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VERSICAN OVEREXPRESSION IN CUTANEOUS MALIGNANT MELANOMA

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Abstract
Objective: Tumor growth regulation by extracellular matrix components has been one of the main topics on tumor biology in the last years. We aimed to investigate the protein expression pattern of decorin and versican in superficial spreading melanoma (SSM) and its precursors.
Patients and methods: Paraffin-embedded sections of benign nevi (BN), dysplastic nevi (DN), and primary SSM were assessed. Immunohistochemistry was performed for decorin and versican antibodies.
Results: We investigated 64 patients with BN (n = 29), DN (n = 15), and SSM (n = 20) with a median Breslow thickness of 0.8 mm (0.2 – 4.6 mm). We did not observe decorin or versican immunoreactivity in melanocytes but in peritumoral stroma. Kruskal-Wallis ANOVA did not reveal significant differences of decorin expression between the groups investigated (P = 0.19). However, compared to BN and DN median expression of versican was significantly increased in SSM (P = 0.016 and P = 0.019, respectively). Decorin as well versican expression of SSM did not significantly correlate with Breslow tumor thickness or Clark level.
Conclusion: Our data indicate that decorin is not differentially expressed in peritumoral stroma of SSM, DN, BN, and thus unlikely of pathogenetic significance in melanoma transformation and/or progression. By contrast, we have demonstrated that SSM is associated with a significant overexpression of peritumoral versican suggesting a role for versican in the pathogenesis of melanoma.

Key words: Malignant melanoma; Melanocytic nevus; Proteoglycans; Decorin; Versican.

INTRODUCTION

Cutaneous malignant melanoma (MM) is a tumor developing by transformation of melanocytes. Its worldwide incidence and mortality rate in fair-skinned populations are on the rise. Presence of metastases carries a severe prognosis because efficacious systemic treatments are still lacking. An earlier differentiation and detection of primary melanoma may improve prognosis. To this aim, markers identifying pre-malignant and malignant lesions are needed. Moreover, understanding the molecular bases of oncogenicity in melanocytic proliferation may contribute to the development of efficacious therapies. Benign and atypical moles have been shown to exist in clinical and histologic contiguity with cutaneous MM, in particular superficial spreading melanoma (SSM) and lentigo maligna melanoma, suggesting that these melanocytic nevi are also susceptible to malignant transformation [1-3].

Tumor growth regulation by extracellular matrix components has been one of the main topics on tumor biology in the last years. Proteoglycans (PGs) comprise a group of extracellular matrix macromolecules which play an important role in matrix biology. PGs are known to be macromolecules with a core protein to which one or more glycosaminoglycan side chains are covalently linked. The molecules are major components of the extracellular matrix and comprise a large family which can be divided into two main branches – small and large. Versican is a large chondroitin sulfate PG produced by several tumor cell types. The expression of increased amounts of versican in the extracellular matrix may play a role in tumor cell growth, adhesion and migration. Decorin, a member of the family of small leucin-rich PGs, has originally been described as a secreted PG component of the connective tissues, and has been implicated in the negative regulation of cell proliferation directly or via interactions with tumor necrosis factor-β [4-6]. The present study was designed to systematically investigate protein expression of decorin and versican in benign and malignant melanocytic skin lesions.

MATERIAL AND METHODS

PATIENTS

Paraffin-embedded sections of benign nevi (BN), dysplastic nevi (DN), and primary SSM patients were subsequently collected in the Department of Dermatology, Ruhr-University Bochum (Germany). We only included patients aged between 30 and 60 years. Clinical data including Breslow's vertical tumor thickness and stage of disease according to the AJCC 2002 system were recorded from the original reports of MM patients [1]. Diagnosis and staging of nevi and melanoma had been performed on the basis of clinical, histopathological, ultrasonic, and computed tomographic findings, respectively. This study adhered to the Declaration of Helsinki and ethics approval for research was obtained from the local review board of the Ruhr-University Bochum (Germany).

Immunohistology

Immunohistochemical staining was performed for decorin and versican as follows: 4 µm paraffin-embedded sections were mounted on silanized slides and stored for 1 hour in a humid chamber at 60 °C. Sections were deparaffinized in xylene and washed with 100%, 96%, 70%, and 50% ethanol for 5 minutes each and rinsed with demineralized water. Sections were covered with 200 µl monoclonal anti-mouse Decorin antibody (R&D, Wiesbaden Nordenstadt, Germany) at a dilution of 1:250 and monoclonal anti-mouse versican antibody (Zytomed, Berlin, Germany) at a dilution of 1 : 500 for 30 minutes each at 25°C in a DAKO (Hamburg, Germany) autostainer, respectively. As a secondary antibody, ChemMate^{Tm} Link, Biotinylated Secondary Antibodies (DAKO) containing anti-mouse and anti-rabbit immunglobulins were used. After washing with Wash Buffer 10 x (DAKO) for two minutes, streptavidin-alkaline phosphatase (DAKO) was used as enzyme for 30 minutes. Chromogen red (Red permanent, DAKO), was used for visualization before counterstaining with hematoxylin and mounting in Mowiol (Roche Molecular Biochemicals, Mannheim, Germany). Specificity testing was performed by blocking of the primary antibody and negative control staining was performed by omitting the primary antibody. All immunohistochemical slides were separately evaluated by the same observer for patterns of immunohistochemical labeling. Expression of decorin and versican was assessed in melanocytes and the peritumoral stroma. Microscopic evaluation (magnification x 100) was an analysis in a coincidental order of the tumor entities assessed. Three randomly chosen fields of view were assessed within the tumor area. Staining for decorin and versican was semi-quantitatively assessed in the dermis using a simple score; 0 = none; 1 = slight; 2 = moderate; 3 = strong.

Statistical Analysis

Data analysis was performed using the statistical package MedCalc Software (Mariakerke, Belgium). Distribution of data was assessed by the D`Agostino-Pearson test. Normally distributed data were expressed as means ± SD, non-normally distributed data as median (range). The one-way ANOVA, the Kruskal-Wallis ANOVA including Mann-Whitney post-hoc test for independent data and the Chi-square test were used for analysis of normally and non-normally distributed data, respectively. The Spearman's coefficients of correlation (r) was also analyzed. A P-value < 0.05 was regarded as statistically significant.

Results

In total, we assessed 64 patients with histopathologically proven benign nevi (BN; n = 29), dysplastic nevi (DN; n = 15), and SSM (n = 20) with a median Breslow thickness of 0.8 mm (0.2 – 4.6 mm). Fourteen patients with SSM had stage IA or IB disease, three had stage IIA or IIB, one had IIIA, one IIIB, and one patient had stage IV. With regard to the tumor localization, there was no significant difference between the groups (P = 0.09). As shown in Table 1, gender and age distribution did not significantly differ between the groups investigated (P = 0.53 and P = 0.1, respectively). Most lesions were on the lower extremities

Table 1. Showing clinical characteristics and lesional protein expression of decorin and versican in patients with benign nevi (BN), dysplastic nevi (DN), and superficial spreading melanoma (SSM).

Diagnosis	**Gender** (m/f)	**Age** (years)	**Localization of tumor**	**Decorin expression** median (range)	**Versican expression** median (range)
BN (n = 29)	11/18	43.4 ± 8.4	A = 0 B = 8 C = 11 D = 3 E = 7	1 (0 – 3)	0 (0 – 2)
DN (n = 15)	5/10	45.5 ± 8.4	A = 1 B = 6 C = 2 D = 0 E = 6	2.3 (0 – 3)	0 (0 – 2)
SSM (n = 20)	9/11	48.9 ± 8.6	A = 0 B = 8 C = 4 D = 0 E = 8	2.6 (0 – 3)	1.2 (0 – 3)
Differences between groups	P = 0.53	P = 0.1	P = 0.09	P = 0.19	P = 0.014* BN vs. SSM, P = 0.016* DN vs. SSM, P = 0.019*

A, head; B, upper trunk; C, lower trunk; D, upper extremities; E, lower extremities. *, statistically significant.

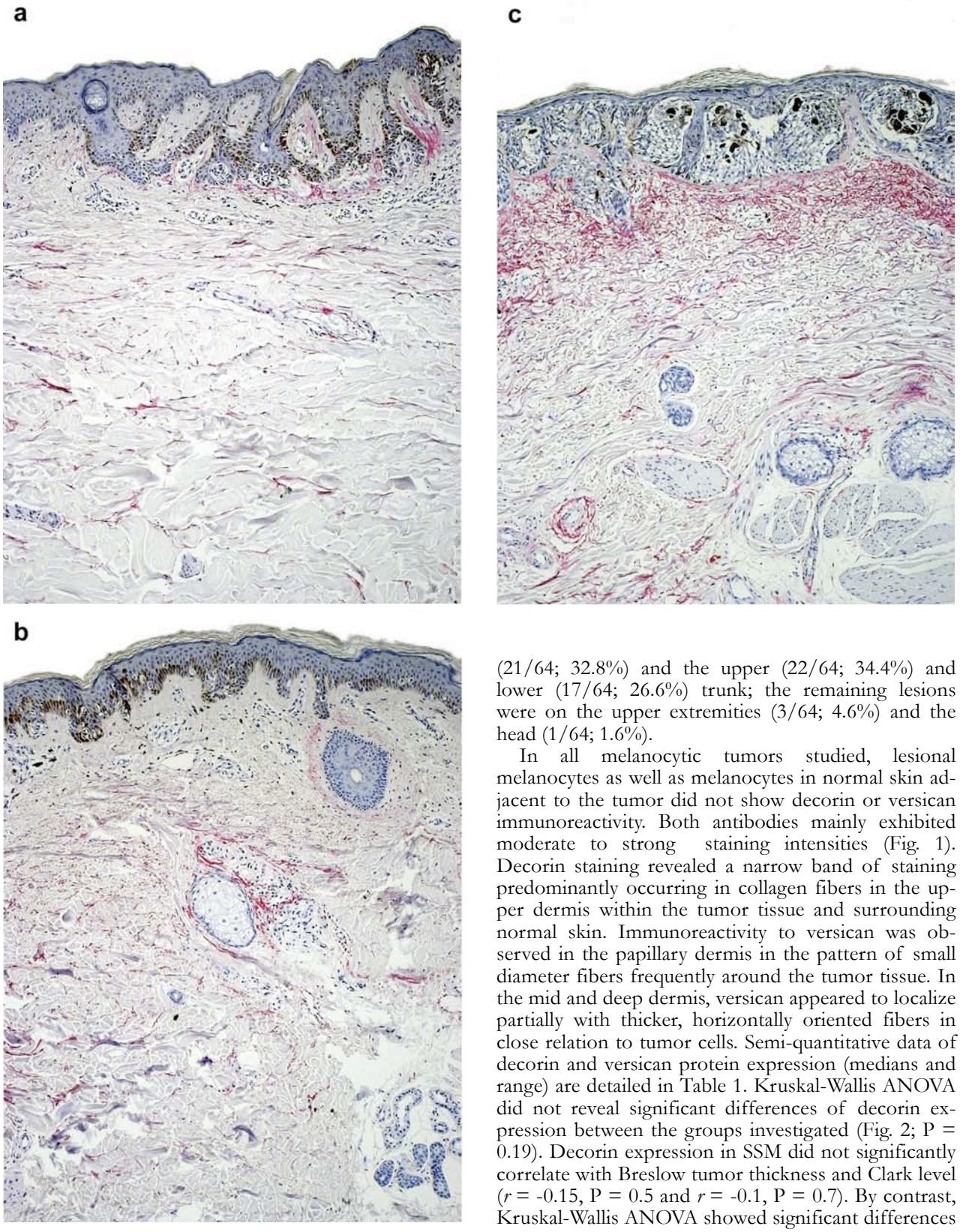

Fig. 1. Immunohistochemical analysis (hematoxylin, x 200) for versican antibody in superficial spreading melanoma (SSM), dysplastic nevus (DN), and benign nevus (BN). Slight peritumoral versican staining in a BN (A) and a DN (B). Relatively strong peritumoral versican staining in a SSM (C) with 1 mm Breslow tumor thickness. These immunohistochemical images are representative for the whole outcome of the study.

(21/64; 32.8%) and the upper (22/64; 34.4%) and lower (17/64; 26.6%) trunk; the remaining lesions were on the upper extremities (3/64; 4.6%) and the head (1/64; 1.6%).

In all melanocytic tumors studied, lesional melanocytes as well as melanocytes in normal skin adjacent to the tumor did not show decorin or versican immunoreactivity. Both antibodies mainly exhibited moderate to strong staining intensities (Fig. 1). Decorin staining revealed a narrow band of staining predominantly occurring in collagen fibers in the upper dermis within the tumor tissue and surrounding normal skin. Immunoreactivity to versican was observed in the papillary dermis in the pattern of small diameter fibers frequently around the tumor tissue. In the mid and deep dermis, versican appeared to localize partially with thicker, horizontally oriented fibers in close relation to tumor cells. Semi-quantitative data of decorin and versican protein expression (medians and range) are detailed in Table 1. Kruskal-Wallis ANOVA did not reveal significant differences of decorin expression between the groups investigated (Fig. 2; $P = 0.19$). Decorin expression in SSM did not significantly correlate with Breslow tumor thickness and Clark level ($r = -0.15$, $P = 0.5$ and $r = -0.1$, $P = 0.7$). By contrast, Kruskal-Wallis ANOVA showed significant differences of versican expression between the groups investigated (Fig. 3; $P = 0.014$). Compared to BN and DN median expression of versican was significantly increased in SSM ($P = 0.016$ and $P = 0.019$, respectively). There was no significant correlation between versican expression, Breslow thickness and Clark level of melanomas ($r = -0.006$, $P = 0.98$ and $r = -0.87$, $P = 0.71$).

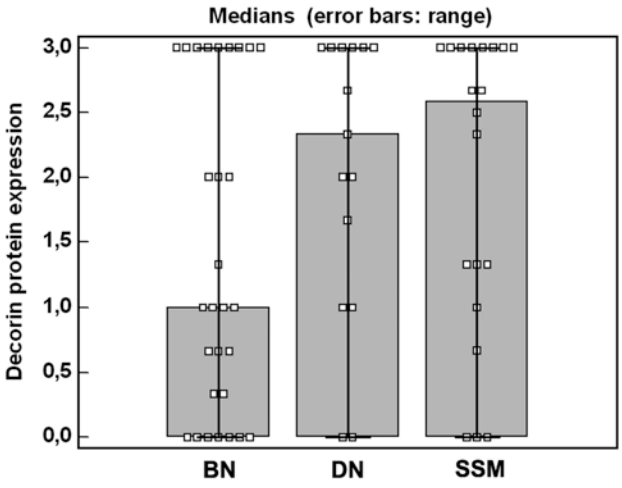

Fig. 2. Showing no significant differences of decorin expression between benign nevi (BN), dysplastic nevi (DN), and superficial spreading melanoma (SSM; Kruskal-Wallis ANOVA P = 0.19).

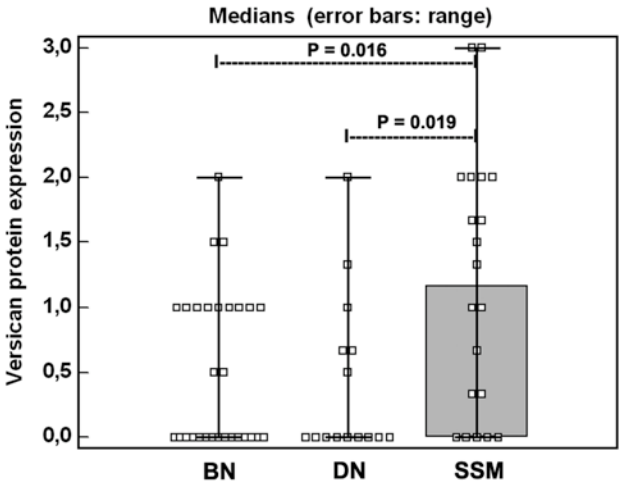

Fig. 3. Significant differences of versican expression between benign nevi (BN), dysplastic nevi (DN), and superficial spreading melanoma (SSM; Kruskal-Wallis ANOVA P = 0.014). Compared to BN and DN median expression of versican was significantly increased in SSM (P = 0.016 and P = 0.019, respectively).

Discussion

The first report on a possible decorin expression in human melanoma came from the research group of Tímár et al. [7, 8] detecting decorin-related antigen epitope in melanoma cell lines. Later, the same group demonstrated the presence of a decorin-like molecule in several human melanoma cell lines, at mRNA as well as protein levels [9]. The expression of this decorin-like molecule did not correlate with the growth and metastatic potential of the tumor cells. However, immunohistochemistry revealed decorin antigen in melanoma but not in BN, suggesting a transformation-related expression pattern [9]. The aforementioned results prompted us to investigate decorin expression in melanoma and its precursor lesions. Neither in benign nor in malignant melanocytes, we observed immunoreactivity for decorin in the present study. Decorin staining of melanocytic skin lesions revealed immunoreactivity predominantly occurring in collagen fibers in the upper dermis within the tumor tissue and surrounding normal skin. Although we observed a trend for increased decorin protein expression in DN and SSM, decorin immunoreactivity of BN, DN, and SSM did not significantly differ. Moreover, we did not observe a correlation between decorin expression and Breslow thickness and Clark level. Similar to our results, Brézillon et al. [10] recently showed that decorin and lumican are located in the dermis and in the peritumoral stroma of melanoma, but are not found in melanoma cells or dense tumor tissue. They hypothesized that lumican, another small PG, may regulate vertical progression of human melanoma [10].

The presence of versican in the extracellular matrix plays a role in tumor cell growth, adhesion and migration, which could be changed by altering the ratio between versican isoforms. Domenzain et al. [11, 12] have shown that overexpression of the V3 isoform of versican in human melanoma cell lines markedly reduces cell growth in vitro and in vivo, since V3-overexpressing cultured cells as well as primary tumors arising from these cells grow slower than their vector-only counterparts. Touab et al. [13] investigated several human melanoma cell lines. They found that versican isoforms are expressed in undifferentiated cell lines but not differentiated cells. Moreover, they observed a negative immunoreactivity for versican in BN, a weakly to strongly positive in DN, and intensively positive in primary melanomas and metastatic melanomas [13]. Similar results were also found in animal studies [14]. Moreover, Touab et al. [15] suggested that versican protein expression may be of value for distinguishing DN from BN and for distinguishing severe DN from mild and moderate DN. In contrast to our observations, however, Touab et al. [11, 13] and other authors [11, 12] described versican immunoreactivity of melanoma cells. Normally, versican has a close association with elastic fibers and is mainly expressed in the papillary and reticular dermis of adult skin [4-6]. Although we did not observe versican immunoreactivity in melanocytes, we found a significant increase of versican expression in the peritumoral stroma of SSM when compared to BN and DN. However, versican immunoreactivity did not correlate with Breslow tumor thickness or Clark level.

It has previously been shown that decorin and versican are differentially expressed in photoaged and chronologically aged skin [4, 16-18]. Therefore, we included only patients aged between 30 and 60 years. Indeed, we have shown that age, gender, and tumor localization did not significantly differ between the groups investigated. Hence, we minimized any bias of decorin and versican results that might have been caused by variations in age, gender, and anatomic site. Of course, however, our data are limited given the absence of mRNA results, survival data, and functional studies. In conclusion, we did not observe decorin or versican immunoreactivity in melanocytes. Our data

indicate that decorin is not differentially expressed in peritumoral stroma of SSM, DN, BN, and thus unlikely of pathogenetic significance in melanoma transformation and/or progression. By contrast, we have demonstrated that SSM is associated with a significant overexpression of peritumoral versican suggesting a role for versican in the pathogenesis of melanoma.

Conflict of Interest Statement: All authors disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within that could inappropriately influence (bias) this work.

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