Acta Scientific CANCER BIOLOGY (ISSN: 2582-4473)

Volume 5 Issue 11 November 2021

Research Article

Microvessel Density in Cutaneous Melanocytic Neoplasia

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DOI: 10.31080/ASCB.2021.04.0353

Received: June 24, 2021

Published: October 27, 2021

Abstract

Background: The prognostic significance of microvessel density (MVD) in cutaneous melanoma is controversial according to conducted relevant studies. The aim of our study was to reveal the possible correlation between immunohistochemical indicators of proliferation and MVD investigating Ki-67, PECAM-1 and EGFR in cutaneous melanocytic neoplasia

Materials and Methods: The study enrolled 33 patients with cutaneous melanocytic neoplasia (18 patients with benign, including 6 atypical nevus and 15 patients with malign neoplasia) retrospectively. The immunohistochemical examination has been performed on paraffin-embedded sections of melanocytic neoplasia's tissue (dysplastic nevi and melanoma) using monoclonal antibodies against Ki-67, VEGF and CD31/PECAM-1.

Results: Moderate proliferative activity was seen in 10 malign melanoma cases (66.7%) and in 4 cases (22.2%) of benign melanocytic neoplasia. It should be noted that the all the latter cases were in dysplastic nevi subgroup. Low neoplastic activity was seen only in other 14 cases (77.8%) of benign melanocytic nevi ($p < 0.01$). VEGF-expression was positive in all melanoma cases and in 2 cases of benign melanocytic nevi ($p < 0.001$). MVD was significantly different in melanoma and benign melanocytic nevi cases ($p < 0.01$). A direct strong correlation between Ki-67 expression and MVD in cutaneous melanocytic neoplasia was found ($r = +0.78$).

Conclusion: High PECAM-1 expression in cutaneous melanocytic neoplasia, that is a surrogate marker of tumor neoangiogenesis, is correlated with higher proliferative activity of melanocytic cells. Such tumors theoretically can have higher metastatic potential not only due to high proliferative activity of melanoma cells also because of having abundant microvessels that are essential for making contact between malignant cells and bloodstream for developing hematogenic metastasis. Further investigations are needed to clarify the prognostic significance of PECAM-1 expression skin melanoma tissue.

Keywords: Melanoma; Melanocytic Neoplasia; Microvessel Density

Introduction

Melanoma is defined as the malignant neoplasia deriving from pigment melanocytes and is located mainly (in > 90% of the cases) in the skin [1]. Although cutaneous melanoma represents approximately 3% of all skin cancers, 80% of skin cancer-related deaths are related to skin melanoma [2]. Moreover, the global incidence of cutaneous melanoma has an increasing trend over the past decades, with substantial variations being reported between different geographic areas, populations, and genders [3-5]. Several studies have attempted to identify prognostic factors that included various

clinical and morphological characteristics [6,7]. A substantial number of studies has investigated the prognostic significance of MVD in cutaneous melanoma, with inconclusive results [8-11].

Aim of the Study

The aim of our study was to reveal the possible correlation between immunohistochemical indicators of proliferation and MVD investigating Ki-67, PECAM-1 and EGFR in cutaneous melanocytic neoplasia.

Materials and Methods

The study retrospectively enrolled 33 patients with cutaneous melanocytic neoplasia - 18 patients with benign (age range 20 - 60 years), 15 patients with malign neoplasia age range (20 - 60 years). In 6 of patents with benign melanocytic neoplasia the nevus met the criteria of dysplastic nevi according to the Dutch Working Group. The Dutch Working Group has used the following criteria for atypical nevi: 1) ≥ 5 mm in diameter, 2) vague border, 3) asymmetric shape, 4) irregular pigmentation, and 5) red hue [12]. The Breslow depth of the melanomas comprised 1.3 - 4.5 mm, the Clark level corresponded to levels II-IV. The melanoma stages based on the AJCC staging system corresponded to the stages II-III.

The immunohistochemical examination has been performed on paraffin-embedded sections of melanocytic neoplasia's tissue (dysplastic nevi and melanoma) using monoclonal antibodies against Ki-67, VEGF and CD31/PECAM-1. For assessment of the proliferative activity the number of Ki-67-positive melanocytic cells per 200-300 neoplastic cells was calculated. ≤ 20% of Ki-67-positive neoplastic cells was assessed as low proliferative activity, 21 - 50% as moderate proliferative activity and ≥ 51% as high proliferative activity according to the more commonly used method. The melanocytic neoplastic tissue was considered positive when > 25% of neoplastic cells were stained with anti-VEGF monoclonal antibody. VEGF-expression was evaluated only in tumor cells. Melanoma cases were classified into three groups according to the number of VEGF+ tumor cells: < 25%, 25 - 50%, and > 50%. MVD (microvessel/field) was assessed according to Weidner., *et al*. [13]. Malignant melanomas were frequently heterogenous in their neovascularization and MVD of the tissue specimens was assessed in the area with the highest number of microvessels staining for CD31/PECAM-1. Single endothelial cells or clusters of endothelial cells positive for CD31 were considered as a vessel. Vessels with muscular walls were not counted. The areas of highest neovascularization were found by scanning of tissue specimens at low power (40×). After the area of highest neovascularization was identified discrete microvessels were counted on a 200× field (20× objective lens and 10× ocular lens; 0,7386 mm^2 per field). Microvessel counts were determined without knowledge of tumor stage, the patient's outcome, or any other relevant parameters.

Differences between nonparametric variables were estimated by Pearson chi-square test. Differences between parametric variables calculated by Student test. Correlation between parametric variables estimated by Pearson correlation coefficient. All statistical analyses were performed by Statistica 7.0 (StatSoft Inc., USA). Statistical significance was considered at the level of $p < 0.05$.

Results

High proliferative activity was seen in 5 cases (33.3%) of malign melanoma and was not encountered in benign melanocytic nevi. Moderate proliferative activity was seen in 10 malign melanoma cases (66.7%) and in 4 cases (22.2%) of benign melanocytic neoplasia. It should be noted that the all the latter cases were in dysplastic nevi subgroup. Low neoplastic activity was seen only in other 14 cases (77.8%) of benign melanocytic nevi. The mean percentage of proliferative active cells comprised 47.5% (41-55%) for malign melanoma cases and 12.5% (5-25%) for benign melanocytic nevi ($p < 0.01$).

VEGF-expression was positive in all melanoma cases and in 2 cases of benign melanocytic nevi (both of them were dysplastic nevi). Thus VEGF-expression was significantly different between the malignant and benign melanocytic neoplasia ($p < 0.001$).

PECAM-1 expression as a membranous staining was demonstrated in al malign melanoma cases (100%), but just in 7 cases (38.9%) of benign melanocytic neoplasia (Figure 1), including all 6 cases of dysplastic melanocytic nevus ($p < 0.01$). Lack of PECAM-1 expression was seen only in cases of benign melanocytic nevus.

MVD was significantly different in melanoma and benign melanocytic nevi cases. So, the highest number of microvessels varied between 5-20/field of view in melanoma cases (mean 12.0/field or 16.2/ mm^2), no more than 4/field (5.4/ mm^2) in the cases of benign melanocytic nevus ($p < 0.01$).

Figure 1: Immunohistochemical staining of skin melanoma by PECAM-1 monoclonal antibody (×200).

We found a direct correlation between Ki-67 expression (Figure 2) and MVD in cutaneous melanocytic neoplasia (r = +0.78).

Figure 2: Immunohistochemical staining of skin melanoma by Ki-67 monoclonal antibody (×200).

Discussion

The relationship between tumor angiogenesis with the grade of tumor differentiation and prognosis for survival has been proven repeatedly [14]. Formation of new blood vessels from the endothelium of the existing vasculature is essential for neoangiogenesis [15]. The angiogenic process depends upon the balance between many stimulatory and inhibitory factors. MVD is a marker which expressly reflects tumor angiogenesis and has been examined as a potential prognostic marker in numerous tumors [16]. PECAM-1 proved to be a more reliable marker for neoangiogenesis than antibodies to von Willebrand Factor. Although both normal and tumor vessels exhibited prominent staining for PECAM-1, different patterns of endothelial reactivity were observed [17].

Angiogenesis has, also, been recognized as a key determinant factor in cancer growth and metastases development in solid tumors, such as breast, gastric, colorectal, and pancreatic tumors [1]. According to the literature, extensive angiogenesis in cutaneous melanomas displayed 69% risk of relapse and 42% mortality rate, when compared with vascularity absent tumors (33% and 12% respectively) [7]. A substantial number of researches has studied the prognostic value of MVD in cutaneous melanoma, with inconclusive results. More specifically, although many reports directly correlate tumor MVD and survival rates [8,9], Hillen., *et al*. [10] found that microvessel density is not associated with tumor stage or survival. Furthermore, a meta-analysis by Pastushenko., *et al*. [11] concluded that MVD does not have a prognostic value for melanoma.

We found a direct correlation between proliferative activity of melanocytic neoplasia and MVD. As a direct reflection of this we also found higher MVD in malignant melanocytic neoplasia compared to benign ones. Considering that hematogenic metastases is committed via vessels in the tumor tissue theoretically higher MVD may be associated with higher metastatic potential of malignant melanoma. Whether associated PECAM-1-expression in melanoma tissue with high MVD may play role in transendothelial migration of melanoma cells (as the first step in the development of hematogenic metastasis) like leucocyte trafficking across endothelium is expecting its answer in future studies.

Conclusion

High PECAM-1 expression in cutaneous melanocytic neoplasia, that is a surrogate marker of tumor neoangiogenesis, is correlated with higher proliferative activity of melanocytic cells. Such tumors theoretically can have higher metastatic potential not only due to high proliferative activity of melanoma cells also because of having abundant microvessels that are essential for making contact be-

tween malignant cells and bloodstream for developing hematogenic metastasis. Further investigations are needed to clarify the prognostic significance of PECAM-1 expression skin melanoma tissue.

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