Harran Üniv Vet Fak Derg, 2021; 10 (1): 43-49 *Research Article*
DOI:10.31196/huvfd.859433

Immunohistochemical assessment of S100, Vimentin, PCNA, p53 and MMP-9 expressions in bovine melanomas

Emin KARAKURT[1,a,*], Uğur AYDIN[2,b], Enver BEYTUT[1,c], Engin KILIÇ[2,d], Serpil DAĞ[1,e], Hilmi NUHOĞLU[1,f], Uğur YILDIZ[2,g], Ayfer YILDIZ[3,h], Emre KURTBAŞ[3,i]

[1]Kafkas University, Faculty of Veterinary Medicine, Department of Pathology, Kars, Turkey.
[2]Kafkas University, Faculty of Veterinary Medicine, Department of Surgery, Kars, Turkey.
[3]Kafkas University, Institute Health Sciences, Kars, Turkey.
[a]ORCID: 0000-0003-2019-3690 , [b]ORCID: 0000-0001-5756-4841 , [c]ORCID: 0000-0003-3360-2940 ,
[d]ORCID: 0000-0001-8126-3918 , [e]ORCID: 0000-0001-7667-689X , [f]ORCID: 0000-0003-2530-2542 ,
[g]ORCID: 0000-0002-4782-1012 , [h]ORCID: 0000-0002-6569-5435 , [i]ORCID:0000-0002-9752-194X

Geliş Tarihi: 12.01.2021 **Kabul Tarihi: 10.03.2021**

Abstract: This study aimed to evaluate the expression of PCNA, p53, MMP-9, Vimentin and S100 immunohistochemically and determine the aggressiveness in diagnosis of bovine melanomas. The material of this study consisted of melanoma biopsy samples taken from 10 cattle brought to our department. Tissue samples from cattle were fixed in 10% buffered formalin solution. After routine procedures paraffin blocks were cut sections of 5 µm thickness. For bleaching, some heavily pigmented tumour sections were deparaffinized, hydrated, and incubated in 10% solution of hydrogen peroxide (H_2O_2) for 5 hours at 65ºC until sections appeared clear and Hematoxylin & Eosin (H&E) staining was applied to the sections to detect histopathological changes. Sections were examined and photographed under a light microscope. Avidin-Biotin Peroxidase was used as the immunohistochemical method. We observed that the tumoral mass was solitary, firm, hairless, oval-round shaped and quite large. We detected spindle and epithelioid type tumoral cells containing a lot of large brownish-black granular melanin pigments in their cytoplasm. All melanoma cases were immune positive for S100, Vimentin, PCNA, p53 and MMP-9 expressions. In conclusion, we think that these immunohistochemical markers are quite convenient in evaluating the prognosis and diagnosis of bovine melanomas.
Keywords: *Bovine, immunohistochemistry, melanoma, prognosis.*

Sığır melanomlarında S100, Vimentin, PCNA, p53 ve MMP-9 ekspresyonlarının immunohistokimyasal olarak değerlendirilmesi

Özet: Bu çalışmada, sığır melanomlarının agresifliğini ve tanısı için PCNA, p53, MMP-9, Vimentin ve S100 ekspresyonlarının immünohistokimyasal olarak değerlendirilmesi amaçlanmıştır. Bu çalışmanın materyali bölümümüze getirilen 10 sığırdan alınan melanom biyopsi örneklerinden oluşmuştur. Sığırlardan alınan doku örnekleri, %10'luk tamponlu formalin solüsyonunda tespit edildi. Rutin prosedürlerden sonra hazırlanan parafin bloklardan 5 µm kalınlığında kesitler alındı. Ağartma için bazı ağır pigmentli tümör kesitleri deparafinize edildi, hidratlandı ve % 10'luk hidrojen peroksit (H_2O_2) çözeltisi içinde 65ºC'de 5 saat süreyle, kesitler berrak hale gelene kadar inkübe edildi ve kesitlere histopatolojik değişikliklerin tespit edilmesi için Hematoksilen & Eozin (H&E) boyaması yapıldı. Kesitler ışık mikroskobu altında incelendi ve fotoğraflandı. İmmünohistokimyasal yöntem olarak Avidin-Biotin Peroksidaz yöntemi kullanıldı. Tümöral kitlenin soliter, sert, tüysüz, oval-yuvarlak şekilli ve oldukça büyük olduğunu gözlemledik. Sitoplazmalarında çok sayıda büyük kahverengimsi siyah granüler melanin pigmenti içeren iğsi ve epiteloid tipi tümör hücrelerini tespit ettik. Tüm melanom vakaları S100, Vimentin, PCNA, p53 ve MMP-9 ekspresyonları için immün pozitifti. Sonuç olarak, bu immünohistokimyasal belirteçlerin sığır melanomlarının prognozunda ve teşhisinde oldukça kullanışlı olduğunu düşünmekteyiz.
Anahtar Kelimeler: *İmmunohistokimya, melanom, prognoz, sığır.*

Introduction

A common consensus of the terminology in denomination of melanocytic tumors in both human and veterinary medicine has not been determined (Smith et al., 2002). Melanoma is the term used for all malignant melanocytic tumors, while melanocytoma refers to benign tumors (Madheswaran et al., 2019). Melanocytic neoplasms are common in dogs, gray horses and cats, they are rarely observed in large animals such as cattle (Bhadaniya et al., 2015; Vijayakumar et al., 2020). Melanocytomas, like in melanomas, is very rare in cattle (Madheswaran et al., 2019). Melanomas originate from neuroectodermal melanoblasts or melanocytes, which would have migrated initially through epidermal-dermal junctions of skin, follicles, and then to dermis during embryonic

development and constitute approximately 2% of all bovine tumors (Vadalia et al., 2016; Beytut, 2018). Melanocytic tumors may form in red, gray or black-skinned cattle under two years of age, or some of these tumors may be congenital (Javanbakht et al., 2014). There is no breed or gender predisposition for melanomas. However, the incidence of melanomas is high in Aberdeen Angus (Garma-Aviña et al., 1981). While tumors are mostly localized in the jaw, maxilla, trunk, udder, prepuce, lips, they are rarely detected in the interdigital areas or eyes (Chandrashekaraiah et al., 2014; Javanbakht et al., 2014). Regardless of whether these tumors are caused by chemical carcinogens or by ultraviolet rays, melanomas of all species exhibit similar biological characteristics due to their frequent recurrence and tendency to metastasize to regional lymph nodes (Babić et al., 2009). Melanomas are classified according to the dominant cell types as follows; epithelioid, spindle cell melanomas, whorled or dendritic type (Brito et al., 2009). Melanomas are mostly solitary and rarely seen in multiple foci and frequently metastasize to regional lymph nodes and organs such as lungs, spleen and liver (Mesarić et al., 2002). Tumor diagnosis is made by histological and cytological evaluations and the presence of melanin pigment is an important parameter for diagnosis (Beytut et al., 2018; Madheswaran et al., 2019).

S100 protein is an effective marker for diagnosing melanocytic tumors of humans and animals, although its functions in melanogenesis are not fully known (Ramos-Vara et al., 2011; Ramos-Vara et al., 2014; Sabattini et al., 2018). Vimentin is an intermediate filament that is expressed from mesenchymal and neuroectodermal cells in normal tissues. Tumor markers such as S100 together with vimentin are used to provide the gold standard in the diagnosis of malignant melanomas (Koenig et al., 2001; Ozyildiz et al., 2012). Proliferating cell nuclear antigen (PCNA) is a 36 kD nuclear polypeptide, expressed mainly in the S-phase in the proliferating cell cycle, which is a key factor for control of DNA duplication and cell division, therefore there is a positive correlation between PCNA expression and cell proliferation activity (Roels et al., 1999; Qin et al., 2017). Matrix metalloproteinases (MMPs) have been noted to perform functions such as degradation of the extracellular matrix (ECM), stimulating neoangiogenesis, inhibiting macrophage activity and migration, and activating the migration of tumor cells, and their expression is increased in many malignant tumors, including invasive skin melanomas (Aksenenko and Ruksha, 2013; Schmid et al., 2019). p53, which is a tumor suppressor gene, is involved in inhibiting the G1 / S phase of the cell proliferation cycle, and as a result of any mutation or deficiency in this gene, the cell cycle is not blocked and the mutations increase gradually, thus accelerating the selection of neoplastic clones and tumor development (Roels et al., 2000; Roels et al., 2001).

In this study, it was aimed to evaluate the expression of PCNA, MMP-9, p53, Vimentin and S100 immunohistochemically to determine the aggressiveness and diagnosis of bovine melanomas.

Materials and Methods

Animals: The material of this study consisted of melanoma biopsy samples taken from 10 cattle brought to our department.

Ethical Approval: The ethics committee report of this study was obtained from Kafkas University Animal Experimentals Local Ethics Committee (Authorization number: KAU-HADYEK-2020/163).

Histopathological Investigations: Tissue samples from cattle were fixed in 10% buffered formalin solution. After routine procedures paraffin blocks were cut to sections of 5 µm thickness. For bleaching, some heavily pigmented tumor sections were deparaffinized, hydrated, and incubated in 10% solution of hydrogen peroxide (H_2O_2) for 5 hours at 65°C until sections appeared clear and Hematoxylin & Eosin (H&E) staining was applied to the sections in order to detect histopathological changes. Sections were examined and photographed under a light microscope.

Immunohistochemical Investigations: Avidin-Biotin Peroxidase method was used as immunohistochemical staining. For immunohistochemical staining, the sections of 4 µm in thickness taken to poly-L-lysine coated slides were deparaffinized and rehydrated in graded alcohols. In order to prevent endogenous peroxidase activity, the sections were treated with 3% hydrogen peroxide solution in Phosphate Buffered Saline (PBS) for 15 minutes. For antigen retrieval, the sections were boiled in Citrat Buffer Solution (pH 6) for 25 min in the microwave oven (at 800 watt). To prevent nonspecific staining, the sections were incubated for 10 min with non-immune serum (Thermo Scientific Histostain-Plus IHC Kit, HRP, broad spectrum, REF: TP-125-HL) at room temperature. Diluted antibodies (Vimentin: Thermo Fisher Scientific, SP-20, ready to use, S100: Thermo Fisher Scientific, 4C4.9, ready to use, PCNA: Santa Cruz, sc-56, Dilution Ratio: 1:100, MMP-9: Santa Cruz, sc-393859, Dilution Ratio: 1:100, p53: Novus Bio, SPM590, Dilution Ratio:1:200) were incubated for overnight (+ 4 °C in the refrigerator). The sections were washed 3 times in PBS solution for 5 minutes, and the biotinylated secondary

antibody (Thermo Scientific, Histostain-Plus IHC Kit, HRP, broad spectrum, REF: TP-125-HL) was applied to them at room temperature for 10 minutes. After washing in PBS (3-5 min), all sections were incubated with peroxidase-bound Streptavidin (Thermo Scientific, Histostain-Plus IHC Kit, HRP, broad spectrum, REF: TP-125-HL) for 10 minutes at room temperature. A solution of 3.3-diaminobenzidine tetra hydrochloride (DAB) (Thermo Scientific, REF: TA-125-HD) was used as a chromogen for 15 minutes. The sections were treated with Mayer's Hematoxylin for 30 seconds and washed in running water for 5 min. The sections incubated with AEC (Thermo Scientific, REF: TA-060-SA) were covered with a special transparent adhesive without passing through alcohol and xylene. Primary antibodies were omitted from the negative control sections and were treated with diluted normal serum. The slides prepared after the covering were examined under a light microscope (Olympus Bx53) and photographed via the Cell^P program (Olympus Soft Imaging Solutions GmbH, 3,4). Analyzes of the images were done with Image J Program. Immunopositivity was evaluated using a semiquantitative grading scheme based on the number of cells exhibiting specific labeling for the Vimentin, S100, PCNA, MMP and p53 markers in 3 representative fields (40× objective): (+) mild labeling of 1%–10% of cells; (++) moderate labeling of 11%–59% of cells; and (+++) severe labeling of >60% of cells (Beytut et al., 2018).

Results

Macroscopical Results: We observed that the tumoral mass was solitary, firm, hairless, oval-round shaped and quite large. We found that the cross-sectional surfaces of melanomas are dark black in patches of brown. Besides, we detected the presence of hemorrhagic and ulcerated areas in places. (Figure 1 a-b).

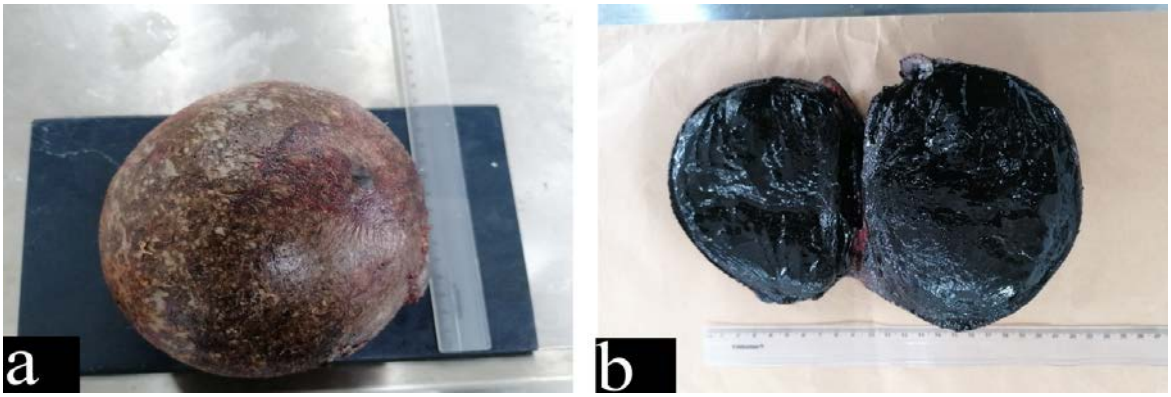

Figure 1. a: Macroscopic view of the tumoral mass, b: Severe dark black appearance on the cross-sectional face of the mass

Microscopical Results: In histopathological examinations, the presence of spindle and epithelioid shaped tumoral cells containing a lot of large brownish-black granular melanin pigments in their cytoplasm is spotted. Melanoma cells were arranged in layers in a band-like pattern especially in the dermis. The nuclei of some tumoral cells were masked because of the excess pigment accumulated. We have demonstrated the existence of a few mitotic figures. Another important finding was that mononuclear cellular infiltrations were detected around some vessels (Figure 2 a-b).

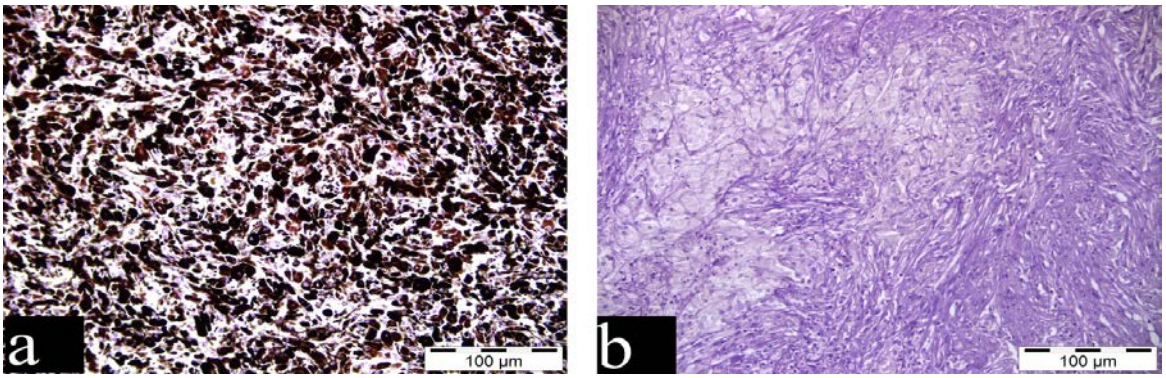

Figure 2. a: Tumor cells containing brownish to black melanin granules in their cytoplasm, unbleached, H&E, Bar=100 µm, **b:** Spindle and epithelioid cell type melanoma, bleached, H&E, Bar=100 µm.

Immunohistochemical Results: Information on the severity of S100, Vimentin, PCNA, p53 and MMP-9 expressions of all cases is given in Table 1. All melanoma cases were immune positive for S100, Vimentin, PCNA, MMP-9 and p53 expressions. Diffuse S100 immunoreactivity was detected especially in both the cytoplasm and nucleus of the epithelial type tumoral cells while varying degrees of vimentin immunoreactivity was detected in the cytoplasm of the spindle type tumoral cells. The expression of vimentin was moderate while the expression of S100 was quite intense. We detected a dark red PCNA positive reaction in the nuclei of epithelial type neoplastic cells. We also observed mild MMP-9 positive reactions mostly in the cytoplasm of epithelial type neoplastic cells. In addition to cytoplasmic reactions, we also detected MMP-9 immunoreactivity in the nuclei of tumoral cells. We detected diffuse p53 expressions in the cytoplasm and nuclei of neoplastic epithelial and spindle type melanoma cells. (Figure 3 a-e).

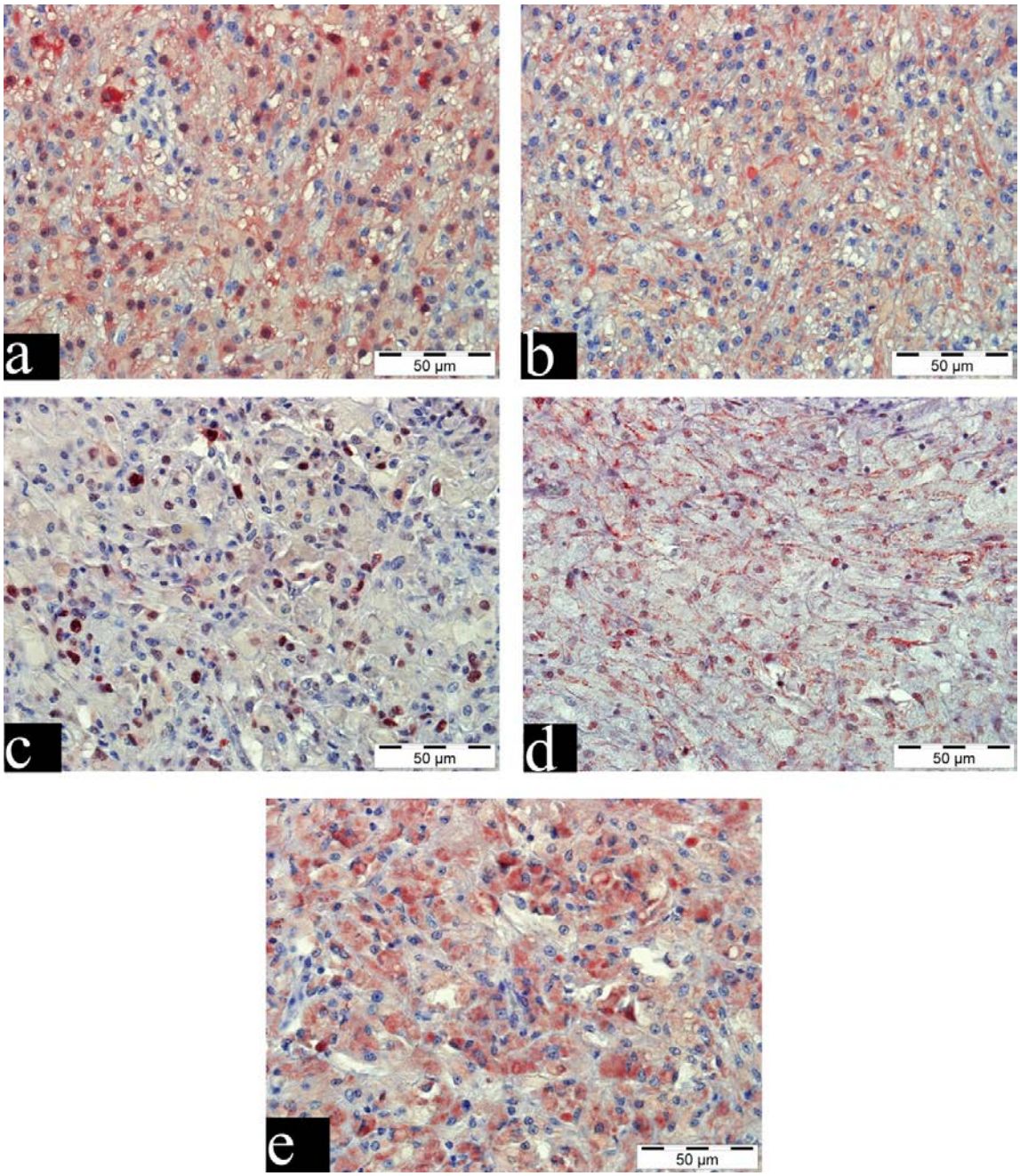

Figure 3. a: Strong S100 immunoreactivity in the cytoplasm and nucleus of the tumoral cells, IHC, Bar=50 µm, **b:** Moderate vimentin expressions in the cytoplasm of the tumoral cells, IHC, Bar=50 µm, **c:** Moderate PCNA positive reactions in the nuclei of tumor cells, IHC, Bar=50 µm, **d:** Moderate MMP-9 expressions in the cytoplasm and nucleus of the tumoral cells, IHC, Bar=50 µm, **e:** Diffuse p53 expressions in the cytoplasm and nucleus of the tumoral cells, IHC, Bar=50 µm.

Table 1. Information on the severity of S100, Vimentin, PCNA, p53 and MMP-9 expressions and metastasis status of all cases

Case No	S100	Vimentin	PCNA	p53	MMP-9	Metastasis
Case 1	+++	++	+++	+++	+	-
Case 2	+++	++	+++	+++	+	-
Case 3	+++	+++	+++	+++	+	-
Case 4	+++	++	+++	+++	+	-
Case 5	+++	++	++	+++	+	-
Case 6	+++	++	++	+++	+	-
Case 7	+++	++	+++	+++	+	-
Case 8	+++	+	+++	+++	+	-
Case 9	+++	++	++	+++	+	-
Case 10	+++	+++	+++	+++	+	-

Discussion and Conclusion

Diagnosis of melanomas is made based on of their typical macroscopic and microscopic features (Brito et al., 2009). In this study, we observed that the tumoral mass was solitary, firm, hairless, oval-round shaped and quite large following the literature data (Babić et al., 2009; Bhadaniya et al., 2015; Vijayakumar et al., 2020). We found that the cross-sectional faces of melanomas are dark black in patches of brown as reported by Madheswaran et al., 2019. Besides, we detected the presence of hemorrhagic and ulcerated areas in places similar to the literature (Sharma et al., 2010; Chandrashekaraiah et al., 2014; Vadalia et al., 2016). Parallel to previous studies, in the histopathological examination of the melanomas, we detected spindle and epithelioid shaped tumoral cells containing a lot of large brownish-black granular melanin pigments in their cytoplasm (Beytut et al., 2018; Hemanth et al., 2014; Javanbakht et al., 2014; Pazhanivel et al., 2003). We also observed perivascular mononuclear cell infiltrations and mitotic figures as previously reported (Miller et al., 1995; Naveen et al., 2013; Sivadas et al., 1971).

We made Vimentin and S100 staining to achieve the diagnostic standard in bovine melanoma cases where we diagnosed melanoma according to their typical macroscopic and microscopic features. In this study, all melanoma cases were immune positive for S100 and Vimentin, similar to literature (Beytut et al., 2018; Javanbakht et al., 2014; Miller et al., 1995). We observed S100 expressions both in the nucleus and cytoplasm of neoplastic cells, similar to that reported by Javanbakht et al., 2014. According to literature (Beytut et al., 2018; Miller et al., 1995), we also detected positive immunoreactivity of the tumoral cells to vimentin. The fact that all cases were positive for S100 and Vimentin expressions made us think that these markers, although not very specific, could be useful in the diagnosis of bovine melanomas (Beytut et al., 2018; Javanbakht et al., 2014).

Important parameters for the prognosis of the tumor in animals are the degree of pigmentation, PCNA expressions intensity, presence of necrosis, ulceration, inflammation, and p53 expression (Smith et al., 2002). To our knowledge, we could not find any studies in which PCNA, MMP-9 and p53 expressions were investigated immunohistochemically in bovine melanoma cases. Consistent with our results, PCNA immunoreactivity was reported in the nuclei of melanocytic tumoral cells in different types of animals such as horses, mice, cats and dogs (Qin et al., 2017; Roels et al., 1999; Roels et al., 2000). P53 mutations have been reported in approximately 20-40% of human melanoma cases (Smith et al., 2002). In veterinary medicine, Roels et al., 2000 detected strong diffuse p53 expression in six equine melanocytic tumors. They also noted that the severity of their expression was more severe in metastatic cases. In a different study, Roels et al., 2001 observed that in one feline case of malignant melanoma, p53 accumulation together with apoptosis was seen in three metastases. They reported that p53 index and apoptosis index are not directly correlated to survival. According to the literature (Roels et al., 2000; Roels et al., 2001) we also detected diffuse p53 expressions in the cytoplasm and nuclei of melanoma cells. In our study, PCNA and p53 expressions were quite severe, we concluded that bovine melanomas exhibited a rather aggressive character in line with these data. Melanomas frequently metastasize to regional lymph nodes and distant organs such as lungs, spleen and liver (Mesarić et al., 2002). Melanoma progression and metastasis consist of a multi-step process initiated by genetic changes, leading to modulation of cell-cell interactions, whereby tumoral cells can separate from the primary lesion, pass through the ECM and enter the microvasculature and spread

through the bloodstream (Schmid et al., 2019). Aksenenko and Ruksha 2013 suggested that MMP-9 is involved not only in the regulation of ECM, but also in the processes of cell proliferation and neoangiogenesis in skin melanoma. Schmid et al., 2019 reported that canine oral primary melanoma cells showed weak MMP-9 expression. In this study, we also observed mild MMP-9 positive reactions in the cytoplasm and nuclei of neoplastic cells as reported by Schmid et al., 2015. Also, no metastasis was observed in all cases in our study, and we found that mmp-9 expressions were quite weak in all cases. According to the data obtained from our study, we thought that MMP-9 might be effective in the metastasis capacity of melanomas. MMP-9 inhibitors can be used in the treatment of melanomas.

In conclusion, we could not find any literature study evaluating PCNA, p53 and MMP-9 expressions in bovine melanoma cases using immunohistochemical methods. In this respect, we hope that results obtained in our study will contribute to the literature. Besides, we think that these three markers are quite useful in evaluating the aggressiveness of bovine melanomas.

References

Aksenenko MB, Ruksha TG, 2013: Analysis of the application of MMP-9 inhibitor in skin melanoma: experimental study. *Bull Exp Biol Med*, 154 (5), 594-596.

Babić T, Grabarević Ž, Vuković S, Kos J, Matičić D, 2009: Congenital melanoma in a 3-month old bull calf - a case report. *Vet Arh*, 79 (4), 315-320.

Beytut E, Kılıç E, Yayla S, 2018: Histopathological and immunohistochemical evaluation of congenital cutaneous melanomas in calves (3 cases). *Ankara Üniv Vet Fak Derg*, 65 (4), 425-432.

Bhadaniya AR, Trangadia BJ, Prasad MC, Fefar DT, Kalaria VA, Vadaliya JV, Modi KS, 2015: Pathology of melanocytic tumour in cattle. *Indian J Vet Pathol*, 39 (3), 249-250.

Brito MF, França TN, Jabour F, Seixas JN, Andrade G, Oliveira LI, Peixoto, P, 2009: Metastasizing oral melanoma in a cow. *Ciencia Rural*, 39 (4), 1236-1240.

Chandrashekaraiah GB, Ballari SV, Manjunatha K, Chavadhal N, Radder SK, 2014: Malignant melanoma in a Hallikar bullock. *Int J Vet Sci*, 3 (2), 65-67.

Garma- Aviña A, Valli VE, Lumsden JH, 1981: Cutaneous melanomas in domestic animals. *J Cutan Pathol*, 8 (1), 3-24.

Hemanth I, Amaravathi P, Anand Kumar A, Devaratnam J, Bharathi S, Sailaja N, Kamalakar G, Sasidhar Babu N, 2014: Cutaneous melano-fibroma in a bullock – a rare concurrence of melanoma and fibroma. *Int J Sci Environ Technol*, 3 (2), 659-662.

Javanbakht J, Sasani F, Adibhashemi F, Hemmati S, 2014: Comparative histopathological diagnosis of cutaneous melanoma by H&E, special staining and immunohistochemical methods against cutaneous squamous cell carcinoma in horse and bovine. *J Bioanal and Biomed*, 6 (4), 19-23.

Koenig A, Wojcieszyn J, Weeks BR, Modiano JF, 2001: Expression of S100a, vimentin, NSE, and melan A/MART-1 in seven canine melanoma cells lines and twenty-nine retrospective cases of canine melanoma. *Vet Pathol*, 38 (4), 427-435.

Madheswaran R, Shahana S, Gopal K, Sankar P, 2019: A case report of non-systemic highly aggressive melanoma in a cow. *Indian J Vet Pathol*, 43 (2), 124-126.

Mesarić M, Zadnik T, Cerne M, 2002: Malignant melanoma in a cow. *Acta Vet-Beograd*, 52 (1), 59-64.

Miller MA, Weaver AD, Stogsdill PL, Fischer JR, Kreeger JM, Nelson SL, Turk JR, 1995: Cutaneous melanocytomas in 10 young cattle. *Vet Pathol*, 32 (5), 479-484.

Naveen B, Chandrashekar M, Vishwaradhya TM, Shambulinga M, Kumar P, Singh D, Rao S, 2013: Malignant melanoma in indigenous cattle - a case report. *Haryana Vet*, 52, 135-136.

Ozyildiz Z, Ceylan C, Yilmaz R, Ozsoy SY, 2012: Immunohistochemical characterization of perineal melanoma in Kilis goats. *Biotech Histochem*, 87 (6), 408-412.

Pazhanivel N, Ezakial Napolean, Murali Manohar B, Ravi U, 2003: A case of cutaneous melanoma in a bull. *Indian J Anim Res*, 37 (2), 151-152.

Qin J, Li S, Zhang C, Gao DW, Li Q, Zhang H, Jin XD, Liu Y, 2017: Apoptosis and injuries of heavy ion beam and x-ray radiation on malignant melanoma cell. *Exp Biol Med (Maywood)*, 242 (9), 953-960.

Ramos-Vara JA, Frank CB, DuSold D, Miller MA, 2014: Immunohistochemical expression of melanocytic antigen PNL2, Melan A, S100, and PGP 9.5 in equine melanocytic neoplasms. *Vet Pathol*, 51 (1), 161-166.

Ramos-Vara JA, Miller MA, 2011: Immunohistochemical identification of canine melanocytic neoplasms with antibodies to melanocytic antigen PNL2 and tyrosinase: comparison with Melan A. *Vet Pathol*, 48 (2), 443-450.

Roels S, Tilmant K, Ducatelle R, 2001: p53 expression and apoptosis in melanomas of dogs and cats. *Res Vet Sci*, 70 (1), 19-25.

Roels S, Tilmant K, Ducatelle R, 1999: PCNA and Ki67 proliferation markers as criteria for prediction of clinical behaviour of melanocytic tumours in cats and dogs. *J Comp Pathol*, 121 (1), 13-24.

Roels S, Tilmant K, Van Daele A, Van Marck E, Ducatelle R, 2000: Proliferation, DNA ploidy, p53 overexpression and nuclear DNA fragmentation in six equine melanocytic tumours. *J Vet Med A Physiol Pathol Clin Med*, 47 (7), 439-448.

Sabattini S, Renzi A, Albanese F, Fantinati M, Rigillo A, Abramo F, Tornago R, Tortorella G, Massaro M, Pagano TB, Buchholz J, Bettini G, 2018: Evaluation of Ki-67 expression in feline non-ocular melanocytic tumours. *BMC Vet Res*, 14 (1), 309.

Schmid F, Brodesser D, Reifinger M, Forte S, Semp P, Eberspächer-Schweda MC, Wolschek M, Brandt S,

Kleiter M, Pratscher B, 2019: Canine oral primary melanoma cells exhibit shift to mesenchymal phenotype and phagocytic behaviour. *Vet Comp Oncol*, 17 (3), 211-220.

Sharma S, Chaudhary RN, Singh K, 2010: Melanoma in a Hariana cow. *Haryana Vet*, 49, 78.

Sivadas CG, Nair MK, Rajan A, Ramachandran KM, 1971: Congenital melanoma in a calf. A review and case report. *Br Vet J*, 127 (6), 289-293.

Smith SH, Goldschmidt MH, Mcmanus PM, 2002: A comparative review of melanocytic neoplasms. *Vet Pathol*, 39 (6), 651-678.

Vadalia JV, Fefar DT, Patel PB, 2016: Surgical management of malignant melanoma in Kankrej cow. *Intas Polivet*, 17 (1), 98-99.

Vijayakumar S, Lakkawar AW, Kumar R, Alphonse RMD, Nair MG, 2020: Pathomorphological studies on mesenchymal and melanocytic neoplasms of cattle. *VMPH*, 1 (3), 102-107.

***Correspondence:** Emin KARAKURT

Kafkas University, Faculty of Veterinary Medicine, Department of Pathology, Kars, Turkey,.

e-mail: mehmeteminkarakurt@hotmail.com