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Immunohistochemical expression of matrix metalloproteinase-2 (MMP-2) in common melanocytic nevi, dysplastic nevi and primary cutaneous malignant melanomas

Banal melanositik ve displastik nevüsler ile derinin primer melanomlarında immünhistokimyasal matriks metalloproteinaz-2 (MMP-2) ekspresyonu

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ABSTRACT

MMPs play a significant role in the progression of different types of human tumors as well as malignant melanomas. MMP-2, which is also known as 72-kD type IV collagenase is a member of MMPs. The aim of this immunohistochemical study is to evaluate the role of MMP-2 expression in common melanocytic nevi, dysplastic nevi and primary cutaneous malignant melanomas.

Formalin-fixed paraffin-embedded materials from 21 common melanocytic nevi (CMN), 42 dysplastic nevi (DN) and 18 primary cutaneous malignant melanomas (MM) were examined. Standard streptavidin-biotin immunoperoxidase method was used for immunostaining with MMP-2 antibody. Both tumoral and stromal cytoplasmic MMP-2 expressions in melanocytic lesions were scored semiquantitatively.

MMP-2 immunoreactivity was not observed in stromal and/or melanocytic cells in either common melanocytic or dysplastic nevi. Eleven of 18 malignant melanomas expressed MMP-2. Five cases showed positive cytoplasmic staining only in stromal cells, and six cases in both stromal and melanocytic cells. MMP-2 was more commonly expressed at the tumor-stroma interface where significant amount of plasma cells and lymphocytes were observed.

MMP-2 expression does not appear to be related to the pathobiology of benign melanocytic lesions such as common melanocytic nevi and dysplastic nevi. On the other hand, MMP-2 expression by the cells in the tumor stroma, particularly by endothelial cells and fibroblasts, may play a significant role in melanoma pathobiology.

Key words: Common melanocytic nevus, dysplastic nevus, malignant melanoma, immunohistochemistry, MMP-2

ÖZET

Matriks metalloproteinazlar malign melanomlar da içinde olmak üzere, değişik türdeki tümörlerin progresyonunda önemli bir rol oynamaktadır. Tip IV kollagenaz olarak da bilinen MMP-2, metalloproteinaz ailesinin bir üyesidir. Bu immünhistokimyasal çalışmanın amacı, banal melanositik ve displastik nevüsler ile derinin primer melanomlarında MMP-2 ifadesinin önemini değerlendirmektir.

Formalinde fikse edilmiş ve parafine gömülü 21 banal melanositik nevüs, 42 displastik nevüs ve 18 primer deri melanomu bu çalışmaya alınmıştır. MMP-2 antikoru ile immünboyama için standard streptavidin-biotin immünperoksidaz yöntemi kullanılmıştır. Melanositik lezyonlardaki tümöral ve stromal MMP-2 ifadesi semikantitatif olarak skorlanmıştır.

Banal melanositik nevüs ve displastik nevüslerin stromal ya da melanositik hücrelerinde MMP-2 varlığı saptanmamıştır. On sekiz primer deri malign melanomu olgusunun 11'inde MMP-2 varlığı gözlenmiştir. Beş olguda yalnızca stromal hücrelerde sitoplazmik boyanma varken, 6 olguda hem stromal, hem de melanositik hücrede boyanma saptanmıştır. MMP-2, çok sayıda plazma hücresi ve lenfositleri içeren tümör-stroma bileşkesinde daha yoğun olarak boyanmıştır.

MMP-2 ekspresyonu, banal melanositik nevüs ve displastik nevüs gibi benign melanositik lezyonların patobiyolojisi ile ilişkili gibi görünmemektedir. Diğer yandan tümör stromasında bulunan, özellikle endotel hücreleri ve fibroblastlar gibi hücreler tarafından eksprese edilen MMP-2'nin, melanoma patobiyolojisinde önemli bir rolü olabilir.

Anahtar sözcükler: Melanositik nevüs, displastik nevüs, malign melanom, immünhistokimya, MMP-2

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INTRODUCTION

Invasion of tumor cells is a multistep process and has an impact on the biology of neoplastic lesions. During this process tumor cells penetrate into basement membranes and degrade the extracellular matrix by various proteolytic enzymes including matrix metalloproteinases (MMPs) (1-3). It has been also shown that MMPs play a significant role in the progression of different types of human tumors as well as malignant melanomas (2-6).

MMPs are members of the zinc-dependent endopeptidases family which play an important role in tumor biology (7). There are 19 different types of human MMPs categorized into five groups based on their structure and substrate specificity such as collagenases, stromelysins, gelatinases, membrane-type MMPs and metalloelastases. MMP-2, which is also known as 72-kD type IV collagenase is a member of gelatinases (2).

In a report evaluating MMP-2 expression in various melanocytic lesions including CMN, DN as well as in situ, invasive and metastatic melanomas, it was found that the increased number of MMP-2-stained cells were associated with decreased architectural organization and increased atypia (8).

The aim of this immunohistochemical study was to evaluate the role of MMP-2 expression in melanocytic lesions including CMN, DN and different stages of cutaneous MM.

MATERIALS and METHODS

The present study examined tissue specimens from 21 common melanocytic nevi (CMN) (9 compound and 12 intradermal), 42 dysplastic nevi (DN) and 18 primary cutaneous malignant melanomas (MM). Mean ages of patients with CMN, DN and MM were 29.2 (17-41), 22.7 (15-75) and 57.0 (18-80) years respectively. The excisional biopsy specimens were initially fixed in formalin, routinely-processed and embedded in paraffin. H&E-stained slides of each case were collected from pathology archives and reviewed in a blind manner to confirm the original histological diagnosis by two pathologists (BL,UP). New AJCC melanoma staging system (2002) (9) was used for staging of cutaneous malignant melanomas.

Immunohistochemistry

The tissue blocks containing the most representative areas in H&E-stained tissue sections fulfilling the histological criteria were chosen from each case and 5 µ sections were cut into poly-L-lysin coated slides for immunohistochemical staining. Standard streptavidin-biotin immunoperoxidase method was performed with anti MMP-2 antibodies (Ab-4, MS-806-R7, NeoMarkers, Fremont CA, USA).

Briefly, tissue sections were deparaffinized in xylene, rehydrated in alcohol series and immersed in distilled water. Endogen peroxidase activity was blocked using a 0.3% solution of hydrogen peroxide in phosphate buffered saline (PBS) at room temperature for 10 minutes and rinsed with TRIS buffer. Primary antibodies were applied for 60 minutes at room temperature and washed in TRIS buffer. Linking antibody and streptavidin peroxidase complex (DAKO LSAB Kit, K-0675, Carpintera, USA) were added consecutively for ten minutes at room temperature and washed in TRIS buffer. Peroxidase activity was visualized with 0.03% 3,3-diaminobenzidine tetrahydrochloride (DAB) (Sigma Chemical Co, St. Louis, Missouri, USA), which was applied for 5 minutes. The sections were then washed in deionized water and counterstained with Mayer's hematoxylin. In the evaluation of MMP-2 immunostaining, breast carcinoma was used as positive control tissue while the primary antibody was replaced by TRIS buffer in case of negative controls.

Scoring of immunostaining

The most representative areas fulfilling the established histologic criteria were selected and evaluated under a light microscope (Nikon, Tokyo, Japan). Both tumoral and stromal (fib-

roblast or endothelial cells) cytoplasmic MMP-2 expressions in melanocytic lesions were scored semiquantitatively according to a method modified from the study by Hoffman et al (2).

RESULTS

Pathologic T stage (new AJCC melanoma staging system) was evident in 13 of 18 MMs and were enumerated as follows: pT1a, 3 cases; pT2a, 1 case; pT2b, 1 case; pT3a, 2 cases; pT3b, 2 cases; pT4a, 1 case and pT4b, 3 cases. No MMP-2 immunoreactivity was observed in stromal and/or melanocytic cells in either CMN or DN series. Seven (39%) of 18 MM showed no cytoplasmic staining with MMP-2 antibody in stromal or melanocytic cells, whereas five cases (28%) manifested positive cytoplasmic staining only in stromal cells, and six cases (33%) demonstrated positive cytoplasmic staining in both stromal and melanocytic cells (Figure 1). In 11 cases strong cytoplasmic positivity were observed in stromal cells while in 6 of them faint cytoplasmic staining was noticed. Strong MMP-2 expression was observed in four (36%) of 11 positively stained tumors confined mainly to endothelial cells (Figure 2). MMP-2 expression was particularly prominent on the invasive front of the tumor and was more commonly expressed at the tumor-stroma interface where significant amounts of plasma cells and lymphocytes were observed (Figures 3 and 4).

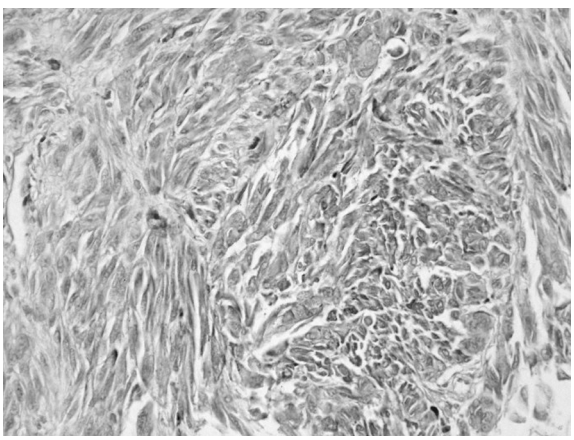

Figure 1. Weak MMP-2 staining in the cytoplasm of melanocytic cells (MMP-2 immunoperoxidase x400).

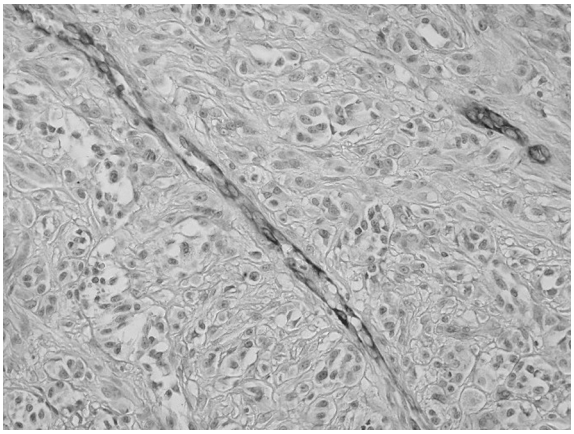

Figure 2. MMP-2 expression confined to endothelial cells in malignant melanoma (MMP-2 immunoperoxidase x200).

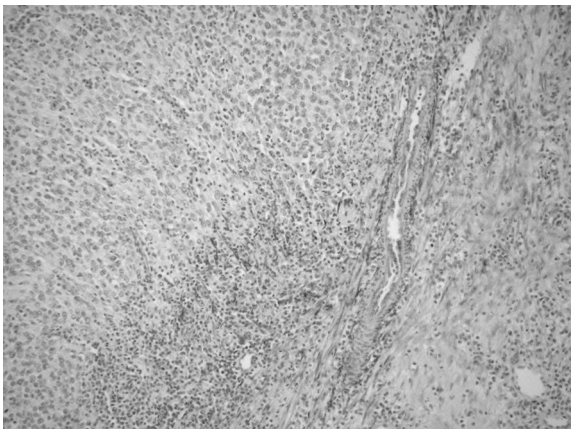

Figure 3. MMP-2 staining in stromal cells at the invasive front of the tumor. Note associated inflammatory response (MMP-2 immunoperoxidase x200).

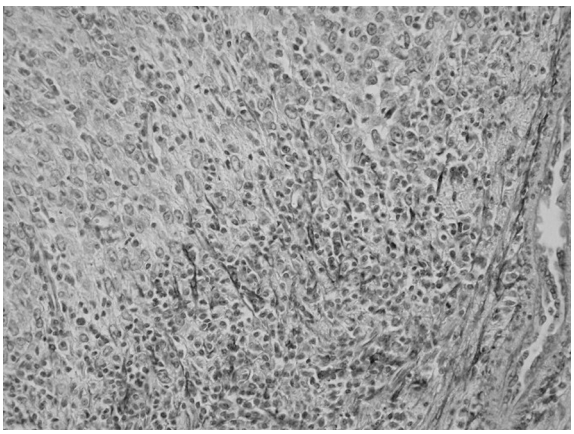

Figure 4. MMP-2 positivity in both endothelial cells and fibroblasts (MMP-2 immunoperoxidase x400).

DISCUSSION

MMPs play an important role in degradation of the extracellular matrix during neoplastic processes. Previous studies have demonstrated that tumor cells as well as various cells in the tumor stroma, including fibroblasts and endothelial cells, expressed MMPs (1,2,10,11).

In English literature, relatively few studies have evaluated MMP-2 expression comparatively in both benign and malignant melanocytic lesions, i.e. CMN, DN and MM (2, 8, 10, 12). Hofmann et al (2) investigated expression of MMP-2, MT1-MMP and TIMP-2 in the melanocytic cells of the xenograft models and in 60 human melanocytic lesions. Consistent with our results, they showed that MMP-2 expression was absent in either common and atypical nevi or in situ melanomas, whereas it was observed in 33 (94%) of 35 malignant melanomas. Authors suggested that the presence of MMP-2 in malignant melanomas might play an important role in the evolution of primary melanoma and might be strongly related with the degree of malignancy. They also demonstrated that MMP-2 was expressed by the cells in the subepidermal nests of primary melanoma and/or in the cells at the tumor-stroma interface of the invasive front of the lesions.

In a study on 118 lesions including CMN, DN, in situ, primary and metastatic melanoma, MMP-2 expression was found in both benign and malignant melanocytic lesions (8). Recently, Simonetti et al (10) evaluated expressions of MMP-2 and MMP-9, and VEGF in different types of cutaneous melanocytic lesions. Contrasting with our results, they showed moderate MMP-2 expression in ordinary benign typical melanocytic nevi and slightly higher MMP-2 expression in atypical melanocytic nevi. Their results were explained by the assumptions that ordinary, benign melanocytic nevi have limited degree of normal connective tissue remodeling. In our study, we did not observe MMP-2 immunoreactivity in stromal and/or melanocytic cells in neither CMN nor DN. Our results suggest that MMP-2 expression does not appear to be related to connective tissue remodeling, and to the interaction between lesional cells and tumor-surrounding stromal cells in both lesions. Simonetti et al (10) also showed strong MMP-2 expression in invasive and in situ melanomas. Similarly, Walker et al (6) demonstrated MMP-1, -2 and -3 expression by melanoma and host tissue cells, especially at the periphery of the tumors, being restricted to less than 10% of all cells.

In our study, we found strong MMP-2 expression in four of 11 melanomas mainly in endothelial cells at the front of the invasive tumor margins. We also observed weak MMP-2 expression in the remaining cases in both endothelial cells and fibroblasts. Recent studies focusing on MMP expression in carcinomas have demonstrated that MMPs are mainly expressed by stromal cells and not by tumor cells (13). These findings suggest that MMP-2 may play a role in the control of local microenvironmental regulation in the stroma surrounding tumoral tissue which results in the support of tumor growth by producing many peptids or supplying providing nutrition by angiogenesis.

Of interest in our study was the finding that in malignant melanomas, MMP-2 was more commonly expressed by stromal fibroblasts and endothelial cells at the invasive front of tumor margins associated with significant amount of plasma cells and lymphocytes. This observation suggests that localized MMP-2 expression appears to be related to the host response at the tumor-stroma interface. Similar observation was reported by Walker et al (6). However, instead of MMP-2 expression they demonstrated MMP-3 expression at the same localization and attributed this finding to a microenviromental regulation, possibly in response to the cellular heterogenity at the tumor margins. This issue remains to be highlighted by further studies.

In conclusion MMP-2 expression does not appear to be related to the pathobiology of CMN

and DN. On the other hand, MMP-2 expression by the cells in the tumor stroma, particularly by endothelial cells and fibroblasts, may have a role in melanoma pathobiology.

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