North Clin Istanb 2024;11(2):158–166
doi: 10.14744/nci.2023.69009

Original Article PATHOLOGY

Evaluation of MMP-9, MMP-13, MMP-21, and TIMP-1 expressions in malign melanom, dysplastic nevi, and banal nevi

Meryem Yuvruk,[1] Rabia Burcin Girgin,[2] Ebru Zemheri[3]

[1]Department of Pathology, Sancaktepe Prof. Dr. Ilhan Varank Training and Research Hospital, Istanbul, Turkiye
[2]Department of Pathology, Inonu University Faculty of Medicine, Turgut Ozal Medical Center, Malatya, Turkiye
[3]Department of Pathology, University of Health Sciences, Umraniye Training and Research Hospital, Istanbul, Turkiye

ABSTRACT

OBJECTIVE: Although the role of MMPs in the pathogenesis of melanoma is known, few studies have investigated their role in the development of nevi and dysplastic nevi. This study aims to search the expression differences of MMP-9, MMP-13, MMP-21, and TIMP-1 between malignant melanoma (MM), intradermal nevi (IDN), and dysplastic nevi (DN).

METHODS: MMP-9, MMP-13, MMP-21, and TIMP-1 antibodies were studied immunohistochemically for 60 cases in our pathology clinic archive between 2013 and 2014.

RESULTS: The MM group had the highest expression percentage and intensity for MMP-9 ($p<0.001$). There was no statistical significance between MMP-13 expression intensities of lesion cells and stromal cells and stromal expression intensities ($p>0.05$). MMP-21 lesion staining intensities in DN and MM compared to IDN were statistically significant ($p=0.001$, $p=0.011$, respectively). For TIMP-1, there was a significant difference between the IDN and the MM group regarding the staining proportion of lesion cells ($p<0.01$). There was a statistically significant difference in all groups according to lesion cells' expression intensity. (IDN-DN $p<0.001$, IDN-MM $p=0.044$, DN-MM $p<0.001$).

CONCLUSION: The following markers can be helpful when lesions cannot be differentiated; increased staining proportions and intensity of MMP-9 in both lesion and stromal cells favor MM in cases where MM and IDN cannot be differentiated. The increased MMP-13 staining proportion of lesion cells can favor DN in cases where the pathologist cannot differentiate DN and MM. Intense expression of MMP-21 by lesion cells can be a potential marker for evaluating the lesion in favor of DN in cases where DN and IDN cannot be differentiated. The high expression intensity of TIMP-1 in lesion cells can favor DN in cases where there is ambiguity between DN and MM. High expression proportion and intensity of stromal cells of TIMP-1 can be useable in favor of MM in cases where MM and DN cannot be differentiated.

Keywords: Dysplastic nevus; intradermal nevus; malignant melanoma; MMP; TIMP-1.

Cite this article as: *Yuvruk M, Girgin RB, Zemheri E. Evaluation of MMP-9, MMP-13, MMP-21, and TIMP-1 expressions in malign melanom, dysplastic nevi, and banal nevi. North Clin Istanb 2024;11(2):158–166.*

The extracellular matrix (ECM) is a dynamic structure that creates a unique environment in the intercellular spaces. It helps hold tissue cells together and acts as a reservoir for many hormones that control cell growth and differentiation [1]. The matrix metalloproteinase (MMP) family is an essential member of the extracellular proteinases. Their most important task is the destruction of the ECM. The MMPs are secreted as

Authors affliation while the work was conducted: Istanbul Medeniyet University, Goztepe Training and Research Hospital.

Received: December 16, 2022 *Revised:* April 24, 2023 *Accepted:* July 07, 2023 *Online:* April 25, 2024

Correspondence: Rabia Burcin GIRGIN, MD. Inonu Universitesi Tip Fakultesi, Turgut Ozal Tip Merkezi, Patoloji Klinigi, Malatya, Turkiye.
Tel: +90 422 341 06 60 e-mail: rabiaburcingirgin@gmail.com

proenzymes from various connective tissue cells such as fibroblasts, osteoblasts, chondrocytes, endothelial cells, macrophages and neutrophils. It has been determined that they participate in many physiological and pathological processes. These enzymes are involved in ECM turnover, tissue remodeling, angiogenesis, morphogenesis, and pathological processes such as inflammation and tumor cell invasion and metastasis [2].

MMPs can be classified into 6 main groups according to their substrate specificity: i) collagenases: MMP-1, MMP-8, MMP-13; ii) gelatinases: MMP-2, MMP-9; iii) stromelysins: MMP-3, MMP-10, MMP-11; iv) matrilysins; v) membrane-type metalloproteinases: MMP-14, MMP-15, MMP-16, MMP-17, MMP-24, MMP-25; vi) other MMPs: MMP-19, MMP-20, MMP-21, MMP-23, MMP-27, MMP-28. These proteins are inhibited by tissue inhibitors specific to metalloproteinases (Tissue inhibitors of matrix metalloproteinases, TIMPs) [3].

Cutaneous melanomas are highly invasive and metastatic tumors. Disruption and remodeling of basement membranes and extracellular matrix by proteolytic enzymes are essential in melanoma cells' migration, invasion, and metastasis forms. Matrix metalloproteinases and their tissue inhibitors play a crucial role in this multistep process. Melanoma cells express multiple members of matrix metalloproteinases and their tissue inhibitors [3]. Although the role of MMPs in the pathogenesis of melanoma is known, few studies investigated their role in the development of nevi and dysplastic nevi. In addition, there are few publications on MMP21 compared to MMP13 and MMP9.

Our study aimed to evaluate the expression of MMP-9, MMP-13, MMP-21 and TIMP-1 in lesion cells and surrounding connective tissue in nevus, dysplastic nevus, and melanoma and the variability of this expression.

Highlight key points

- Increased staining proportions and intensity of MMP-9 in both lesion and stromal cells favor MM in cases where MM and IDN cannot be differentiated.
- Increased MMP-13 staining proportion of lesion cells can favor DN in cases where the pathologist cannot differentiate DN and MM.
- Intense expression of MMP-21 by lesion cells can be a potential marker for evaluating the lesion in favor of DN in cases where DN and IDN cannot be differentiated.
- The high expression intensity of TIMP-1 in lesion cells can favor DN in cases where there is ambiguity between DN and MM.
- High expression proportion and intensity of stromal cells of TIMP-1 can be useable in favor of MM in cases where MM and DN cannot be differentiated.

MATERIALS AND METHODS

Ethical Approval

This clinical study adhered to the tenets of the Declaration of Helsinki and was approved by decision number 2014/0154 on 21/10/2014 by the hospital's Local Ethics Committee.

Study Population

Our study included 60 cases; 20 malignant melanoma, 20 dysplastic nevi, 20 banal nevi, in the archive of our pathology clinic between 2013 and 2014.

Specimen Preparation and Immunohistochemistry

Hematoxylin-eosin sections were prepared and evaluated from paraffin blocks of these cases.

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue samples, 3-µm-thick sections were used. Tissue sections were taken to electrostatically charged slides and dried at 70 °C for at least 1 h. The entire immunohistochemical staining process, including deparaffinization and antigen expression, was performed in a fully automated immunohistochemistry staining device, Leica BOND-MAX Detection System (Leica Biosystems, Melbourne, Australia). A biotinylated, HRP multimer-based, hydrogen peroxide substrate and a ready-to-use kit containing the 3,3-diaminobenzidine tetrahydrochloride chromogen were used for the procedure. The following antibodies were used according to their manufacturer's instructions: MMP-9 (LEICA/Novocastra, NCL-MMP9-439; Clone15 W2, Antigen Retrieval: EDTA 20 min, 1 ml dilution 1:50, 30 min); MMP-13 (THERMO/Pierce, MA5-14238, Clone VIIIA2, Antigen Retrieval: citrate 20 min, 1 ml, dilution: 1:25, 30 min); MMP-21 (ABCAM, ab52817; Clone: EP1277Y, Antigen Retrieval: citrate 20 min, 1 ml, dilution: 1:100, 30 min); TIMP1(LEICA/Novocastra, NCL-TIMP1-485, Clone: 6F6a, Antigen Retrieval: EDTA 20 min, 1 ml, dilution: 1:50, 30 min). The immunohistochemical reaction was graded and scored according to the specific staining sites (nuclear, cytoplasmic, membranous or mixed) and intensities of the relevant antibodies. Expressions of MMP-9, MMP-13, MMP-21, and TIMP-1 immunohistochemical stains in malignant melanoma, dysplastic nevus, and banal nevus were evaluated as the percentage and intensity of staining both in the cells forming the lesion and in the

Table 1. Evaluation of age and gender according to groups

	IDN (n=20)	DN (n=20)	MM (n=20)	p
Age, range, Mean±SD	20–59 39.7±11.4	8–77 38.4±18.5	47–85 71.4±11.8	[a]0.048*
Gender, (%)				[b]0.001
Male	40	70	75	
Female	60	30	25	

SD: Standard deviation; a: Pearson Chi-Square; b: One-Way Anova Test; *: p<0.05; IDN: Intradermal nevus; DN: Dermal nevus; MM: Malignant melanoma.

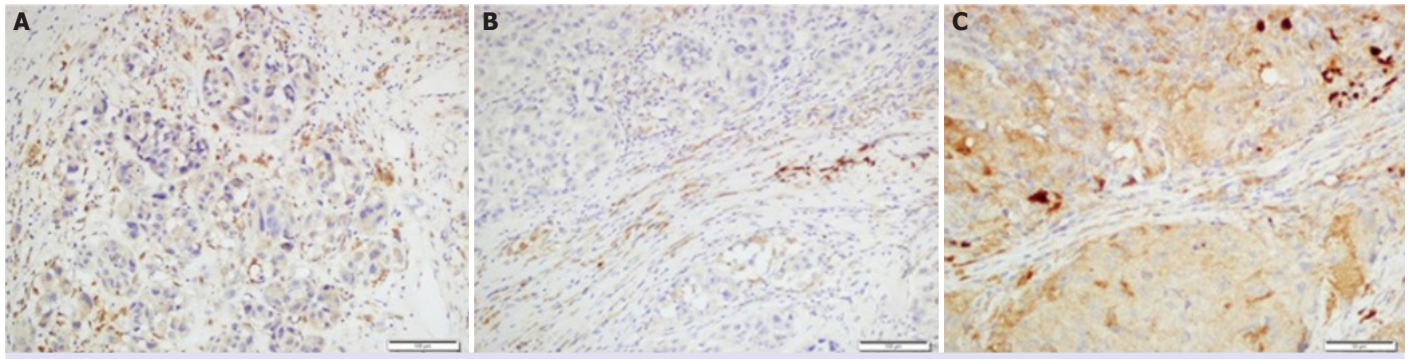

Figure 1. MMP-9 expression. **(A)** Intradermal nevus: 25–50% of lesion cells expressed weakly MMP-9, whereas 25–50% of stromal cells expressed strongly MMP-9. **(B)** Malignant melanoma: Less than 25% of lesion cells expressed weakly MMP-9, whereas 25%-50% of stromal cells expressed strongly MMP-9. **(C)** Malignant melanoma: 50–75% of lesion cells expressed moderately MMP-9, whereas 25%-50% of stromal cells expressed weakly MMP-9.

stroma around the lesion. While making the evaluation, expression rate was grouped as positive in less than 25% cells, positive in 25–50% cells, positive in 51–75% cells, and positive in more than 75% cells. Staining intensity was grouped as none, weak, moderate, and strong.

Statistical Analysis

NCSS (Number Cruncher Statistical System), 2007 (NCSS, LLC Kaysville, Utah, USA) program was used for statistical analysis. While evaluating the study data, in addition to descriptive statistical methods (mean, standard deviation, median, frequency, and ratio), the Oneway Anova test was used to compare the parameters with normal distribution between groups, and the Tukey HDS test was used to determine the group that caused the difference. In addition, the Pearson Chi-Square test, Yates Continuity Correction, Fisher's Exact test, and Fisher Freeman Halton test were used to compare qualitative data. The results were evaluated at the 95% confidence interval with a significance level of p<0.05.

RESULTS

This study evaluated 60 consecutive patients who underwent skin biopsies at our institution between 2013 and 2014. The median age was 49.80±20.85 years (range, 8–85 years). Of these patients, 37 (61.7%) were men and 23 (38.3%) were women (male-to-female ratio: 1.61:1). The gender distribution of the groups was statistically significant. The number of women in the IDN group were found to be significantly higher than the other groups (p=0.048; p<0.05) (Table 1).

Comparison of Expression Proportions and Intensities of MMP-9 in Lesion Cells and Stromal Cells According to Groups

The difference between expression proportions and intensities of MMP-9 in lesion cells and stromal cells according to groups was statistically significant (p=0.001; p<0.001 respectively) (Table 2). The percentage of expression in the IDN group was below 25% and weak (Fig. 1A).

TABLE 2. Comparison of expression proportions and intensities of MMP9, MMP21, and TIMP1 in lesion cells and stromal cells according to groups

	MMP9				MMP21				TIMP1			
	IDN (n=20)	DN (n=20)	MM (n=20)	[a]p	IDN (n=20)	DN (n=20)	MM (n=20)	[a]p	IDN (n=20)	DN (n=20)	MM (n=20)	[a]p
Stained proportion of lesion cells, n (%)												
<25%	40	95	0	0.001**	0	0	0	–	5	0	0	0.027*
25–50%	0	0	15		0	0	0		20	10	0	
50–75%	0	0	0		0	0	0		15	5	0	
>75%	60	5	85		100	100	100		60	85	100	
Staining intensity of lesion cells, n (%)												
None	40	95	0	0.001**	0	0	0	0.001**	5	0	0	0.001**
Weak	60	5	50		0	0	0		25	5	35	
Moderate	0	0	30		45	0	5		35	25	60	
Strong	0	0	20		55	100	95		35	70	5	
Stained proportion of stromal cells, n (%)												
<25%	90	25	5	0.001**	5	0	0	0.001**	0	0	5	0.001**
25–50%	5	35	60		90	95	15		15	95	10	
50–75%	0	5	0		0	0	15		45	5	0	
>75%	5	35	35		5	5	70		40	0	85	
Staining intensity of stromal cells, n (%)												
None	90	25	5	0.001**	5	0	0	0.001**	0	0	5	0.001**
Weak	10	75	50		65	70	5		20	75	70	
Moderate	0	0	20		25	25	55		70	25	25	
Strong	0	0	25		5	5	40		10	0	0	

a: Fisher-Freeman-Halton Test; *: P<0.05; **: P<0.001; IDN: Intradermal nevus; DN: Dermal nevus; MM: Malignant melanoma.

Table 3. Post Hoc (binary) evaluation of MMP9[a], MMP21[b], and TIMP1[b] expressions proportions and intensities in lesion cells and stromal cells according to groups

	MMP9			MMP21			TIMP1		
	IDN-DN	IDN-MM	DN-MM	IDN-DN	IDN-MM	DN-MM	IDN-DN	IDN-MM	DN-MM
Proportion of lesion cells	0.001**	0.001**	0.001**	0.001**	0.011*	1.000	0.078	0.003**	0.231
Staining intensity of lesion cells	0.001**	0.001**	0.001**	1.000	0.001**	0.001**	0.001**	0.044*	0.001**
Stained proportion of stromal cells	0.001**	0.001**	0.150	1.000	0.001**	0.001**	0.001**	0.001**	0.001**
Staining intensity of stromal cells	0.001**	0.001**	0.002**	0.001**	0.011*	1.000	0.002**	0.002**	1.000

a: Yates Continuity Correction Test; b: Fisher Exact Test & Fisher-Freeman-Halton Test; *: P<0.05; **: P<0.001; IDN: Intradermal nevus; DN: Dermal nevus; MM: Malignant melanoma.

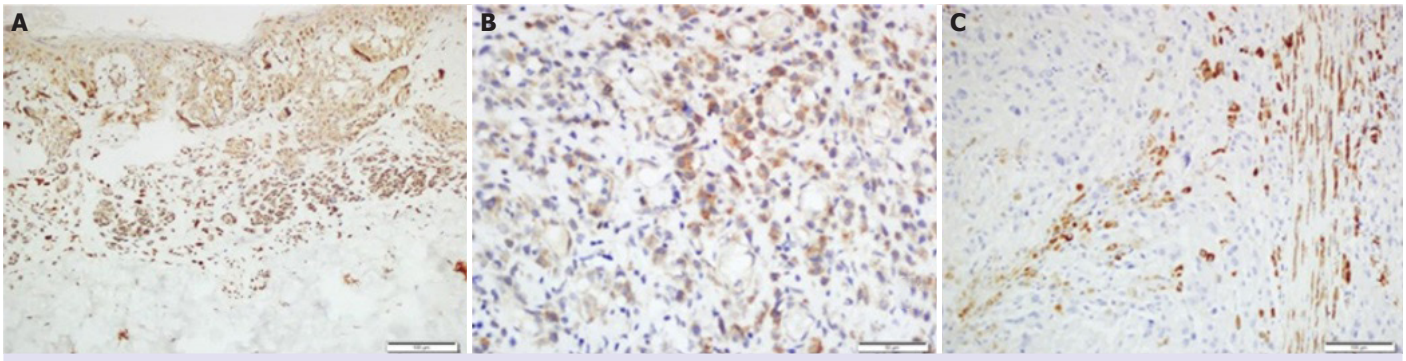

Figure 2. MMP-13 expression. **(A)** Intradermal nevus: 50–75% of lesion cells expressed strongly MMP-13, whereas stromal cells did not express MMP-13. **(B)** Malignant melanoma: 50–75% of lesion cells expressed moderately MMP-13, whereas stromal cells did not express MMP-13. **(C)** Malignant melanoma: 25–50% of both lesion and stromal cells expressed strongly MMP-13.

While more than 75% of lesion cells expressed MMP-9 in the MM group, the expression rate was less than 25% in the DN group. While all cases with moderate and strong intensity of lesion cells were seen in the MM group, no significant or weak expression was observed in the lesion cells in the DN group. More than 75% of the stromal cells in DN and MM expressed MMP-9; weak expression was observed in DN, while moderate and strong expression was observed in MM (Fig. 1B, C).

In the post hoc evaluations calculated according to the expression percentages and intensities of MMP-9 in the lesion and stromal cells, while the DN group had the lowest percentage and intensity, the IDN group followed this. Meanwhile, the MM group was the group with the highest expression percentage and intensity (Table 3).

Comparison of Expression Proportions and Intensities of MMP-13 in Lesion Cells and Stromal Cells According to Groups

The difference between expression proportions and intensities of MMP-13 in lesion cells and stromal cells according to groups was statistically significant (p=0.048; p<0.005 respectively) (Table 2). More than 75% of lesion cells expressed MMP-13 in the DN group, while below 25% in the IDN and MM group (Fig. 2A–C). There was no statistical significance between MMP-13 expression intensities of lesion cells and stromal cells and stromal expression intensities (p>0.05).

Comparison of Expression Proportions and Intensities of MMP-21 in Lesion Cells and Stromal Cells According to Groups

More than 75% of lesion cells expressed MMP-21 in all groups (cannot be calculated statistically). The MMP-21 expression intensity of lesion cells was statistically significant between groups (p<0.001). The expression percentage and intensity of stromal cells were statistically significant (p<0.01). Stromal staining percentages and intensities were low and weak in the IDN and DN groups (Fig. 3A, B). The percentage of stromal expression in MM is more than 75% and moderate/strong intensity (Fig. 3C) (Table 2).

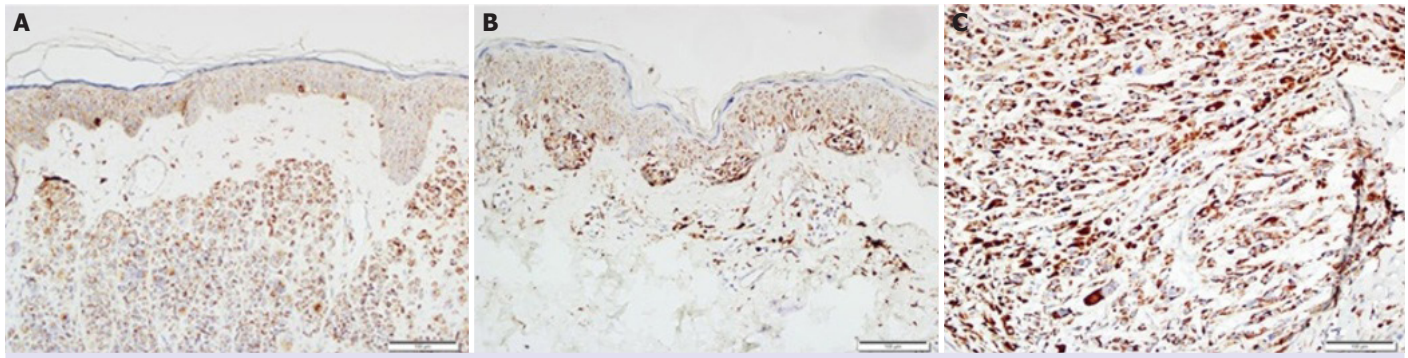

FIGURE 3. MMP-21 expression. **(A)** Intradermal nevus: 50–75% of lesion cells expressed moderately MMP-21, whereas stromal cells did not express MMP-21. **(B)** Displastic nevus: 50–75% of lesion cells expressed moderately MMP-21, whereas 25–50% of stromal cells expressed moderately MMP-21. **(C)** Malignant melanoma: 50–75% of lesion cells expressed strongly MMP-21, whereas stromal cells did not express MMP-21.

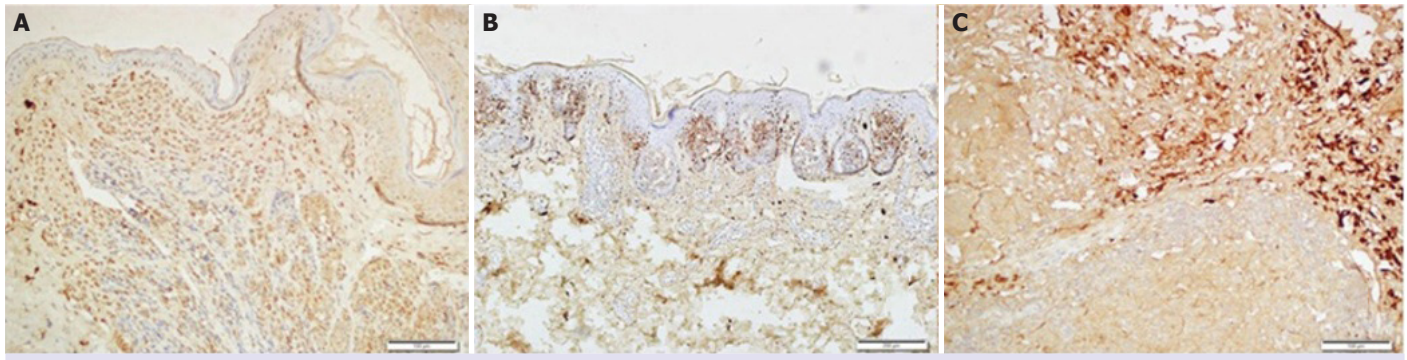

FIGURE 4. TIMP-1 expression. **(A)** Intradermal nevus: 50–75% of lesion cells expressed moderately TIMP-1, whereas 25–50% of stromal cells expressed moderately TIMP-1. **(B)** Displastic nevus: 50–75% of lesion cells expressed strongly TIMP-1, whereas 25–50% of stromal cells expressed strongly TIMP-1. **(C)** Malignant melanoma: 50–75% of lesion cells expressed weakly TIMP-1, whereas 50–75% of stromal cells expressed strongly TIMP-1.

In post hoc evaluation, MMP-21 lesion staining intensities in DN and MM compared to IDN were statistically significant (p=0.001, p=0.011, respectively). There was no statistically significant difference between DN and MM (p>0.05). While the MMP-21 stromal expression percentages and intensities were not statistically significant between the DN and IDN groups (p>0.05), a significant difference was found between IDN and MM groups and DN and MM groups (p<0.01) (Table 3).

Comparison of Expression Proportions and Intensities of TIMP-1 in Lesion Cells and Stromal Cells According to Groups

The TIMP-1 expression proportions and intensities of lesion cells were statistically significant between the groups (p=0.027, p<0.001 respectively). Lesion cell expression intensities are also statistically significant (p<0.01), they are high in IDN and DN (Fig. 4A, B) and moderate in MM. The expression proportion of lesion cells was more than 75% in all cases in the MM group (Fig. 4C). Stromal expression proportion and intensity of TIMP-1 showed a statistical significance between all groups (p<0.01) (Table 2).

The post hoc evaluation revealed a significant difference between the IDN and the MM group regarding the staining proportion of lesion cells (p<0.01). There was a statistically significant difference in all groups according to lesion cells' expression intensity (IDN-DN p<0.001, IDN-MM p=0.044, DN-MM p<0.001). According to the stromal staining proportions, there was a statistically significant difference between all groups (IDN-DN p<0.01; IDN-MM p<0.02, and DN-MM p<0.01). Stromal staining intensity showed statistical significance in IDN-DN and IDN-MM groups (p<0.01 and p=0.002, respectively) (Table 3).

DISCUSSION

The MMP family is an essential member of the extracellular proteinases. Their most important task is the destruction of the ECM that acts as a primary barrier to prevent tumor tissue growth and tumor cell spread [4]. Malignant tumors use MMPs to cross this barrier [4–7]. Some factors that inhibit MMPs have been identified. These are alpha-2-macroglobulin, serum C-reactive protein, and specific MMP-tissue inhibitors (TIMP). TIMP regulates MMP activity during both proenzyme activation and substrate degradation [8]. MMP-9 is secreted by lung alveolar macrophages, monocytes, lymphocytes, polymorphonuclear leukocytes, and keratinocytes. Macrophages and leukocytes use this enzyme to penetrate different tissue compartments in the body during their migration. MMP-9, also known as gelatinase B, is substrate specific for gelatin and type IV basement membrane collagen [2, 8, 9]. MMP-9 expression has been demonstrated in the carcinogenesis and progression of bladder, lung, prostate, colorectal, breast, head and neck, stomach cancers, and osteosarcoma [10–12]. In 2002, a study designed by Kurschat et al. [13] revealed that MMP-2 and MMP-9 were immunohistochemically detected both in melanoma cells and the stroma, but only in the peritumoral area with in situ enzymatic assays. Chen et al. [14] investigated the expression of heparanase and MMP-9 in normal skin, junctional nevus, and cutaneous melanoma immunohistochemically. They found that heparanase and MMP-9 showed similar profiles. They detected weak staining of over 70% in melanoma and over 10% in junctional nevi, while normal skin did not express these markers. Both markers showed high expression in the group with lymph node metastasis. In another publication investigating MMP-9 expression in melanomas at the molecular level, the intragenic hypermethylation of MMP-9 was associated with MMP-9 overexpression and played a role in the development and progression of cancer. In conclusion, increased MMP-9 level was found to support the malignant phenotype [15]. MMP-9 was found as an indicator of invasion in melanoma and the BRAFV600E mutation has a role in melanoma development. In addition, circulating-free DNA BRAFV600E and MMP-9 serum levels were compared in patients receiving dabrafenib treatment; It was concluded that MMP-9 can be used as a prognostic indicator in the evaluation of response to therapy in patients with a diagnosis of melanoma using BRAF inhibitors [16]. In our study, MMP-9 had the lowest lesion expression proportion and intensity in the DN group, while the highest expression proportion and intensity were in the MM group. While the IDN group had the lowest stromal staining proportion and intensity, the MM group had the highest stromal staining proportion and intensity. These findings showed that MMP-9 is active in all benign, invasive or non-invasive malignant lesions. Following the literature, it is more expressed in the cells forming the lesion and in the accompanying stroma in invasive lesions.

MMP enzymes can destruct the extracellular matrix, and these properties play an essential role in invasion of cutaneous malignant melanoma. MMP-13 plays a crucial role in the activation of MMPs. Corte et al. [17] evaluated the expression of MMP-13 in invasive cutaneous malignant melanoma, melanoma in situ and benign lesions. This study found MMP-13 negative in benign lesions, MMP-13 positive in 30% of in situ melanomas and 45% of invasive cutaneous malignant melanoma. Zigrino, who showed that there was MMP's activity at the tumor cell border and in the stroma in his previous study, injected intradermal melanoma cells into mice whose MMP-13 pathway was inactivated entirely and found that inhibition of the MMP-13 pathway caused a significant decrease in both metastasis and local invasion and vascular spread [18]. Meierjohann et al. [19] studied the tumorigenic role of MMPs in melanoma cells and melanocytes and the tyrosine kinase receptor-dependent migration of melanocytes. As a result of the study, they found that the primary mediator stimulating growth in melanoma cells and melanocytes was MMP-13. Therefore, they reported that MMP-13 inhibitors should be considered in the treatment of melanoma [19, 20]. In a recent study, Zamolo et al. [21] investigated the expression of MMP-1, MMP-2, and MMP-13 in cells of melanocytic origin in primary nodular melanoma and dysplastic nevi and the presence of BRAF V600e mutation. MMPs were expressed in more than 30% of nodular melanoma and less than 8% of dysplastic nevi, which was statistically significant. In addition, MMP-1 and MMP-13 were significantly less expressed in BRAF V600 mutated melanomas than in BRAF V600 wild-type melanomas. In our study, the expression of MMP-13 in lesion cells at a proportion of more than 75% was statistically significant in DN than in IDN and MMs. There was no significant difference between IDN, DN and MM in terms of expression intensity of MMP-13 expression in lesion cells. This re-

sult we found differs from that of Zamolo et al. [21] that nodular melanomas showed a significantly higher level of protein expression for all three matrix metalloproteinases compared to DN.

To date, few studies have evaluated the expression of MMP-21 in nevoid lesions. Kuivanen et al. [22] studied MMP-21, MMP-26, MMP-28 in melanoma tissue culture and in vivo. They investigated MMP-21, MMP-28, and MMP-13 expressions immunohistochemically in non-metastatic melanoma, melanoma with micrometastasis and melanoma in situ. Additionally, MMP-21 messenger RNA expression was investigated by the PCR method in all five melanoma cell lines. MMP-21 was more prominently expressed in non-metastatic melanoma than in metastasized ones in both tissues and in vitro studies. Expression was observed more prominently in non-metastatic melanoma than in those with metastasis. While IDN included in this study did not show expression with MMP-21, MM in situ showed expression. MMP-21 was expressed by fibroblasts around melanoma islands in addition to melanoma cell However, endothelial cells and keratinocytes did not express. In conclusion, MMP-21 was found to play a role in the early stages of melanoma progression. Furthermore, immunohistochemical expression of MMP-13 was observed in melanoma cells. Contrary to MMP-21, all cases with sentinel node metastasis showed MMP-13 expression. MMP-21 mRNA was positive in all melanoma cell lines [22]. A study investigating MMPs and their inhibitors in melanoma revealed that MMP-21 was responsible for malignant transformation in MM and was a potential predictive biomarker for cancer progression [3]. Similarly, our results showed that lesion cells and stromal cells expressed MMP-21 at a low proportion and intensity in the IDN and DN groups. However, the expression was detected at a higher proportion and intensity in the MM group.

Bastian et al. [20] investigated the expressions of TIMP and MMPs in melanoma cases with and without regression. The MMPs group of this study included MMP-9 and MMP-13 which were also investigated in our study. MMP-9 was found in tumor cells in melanomas without regression and in tumor cells, few fibroblasts and plasma cells in melanomas with regression. MMP-13 was diffusely positive in melanoma cells, and the melanoma group with regression tumor cells, fibroblasts, endothelial and inflammatory cells expressed MMP-13. In the TIMP group, TIMP-1, TIMP-2, and TIMP-3 were studied; both groups showed intense staining in tumor cells but scattered staining in fibroblasts, endothelial, and plasma cells.

This study is not devoid of limitations. First, the number of patients in our study was too small for generalization of the results thus larger cohorts' studies are needed to support our results. Second, since the follow-up data of the patients are not available, we could not comment on the effects of MMP-9, MMP-13, MMP-21, and TIMP-1, which we evaluated in the study, on the survival and prognosis of the patients.

Conclusion

The current study aimed to investigate whether MMP-9, MMP-13, MMP-21 and TIMP-1 can be used in favor of IDN in evaluating the lesion in cases where DN and MM cannot be distinguished and whether these markers can be used to assess the lesion in favor of DN when DN and MM cannot be distinguished. Our results revealed that increased staining proportions and intensity of MMP-9 in both lesion and stromal cells can be interpreted favor MM in cases where MM and IDN cannot be differentiated. The increased MMP-13 staining proportion of lesion cells can favor DN in cases where the pathologist cannot differentiate DN and MM. Intense expression of MMP-21 by lesion cells can be a potential marker for evaluating the lesion in favor of DN in cases where DN and IDN cannot be differentiated. The high expression intensity of TIMP-1 in lesion cells can favor DN in cases where there is ambiguity between DN and MM. High expression proportion and intensity of stromal cells of TIMP1 can be useable in favor of MM in cases where MM and DN cannot be differentiated.

Ethics Committee Approval: The Medeniyet University, Göztepe Training and Research Hospital Clinical Research Ethics Committee granted approval for this study (date: 21.04.2014, number: 2014/0154).

Authorship Contributions: Concept – MY, RBG, EZ; Design – MY, RBG, EZ; Supervision – EZ; Fundings – MY, EZ; Materials – MY, EZ; Data collection and/or processing – MY, RBG, EZ; Analysis and/or interpretation – MY, RBG, EZ; Literature review – MY, RBG, EZ; Writing – MY, RBG, EZ; Critical review – MY, RBG, EZ.

Conflict of Interest: No conflict of interest was declared by the authors.

Use of AI for Writing Assistance: Not declared.

Financial Disclosure: The Scientific Research Projects Commission of Istanbul Medeniyet University [TTU-2015-653].

Peer-review: Externally peer-reviewed.

REFERENCES

1. Frantz C, Stewart KM, Weaver VM. The extracellular matrix at a glance. J Cell Sci 2010;123:4195–200.
2. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res 2003;92:827–39.
3. Napoli S, Scuderi C, Gattuso G, Bella V Di, Candido S, Basile MS, et al. Functional roles of matrix metalloproteinases and their inhibitors in melanoma. Cells 2020;9:1151.
4. Johansson N, Ahonen M, Kähäri VM. Matrix metalloproteinases in tumor invasion. Cell Mol Life Sci C 2000;57:5–15.
5. Nabeshima K, Inoue T, Shimao Y, Sameshima T. Matrix metalloproteinases in tumor invasion: Role for cell migration. Pathol Int 2002;52:255–64.
6. Belotti D, Paganoni P, Giavazzi R. MMP inhibitors: experimental and clinical studies. Int J Biol Markers 1999;14:232–8.
7. Zucker S, Cao J, Chen WT. Critical appraisal of the use of matrix metalloproteinase inhibitors in cancer treatment. Oncogene 2000;19:6642–50.
8. Apakkan Aksun S, Özmen D, Bayındır O. Metalloproteinases, their inhibitors and related physiological and pathological conditions. [Article in Turkish]. T Klin J Med Sci 2001;21:332–42.
9. Beaudeux JL, Giral P, Bruckert E, Foglietti MJ, Chapman MJ. Matrix metalloproteinases, inflammation and atherosclerosis: therapeutic perspectives. Clin Chem Lab Med 2004;42:121–31.
10. Dos Reis ST, Viana NI, Iscaife A, Pontes-Junior J, Dip N, Antunes AA, et al. Loss of TIMP-1 immune expression and tumor recurrence in localized prostate cancer. Int Braz J Urol 2015;41:1088–95.
11. Mehner C, Hockla A, Miller E, Ran S, Radisky DC, Radisky ES. Tumor cell-produced matrix metalloproteinase 9 (MMP-9) drives malignant progression and metastasis of basal-like triple negative breast cancer. Oncotarget 2014;5:2736.
12. Kunz P, Sähr H, Lehner B, Fischer C, Seebach E, Fellenberg J. Elevated ratio of MMP2/MMP9 activity is associated with poor response to chemotherapy in osteosarcoma. BMC Cancer 2016;16:1–10.
13. Kurschat P, Wickenhauser C, Groth W, Krieg T, Mauch C. Identification of activated matrix metalloproteinase-2 (MMP-2) as the main gelatinolytic enzyme in malignant melanoma by in situ zymography. J Pathol 2002;197:179–87.
14. Chen Y, Chen Y, Huang L, Yu J. Evaluation of heparanase and matrix metalloproteinase-9 in patients with cutaneous malignant melanoma. J Dermatol 2012;39:339–43.
15. Falzone L, Salemi R, Travali S, Scalisi A, McCubrey JA, Candido S, et al. MMP-9 overexpression is associated with intragenic hypermethylation of MMP9 gene in melanoma. Aging (Albany NY) 2016;8:933.
16. Salemi R, Falzone L, Madonna G, Polesel J, Cinà D, Mallardo D, et al. MMP-9 as a candidate marker of response to BRAF inhibitors in melanoma patients with BRAFV600E mutation detected in circulating-free DNA. Front Pharmacol 2018;9:856.
17. Corte MD, Gonzalez LO, Corte MG, Quintela I, Pidal I, Bongera M, et al. Collagenase-3 (MMP-13) expression in cutaneous malignant melanoma. Int J Biol Markers 2005:20:242–8.
18. Zigrino P, Kuhn I, Bäuerle T, Zamek J, Fox JW, Neumann S, et al. Stromal expression of MMP-13 is required for melanoma invasion and metastasis. J Invest Dermatol 2009;129:2686–93.
19. Meierjohann S, Hufnagel A, Wende E, Kleinschmidt MA, Wolf K, Friedl P, et al. MMP13 mediates cell cycle progression in melanocytes and melanoma cells: in vitro studies of migration and proliferation. Mol Cancer 2010;9:1–15.
20. Bastian A, Nichita L, Zurac S. Matrix metalloproteinases in melanoma with and without regression. In: Travascio F, editor. The Role of Matrix Metalloproteinase in Human Body Pathologies. London, UK: Intech; 2017.
21. Zamolo G, Grahovac M, Žauhar G, Vučinić D, Kovač L, Brajenić N, et al. Matrix metalloproteinases MMP-1, MMP-2, and MMP-13 are overexpressed in primary nodular melanoma. J Cutan Patholi 2020;47:139–45.
22. Kuivanen T, Ahokas K, Virolainen S, Jahkola T, Hölttä E, Saksela O, et al. MMP-21 is upregulated at early stages of melanoma progression but disappears with more aggressive phenotype. Virchows Arch 2005;447:954–60.