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Immunohistochemical Analysis of Asprosin and Meteorin-Like Peptide in Melanocytic Nevus and Malignant Melanoma

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Abstract

Introduction: Asprosin (ASP) plays many important roles in the central nervous system, peripheral tissues, and organs, such as appetite, glucose metabolism, insulin resistance, and cell apoptosis. Many experimental and clinical studies have examined the effects of meteorin-like peptide (METRNL) on inflammation. This study aimed to examine the immunohistochemical analysis of ASP and METRNL in healthy skin, melanocytic nevus, and malignant melanoma and to investigate their possible role in melanocyte proliferation and whether ASP and METRNL are potential biomarkers for melanocytic tumor development.

Materials and Methods: A total of 36 skin samples, including 12 benign nevi, 8 dysplastic nevi, 6 malignant melanoma, and 10 healthy skin around lesions removed from breast tissue that was not exposed to the sun as a control group were included in this study. The skin samples were stained immunohistochemically using the avidin-biotin-peroxidase complex method.

Results: ASP and METRNL expressions were detected in the epidermis and hair follicles of healthy skin. No significant difference was detected in ASP and METRNL histoscores in tumor tissues of benign nevus, dysplastic nevus, and malignant melanoma ($p>0.05$).

Conclusion: Current findings suggest that ASP and METRNL may be associated with cell-to-cell interactions between keratinocytes, melanocytes, and inflammatory cells and may be effective in melanocyte functions. Future studies will clarify whether ASP and METRNL are potential biomarkers for melanocytic tumor development.

Key words: Asprosin; meteorin-like peptide; melanocytic nevi; malignant melanoma

Introduction

Adipokines are biologically active proteins secreted mainly by adipocytes, although many adipokines are expressed and secreted also by nonadipocytes. Adiponectin and leptin zinc-α2-glycoprotein can be given as examples of adipokines that are secreted by keratinocytes, and that are responded to by melanocytes. Zinc-α2-glycoprotein plays a role in the regulation of obesity-related inflammatory responses in hepatocytes. It has been reported to have effects such as differentiation and desquamation of keratinocytes, proliferation of melanocytes, and inhibition of melanin production via tyrosinase.(1,2) As an example, leptin has been reported to contribute to the uncontrolled proliferation of melanoma cells by activating the Mitogen-activated protein kinase (MAPK) pathway and can be considered a melanoma growth factor.(3) Asprosin (ASP), a glucogenic adipokine, was identified by Romere et al. in 2016 and is encoded by the fibrillin 1 gene and synthesized by white adipose tissue during fasting.(4) After the synthesis, it is released into the bloodstream and shows an elevated plasma concentration during fasting. It has been shown that peripheral ASP, which crosses the blood-brain-barrier, directly activates orexigenic neurons and increases appetite through hypothalamic interaction and it has been reported to affect glucose metabolism, cell apoptosis, and cardiovascular system.(5,6) It has also been suggested that it exhibits circadian oscillation in healthy

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humans and mice, but is high in both fasting and postprandial periods in patients with type-2 diabetes, thus disrupting the circadian rhythm.(7) Meteorin-like peptide (METRNL), has recently been identified as a new insulin-sensitizing adipokines, and it has been reported that, besides adipose tissue, it is expressed in the skin, oral-pharyngeal mucosa, esophagus, breast, nervous system, pericardium, aorta and activated macrophages.(8,9) Its regulatory role in adipogenesis and obesity has been demonstrated.(10) It has been reported to alleviate inflammation and insulin resistance and induce fatty acid oxidation in skeletal muscle.(11) In patients with coronary artery disease, serum METRNL is negatively correlated with metabolic parameters such as body mass index, total cholesterol, and low-density lipoprotein, and with inflammatory markers such as C-reactive protein, interleukin (IL)1β and IL11. Serum METRNL levels have been reported to be associated with coronary artery disease, thermogenesis-related genes, anti-inflammatory cytokines (IL4/IL13) release, and chronic obstructive pulmonary disease.(12,13,14) Immunohistochemical studies play an important role in the diagnosis of melanocytic lesions. This study aimed to examine the immunohistochemical analysis of ASP and METRNL in healthy skin, melanocytic nevus, and malignant melanoma and to investigate their possible role in melanocyte proliferation and whether ASP and METRNL are potential biomarkers for melanocytic tumor development.

Materials and Methods

The present study was carried out in the Firat University Hospital Departments of Histology and Embryology, Dermatology and Pathology. Written informed consent was obtained from all individual participants included in the study. This study was conducted by the principles of the Declaration of Helsinki. A total of 36 skin samples, including 12 benign nevi, 8 dysplastic nevi, 6 malignant melanoma, and 10 healthy controls, provided by the biological materials archive of the Department of Pathology, were used in the study. Benign nevus, dysplastic nevus and melanoma groups were separated according to histopathological examination. The control group was selected from breast tissue and healthy skin that was completely protected from the harmful effects of sunlight. No samples from patients with diabetes mellitus were included in the study.

Immunohistochemical staining: The skin samples were stained immunohistochemically using the avidin-biotin-peroxidase complex method. For antigen retrieval, the citrate buffer was heated in a 750 W microwave oven at pH 6.0 for 12 (7+5) min.(15) After heating, the samples were allowed to cool at room temperature for 20 min, and each sample was washed three times with phosphate-buffered saline (PBS) (P4417; Sigma-Aldrich, St. Louis, MO), and then incubated in a hydrogen peroxide block (TA-125-HP; Lab Vision Corp, New York, NY) solution for five min to block any endogenous peroxidase activity. The PBS-washed skin was treated with an Ultra V Block (TA-125-UB; Lab Vision Corp.) solution for 5 min to block background staining, and the samples were then incubated with an ASP antibody (FNab09797; Fine Biotech Co, Wuhan, China) and a METRNL antibody (MBS7004241; MyBioSource, San Diego, CA) for 60 min at room temperature in a humid chamber, diluted 1:200. After incubation with primary antibodies, the samples were rinsed three times with PBS for five min, and the sections were then incubated with a biotinylated goat anti-polyvalent (anti-mouse/rabbit IgG) (TP-125-BN; Lab Vision Corp) secondary antibody for 30 min at room temperature in a humid chamber. The sections were rinsed three more times with PBS, followed by incubation with streptavidin peroxidase (TS-125-HR; Lab Vision Corp). A 3-amino-9-ethylcarbazole (AEC) substrate + AEC chromogen (TA015-HAS; AEC Chromogen, TA-002-HAC; Lab Vision Corp) solution was applied, and after labeling (receiving image signals) and observing with a light microscope, the sections were rinsed with PBS. The sections were sealed with the appropriate mounting solution (Large Volume Vision Mount, TA-125-UG; Lab Vision Corp), and the preparations were assessed and photographed under a Leica DM500 (Leica DFC295; Berlin, Germany) microscope. The immunohistochemistry assessment was based on the extent of staining, which measures the percentage of positive cells (0.1: <25%, 0.4: 26-50%, 0.6: 51-75%, and 0.9: 76-100%) and the intensity of staining (0: negative, +0.5: very weak, +1: weak, +2: moderate, +3: strong), and a histoscore was derived. Calculations were made using the following formula: histoscore=extent x intensity.(16)

Ethical approval: This study was approved by Firat University local ethics committee (Date: 2019, Number: 351340).

Statistical analysis : The obtained data were analyzed using the statistical package for the social sciences (SPSS, version 22.0) (Chicago, IL, USA) package program. Numbers and percentages were

used as descriptive statistics for categorical variables, and median, minimum, and maximum values were obtained for numerical variables. In the analysis of numerical variables, since they did not show a normal distribution, the Kruskal-Wallis test was employed to compare the groups. The correlations were calculated using Spearman's rho correlation test. p-value of <0.05 was accepted as statistically significant

Results

Included in the study were skin samples from a total of 36 cases, comprising 21 (58.3%) women and 15 (41.7%) men. The healthy control group included 10 (27.8%) samples, the benign nevi group included 12 (33.3%) samples, the dysplastic nevi group included 8 (22.2%) samples, and the malignant melanoma group included six (16.7%) samples. There were two (5.6%) junctional, 4 (11.1%) compound and 6 (16.7%) dermal nevi samples in the benign nevi group. When scored according to age, there were samples from 5 (13.9%) cases in the <20 years age group, 21 (58.3%) cases in the 20-49 years age group, seven (19.4%) cases in the 50-79 years age group, and 3 (8.3%) cases in the ≥ 80 years age group. The mean age was 40 (18-78) years in the control group, 31 (15–88) years in the benign nevi group, 29.5 (18-41) years in the dysplastic nevi group, 76 (35-87) years in the malignant melanoma group and 33.5 (15-88) years overall. Of all the study samples, 21 (58.3%) were collected from areas without sun exposure, and 15 (41.7%) from areas with sun exposure. Of the melanocytic nevi group, 11 (42.3%) were collected from areas without sun exposure, and 15 (57.7%) from areas with sun exposure.

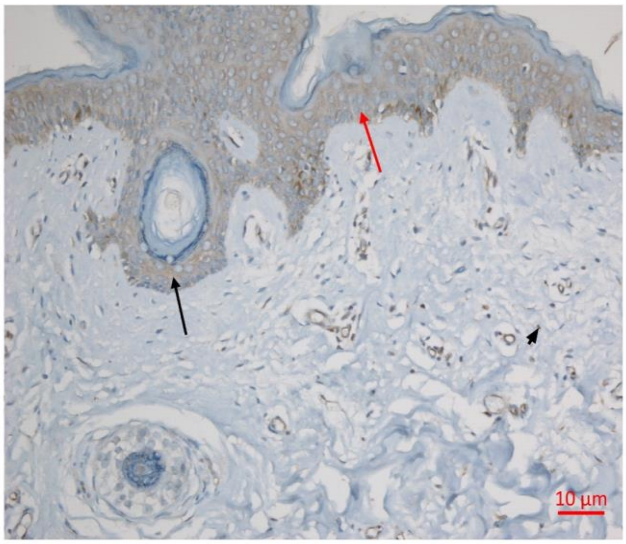

Figure 1. ASP expression in the epidermis (red arrow), hair follicles (black arrow) and inflammatory cell (black arrow) in the control

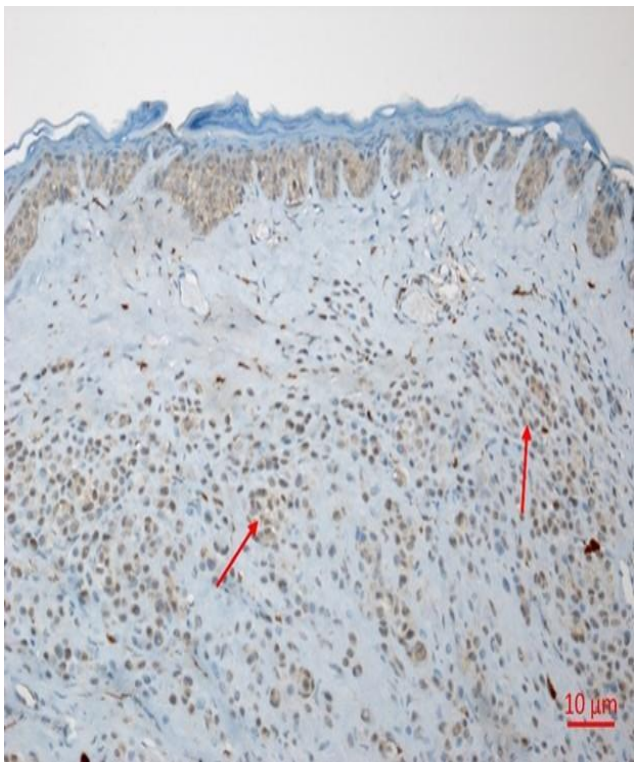

Figure 2: ASP expression in the tumor tissue of benign melanocytic nevi (red arrow)

Asprosin expression: Asprosin expression was detected in the epidermis and hair follicles in the control in the current study (Figure 1). ASP histoscores for the epidermis were higher in the benign nevi (Figure 2) than in the control (p=0.009). There was no difference in the ASP histoscores for tumor tissues between benign nevi, dysplastic nevi, and malignant melanoma (Table 1) (p>0.05). The histoscores for inflammatory cells, in turn, were higher in the malignant melanoma (Figure 3) than in the control (p=0.041). The histoscores for hair follicles were higher in the benign nevi than in the dysplastic nevi (p=0.038). The histoscores for subcutaneous tissues were higher in the malignant melanoma than in the benign nevi (p=0.017). ASP histoscores for sebaceous glands were higher in the 50-79 years age than in the <20 years age, while ASP histoscores for eccrine glands were higher in the ≥80 years age of than in the 20-49 years age (p=0.021, p=0.038, respectively). ASP histoscores of the six samples in the malignant melanoma was 1.35 (0.4-1.8) for the epidermis, 1.5 (0.8-2.7) for hair follicles, 1.5 (0.8-2.7) for sebaceous glands, 1.8 (0.8-2.7) for eccrine glands, 0.4 (0.2-0.6) for subcutaneous tissues, 1.5 (0.4-1.8) for inflammatory cells, 1.8 (0.8-2.7) for vascular structures and 1.8 (1.8-2.7) for tumor tissues. There was no difference in the histoscores of the

Table 1: Asprosin histoscores of the groups

	Control	BN	DN	MM	p
Epidermis	0.4 (0.2-0.9)[a]	1.05 (0.4-1.8)[a]	0.9 (0.2-1.8)	1.2 (0.4-1.8)	a=0.009
Hair follicles	0.4 (0.2-0.4)[a]	1.2 (0.6-2.7)[a,b]	0.6 (0.2-1.2)[b]	0.9 (0.4-1.2)	a=0.000
					b=0.038
Sebaceous glands	1.2 (0.2-1.2)	0.9 (0.6-1.8)	1.2 (0.2-1.8)	1.5 (0.4-1.8)	
Eccrine glands	0.9 (0.2-0.9)	1.2 (0.4-1.8)	1.05 (0.2-1.8)	1.2 (0.4-1.8)	
Subcutaneous tissue	0.4 (0.1-0.4)	0.1 (0.0-1.2)[a]	0.3 (0.2-0.4)	0.6 (0.4-0.9)[a]	a=0.017
Inflammatory cells	0.6 (0.2-1.8)[a]	0.9 (0.2-2.7)	0.6 (0.2-1.2)	1.8 (0.6-2.7)[a]	a=0.041
Vascular structures	1.8 (0.2-1.8)	1.2 (0.2-2.7)	1.0 (0.2-2.7)	1.8 (0.6-2.7)	
Tumor		1.8 (0.2-2.7)	1.2 (0.6-1.8)	1.8 (0.6-2.7)	

BN: Benign Nevus, **DN:** Dysplastic Nevus, **MM:** Malignant Melanoma

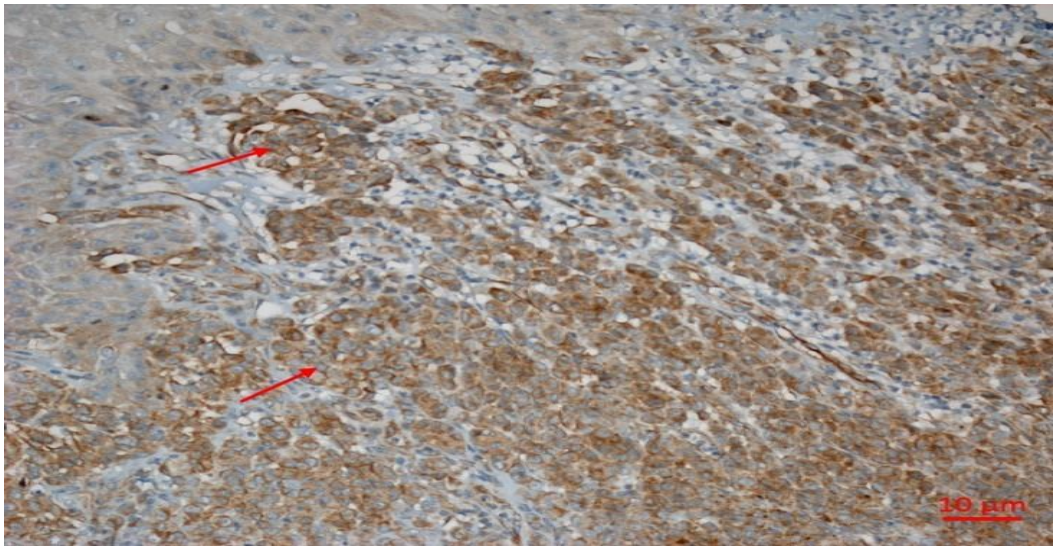

Figure 3: ASP expression in the tumor tissue of malignant melanoma (red arrows)

Table 2: Asprosin and meteorin-like peptide histoscores of the samples according to the sun exposure

Melanocytic Nevus and Malignant Melanoma					
Cutaneous Component	Sun Exposure (-)		Sun Exposure (+)		p
	ASP	METRNL	ASP	METRNL	
Epidermis	1.2 (0.2-1.8)	1.2 (0.6-2.7)	0.9 (0.4-1.8)	1.2 (0.4-2.7)	
Hair follicles	0.8 (0.2-1.8)	0.9 (0.2-2.7)	0.9 (0.4-2.7)	1.2 (0.2-2.7)	p=0.048*
Sebaceous glands	1.2 (0.2-1.8)	0.9 (0.4-2.7)	1.2 (0.4-1.8)	1.2 (0.2-2.7)	
Eccrine glands	1.2 (0.2-1.8)	1.2 (0.4-2.7)	1.2 (0.4-1.8)	1.2 (0.2-2.7)	
Subcutaneous tissue	0.4 (0.0-0.6)	0.9 (0.2-1.2)	0.4 (0.0-1.2)	0.9 (0.2-0.9)	
Inflammatory cells	0.6 (0.2-1.2)	1.2 (0.4-2.7)	1.2 (0.4-2.7)	1.2 (0.2-2.7)	p=0.005*
Vascular structures	0.9 (0.2-2.7)	0.9 (0.2-2.7)	1.8 (0.4-2.7)	1.2 (0.2-2.7)	
Tumor	1.8 (0.4-1.8)	1.8 (0.4-2.7)	1.8 (0.2-2.7)	1.8 (0.2-2.7)	

* **ASP:** Asprosin, **METRNL:** Metrin-like Peptide

Table 3: Asprosin and meteorin-like peptide histoscores in samples without sun exposure

Sun Exposure (-)					
Cutaneous Component	**ASP**		**METRNL**		
	Control	**MN and MM**	**Control**	**MN and MM**	**p**
Epidermis	0.4 (0.2-0.9)	1.2 (0.2-1.8)	0.45 (0.2-0.8)	1.2 (0.6-2.7)	p=0.021* p=0.000¤
Hair follicles	0.4 (0.2-0.4)	0.8 (0.2-1.8)	0.0 (0.0-0.0)	0.9 (0.2-2.7)	p=0.005* p=0.000¤
Sebaceous glands	1.2 (0.2-1.2)	1.2 (0.2-1.8)	0.3 (0.2-0.4)	0.9 (0.4-2.7)	p=0.000¤
Eccrine glands	0.9 (0.2-0.9)	1.2 (0.2-1.8)	0.6 (0.2-0.6)	1.2 (0.4-2.7)	p=0.008* p=0.002¤
Subcutaneous tissue	0.4 (0.1-0.4)	0.4 (0.0-0.6)	0.0 (0.0-0.0)	0.9 (0.2-1.2)	p=0.000¤
Inflammatory cells	0.6 (0.2-1.8)	0.6 (0.2-1.2)	0.6 (0.2-0.6)	1.2 (0.4-2.7)	p=0.001¤
Vascular structures	1.8 (0.2-1.8)	1.8 (0.4-1.8)	0.4 (0.2-0.6)	0.9 (0.2-2.7)	

* **ASP:** Asprosin, ¤ **METRNL:** Metrin-like, **MN:** Melanocytic nevus, **MM:** Malignant melanoma

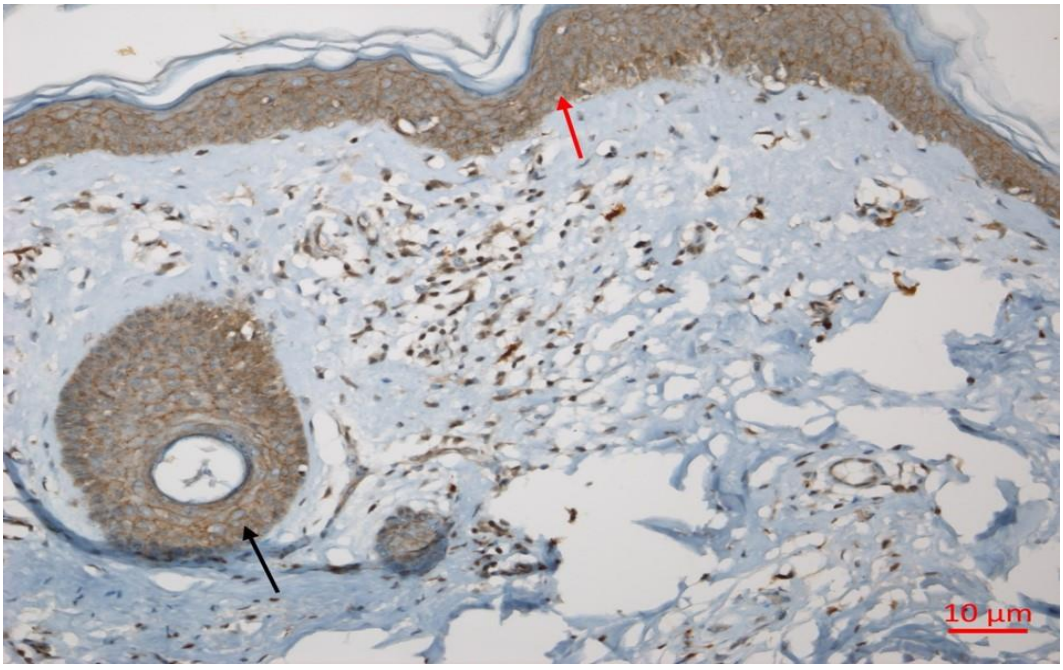

Figure 4. METRNL expression in the epidermis (red arrow) and hair follicles (black arrow) in the control

epidermis, hair follicle, sebaceous and eccrine gland, subcutaneous tissue, inflammatory cell, vascular structure, and tumor tissues, according to Breslow's thickness (1 mm<, 1.01-2 mm, 2.01-4 mm, >4 mm) (p>0.05). In the melanocytic nevi and malignant melanoma, ASP histoscores were higher in the sun-exposed hair follicles and inflammatory cells than in the hair follicle and inflammatory cells without sun exposure (p=0.048, p=0.005, respectively) (Table 2). ASP histoscores for the epidermis, hair follicles, and eccrine glands were higher in the melanocytic nevi and malignant melanoma without sun exposure than in the control (p=0.021, p=0.008, p=0.005, respectively) (Table 3).

Meteorin-like peptide expression: Meteorin-like peptide expression was detected in the epidermis and hair follicles in the control in the current study (Figure 4). METRNL histoscores for the epidermis, hair follicles, and sebaceous glands were higher in the benign nevi, dysplastic nevi, and malignant melanoma (Figure 5) than in the control (p=0.003, p=0.003, p=0.046, p=0.001, p=0.003, p=0.000, p=0.030, p=0.014, p=0.001, respectively). There was no difference in the METRNL histoscores of the tumor tissues between the benign nevi, dysplastic nevi, and

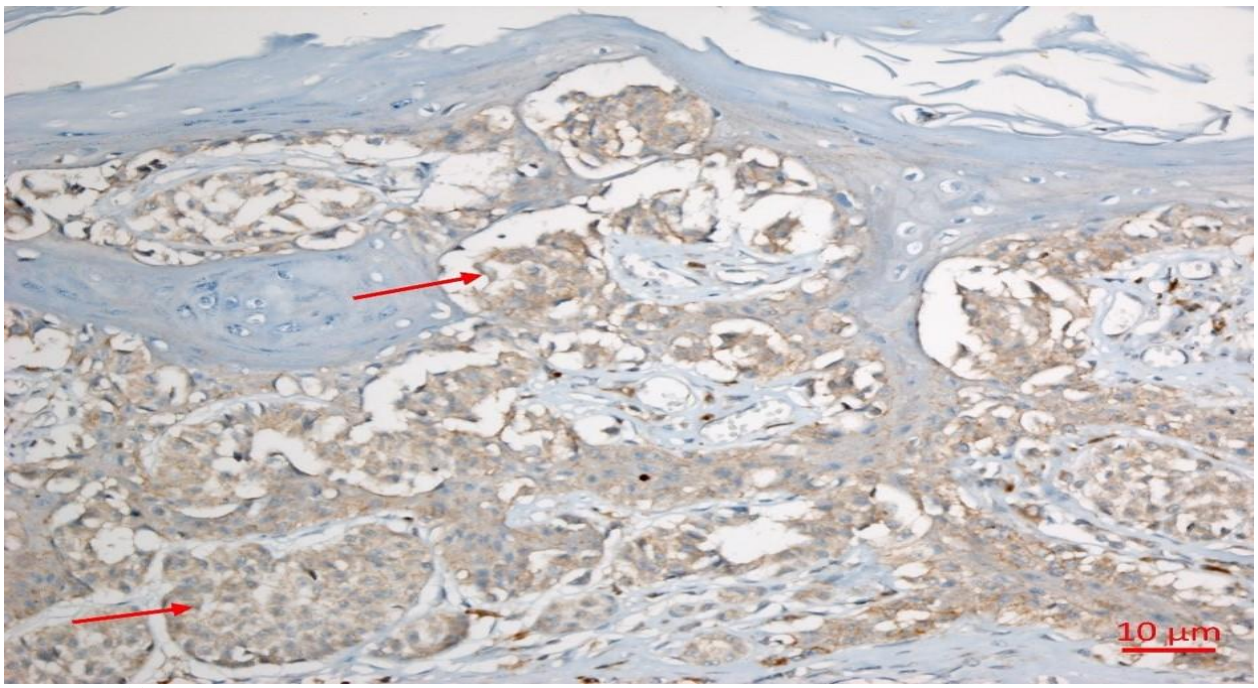

Figure 5. METRNL expression in the tumor tissue of malignant melanoma (red arrows)

Table 4: Meteorin-like peptide histoscores of the groups

Cutaneous Component	Control	BN	DN	MM	p
Epidermis	0.45 (0.2-0.8)[a,b,c]	1.2 (0.6-2.7)[a]	1.2 (0.6-2.7)[b]	1.35 (0.4-1.8)[c]	a=0.003 b=0.003 c=0.046
Hair follicles	0.0 (0.0-0.0)[a,b,c]	1.05 (0.2-1.8)[a]	0.9 (0.2-2.7)[b]	1.5 (0.8-2.7)[c]	a=0.001 b=0.003 c=0.000
Sebaceous glands	0.3 (0.2-0.4)[a,b,c]	0.9 (0.2-1.8)[a]	1.05 (0.4-2.7)[b]	1.5 (0.8-2.7)[c]	a=0.030 b=0.014 c=0.001
Eccrine glands	0.6 (0.2-0.6)[a]	1.2 (0.2-2.7)	1.05 (0.4-2.7)	1.8 (0.8-2.7)[a]	a=0.002
Subcutaneous tissue	0.0 (0.0-0.0)[a,b]	0.9 (0.2-0.9)[a]	0.9 (0.2-1.2)[b]	0.4 (0.2-0.6)	a=0.000 b=0.001
Inflammatory cells	0.6 (0.2-0.6)[a]	1.05 (0.2-2.7)[a]	1.05 (0.4-2.7)	1.5 (0.4-1.8)	a=0.043
Vascular structures	0.4 (0.2-0.6)[a]	0.7 (0.2-2.7)	0.9 (0.2-1.8)	1.8 (0.8-2.7)[a]	a=0.007
Tumor		1.5 (0.2-2.7)	1.8 (0.4-2.7)	1.8 (1.8-2.7)	

BN: Benign Nevus, **DN:** Dysplastic Nevus, **MM:** Malignant Melanoma

malignant melanoma (p>0.05) (Table 4). There was no difference in METRNL histoscores of the skin samples with and without sun exposure in the melanocytic nevi and malignant melanoma (p >0.05) (Table 2). METRNL histoscores for the epidermis, hair follicles, sebaceous glands, eccrine glands, subcutaneous tissues, and inflammatory cells were higher in the melanocytic nevi and malignant melanoma groups without sun exposure than in the control (p=0.000, p=0.000, p=0.000, p=0.002, p=0.000, p=0.001, respectively) (Table 3). METRNL histoscores of the six samples in the malignant melanoma were 1.35 (0.4-1.8) for the epidermis. 1.5 (0.8-2.7) for hair follicles, 1.5 (0.8-2.7) for sebaceous glands, 1.8 (0.8-2.7) for eccrine glands, 0.4 (0.2-0.6) for subcutaneous tissues, 1.5 (0.4-1.8) for inflammatory cells, 1.8 (0.8-2.7) for vascular structures, and 1.8 (1.8-2.7) for tumor tissues. There was no difference in histoscores between the epidermis, hair follicles, sebaceous

and eccrine glands, subcutaneous tissues, inflammatory cells, vascular structures, and tumor tissues according to the Breslow's thickness (1 mm<, 1.01-2 mm, 2.01-4 mm, >4 mm) (p>0.05). The histoscores of the ASP and METRNL expressions in the melanocytic nevi and malignant melanoma samples did not differ between the groups, genders, or age (p=0.168, p=0.885, p=0.332, p=0.164, p=0.621, p=0.266, respectively).

Discussion

The present study identified ASP expression in the epidermis, hair follicles, sebaceous and eccrine glands, subcutaneous tissue, inflammatory cells, vascular structures, and melanocytic tumor tissues in the healthy skin, melanocytic nevi, and malignant melanoma. ASP histoscores for the epidermis were higher in the benign nevi than in the control. METRNL expression was detected in the epidermis and hair follicles in the control. METRNL histoscores for the epidermis, hair follicles, and sebaceous glands were higher in the benign nevi, dysplastic nevi, and malignant melanoma than in the control. There was no difference in the ASP and METRNL histoscores for tumor tissues between benign nevi, dysplastic nevi, and malignant melanoma. Asprosin expression in the epidermis was higher in the benign melanocytic nevi, while ASP expression in the hair follicles was higher in both the benign and dysplastic nevi. It has long been known that keratinocytes make multiple contacts with melanocytes.(15) The migration, proliferation, and differentiation of melanoblasts are regulated mainly by the dorsal neural tube, ectoderm, and keratinocyte-secreted factors, such as Wingless-type (Wnt)/protein, endothelin-3, and stem cell factors.(17) The findings of the present study suggest that ASP may contribute to the relationship between keratinocytes and melanocytes. Mitogen-activated protein kinase (MAPK) signaling pathways regulate various essential cellular processes, including cell proliferation, differentiation, senescence, survival, transformation, and migration. The MAPK subgroup includes extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38/stress-activated protein kinase (p38/SAPK). The MAPK/ERK signaling pathway plays a crucial role in cancer development by stimulating cell proliferation and migration. These pathways also have significant functions in melanoma development, with MAPK/ERK signaling typically initiated by the activation of a growth factor/cytokine receptor tyrosine kinase.(18) Dermal fibroblasts have been reported to increase the chemotaxis of tumor cells via the MAPK pathway.(19) According to another study, ASP protects the cardiac muscle from oxidative stress-induced apoptosis by activating the ERK1/2-SOD2 pathway.(20) The elevating of ASP expression identified both in the benign nevi and malignant melanoma in the present study suggested that one source of ASP may be melanocytes. The ability of ASP to function through the ERK pathway suggests that ASP may affect melanocyte functions through this pathway. The present study also identified ASP expression in the subcutaneous adipose tissue of the healthy skin. ASP expression in subcutaneous tissue was similar in the healthy skin and the melanocytic nevi, while the highest value was identified in the malignant melanoma. Fibrillin-1 is an extracellular matrix protein that is expressed in cells of mesenchymal origin, and that regulates the bioavailability of transforming growth factor β (TGFβ).(21) It has been reported that TGFβ is secreted by cancer cells, stromal fibroblasts, and other cells in the tumor microenvironment, and promotes cancer progression, and creates an immunosuppressive environment by shaping the tumor structure and suppressing the antitumor activities of immune cells.(22) It has been reported that ASP, a derivative of fibrillin-1, may contribute to the invasion of malignant melanocytes into the subcutaneous tissues through TGFβ, although no relationship between Breslow's thicknes. Further studies examining a greater number of melanoma samples would produce clearer findings. The present study also identified ASP expression in inflammatory cells, the relationship between which has been demonstrated in several studies. The in vitro study by Lee et al. found that ASP upregulated the toll-like receptor 4/JNK-mediated pathway.(23) In addition, ASP was shown to cause impaired insulin sensitivity in skeletal muscle through the stress and inflammation associated with the endoplasmic reticulum activated by protein kinase-Cδ.(24) Accordingly, it is likely that inflammatory cells secrete ASP or respond to ASP in the environment through the receptor. Another finding of the present study is that the rate of ASP expression in inflammatory cells is much higher in malignant melanoma than in healthy skin. Melanocytes are phagocytic cells that can respond to inflammations in the epidermis with (increased/decreased) pigment production.(25) The high rate of ASP in inflammatory cells in the microenvironment of malignant lesions may contribute to the triggering of inflammatory cells

by increased/impaired ASP release from malignant melanocytes. The relationship between ASP expression and sunlight exposure was also examined in the present study. ASP expression was higher in the epidermis, hair follicles, and eccrine glands in the melanocytic nevi and malignant melanoma without sunlight exposure, and in the hair follicles and inflammatory cells in those with sunlight exposure. While no studies have explored the relationship between ASP and sunlight, the proliferation of epidermal melanocytes and melanin production are known defenses against DNA damage from ultraviolet radiation.(26) In the present study increased ASP expression in inflammatory cells in areas exposed to sunlight was a remarkable finding. In the present study, METRNL expression was identified in the epidermis, sebaceous, and eccrine glands, and in the inflammatory cells and vascular structures in healthy skin. In an experimental study by Ushach et al. using cell cultures taken from human skin, METRNL expression was demonstrated in inflammatory and neoplastic diseases, and METRNL was found to be expressed by resting fibroblasts and interferonγ (IFNγ)-treated keratinocytes.(8) In the present study, METRNL expression in the epidermis and epidermal appendages, indicates keratinocytes and supports the findings of previous studies. METRNL expression in the epidermis, hair follicles, and sebaceous glands was higher in benign and dysplastic nevi and malignant melanoma than in healthy skin. METRNL was identified previously as a neurotropic factor and was named Cometin. It has also been reported to function through the Janus kinase (JAK)-signal transducer and the activator of the transcription-3 and MAPK/ERK pathways in processes such as neuroblast migration.(27) This discovery has led to the idea that METRNL expressed in keratinocytes may be one of the factors involved in the proliferation of melanocytes. Kocaman et al. identified a high level of METRNL expression in malignant mesothelioma and suggested that METRNL could be a marker in differentiating malignant mesothelioma from benign diseases.(28) METRNL expression did not differ in benign nevi, dysplastic nevi, and malignant melanoma in the present study. Since METRNL is expressed more in subcutaneous adipose tissues than in visceral adipose tissues, it is also known as subfatin.(10) In the present study, METRNL expression could not be identified in subcutaneous adipose tissues and hair follicles in healthy skin, and while there was METRNL expression in subcutaneous tissues and hair follicles in the melanocytic nevi and malignant melanoma, they were not different from each other. The present study observed elevated METRNL expression in the inflammatory cells in the microenvironment in healthy skin, melanocytic nevi, and malignant melanoma. The study by Ushach et al. identified an association between METRNL and innate and acquired immunity, as well as its role in inflammatory responses. It has been shown that METRNL expression in cell cultures is strongly stimulated by macrophage colony-stimulating factors, and IL10 is produced. Overexpression of METRNL has been associated with innate immunity, particularly in psoriasis.(8) The findings obtained in this study suggest that METRNL may mediate the relationship between inflammatory cells and melanocytes.Wingless-type/β-catenin signaling pathway is known to be associated with melanocyte differentiation and melanoma development.(29) Wnt/β-catenin pathway is more frequently activated in melanocytic lesions without chronic sun damage.(30) The present study found METRNL expression to be the same in melanocytic nevus and malignant melanoma with sunlight exposure.

Study limitations: The small number of samples included in this study is a limitation of the study.

Conclusion

In this study, both ASP, and METRNL expressions were detected in healthy skin, melanocytic nevus, and malignant melanoma. Current findings suggest that ASP and METRNL may be associated with cell-to-cell interactions between keratinocytes, melanocytes, and inflammatory cells and may be effective in melanocyte functions. Future studies will clarify whether ASP and METRNL are potential biomarkers for melanocytic tumor development.

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