Histol Histopathol (2016) 31: 453-459

http://www.hh.um.es

Histology and Histopathology

From Cell Biology to Tissue Engineering

Immunohistochemical analysis of CD146 expression in canine skin tumours

S. Abou Asa[1,2], Sh. Anwar[1], T. Yanai[1] and H. Sakai[1]

[1]Laboratory of Veterinary Pathology, Faculty of Applied Biological Sciences, Gifu University, Gifu, Japan and [2]Department of Veterinary Pathology, Faculty of Veterinary Medicine, Kafr El-Sheikh University, Egypt

Summary. CD146, a cell adhesion molecule, is overexpressed in a variety of carcinomas, including melanoma, prostate cancer, epithelial ovarian cancer, and breast cancer. The level of expression is directly correlated with tumour progression and metastatic potential. The most commonly affected organ for both neoplastic and non-neoplastic tumours is the skin. The objective of this study is to investigate the immunohistochemical expression of CD146 in canine skin tumours of epidermal or follicular origin in 53 squamous cell carcinomas (SCCs), 9 squamous papillomas, 7 infundibular keratinizing acanthomas (IKA), 21 trichoepitheliomas, 13 trichoblastomas, and 3 pilomatricomas. Immunohistochemical results showed that SCCs (90.6%), squamous papilloma (33.3%), IKA (85.7%), trichoepithelioma (85.9%), trichoblastoma (30.8%) and pilomatricoma (100%), respectively, were positive for CD146. The significant expression of CD146 in SCCs supports its importance as a useful treatment target. CD146 could also be used in differentiation of trichoepithelioma and trichoblastoma.

Key words: CD146, Canine, Skin tumours, Immunohistochemistry

Offprint requests to: Hiroki Sakai, Laboratory of Veterinary Pathology, Department of Veterinary Medicine, Faculty of Applied Biological Sciences, Gifu University, 1-1 Yanagido, Gifu, 501-1193, Japan. e-mail: shiroki@gifu-u.ac.jp

DOI: 10.14670/HH-11-690

Introduction

Cluster of differentiation 146 (CD146) is a cell adhesion molecule (CAM) belonging to the immunoglobulin superfamily (IgSF) (Lehman et al., 1987). Human CD146 has previously been designated by several synonyms including MUC18 (Lehman et al., 1987, 1989), A32 antigen (Shih et al., 1994), S-Endo-1 (Bardin et al., 1996), melanoma CAM (MCAM or Mel-CAM) (Rummel et al., 1996), metastasis CAM (MET-CAM) (Wu et al., 2011), and haemopoietic CAM (HEMCAM) (Vainio et al., 1996). The avian homologue of CD146 has been named gicerin (Taira et al., 1994). CD146 was originally identified as a marker for melanoma (MCAM) because of its overexpression in metastatic lesions and advanced primary tumours, but not in benign lesions (Lehman et al., 1987; Shih et al., 1994). CD146 has been included in the group of new endothelial antigens, and its immunoreactivity in normal adult tissues has been consistently demonstrated in the endothelium, smooth muscle, Schwann cells, and ductal and myoepithelial cells of salivary glands (Shih et al., 1998). CD146 is functionally involved in focal adhesion, cytoskeletal organisation, intercellular interactions, cell shape maintenance, cellular migration, and proliferation control (Shih, 1999). Increasing evidence has demonstrated that CD146 is overexpressed in a variety of carcinomas, in addition to melanomas (Zeng et al., 2012). Tumoral invasion and metastasis have been correlated to CD146 expression in melanoma cells. However, in other tumours, such as ductal breast carcinoma, its expression seems to be inversely associated with a more aggressive behaviour (Shih,

1999). CD146 has been associated with malignant transformation and metastatic potential in prostate carcinoma (Wu et al., 2011), and is considered to be a potential marker for tumour diagnosis, prognosis, and treatment. The majority of studies support that CD146 promotes tumour growth, angiogenesis, and metastasis (Ouhtit et al., 2009), and is a promising target for tumour therapy (Bu et al., 2006; Wellbrock and Fiedler, 2012). The skin is continuously exposed to a wide variety of chemical, physical, and other environmental factors and is prone to neoplastic proliferation. In dogs, approximately 30% of all neoplasms occur in the skin (Kaldrymidou et al., 2002).

To the best of our knowledge, there are no previous reports on the expression of CD146 in canine skin neoplasms. In this study, the immunohistochemical expression of CD146 was investigated using tumour samples of squamous cell carcinoma (SCCs), squamous papilloma, infundibular keratinizing acanthoma (IKA), trichoepithelioma, trichoblastoma, and pilomatricoma.

Materials and methods

Tumour samples

Surgically removed tissue samples from the archives of the Department of Veterinary Pathology, Gifu University, in the period between January 2011 and December 2013 were used in this study. These included 5 normal canine skin (1 head, 2 limb, and 2 breast skin) samples that were taken from normal skin or after mammary gland reduction (mastectomy) and 106 tumour samples consisted of cutaneous squamous cell carcinoma, (30 well-differentiated, 17 moderately differentiated, and 6 poorly differentiated SCCs) (n=53), squamous papilloma (n=9), infundibular keratinizing acanthoma (IKA) (n=7), trichoepithelioma (n=21), trichoblastoma (n=13), and pilomatricoma (n=3). The diagnosis of each tumour had been previously confirmed by haematoxylin and eosin staining, according to the World Health Organization histological classification and updates (Goldschmidt et al., 1998; Gross et al., 2005).

Immunohistochemical analysis of CD146

Immunohistochemical staining of CD146 was performed on all tissue samples using 4-µm thick paraffin-embedded sections. The sections were deparaffinised in xylene and rehydrated in graded ethanol. For antigen retrieval, the sections were immersed in Target Retrieval Solution High pH (Dako®) and autoclaved at 121°C for 15 minutes. Endogenous peroxidase was blocked by incubation in 0.3% H_2O_2 in methanol for 20 minutes at room temperature (RT). To prevent the binding of non-specific proteins, the sections were treated with Protein Block Serum Free (Dako®) for 30 minutes at RT. Immunolabelling of CD146 was performed on all samples, using anti-CD146 (rabbit monoclonal antibody ([EPR3208] (ab75769); Abcam, Cambridge, UK), at a dilution of 1:250, overnight in a humidified chamber at 4°C. The sections were washed with phosphate-buffered saline (PBS) and visualised with EnVision+ System, HRP-labelled polymer anti-rabbit (Dako®). After washing 3 times with PBS, 3,3-diaminobenzidinetetrahydrochloride (Liquid DAB + Substrate Chromogen System, Dako®) was added to the sections. The sections were then washed in distilled water, counterstained with Mayer's haematoxylin, dehydrated in an alcohol gradient, cleared with xylene, and mounted for examination under light microscope.

Scoring of immunoreactivity and statistical analysis

The percentage of neoplastic cells with positive labelling of CD146 was determined by counting 1000 cells observed under 10 high power fields (× 400) for each tumour section. The immunoreactivity was divided into four categories based on the percentage of neoplastic cells showing positive labelling: negative (0-10% positive cells), weakly positive (10-25% positive cells), moderately positive (25-50% positive cells) and strongly positive (>50% positive cells). A chi-square test was used to compare the proportion of CD146 immunoreactivity among different types of skin tumours, and a P value <0.05 was considered statistically significant.

Results

In normal canine skin, vascular smooth muscle and endothelial cells within the dermis and the cytoplasm of epidermal cells including basal cell, spinous, and melanocytes cells positively expressed CD146. Sebocytes were moderately positive, and arrector pili muscle cells and ductal cells were strongly positive for CD146 (Fig. 1A,B). CD146 was identified in the external and internal root sheath cells of hair follicles, and the intensity was stronger in the internal root sheath cells. However, pilomatrical cells were negative for CD146 (Fig. 1C). In the positive neoplastic cells, the staining was localised in the membrane and cytoplasm. In SCCs samples, 5/53 (9.4%) were negative. The neoplastic cells showed negative staining of CD146 or very few positively stained cells within the tumour area while the endothelium and smooth muscle of blood vessels showed reactivity, which is considered as an internal control for the immunoreaction. In addition, reactivity was observed in the epithelium of some hair follicles, while no reaction was seen in the basal or suprabasal keratinocytes. In 4 out of 53 (7.5%) cases, CD146 expression was weakly positive, accompanied with slight immunoreactivity in some suprabasal keratinocytes. In 2 (3.8%) cases, the expression was moderately positive. The majority of SCCs, i.e. 42 out of 53 (79.2%) cases showed strongly positive expression of CD146, showing intense cytoplasmic and membranous staining (Fig. 1D,E) in the epidermal epithelium and

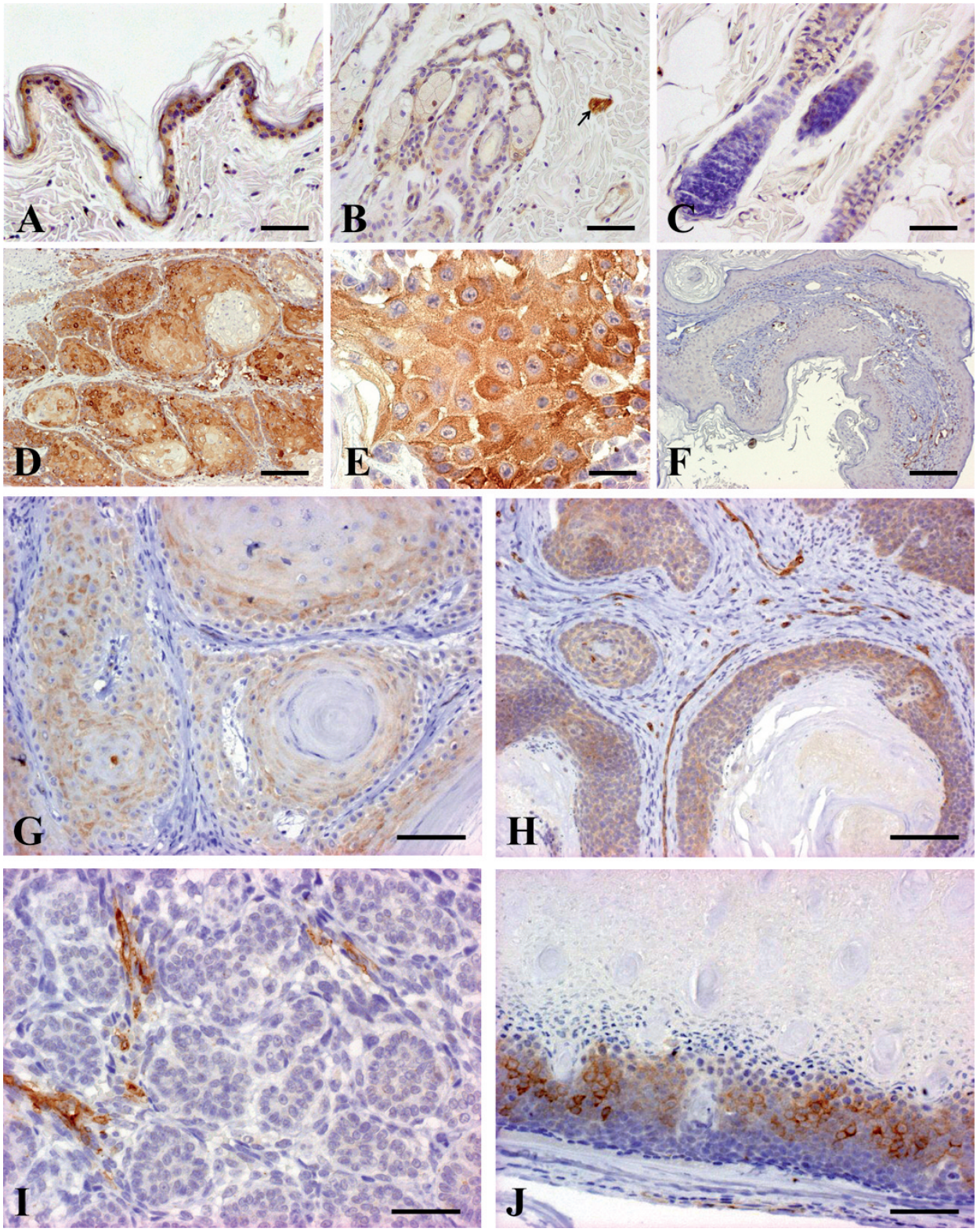

Fig. 1. Immunohistochemistry of CD146 in normal canine skin and different skin tumors. **A.** Epidermis, showing homogenous positive epidermal cells. **B.** Sebaceous gland, where sebocytes are moderately positive. Ductal cells and arrector pili muscle cells are strongly positive for CD146 (arrow). **C.** Hair follicles, showing negative pilomatrical cells while follicular epithelial cells are positive. **D, E.** Well-differentiated SCC, showing strong cytoplasmic immunoreactivity, where all neoplastic cells were positive. **F.** Squamous papilloma, showing negatively stained squamous epithelium and positively stained blood vessels in the fibrovascular core. **G.** IKA, concentrically lamellated keratin-filled crypts lined with strongly stained squamous cells. **H.** Trichoepithelioma, showing strong reactivity in proliferating nests of neoplastic cells. **I.** Trichoblastoma, showing negatively stained neoplastic cells. **J.** Pilomatricoma, moderately membranous immunolabelled basophilic basaloid cells, with gradually degenerated negatively stained ghost cells. Scale bar: A, B, C, E, I, J, 50 µm; D, F, 200 µm; G, H, 100 µm.

Table 1. Immunoreactivity of CD146 in canine skin tumours.

Tumor samples	Expression (%)			
	Negative	Positive		
	(0-10)	Weak (10-25)	Moderate (25-50)	Strong (>50)
SCCs* (n=53)	5(09.4)	4(07.5)	2(03.8)	42(79.2)
Papilloma (n=9)	6(66.7)	2(22.2)	1(11.1)	0(00.0)
IKA* (n=7)	1(14.3)	0(00.0)	3(42.9)	3(42.9)
Trichoepithelioma (n=21)	4(19.0)	5(23.8)	4(19.0)	8(38.1)
Trichoblastoma (n=13)	9(69.2)	3(23.1)	1(07.7)	0(00.0)
Pilomatricoma (n=3)	0(00.0)	1(33.3)	2(66.7)	0(00.0)

* SCC: squamous cell carcinoma, IKA: intracutaneous keratinizing acanthoma.

infiltration of positively stained inflammatory cells, mainly macrophages and neutrophils. In poorly differentiated SCCs, 6/53 (11.3%) cases showed focal and weak staining, while in well- and moderately differentiated SCCs, no difference in intensity was observed. The reaction tended to be intense and diffused throughout the neoplastic cells. In squamous papillomas, 6/9 (66.7%) cases were negative for CD146 expression (Fig. 1F). However, a positive reaction was seen in the endothelium of blood vessels in the fibrovascular core, with 2/9 (22.2%) and 1/9 (11.1%) cases being weakly and moderately positive stained, respectively. In IKA, only one case 1/7 (14.3%) was negative, except for the staining of small blood vessels and inflammatory cells, mainly neutrophils. In 3/7 (42.9%) cases each, moderate and strong expression of CD146 (Fig. 1G) was observed, with no staining of basal or suprabasal keratinocytes. Reactivity was observed only in the epithelium of some hair follicles. The proportion of SCCs cases showing positive immunoreactivity for CD146 was significantly higher than that of papillomas (P=0.00), while no significant difference was detected between SCCs and IKA (P=0.085). In trichoepithelioma, 4/21 (19%) cases were negative for CD146 expression, with no reactivity seen in the epidermal keratinocytes. Reactivity was only seen in the follicular epithelium, arrector pili muscle, and some macrophages. About 5/21 (23.8%) cases were weakly positive for CD146, with slight reactivity in suprabasal keratinocytes. About 4/21 (19.0%) cases were moderately positive for CD146 where the immunolabelling was mainly seen in the prickle cells rather than in the hair matrical cells. The remaining 8/21 (38.1%) cases strongly expressed CD146 (Fig. 1H), accompanied by infiltration of positively stained macrophages and neutrophils. Staining of suprabasal keratinocytes, hair follicle epithelium, endothelial lining of small blood vessels, and tunica media of medium and large sized blood vessels was frequently observed. In trichoblastoma, 9/13 (69.2%) cases negatively expressed CD146 (Fig. 1I), with weak reactivity seen in some suprabasal keratinocytes or hair follicle and the main stromal reactivity was seen in the endothelial lining of small blood vessels. About 3/13 (23.1%) cases were weakly positive where some neoplastic cells showed immunoreactivity scattered throughout the tumour area. Only 1 case out of 13 (7.7%) showed moderate staining of CD146 in some stromal cells, and strong reactivity in the suprabasal keratinocytes and hair follicle epithelium. The proportion of trichoepithelioma cases showing positive immunoreactivity for CD146 was significantly higher than that of trichoblastoma cases (P=0.01). In pilomatricoma, 1/3 (33.3%) cases showed weak immunolabelling and the remaining 2 cases (66.7%) showed moderate staining of the neoplastic basophilic basaloid cells (Fig. 1J). Towards the centre of the tumour, the shadow cells were negative for CD146. The immunoreactivity of CD146 in the different tumours is summarized in (Table 1).

Discussion

Cell adhesion molecules (CAMs) are involved in an extensive range of physiological processes, including cell-cell and cell-matrix interactions, cell migration, cell cycle, and signalling as well as morphogenesis during development and tissue regeneration. Increasing evidence has highlighted the fundamental role of CAMs in a variety of pathological progressions, such as cancer, inflammation, pathogenic infections, and autoimmune disease (Trzpis et al., 2007). Changes in their expression are often associated with changes in cellular morphology and tissue architecture. Alterations in intercellular adhesion are hallmarks of malignant cells and are thought to contribute to deranged cellular interactions characteristic of cancer (Hart and Easty, 1991; Albelda, 1993; Okegawa et al., 2004; Makrilia et al., 2009). During the process of tumor invasion and metastasis, adhesive interactions occur between tumor cells, and between tumor and normal cells in the stroma (Herlyn and Austin, 1993). Deregulation of CAM expression has been frequently observed during the process of tumor progression. CD146 is a cell-cell or cell-matrix adhesion molecule that was first described in melanoma (Lehman et al., 1989). Previous studies have indicated that CD146 expression correlates with the malignant progression and metastatic potential of human melanoma cells (Luca et al., 1993; Luo et al., 2012). Expression of CD146 has been observed in certain normal human tissues and numerous malignancies, such as non-small cell lung cancer, gallbladder adenocarcinoma, and gastric cancer (Kristiansen et al., 2003; Liu et al., 2012; Wang et al., 2012). This study demonstrated that CD146 was significantly overexpressed in SCCs where 48/53 (90.6%) cases were positive. In IKA, which is a rare epithelial benign follicular canine neoplasm that evolves rapidly, forming a solitary or multiple firm flask-shaped cystic nodules with keratin in their centres (Della Salda et al., 2002), 6/7 (85.7%) cases significantly expressed CD146 in contrast to squamous papilloma, where 3/9 (33.3%) cases were positive. In trichoepithelioma, 17/21

(80.95%) cases expressed CD146 in contrast to trichoblastoma, where 4/13 (30.8%) cases were positive. CD146 can be used to differentiate between trichoepithelioma and trichoblastoma. Trichoepthelioma include approximately 80% of hair follicle tumours in domestic dogs, especially golden retrievers, basset hounds, and German shepherds (Suedmeyer and Williams, 2005). In pilomatricoma, despite the weak to moderate expression of CD146 in the three examined cases, there is no significance of expression because of the small sample size. Additionally, pilomatricoma is an uncommon follicular skin tumour originating from matrix cells of the hair bulb described in dogs, cats, and human beings (Du Vivier and McKee, 1993; McKee, 1999). Moreover, the incidence of its malignant counterpart (matrical carcinoma or malignant pilomatricoma) is extremely low (Goldschmidt and Hendrick, 2002). Squamous cell carcinoma is one of the most common neoplasms in dogs, being the second most common skin tumour as well as the second most common malignancy of the oral cavity (Gardner, 1996). Canine SCC shares several similarities with the human disease (Moulton, 1990; Dhaliwal et al., 1998). In humans, it is a malignant tumour that can arise from the epidermis or from a mucosal surface (principally the oral cavity). In both species, tumour prevalence increases with advancing age, and there is no breed or racial predilection. In addition, tumours can arise from a variety of anatomic sites in both humans and dogs (Buckman et al., 1998; Salashe, 2000). SCC of the skin in dogs is a locally invasive tumour that demonstrates a predilection for the feet, face, and abdomen (Kraegel and Madewell, 2000). Numerous genetic alterations have been described in SCC sub-types, although the molecular mechanisms contributing to tumour initiation and progression are still poorly understood. SCCs share many phenotypic and molecular characteristics with each other (Pisani et al., 2002; Travis, 2002; Farhadieh et al., 2009). Despite advances in diagnostic methods and combined treatment modalities of SCC in humans, the survival rate has not improved significantly over the last 30 years due to a lack of reliable early diagnostic biomarkers and a limited number of molecularly targeted therapeutic strategies (Agada et al., 2009). Improved understanding of the mechanisms of SCC tumorigenesis, metastases, and treatment failure may have a significant impact on a better understanding of the cellular and molecular pathways, which in turn would help develop more efficient therapies in humans and canines. To the best of our knowledge, there is no report on the expression of CD146 in canine skin tumours. In humans, a few studies investigated CD146 expression (Schon et al., 2005), which showed focal moderate to strong expression of CD146 within the tumour nests of 8/11 human basal cell carcinomas as well as 6/6 squamous cell carcinomas; these findings support our results with regard to the significant expression of CD146 in SCCs. (Wolfgang et al., 2000) reported that CD146 expression in human epithelial skin tumours was heterogenous, where 1/4 lesions of Bowen's disease and 0/2 keratoacanthoma showed anti-CD146 reactivity. In basal cell carcinoma (n=15), the result was variable and in SCCs, 4/8 were negative for CD146. In parotid mucoepidermoid carcinoma (MEC), CD146 expression was observed in 92.7% of the cases but not in 10 oral SCCs. CD146 can be a useful marker in differentiating between high grade MEC and SCC (Pires et al., 2003). CD146 is required in VEGF-induced VEGFR-2 phosphorylation, AKT/p38 MAPKs/NF-κB activation, and promotion of endothelial cell migration and microvascular formation (Jiang et al., 2012). CD146 expression has been found to be correlated with the aggressiveness and development of metastasis, and is a predictor of worse prognosis in certain cancer types (Shih et al., 1998). CD146 is associated with an advanced tumour stage in melanoma, prostate cancer, ovarian cancer, and triple-negative breast cancer (Melnikova et al., 2009; Zeng et al., 2012).

In conclusion, overexpression of CD146 was observed in canine SCCs and could be a useful therapeutic target. New markers discovered in canine SCCs may shed some light on this type of cancer in humans. Further studies are warranted to determine its significance and mechanism of action. In contrast, the expression of CD146 was minimal in squamous papillomas. The significant difference in CD146 expression between trichoepithelioma and trichoblastoma could be useful in differential diagnosis. In this study, the significance of CD146 expression in pilomatricoma could not be determined because of lack of samples.

References

Agada F.O., Patmore H., Alhamarneh O., Stafford N.D. and Greenman J. (2009). Genetic profile of head and neck squamous cell carcinoma: clinical implications. J. Laryngol. Otol. 123, 266-272.

Albelda S.M. (1993). Role of integrins and other cell adhesion molecules in tumor progression and metastasis. Lab. Invest. 68, 4-17.

Bardin N., Frances V., Lesaule G., Horschowski N., George F. and Sampol J. (1996). Identification of the S-Endo 1 endothelial-associated antigen. Biochem. Biophys. Res. Commun. 218, 210-216.

Bu P., Gao L., Zhuang J., Feng J., Yang D. and Yan X. (2006). Anti CD146 monoclonal antibody AA98 inhibits angiogenesis via suppression of nuclear factor-kappa B activation. Mol. Cancer Ther. 5, 2872-2878

Buckman S.Y., Gresham A., Hale P., Hruza G., Anast J., Masferrer J. and Pentland A.P. (1998). COX-2 expression is induced by UVB exposure in human skin: implications for the development of skin cancer. Carcinogenesis 19, 723-729.

Della Salda L., Preziosi R., Mazzoni M. and Marcato P.S. (2002). Cell proliferation patterns in canine infundibular keratinizing acanthoma and well differentiated squamous cell carcinoma of the skin. Eur. J. Histochem. 46, 165-172.

Dhaliwal R.S., Kitchell B.E. and Maretta S.M. (1998). Oral tumors in dogs and cats. Part I. Diagnosis and clinical signs. Compend. Continuing. Educ. 20, 1011-1021.

Du Vivier A. and McKee P. (1993). Benign tumours of the skin. In: Atlas of clinical dermatology. Du Bivier A. (ed). Gower Medical Publishing, London, 8.11-8.12.

Farhadieh R.D., Salardini A., Yang J.L., Russell P. and Smee R. (2009). Diagnosis of second head and neck tumors in primary laryngeal SCC is an indicator of overall survival and not associated with poorer overall survival: a single center study in 987 patients. J. Surg. Oncol. 101, 72-77.

Gardner D.G. (1996). Spontaneous squamous cell carcinomas of the oral region in domestic animals: a review and consideration of their relevance to human research. Oral Dis. 2, 148-154.

Goldschmidt M.H. and Hendrick M.J. (2002). Tumors of the skin and soft tissues. In: Tumors in domestic animals. 4th edition. Meuten D.J. (ed). Iowa State Press, 61-63.

Goldschmidt M.H., Dunstan R.W., Stannard A.A., von Tscharner C., Walder E.J. and Yager J.A. (1998). Histological classification of epithelial and melanocytic tumors of the skin of domestic animals. World Health Organization, International Histological Classification of Tumors of Domestic Animals, Armed Forces Institute of Pathology, American Registry of Pathology, Washington, DC, 20-2.

Gross T.L., Ihrke P.J., Walder E.J. and Affolter V.K. (2005). Skin diseases of the dog and cat: Clinical and histopathologic diagnosis. 2nd ed. Oxford: Blackwell Publishing Company.

Hart I.R. and Easty D. (1991). Tumor cell progression and differentiation in metastasis. Semin. Cancer Biol. 2, 87-95.

Herlyn M. and Austin R.G. (1993). Molecular and cellular biology of melanoma, Landes Company, 1-102.

Jiang T., Zhuang J., Duan H., Luo Y., Zeng Q., Fan K., Yan H., Lu D., Ye Z., Hao J., Feng J., Yang D. and Yan X. (2012). CD146 is a coreceptor for VEGFR-2 in tumor angiogenesis. Blood 11, 2330-2339.

Kaldrymidou H., Leontides L., Koutinas A.F., Saridomichelakis M.N. and Karayannopoulou M. (2002). Prevalence, distribution and factors associated with the presence and the potential for malignancy of cutaneous neoplasms in 174 dogs admitted to a clinic in northern Greece. J .Vet. Med. A. Physiol. Pathol. Clin. Med. 49, 87-91.

Kraegel S.A. and Madewell B.R. (2000). Tumors of the skin. In: Textbook of Veterinary Internal Medicine. Diseases of the Dog and Cat. 5th ed. Ettinger S.J. and Feldman E.C. (eds). Philadelphia, WB Saunders, 524-528.

Kristiansen G., Yu Y., Schluns K., Sers C., Dietel M. and Petersen I. (2003). Expression of the cell adhesion molecule CD146/MCAM in non-small cell lung cancer. Anal. Cell. Pathol. 25, 77-81.

Lehmann J.M., Holzmann B., Breitbart E.W., Schmiegelow P., Riethmüller G. and Johnson J.P. (1987). Discrimination between benign and malignant cells of melanocytic lineage by two novel antigens, a glycoprotein with a molecular weight of 113,000 and a protein with a molecular weight of 76,000. Cancer Res. 47, 841-845.

Lehmann J.M., Riethmuller G. and Johnson J.P. (1989). MUC18, a marker of tumor progression in human melanoma, shows sequence similarity to the neural cell adhesion molecules of the immunoglobulin superfamily. Proc. Natl. Acad. Sci. USA 86, 9891-9895.

Liu W.F., Ji S.R., Sun J.J., Zhang Y., Liu Z.Y., Liang A.B. and Zeng H.Z. (2012). CD146 Expression correlates with epithelial-mesenchymal transition markers and a poor prognosis in gastric cancer. Int. J. Mol. Sci. 13, 6399-6406.

Luca M., Hunt B., Bucana C.D., Johnson J.P., Fidler I.J. and Bar-Eli M. (1993). Direct correlation between MUC18 expression and metastatic potential of human melanoma cells. Melanoma Res. 3, 35-41.

Luo Y., Zheng C., Zhang J., Lu D., Zhuang J., Xing S., Feng J., Yang D. and Yan X. (2012). Recognition of CD146 as an ERM-binding protein offers novel mechanisms for melanoma cell migration. Oncogene 31,306-321.

Makrilia N., Kolliasa A., Manolopoulosb L. and Syrigos K. (2009). Cell adhesion molecules: Role and clinical significance in cancer. Cancer invest. 27, 1023-1037.

McKee P. (1999). Essential skin pathology. Mosby International Ltd, London.

Melnikova V.O., Balasubramanian K., Villares G.J., Dobroff A.S., Zigler M., Wang H., Petersson F., Price J.E., Schroit A., Prieto V.G., Hung M.C. and Bar-Eli M. (2009). Crosstalk between protease-activated receptor 1 and platelet-activating factor receptor regulates melanoma cell adhesion molecule (MCAM/MUC18) expression and melanoma metastasis. J. Biol. Chem. 284, 28845-28855.

Moulton J.E. (1990). Tumors of the skin and soft tissues. In: Tumors in domestic animals. 3rd ed. Moulton J.E. (ed). University of California Press. Berkeley, Los Angeles. 23-87.

Okegawa T., Pong R.C., Li Y. and Hsieh J.T. (2004). The role of cell adhesion molecule in cancer progression and its application in cancer therapy. Acta. Biochim. Pol. 51, 445-457.

Ouhtit A., Gaur RL., Abd Elmageed Z.Y., Fernando A., Thouta R., Trappey A.K., Abdraboh M.E., El-Sayyad H.I., Rao P. and Raj M.G. (2009). Towards understanding the mode of action of the multifaceted cell adhesion receptor CD146. Bioch. Biophys. Acta 1795, 130-136.

Pires F.R., Shih L.M., Cruz P.D., Almeida O.P and Kowalski L.P. (2003). Mel-CAM (CD146) expression in parotid mucoepidermoid carcinoma. Oral Oncol. 39, 277-281.

Pisani P., Bray F. and Parkin D.M. (2002). Estimates of the world-wide prevalence of cancer for 25 sites in the adult population. Int. J. Cancer 97, 72- 81.

Rummel M.M., Sers C. and Johnson J.P. (1996). Phorbol ester and cyclic AMP-me diated regulation of the melanoma-associated cell adhesion molecule MUC18/MCAM. Cancer Res. 56, 2218-2223.

Salashe S.J. (2000). Epidemiology of actinic keratoses and squamous cell carcinoma. J. Am. Acad. Dermatol. 42, S4-7.

Schon M., Kahne T., Gollnick H. and Schon M.P. (2005). Expression of gp130 in tumors and inflammatory disorders of the skin: formal proof of its identity as CD146 (MUC18, Mel-CAM). J. Invest. Dermatol. 125, 353-363.

Shih I.M. (1999). The role of CD146 (Mel-CAM) in biology and pathology. J. Pathol. 189: 4-11.

Shih I.M., Elder D.E., Hsu M.Y. and Herlyn M. (1994). Regulation of Mel-CAM/MUC18 expression on melanocytes of different stages of tumor progression by normal keratinocytes. Am. J. Pathol. 145, 837-845.

Shih I.M., Nesbit M., Herlyn M. and Kurman R.J. (1998). A new Mel-CAM (CD146)-specific monoclonal antibody, MN-4, on paraffin-embedded tissue. Mod. Pathol. 11, 1098-1106.

Suedmeyer W.K. and Williams F. (2005). Multiple trichoepitheliomas in an alpaca (*Lama pacos*). J. Zoo Wildl. Med. 4, 706-708.

Taira E., Takaha N., Taniura H., Kim C.H. and Miki N. (1994). Molecular cloning and functional expression of gicerin, a novel cell adhesion molecule that binds to neurite outgrowth factor. Neuron 12, 861-872.

Travis W.D. (2002). Pathology of lung cancer. Clin. Chest. Med. 23, 65-

81.
Trzpis M., McLaughlin P.M., de Leij L.M. and Harmsen M.C. (2007). Epithelial cell adhesion molecule: more than a carcinoma marker and adhesion molecule. Am. J. Pathol. 171, 386-395.
Vainio O., Dunon D., Aissi F. Dangy J.P., McNagny K.M. and Imhof B.A. (1996). HEMCAM, an adhesion molecule expressed by c-kit+ hemopoietic progenitors. J. Cell Biol. 135, 1655-1668.
Wang W., Yang Z.L., Liu J.Q., Jiang S. and Miao X.Y. (2012). Identification of CD146 expression, angiogenesis, and lymphangiogenesis as progression, metastasis, and poor prognosis related markers for gallbladder adenocarcinoma. Tumour Biol. 33, 173-182.
Wellbrock J. and Fiedler W. (2012). CD146: a new partner for VEGFR2. Blood 120, 2164-2165.
Wolfgang W., Michael R., Michael M., Christoph M., Jozef B., Alexandra G., Gottfried B., Adrian T., Otto M. and Erwin T. (2000). Keratinocytes express the CD146 (Muc18/S-Endo) antigen in tissue culture and during inflammatory skin diseases. J. Invest. Dermatol. 115, 219-224.
Wu G.J., Wu M.W., Wang C. and Liu Y. (2011). Enforced expression of METCAM/MUC18 increases tumorigenesis of human prostate cancer LNCaP cells in nude mice. J. Urol. 185, 1504-1512.
Zeng Q., Li W., Lu D., Wu Z., Duan H., Luo Y., Feng J., Yang D., Fu L. and Yan X. (2012). CD146, an epithelial-mesenchymal transition inducer, is associated with triple-negative breast cancer. Proc. Natl. Acad. Sci. USA 109, 1127-1132.

Accepted November 17, 2015