFR-2 is related with greater density of microvessels in the tumor.
2. The highest level of VEGF-A expression in tumor tissue can be observed in high-grade gliomas.
3. The highest level of VEGFR-2 expression in tumor tissue can be observed in metastatic tumors and high-grade gliomas.
4. The greatest average MVD in tumor tissue can be observed in high-grade gliomas.
5. The level of VEGF-A, VEGFR-2 and MVD expression in tumors of the central nervous system may be the indicator for intensification of neoangiogenesis.
6. A high level of VEGF-A, VEGFR-2 and MVD expression in tumors of the central nervous system shows a positive correlation with a high level of tumor malignancy.
7. Measuring the VEGF-A, VEGFR-2 and MVD expression levels in tumor tissue may be an additional prognostic factor in tumors of the central nervous system. Comparing obtained

results to the routine examinations may translate into more accurate choice of treatment and evaluation of patient's overall survival.

Conflict of interest

All authors declare no conflicts of interest in this paper.

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