Significance of vascular endothelial growth factor expression in skin melanoma

Značaj ekspresije vaskularnog endotelnog faktora rasta kod melanoma kože

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Abstract

Background/Aim. Melanoma is a heterogeneous disease of skin and mucous membranes which shows significant increase in incidence worldwide in the past decades. In the process of forming new blood vessels stimulators of angiogenesis participate. There is an increase production of vascular endothelial growth factor (VEGF-C and VEGF-D), which expression cause change of endothelial cells, and higher degree of tumor's aggressiveness. The aim of this research was to determine the level of VEGF expression in skin melanoma in different body regions and in different primary stages of the disease. Methods. The research was conducted on biopptic materials of skin in 39 patients. On excision-made materials a routine histological preparation was done and following parameters were determined: histological type, alteration thickness (according to Breslow), Clark level, TNM (Tumor Nodus Metastasis) stage (pT), alteration width, thickness of lymphocytic infiltration in the tumor, mitotic index, phase of the tumor growth, presence of ulcerations, cellular type of the tumor, localization and level of VEGF expression. Results. Analysis confirmed that 61.54% of skin melanoma showed a high VEGF expression. Nodular and acral lentiginous melanomas showed more frequently a high level of VEGF expression, while superficial spreading melanoma showed a lower level of VEGF expression (p = 0.032, p < 0.05). A higher level of expression was present in thicker melanomas (higher in the Breslow stage; p = 0.011, p < 0.05). The width of the lesion did not have an influence on the level of VEGF expression in melanoma (U = 142,000, p = 0.273). Conclusion. Melanomas show a higher level of VEGF expression. Nodular and acral lentiginous types of melanoma show a high level of VEGF expression, while superficial spreading melanoma shows a lower level of VEGF expression. Melanomas in higher-stage disease (Breslow, Clark, pTNM) show a higher level of VEGF expression.

Key words: melanoma; neoplasm staging; angiogenesis inducing agents; vascular endothelial growth factor; histological techniques.

Apstrakt

Uvod/Cilj. Melanom je heterogen oboljenje kože i sluznića koje u posljednjim decenijama pokazuje izrazito povećanje incidencije u celom svetu. U procesu nastanka novih krivnih sudova učestvuju stimulatori angiogeneze. Povećava se produkcija vaskularnog endotelnog faktora rasta (VEGF-C i VEGF-D) čijom ekspresijom dolazi do promene endotelnih čelija što za posledicu može imati veći stepen agresivnosti tumora. Cilj istraživanja bio je da se utvrdi stepen ekspresije VEGF u melanomima kože različitih regija u različitim primarnim stadijima bolesti. Metode. Istraživanje je obuhvatalo biopsijske materijale kože 39 bolesnika. Na materijalima koji su dobijeni ekszicijom urađena je rutinska histo-loška obrada i određeni su sledeći parametri: histo-loški tip, debljina promene (Breslow), Clark nivo, TNM (tumor nodus metastaza) stadijum (pT), širina promene, gustina lmfokitnog infiltrata u tumoru, mitotski indeks, faza rasta tumora, prisustvo ulceracije, čelijski tip tumora, lokalizacija i stepen ekspresije VEGF. Rezultati. Analizom je utvrđeno da 61,54% melanoma kože pokazuje visoku ekspresiju VEGF. Nodularni i akralni lentiginozni tip melanoma češće pokazuju visok stepen ekspresije VEGF, a površinski šireći melanom češće niski stepen ekspresije VEGF (p = 0,032, p < 0,05). Viši stepen ekspresije prisutan je u melanomima koji su deblji (viši stadijum prema Breslow) (p = 0,011, p < 0,05). Širina lezije ne utiče na stepen ekspresije VEGF u melanomu (U = 142,000, p = 0,273). Zaključak. Melanomi pokazuju visok stepen ekspresije VEGF. Nodularni i akralni lentiginozni tip melanoma pokazuju visoku ekspresiju VEGF, a površinski šireći melanom pokazuje nisku ekspresiju VEGF. Melanomi u višem stadiju bolesti (Breslow, Clark, pT) pokazuju veći stepen ekspresije VEGF.

Ključne reči: melanom, maligni; neoplazme, određivanje stadijuma; angiogeneza, induktori; faktori rasta endotelna krivnih sudova; histološke tehnike.

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Introduction

Melanoma is a heterogeneous disease of the skin and mucous membranes which shows a significant increase in worldwide incidence in the past decades (from 2.7 to 6.0 on 100 000 residents per year in men and from 4.6 to 8.5 on 100 000 residents in women). The total incidence is the highest in Australia and between 1980 and 1990 it tripled. Risk factors for the onset and development of melanoma are numerous, but the most frequently mentioned is exposure to sunlight. Melanoma is more likely to occur on the head, neck and trunk in men and the lower leg in women. The two phases of tumor progression are described: radial and vertical phase of the melanoma growth. On the basis of clinical and biological characteristics the World Health Organization (WHO) has offered the classification of melanoma where are (because of the frequency) particularly described the superficial spreading melanoma, nodular and acral lentiginous melanoma.

Angiogenesis implies the creation of new capillary blood vessels from the existing vascular network and is a very complex process that involves extravasation of proteins and plasma, decomposition of extracellular matrix, migration and proliferation of endothelial cells and formation of capillary tubes. All processes that occur during this angiogenic cascade are regulated by various factors, stimulators and inhibitors whose balance limits the process.

Stimulators of angiogenesis are: growth factor of endothelial cells of blood vessels (VEGF), basic and acidic fibroblast growth factors (b-FGF, aFGF, FGF-2, FGF-1), endothelial cell growth factor originating from platelets (PD-ECGF), angiopoietin-1 and others. VEGF is a soluble homodimeric glycoprotein that binds to the receptors with tyrosine kinase activity on endothelial cells. Five subtypes of VEGF with different molecular weight are described. Among them, VEGF-B (23 kd) and VEGF-C (34 kd) have a significant place. Angiogenesis in the skin is regularly associated with an increased expression of VEGF in epidermal keratinocytes. Explanation of why melanoma spreads faster in lymphogenic than hematogenic pathway is given by the information about the increased production of factors VEGF-C and VEGF-D, whose expression changes endothelial cells in the lymph vessels and consequently there is an increase of the degree of tumor progression. VEGF is required in the process of angiogenesis because it represents a specific mitogen for endothelial cells, allows their migration and increases the permeability of blood vessels.

Data from the literature indicate a low level of expression of VEGF in benign melanocyte alterations (nevi). The authors suggest that increased expression of VEGF in dysplastic nevi may be an indicator of preneoplastic alterations in melanocyte lesions. A group of authors stated that estimation of VEGF expression might help in differential diagnosis among dysplastic nevi and melanoma and that VEGF may be a “candidate for targeted treatment”. Piscane and Risio have found expression of VEGF and VEGFR-2 in benign and malignant melanocyte alterations. They have found that cytoplasmatic and nuclear membrane expression of VEGFR-2 is associated with progression of melanocyte lesions to invasive melanoma.

Melanocyte alterations show expression of VEGF, regardless of clinical behavior: benign melanocyte alterations more often indicate a low level of VEGF expression and malignant melanocyte alterations more often show a high level of VEGF expression.

Inhibitors of angiogenesis are thrombospondins (TSP), angiostatin, platelet factor 4 (PF 4), angiostatic steroids and some other substances.

Numerous diseases in dermatology, including malignant neoplasms, have factors of stimulation or inhibition of angiogenesis as an important component, which could in the future enable the effective treatment of patients suffering from these diseases. Targeted treatment with angiogenesis inhibitors decreases the possibility of creating new blood vessels in tumor, and thus indirectly affects tumor cells and slows tumor growth and development.

Angiogenic factors exhibit their effects on endothelial cells directly or indirectly. Highly vascularized tumors have a higher metastatic potential compared to less vascularized tumors, and researches show that the number of metastases is directly proportional to the number of released tumor cells in circulation. Angiogenesis is a necessary step for the onset and for the end of the metastatic process. With vascularization of the primary tumor begins expansive growth and tumor gets metastatic potential.

The aim of this study was to determine the level of VEGF expression in melanoma of the skin of different regions, and to determine the correlation between the level of VEGF expression and morphologic prognostic parameters (histological type, ulceration, inflammatory infiltration density, mitotic index, stage of disease, stage of growth and cell type).

Methods

The research included biopptic materials of the skin of 39 patients, performed in the Clinical Center of Banja Luka in the period from 2004 to 2007. Histological analysis established the diagnosis of skin melanoma (primary skin melanoma).

In all subjects we determined: histological type by analysing of histological samples according to the WHO histological classification; thickness change (Breslow), measured vertically in millimeters from the granular layer of the epidermis to the place of deepest invasion, or from the base of the defect to the place of deepest invasion; Clark level histologically and by the layers of tumor location (level I to level V); pTNM stage on the basis of histological analysis and insight into the history of the disease, according to the 7th pTNM classification; width of the lesion microscopically in millimeters, from one side edge of the alteration to the other side edge; tumor infiltration by lymphocytes (absent – no lymphocytes in the tumor stroma; rare infiltrate present – from 1 to 10 lymphocytes on one visual field in high magnification; medium dense infiltration pres-
ent – from 11 to 20 lymphocytes on one visual field in high magnification; dense infiltration present – more than 20 lymphocytes on one visual field in high magnification). Assessment was done in the stroma of the tumor and on the border of the tumor and surrounding tissue; mitotic index: the number of mitosis was determined in 10 visual fields in high magnification. The width of visual field was 1.4 mm. Visual fields which were quantified represent the peripheral parts of the tumor (to the surrounding tissue); estimation of growth phase: radial growth phase – present or absent (lentigo malignant, acral lentiginous, superficial spreading type); vertical growth phase – present or absent (expansive tumor clusters located in the papillary and / or reticular dermis) 7; the presence of ulceration: assessed histologically based on the continuity of the epidermis above the lesion (present or absent ulceration); cell type of the tumor: epitheloid cells, spindle cells, mixed type (epitheloid + spindle cells); localization: alterations were classified according to localization into the following subgroups: head and neck, trunk, extremities; expression of VEGF: assessment of expression from 0 to 3.

Epitope demasking was performed by pretreatment in a microwave oven and by soaking of slides in Target Retrieval Solution pH 9.0 (Daco S2367). As the primary antigen we used commercial mouse monoclonal anti-human VEGF antibody (Daco M7273), the concentration of VG1 with 1:25 dilution. For visualization we used the LSAB + (Daco K0690) system and chromogen DAB Liquid (K3466).

The presence or absence of factors and the intensity of their presence was valued by a semiquantitative scale from 0 to 3, taking as an internal control level of immunostaining of keratinocytes.

Quantification was as follows: score 0 – no difference in immunostaining for VEGF between melanocytes and keratinocytes; score 1 to 3 – a higher level of VEGF expression in tumor cells compared to keratinocytes; score 1 – less than 25% tumor cells showed expression of higher intensity compared with the level of staining of keratinocytes; score 2 – 25 to 75% of tumor cells showed expression of higher intensity compared with the level of staining of keratinocytes; score 3 – more than 75% tumor cells showed expression of higher intensity compared with the level of staining of keratinocytes.

The results were analyzed by methods of descriptive and correlative statistics. Statistical analysis was performed using the SPSS software version 15.0, and the following tests were applied: $\chi^2$ and the related methods of analysis of categorical variables (Fisher's exact test, Kendall's Tau test) and Mann Whitney U- test.

Results

Our research was done on bioptic materials of 39 patients (23 women and 16 men) performed in the Clinical Center of Banja Luka in the period from 2004 to 2007. Based on the analyzed materials a diagnosis of primary melanoma of the skin was established.

The youngest examinee was 18 years old and the oldest one 85 years. The average age of all examinees was 58.66 years. Melanomas in our material belonged to the following histological types: nodular type in 31 (79.49%) cases, superficial spreading melanoma of the in 6 (15.38%) cases and acral lentiginous type in 2 (5.13%) cases.

Characteristics of examinees are presented in Table 1.

The study found that a low level of expression of VEGF was present in 38.46% of the cases – level 0 and 1, while a high level of expression was present in 61.54% of the cases – level 2 and 3 (Table 2) (Figures 1 and 2). More often, a high expression of VEGF was verified (score 2 and 3 – $\chi^2 = 8.487$ with a df 3; $p = 0.037$, $p < 0.05$).

Table 2 presents the relationship between histologic type of melanoma and expression of VEGF in accordance with the WHO classification. There was a statistically significant difference in the level of VEGF expression in melanoma due to the histological type. Nodular and acral lentiginous type of melanoma more often showed a high level of expression of VEGF, while superficial spreading melanoma often showed a low level of VEGF expression ($\chi^2 = 6.858$ with a degree of freedom df = 2, $p = 0.032$, $p < 0.05$).

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The presence of defects (ulcerations) in the epidermis was verified in 22 (56.41%) of the cases. Table 2 shows the relationship between VEGF expression and the presence or absence of ulcerations. There was a statistically significant difference in the level of VEGF expression and the presence of ulcerations – $\chi^2 = 4.545$ with a degree of freedom (df) 1 where $p = 0.033$ ($p < 0.05$). In the cases of verified ulcerations, the level of VEGF expression was mainly high score, 2 and 3.

The average value of Breslow level (thickness of lesion) in melanoma with the level of immunostaining 0 for VEGF was 4.25 mm, with the level 1 of immunostaining for VEGF was 5.37 mm, with the level 2 was 4.23 mm, and for melanomas with the level 3 of immunostaining for VEGF mean value of Breslow level was 14.51 mm (Table 3). Based on the analysis by $\chi^2$ test we could conclude that there was a statistically significant difference in the level of VEGF expression and thickness of the lesion in skin melanomas – $\chi^2 = 11.211$ with the degree of freedom df = 3 where $p = 0.011$ ($p < 0.05$). A higher level of expression was present in thicker melanomas (higher level according to Breslow).

In melanoma with level 0 of immunostaining of VEGF, level of invasion according to Clark was IV in one of the cases, and V in the other one. In melanomas with the level of immunostaining 1 for VEGF, the level of invasion according to Clark was II in 3 of the cases (23.07%), III in 4 of the cases (30.76%), IV in 3 cases (23.07%). In melanomas with level 2 of immunostaining for VEGF, the level of invasion according to Clark was II in 2 of the cases (15.38%), III in 4 of the cases (30.76%), IV in 5 of the cases (38.46%), and V in 2 of the cases (15.38%).

Table 1

| Characteristics | Years | Number of examinees |
|-----------------|-------|---------------------|
| Age             | average 58.66 |
| Localization    |       |
| head and neck   | 13    |
| trunk           | 11    |
| extremities     | 15    |
| Histologic type of melanoma |       |
| nodular         | 31    |
| superficial spreading | 6    |
| acral lentiginous | 2    |
| Ulceration      |       |
| present         | 22    |
| absent          | 17    |
| Mitotic activity /10HPV |       |
| 0–5             | 15    |
| 6–10            | 11    |
| >10             | 13    |
| Clark           |       |
| I               | 0     |
| II              | 5     |
| III             | 8     |
| IV              | 14    |
| V               | 12    |
| Breslow         |       |
| I (0–0.75 mm)   | 2     |
| II (0.76–1.50 mm) | 5    |
| III (>1.50 mm)  | 32    |
| TNM             |       |
| pT1a            | 2     |
| pT1b            | 1     |
| pT2a            | 4     |
| pT2b            | 2     |
| pT3a            | 4     |
| pT3b            | 6     |
| pT4a            | 5     |
| pT4b            | 15    |
| Growth phase    |       |
| radial          | 1     |
| vertical        | 25    |
| vertical and radial | 13   |
| Level of VEGF expression |       |
| 0               | 2     |
| 1               | 13    |
| 2               | 11    |
| 3               |       |

TNM – Tumor Nodus Metastasis; VEGF – vascular endothelial growth factor
In melanomas with the degree 3 of immunostaining for VEGF, the level of invasion according to Clark was IV in 6 of the cases (54.54%), and V in remaining 5 of the cases (45.45%). Based on the analysis by Kendall's tau b test, no statistically significant difference was found in the level of VEGF expression and level of invasion according to Clark (Kendall's tau \( b = 0.244, p = 0.063\)).

Table 2 presents the results of the level of expression of VEGF and the TNM (pT) stage of melanoma. A higher level of expression was present in melanoma in advanced stage of the disease. Statistical analysis using Kendall's tau c test showed that there was a statistically significant difference in the level of expression of VEGF and pT stage of melanoma (Kendall's tau \( c = 0.259, p = 0.050 \) and \( p < 0.05\)). Melanomas in higher pT stage of the disease showed a higher expression of VEGF (score 2 and 3).

The average value of width of lesion for melanomas with the level of immunostaining 0 for VEGF was 8.6 mm. The average width of melanoma lesion with the level 1 of immunostaining for VEGF was 14 mm, with level 2 mean lesion width was 9.91 mm, and in melanoma with the level 3 of immunostaining for VEGF average lesion width was 28.47 mm.

### Table 2

| Prognostic parameters in melanoma | Level of immunostaining for VEGF |
|-----------------------------------|----------------------------------|
|                                   | 0 (5.13%) | 1 (33.33%) | 2 (33.33%) | 3 (28.21%) |
| Histologic type                   |          |            |            |            |
| nodular                           | 2 (6.45%)| 8 (25.81%) | 11 (35.48%)| 10 (32.26%)|
| superficial spreading acral       | 5 (83.33%)| 1 (16.67%) |            |            |
| lentiginous                       | 1 (50%)  | 1 (50%)    |            |            |
| Presence of ulceration            |          |            |            |            |
| present                           | 1 (4.54%)| 5 (22.73%) | 7 (31.82%) | 9 (40.91%) |
| absent                            | 1 (5.88%)| 8 (47.06%) | 6 (35.29%) | 2 (11.77%) |
| TNM                               |          |            |            |            |
| pT1a                              | 2 (15.38%)|            |            |            |
| pT1b                              | 1 (7.69%) |            |            |            |
| pT2a                              | 2 (15.38%)| 2 (15.38%) |            |            |
| pT2b                              | 1 (7.69%) | 1 (7.69%)  |            |            |
| pT3a                              | 1 (50%)  | 2 (15.38%) | 1 (9.09%)  |            |
| pT3b                              | 5 (38.46%)|            | 1 (9.09%)  |            |
| pT4a                              | 3 (23.07%)| 1 (7.69%)  | 1 (9.09%)  |            |
| pT4b                              | 1 (50%)  | 4 (30.76%) | 2 (15.38%) | 8 (72.72%) |
| Density of lymphocytic infiltrate |          |            |            |            |
| dense infiltrate                   | 2 (8%)   | 12 (48%)   | 5 (20%)    | 6 (24%)    |
| medium dense infiltrate           | 1 (12.5%)| 4 (50%)    | 3 (37.5%)  | 2 (40%)    |
| rare infiltrate                   | 3 (60%)  |            |            |            |
| Mitotic activity                  |          |            |            |            |
| 0–5/10HPF                         | 8 (53.33%)| 5 (33.33%) | 2 (13.34%) |            |
| 6–10/10HPF                        | 1 (9.09%)| 3 (27.27%) | 4 (36.37%) | 3 (27.27%) |
| > 10/10HPF                        | 1 (7.96%)| 2 (15.38%) | 4 (30.77%) | 6 (46.16%) |
| Growth phase                      |          |            |            |            |
| radial                            | 1 (4%)   | 6 (24%)    | 11 (44%)   | 7 (28%)    |
| vertical                          | 1 (100%) |            |            |            |
| vertical and radial               | 1 (7.69%)| 6 (46.16%) | 2 (15.38%) | 4 (30.77%) |
| Cell type                         |          |            |            |            |
| epitheloid                        | 1 (3.22%)| 12 (38.71%)| 10 (32.26%)| 8 (25.81%) |
| spindle                           | 1 (20%)  | 1 (20%)    | 1 (20%)    | 2 (40%)    |
| mixed                             | 2 (66.67%)|            | 1 (33.33%) |            |
| Localization                      |          |            |            |            |
| head and neck                     | 1 (7.69%)| 7 (53.85%) | 3 (23.07%) | 2 (15.39%) |
| trunk                             | 5 (45.46%)|            | 3 (27.27%) | 3 (27.27%) |
| extremities                       | 1 (6.67%)| 1 (6.67%)  | 7 (46.66%) | 6 (40%)    |

### Table 3

| Characteristics of melanoma | Level of immunostaining for VEGF |
|-----------------------------|----------------------------------|
|                            | 0 (5.13%) | 1 (33.33%) | 2 (33.33%) | 3 (28.21%) |
| Average thickness (mm)      | 4.25      | 5.37       | 4.23       | 14.51      |
| Average width (mm)          | 8.6       | 14         | 9.91       | 28.47      |

VEGF – vascular endothelial growth factor

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of melanoma. Analysis by Mann-Whitney test, we concluded that there was no statistically significant difference in the level of expression of VEGF and width of lesions in melanomas (U = 142.000, p = 0.273).

In the analyzed material inflammation infiltrate was present in 38 (97.44%) cases. Inflammation infiltrate was generally dense (Table 2). In case of low expression of VEGF (score 0 and 1) there was usually dense inflammation infiltrate in 14 (93.33%) of the cases. Dense inflammation infiltrate was also present in most of the cases of high expression of VEGF (score 2 and 3), in 11 (47.83%) of the cases. On the basis of statistical analysis it was concluded that there was a statistically significant difference in the level of expression of VEGF and the density of lymphocyte infiltrates – $\chi^2 = 8.555$ with a degree of freedom (df) 2 where $p = 0.014$ ($p < 0.05$). The low level of expression of VEGF was more often verified in melanomas with dense lymphocyte infiltrate, and high level of expression was verified in melanomas with rare lymphocyte infiltrate.

In our research measuring of mitotic activity in 10 consecutive visual fields in high magnification was done. Regarding the mitotic activity, alterations were divided into three categories: 0 to 5/10HPF, from 6 to 10/10HPF, and 11 and over /10HPF. Relationship of VEGF expression and mitotic activity of tumor cells was shown in Table 2. Based on Kendall’s tau-c test, we found that there was no statistically significant difference in the level of VEGF expression in relation to the level of mitotic activity (Kendall's tau $c = t = 0.256, p = 0.060$).

Melanocyte malignant alterations (melanomas) in our material were in advanced stages, and in most cases were in vertical growth phase. Dominantly vertical growth phase was verified in 25 (64.1%) of the cases. The presence of vertical and radial growth phase was verified in 13 (33.33%) of the cases. In one of the cases (2.56%) only radial growth phase was verified. Table 2 shows the relationship between the level of VEGF expression and stage of tumor growth. On the basis of statistical analysis we concluded that there was a statistically significant difference in the level of VEGF expression and stage of tumor growth. On the basis of statistical analysis we concluded that there was a statistically significant difference in the level of VEGF expression and stage of tumor growth. On the basis of statistical analysis we concluded that there was a statistically significant difference in the level of VEGF expression and stage of tumor growth.

In our research nodular and acral lentiginous types of melanoma more often showed a high level of expression of VEGF, and superficial spreading melanoma more often had a low level of expression of VEGF. The results are in line with the literature data.

In our research there was a statistically significant difference in level of VEGF expression and the presence of ulceration and thickness according to Breslow. Boone et al. 34 in their study did not manage to prove a positive correlation between expression of VEGF-C factor and the presence of ulceration, tumor thickness according to Breslow, and the level of invasion according to Clark.

In our material there were malignant alterations in Clark level I. A higher number of melanoma cases showed a high expression of VEGF (score 2 and 3) in 24 (61.54%) of the cases. Cases with higher degree of expression are generally in higher Clark level. Based on the analysis by Kendall's tau-b test, there was no statistically significant difference in the level of VEGF expression and level of invasion according to Clark. Salven et al. 35 also failed to found differences in manifestation of VEGF measured by immunohistochemical methods between smaller and larger primary melanomas. However, this is not what all authors agree about: Redondo et al. 36 believe that the more the Clark's or Breslow's level increases, the percentage of positive immunostaining for VEGF also increases, linking it with the development of the primary tumor, but no prognostic research was done.

Discussion

This study included a total of 39 melanomas. A low level of expression of VEGF was present in 38.46% of the cases (score 0 and 1), while a high expression was present in 61.54% of the cases (score 2 and 3). Caraza and Peyrì 31 in their research state that the majority of melanomas show a lower level of expression (score 0 and 1), which is different compared with our results. A logical explanation of this difference is that these authors have studied a selected group of melanomas (“thin melanoma”, ie Breslow less than 1 mm), while in our research we studied the unselected group of melanomas. As an alternative, two different research groups describe alternative mechanisms for traditional angiogenesis which melanoma cells use to reach the vascular circulation: Leeners tells about “vascular co-option” or that melanoma uses the blood vessels that existed earlier in the animal model of cerebral metastatic melanoma, and Folberg describes “vasculogenic mimicry”, which primary cutaneous melanoma use to create three-dimensional structures that function as blood vessels, without a real angiogenesis.

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Melanocyte malignant alterations (melanoma) in our material were in advanced stages, and in most of the cases were in the vertical growth phase. Dominantly vertical growth phase was verified in 25 (64.1%) of the cases. On the basis of statistical analysis, there was a significant difference in the level of VEGF expression and growth phase of melanoma. Melanoma with a vertical growth phase showed a higher level of VEGF expression. Looking at the value of immunostaining of VEGF against the Breslow level, a very important item was found: melanomas in radial stage changing to the malignant eclipse manifested less VEGF, which was significantly different measured by the precise Fisher test \( (p = 0.002) \) compared to already penetrated melanomas 36. Erhard et al. 37 describe the same findings: larger angiogenesis in the vertical phase to the radial one and higher manifestation of VEGF. Later, Bayer-Garner et al. 37 found a less manifestation of VEGF measured by immunohistochemical methods in melanomas in radial phase compared to melanomas in vertical phase found. VEGF was not the only angiogenic factor for which these data were observed. Bachelot et al. 22, using in situ hybridization, showed low manifestation of bFGF-a7 in melanomas in situ, compared with its higher manifestation in invasive melanomas. In this way, both studies support the importance of these factors in transition of melanoma from radial to vertical growth phase. Also it should be emphasized that bFGF and VEGF act synergically as activators in angiogenic process 38. VEGF would thus often been absent or could be manifested minimally in skin melanoma in radial stage. These melanomas are indolent and their extirpation assumes the probability of healing of almost 100%. The reason is their inability to form metastases. At a time when neoplastic cells manage to manifest angiogenic factor, this will provide them with a favorable microenvironment by increasing vascular permeability in melanoma as a necessary precondition for the development of the tumor. In addition, a factor directly stimulates angiogenesis and the arrival of multiple macromolecules essential for the metastatic cascade due to the increase of permeability. Even hypothetically speaking, VEGF could even more stimulate autocrine growth of melanoma cells if they exhibit kdr receptor, a phenomenon that was previously described in medical literature (specifically, in A375P line of melanoma in humans and its derivatives A375MM) 39.

**Conclusion**

Melanomas show a high level of VEGF expression. Nodular and acral lentiginous types of melanoma show a high expression of VEGF, and superficial spreading melanoma shows a low expression of VEGF. Melanomas with ulceration, with rare inflammation infiltrate in the stroma, high mitotic index, and melanomas in a higher stage of disease (Breslow, Clark, pTNM) show a higher level of expression of VEGF.

Melanomas in radial phase of growth show a low level of expression of VEGF and melanomas in vertical growth phase showed a high level of expression of VEGF. Epithelial-cellular melanomas and melanomas localized on extremities show a higer level of VEGF expression.

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