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Tumour-Associated Macrophages (TAMs) and Cox-2 Expression in Canine Melanocytic Lesions

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1. Introduction

Melanoma is a devastating disease frequently encountered within both veterinary and human medicine. Melanocytic tumours are relatively common in dogs and represent 4 to 7% of all canine neoplasms. It is the most common malignant neoplasm of the oral cavity and the second most frequent subungual neoplasm. Classically, body location is one criteria used to establish a prognosis of these tumours (Martínez et al., 2011; Smith et al., 2002). Cutaneous canine melanocytic lesions are usually benign. However, the canine oropharyngeal, uveal, and mucocutaneous neoplasms are aggressive and have high metastatic potential (Marino et al., 1995; Smith et al., 2002; Spangler & Kass, 2006).

In other animals species melanocytic tumours were also seen, but in cats it is not as common and carries a poor prognosis (Dorn&Priester, 1976; Goldschmidt, 1985). In white or gray horses melanoma is very common, considered almost inevitable (Johnson, 1998; Valentine, 1995). In pigs, melanoma is very frequent in some breeds, as Sinclair (Berkelhammer&Hook, 1980; Hook et al., 1982; Misfeldt & Grimm, 1994).

Generally, cutaneous melanoma in domestic animals does not share the same degree of diversity described in the medical literature. Canine cutaneous melanoma can be either benign (melanocytoma) or malignant (melanoma) and are most common in older animals with darker coats. The benign form is often referred to as melanocytic nevus or melanocytoma, and is well-defined, firm, dome-shaped, with less than 2 cm in diameter and mobile. Malignant melanomas are less frequent in the skin and appear ulcerated with a rapid grow. Most oral and mucocutaneous (except eyelid) and 50% of nail bed (subungual) melanomas are malignant (Smith et al., 2002)

Unlike humans, dogs do not seem to develop malignant melanomas due to exposure to ionizing solar radiation. With the exception of gray horses, malignant transformation of benign lesions is very uncommon in animals, and most melanomas are believed to arised "*de novo*" from melanocytes in the epidermis, dermis, ocular epithelium, and oral epithelium (Conroy, 1967; Smith et al., 2002). However, little is known about initiation of most animal melanomas. Breed and familial clustering in domestic animals suggest that genetic susceptibility, possibly resulting in a greater frequency of spontaneously mutated cells, may

be critical to initiation of many of these tumours (Goldschmidt, 1985; Smith et al., 2002). Conroy described two canine cases of melanoma that arose from junctional or dermal pigmented nevi (hamartomas), although in neither case was metastatic behavior observed (Conroy, 1967). There is a single report of a primary melanoma in the skin of a dog that originated from a subcutaneous melanocytoma (Mulligan, 1961).

Cutaneous melanoma is the group of melanocytic lesions that constitute the big challenge to pathologist. The majority of the cases are benign but can be find malignant melanomas with elevated aggressiveness. Mitotic rate is highly predictive of the degree of malignancy and a mitotic rate of less than 3 per 10 high-power fields is strongly associated with benign behavior (Smith et al., 2002). Additional analysis by flow cytometry has shown a correlation between DNA ploidy and malignancy (Bolon et al., 1990). In the last years, several prognostic factors related to proliferation (Laprie et al., 2001; Roels et al., 1999), angiogenesis (Mukaratirwa et al., 2006; Taylor et al., 2007), apoptosis (Roels et al., 2001), and others have been investigated.

The incidence of melanocytic lesions is increasing among canine population. Canine malignant melanoma could have an aggressive behavior, metastasize early in the course of the disease and is resistant to most current therapeutic regimens. The role of a vaccine and the search of novel therapeutic tools are essential in the fight against this devastating disease. Furthermore, the similarities between human and canine melanoma make spontaneous canine melanoma an excellent disease model for studying the correspondent human disease (Bergman et al., 2006; Smith et al., 2002; von Euler et al., 2008).

Inflammation in tumour stroma greatly influences its development. In human cancer it has been described that tumour associated macrophages (TAMs) may promote tumour cell invasiveness and potentiate metastatic diffusion. Among the various inflammatory mediators generated by TAM, assume particular relevance the arachidonic acid metabolites, which are known to influence several biological responses involved in tumour progression, such as inflammatory and immune reactions, haemostasis and angiogenesis. Although the scientific evidence of an association between TAM and histological aggressiveness in human melanoma (Bianchini et al., 2007; Brocker et al., 1987), in the dog there are no studies concerning this subject.

Cyclooxigenase (-1 and -2) are isoenzymes that promote the transition of arachidonic acid in to different prostanoids. These isoenzymes are also the main targets of the NSAIDs (non steroid anti-inflammatory drugs). Several studies have been suggested the potential role of NSAID blocking particularly cyclo-oxygenases-2 (Cox-2) in the prevention and treatment of malignant tumours in humans (Cerella et al., 2010; Dubois et al., 1998). In dogs, there are evidences of a strong relationship between Cox-2 expression and malignancy in several types of cancers (bladder, skin, intestinal, mammary, bone, nasal) (Mohammed et al., 2004; Queiroga et al., 2007). Cox-1 and Cox-2 expression in canine melanocytic lesions has been recently published by the authors, describing that Cox-2 expression was restricted to the malignant melanoma group, being found in 11 of the 20 cutaneous malignant melanomas, in all oral malignant melanomas and in one of two ocular malignant melanomas analyzed. Authors also found that Cox-2 labeling was particularly intense in the more aggressive oral tumours (Pires et al., 2010). In human melanoma, Cox-2 has been implied in tumour progression (Chwirot&Kuzbicki, 2007; Kuzbicki et al., 2006).

The aims of the present work were: a) to describe, the number and the distribution of TAMs in canine melanocytic lesions; b) to investigate associations between TAMs and several clinicopathological characteristics; c) investigate the possible association between Cox-2 expression and the presence of TAMs in these tumours.

2. Material and methods

2.1 Tissue processing and tumour classification

Thirty seven melanocytic tumours, obtained from the UTAD Histopathology Laboratory archives were included. For the histopathologic study, 4-µm-thick tissue sections were stained with the hematoxylin and eosin with and without blanching by incubation in 0,25% potassium permanganate for 30–60 minutes, depending on the amount of pigment and then by incubation in 0,1% oxalic acid for 5–8 minutes. Each sample was re-examined by two independent pathologists (IP and JP) in order to confirm the diagnosis, according to the World Health Organization International Histological Classification of Tumours of Domestic Animals criteria (Goldschmidt et al., 1998).

2.2 Clinicopathological evaluation

The following clinicopathological features were evaluated: histological type-melanocytoma (benign), melanoma (malign); presence of ulceration; presence of necrosis; mitotic index; nuclear grade; degree of pigmentation, presence of aberrant tumoural cells; stroma; and tumoural vascular invasion. Mitotic index was calculated by counting all the mitosis present in 10 high power fields (HPF) (400x): mitotic index I (<3 mitosis in 10 HPF); mitotic index II (3–5 mitosis in 10 HPF) or mitotic index III (>5 mitosis in 10 HPF). For nuclear grade, the following grades were defined: (i) nuclear grade I when the nuclei had minimal variations in their shape and size compared to normal nuclei; (ii) nuclear grade II consisted of moderate alterations of nuclear shape; and (iii) nuclear grade III consisted of the nuclei that were irregular and larger than normal (Queiroga et al., 2010).The degree of pigmentation was estimated using a subjective scale from scant (pigment in fewer than 30% of cells), moderate (pigmentation in 31–80% of cells), and abundant (pigment in more than 80% of cells). The amount of stroma was categorized in: scant, moderate, and abundant (Ramos-Vara et al., 2000).

2.3 Immunohistochemistry

For immunohistochemical studies, 3-µm sections were cut from each specimen and mounted on silane-coated slides. MAC 387 (for macrophage immunolabelling) and Cox-2 immunoexpression were carried out by the streptavidin-biotin-peroxidase complex method, with a commercial detection system (Ultra Vision Detection System; Lab Vision Corporation, Fremont, USA) following the manufacturer's instructions, with and without blanching. Antigen retrieval was by microwave treatment in citrate buffer (pH 6.0) three times for 5 min each in a 750 W microwave oven, followed by cooling for 20 min at room temperature.

Primary antibodies were: MAC 387 (AbDSerotec, MorphoSys UK Ltd., Kidlington, Oxford,U.K.; Clone MCA 874G) diluted 1 in 100 in PBS and applied for 1 h at room temperature and COX-2 (Transduction Laboratories, Lexington, Kentucky; clone 33) diluted 1 in 40 in phosphate buffered saline (PBS; pH 7.4, 0.01 M) and applied overnight at 4°C.

Immunoreaction was visualized by incubation with 3,3'-diaminobenzidine tetrahydrochloride (DAB) at 0.05% with 0.01% H_2O_2 as the final substrate for 5 minutes. After a final washing in distilled water, the sections were counterstained with haematoxylin, dehydrated, cleared and mounted.

The primary antibody was replaced by PBS and by an irrelevant antibody for negative controls. Positive controls consisted of canine epidermis and liver for MAC 387 and sections from *macula densa* of young normal canine kidney for Cox-2.

2.4 Immunohistochemistry evaluation

Positivity was indicated by the presence of distinct brown cytoplasmic labeling. Immunoreactivity was evaluated ''blindly'' by two observers (IP e FLQ).

TAMs were counted in the three regions with more intense and homogeneous positivity of each of counting areas (Hussein et al., 2009). In these regions, we counted all labeled cells, evaluating a total of ten high-power fields (HPF), (400x magnification power). TAM were then categorized in three classes: 1: <20 positive macrophages (sparse); 2: 20-100 (moderate), 3: >100 positive macrophages (intense) (Piras et al., 2005).

Positive Cox-2 expression was defined when more than 10% of the tumour cells showed positive staining. The staining intensity was not scored in this method.

2.5 Statistical analysis

The statistical software SPSS version 12.0 was used for statistical analysis. The Chi-square test and the Fisher's exact test were used for studying categorical variables. In all statistical comparisons, $p < 0.05$ was accepted as denoting significant differences.

3. Results

3.1 Tumours

From the 37 tumours included in the study, 8 cases were classified as melanocytomas (benign tumours) and 29 cases as malignant melanomas (malignant tumours, Fig.1).

Fig. 1. Cutaneous malignant melanoma in a German Shepard dog.

3.2 Tumour- associated macrophages (TAMs) in canine melanocytic tumours

MAC 387 immunostaining was always observed in the cytoplasm of macrophages in a diffuse and homogeneous pattern. The upper layers of epidermis also stained with MAC 387.

TAMs were observed in all the samples analyzed (n=37). As shown in Table 1, all the benign lesions had sparse macrophage infiltration, while malignant melanomas presented a

moderate or intense infiltration (Fig.2 and Fig.3). The number of tumoural-associated macrophages in malignant melanoma was significantly higher than the mean values in the benign counterparts (p=0,002).

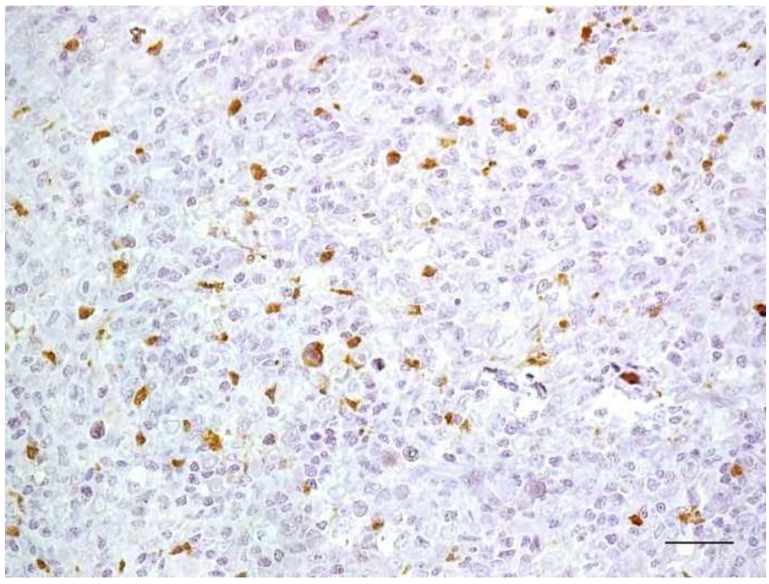

Fig. 2. Canine malignant melanoma showing a moderate number of TAMs. IHC. Bar, 30 mm.

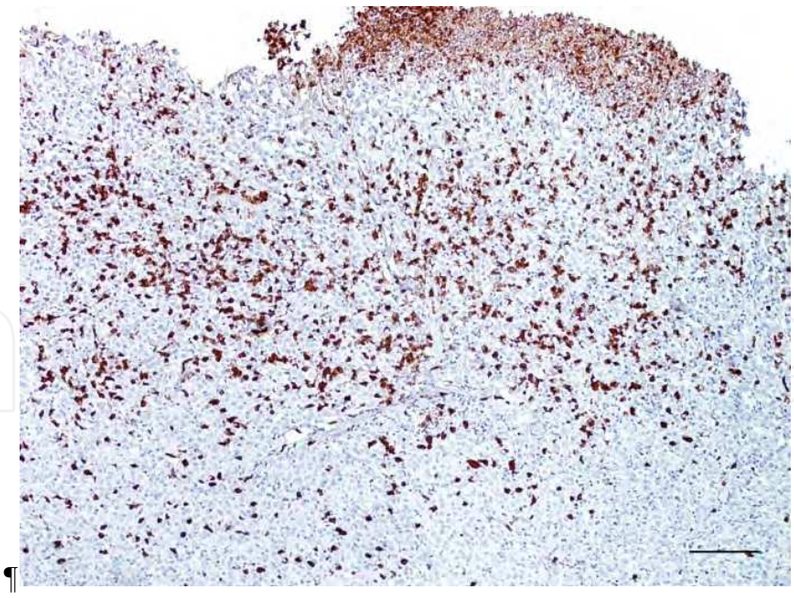

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Fig. 3. Intense infiltration of macrophages in a cutaneous melanoma. IHC. Bar, 120 µm.

3.3 Cox-2 expression in canine melanocytic tumours

The expression of COX-2 was absent in 18/37 (48,6%) of the canine melanocytic tumours (Fig. 4). None of the 8 benign tumours expressed Cox-2 in tumoural cells. Among the

malignant tumours, COX-2 was expressed by 19 of the 29 melanomas (65,5%), (Fig. 5 e Fig. 6). There was a significant difference in COX-2 expression between melanocytomas and malignant melanomas ($p < 0,001$).

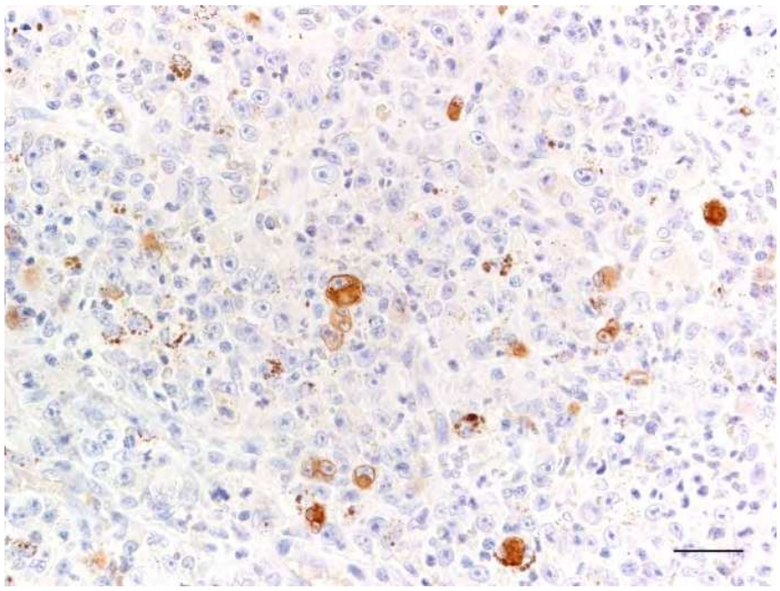

Fig. 4. Cutaneous malignant melanoma considered negative to Cox-2 (less than 10% of positive cells). IHC. Bar, 30 µm.

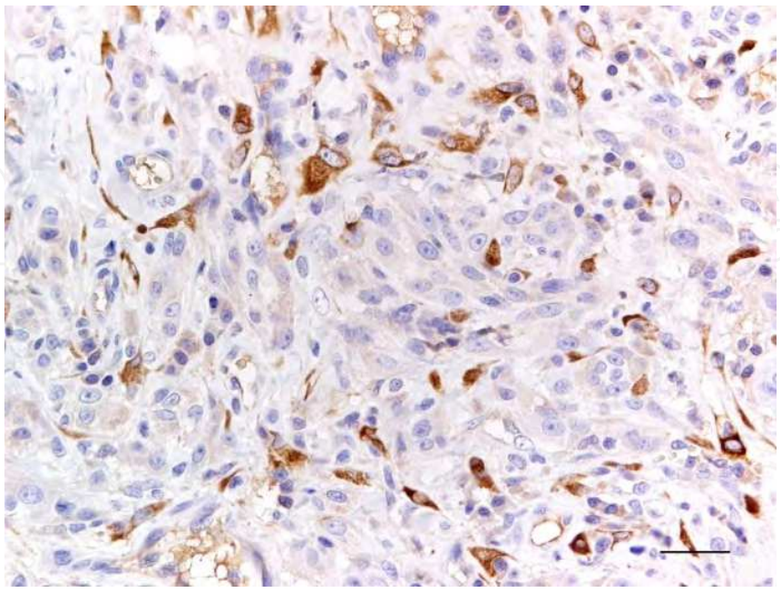

Fig. 5. Cox-2 expression in canine malignant melanoma. IHC. Bar, 30 µm.

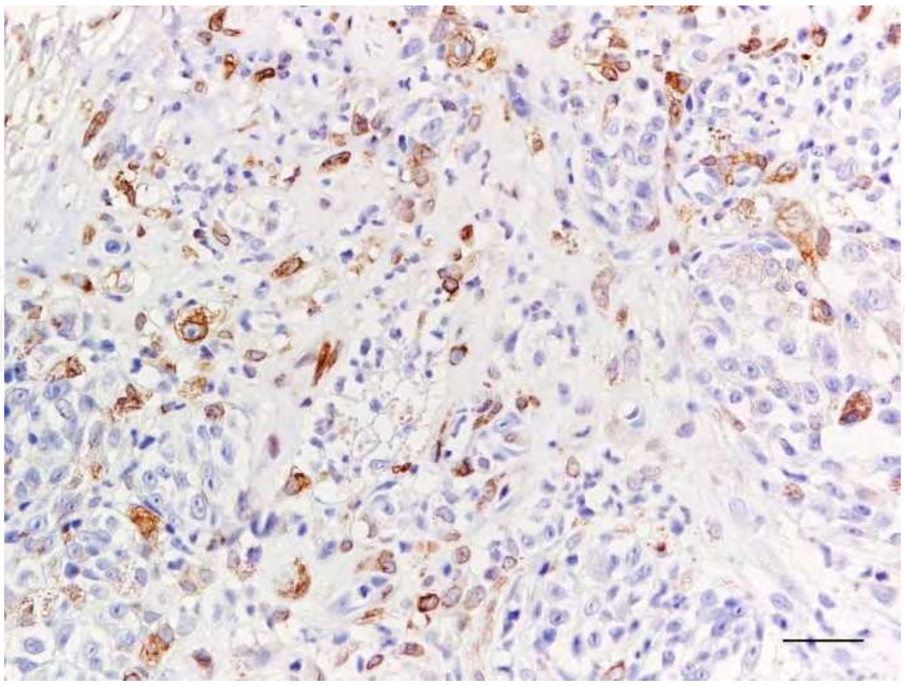

Fig. 6. Cox-2 expression in canine melanoma; positive cells invading epidermis. IHC. Bar, 30 μm.

3.4 Association between TAMs and COX-2 and clinicopathological features in canine melanocytic tumours

Table 1 presents the clinicopathological variables analyzed and its association with TAM and Cox-2 expression in canine melanocytic tumours.

The number of TAMs presented a statistical significant association with the presence of ulceration (p<0,001), presence of necrosis (0,001), nuclear grade (p=0,046), degree of pigmentation (p<0,001), tumoural aberrant cells (p=0,014), stroma (p=0,018) and tumoural embolus (p=0,011). Canine melanocytictumours with ulceration, necrosis, with a high nuclear grade, less pigmented, with the presence of tumoural aberrant cells, with a scarce or moderate stroma and with vascular invasion have a high number of macrophages associated to the tumour.

COX-2 expression was associated with ulceration (p=0,005), necrosis (p=0,003), mitotic index (p=0,029), nuclear grade (p=0,035) and degree of pigmentation (p=0,001). Cox-2 expression was observed in tumours with epithelium ulceration, necrosis, high mitotic index and nuclear grade and in less pigmented neoplasms.

3.5 Association of TAMs and COX-2 in canine melanocytic tumours

There was a significant association between the number of TAMs and COX-2 expression in canine melanocytic tumours (p=0,006). Cox2 positive tumours had a high number of TAMs. Among Cox- negative tumours, only 3 out of 18 tumours had a moderate or abundant number of TAMs. Considering only malignant melanomas, in spite of Cox-2 positive tumours had more TAMs than Cox-2 negative tumours, the association was not statistical significant.

Clinicopathological features	TAMs				Cox-2		
	1	2	3	p	-	+	p
Histological classification							
Melanocytoma	8	0	0	**0,020**	8	0	**0,001**
Melanoma	13	10	6		10	19	
Ulceration							
Absent	15	1	0	**<0,001**	12	4	**0,005**
Present	6	9	6		6	15	
Necrosis							
Absent	18	6	0	**0,001**	16	8	**0,003**
Present	3	4	6		2	11	
Mitotic index							
I	9	2	10	0,308	9	2	**0,029**
II	2	1	7		1	3	
III	10	7	5		8	14	
Nuclear grade							
I	10	3	0	**0,046**	10	3	**0,035**
II	2	3	2		5	8	
III	3	4	4		3	8	
Degree of pigmentation							
Scant	0	10	11	**<0,001**	1	11	**0,001**
Moderate	10	1	2		6	6	
Abundant	11	1	0		11	2	
Aberrant cells							
Absent	19	7	2	**0,014**	16	12	0,068
Present	2	3	9		2	7	
Stroma							
Scarse	3	7	2	**0,018**	4	10	0,154
Moderate	16	3	0		13	8	
Abundant	2	0	0		1	1	
Vascular invasion							
Absent	19	8	2	**0,011**	16	13	0,232
Present	2	2	4		2	6	

Table 1. Association of clinicopathological variables with TAMs and Cox-2 in canine melanocytic tumours.

Clinicopathological features	TAMs 1	2	3	p	Cox-2 -	+	p
Ulceration							
Absent	7	1	0	**0,016**	4	4	0,390
Present	6	9	6		6	15	
Necrosis							
Absent	10	6	0	**0,007**	8	8	0,114
Present	3	4	6		2	11	
Mitotic index							
I	1	2	0	0,760	1	2	**0,029**
II	2	1	1		1	3	
III	10	7	5		8	14	
Nuclear grade							
I	1	8	3	0,235	2	3	0,814
II	2	3	4		5	8	
III	10	2	4		3	8	
Degree of pigmentation							
Scant	0	7	5	**<0,001**	1	11	**0,031**
Moderate	9	1	1		5	6	
Abundant	4	2	0		4	2	
Aberrant cells							
Absent	11	7	2	0,08	8	12	0,431
Present	2	3	4		2	7	
Stroma							
Scarse	3	7	4	0,165	4	10	0,555
Moderate	9	3	2		6	8	
Abundant	1	0	0		0	1	
Vascular invasion							
Absent	11	8	2	**0,049**	8	2	0,675
Present	2	2	4		13	6	

Table 2. Association of clinicopathological variables with TAMs and Cox-2 in canine malignant melanomas.

4. Discussion

Melanoma is relatively common in dogs, accounting for 3% of all neoplasms and up to 7% of all malignant tumours. Melanocytic neoplasms that arise in the oral cavity, one of the most frequent localization of melanocytic tumours in dog, are virtually always considered malignant and constitute the most common oral malignant neoplasm (Smith et al., 2002). Much work is currently underway in to try and identify specific tumour markers, associated with malignancy. However, more studies are needed to differentiating canine benign from malignant melanocytic neoplasms or predicting survival times.

4.1 TAMs in canine melanocytic tumours

It has long been thought that inflammation and carcinogenesis are related (Le Bitoux&Stamenkovic, 2008). Macrophages belong to the innate immune system and as such

constitute one of the first barriers against infection. Depending on the activation state of the macrophage, this antigen presentation may trigger a full-blown active immune response, or may suppress a potential immune reaction (Jager et al., 2011). The role of macrophages in tumour growth and development is complex and multifaceted. Whilst there is limited evidence that TAMs can be directly tumouricidal and stimulate the anti-tumour activity of T cells, there is now contrasting evidence that tumour cells are able to block or evade the activity of TAMs at the tumour site. In some cases, tumour-derived molecules even redirect TAMs activities to promote tumour survival and growth (Bingle et al., 2002).

In our study, TAMs were observed in all tumours analyzed. Melanocytomas presented few macrophages, while malignant melanoma showed generally moderate or intense macrophage infiltration. Higher number of TAMs has been associated with malignancy in different types of human tumours (Siveen&Kuttan, 2009), as breast carcinomas (Leek et al., 1996) and gliomas (Nishie et al., 1999). In human cutaneous melanoma (Torisu et al., 2000), uveal melanoma (Toivonen et al., 2004) and sinonasal melanoma (Shi et al., 2010) a high number of TAMs was closely associated with bad tumour phenotypes. These observations were in accordance with our results in canine melanocytic lesions.

In canine melanocytic tumours, a higher number of TAMs appears associated, in a statistical significant way, with ulceration ($p<0,001$), necrosis (0,001), nuclear grade ($p=0,046$), degree of pigmentation ($p<0,001$), tumoural aberrant cells ($p=0,014$), stroma ($p=0,018$) and tumoural embolus ($p=0,011$). Even considering only malignant melanomas, an association was noted between TMAs and ulceration ($p=0,016$), histological necrosis ($p=0,007$), degree of pigmentation ($p=<0,001$) and presence of vascular invasion by tumoural cells ($p=0,049$). Interestingly most of these characteristics are classically linked to higher tumoural aggressiveness and poor clinical prognosis in these neoplasias. TAMs could constitute an important marker of canine melanocytic aggressiveness; however, studies with prognostic are needed to clarify this subject.

The statistical significant association observed between tumoural necrosis and a high number of TAMs in malignant melanomas is not surprising. Indeed, in human cancer, evidence has emerged for a symbiotic relationship between tumour cells and TAMs. The pathways involved in this crosstalk could imply a response to micro-environmental factors such as hypoxia, as well as various growth factors and enzymes that stimulate tumour angiogenesis (Bingle et al., 2002). Many macrophage products released in the tumour stroma can directly stimulate the growth of tumour cells and/or promote tumour cell migration and metastasis. These include, for instance, the epidermal growth factor (EGF), cytokines like IL-6 and TNF, as well as chemokines such as CXCL12. TAMs contribute to tumour progression also by producing several factors which enhance neo-angiogenesis and the dissolution and remodeling of the interstitial matrix. Moreover TAMs are a source of potent immunosuppressive molecules, such as IL-10 and PGE2, contributing to the tumour immune-evasion (Allavena et al., 2008; Mantovani et al., 2002; Van Ginderachter et al., 2006). It is probably that similar mechanisms occur also in canine malignant melanomas.

4.2 Cox-2 labeling in canine melanocytic tumours

Cox-2 is a key enzyme controlling the conversion of arachidonic acid to prostaglandin H2, the precursor of various molecules, including prostaglandins, prostacyclins and tromboxanes. Cox-2 is commonly undetectable in normal tissues, but can be induced through several stimuli, including mitogens, growth factors, hormones, and cytokines (Dubois et al., 1998). In recent years, many molecular pathways have been suggested to

explain how increased Cox-2 and the resultant prostaglandin overproduction might contribute to carcinogenesis (Hu et al., 2009; Singh-Ranger et al., 2008; Wu&Liou, 2009). These pathways included stimulation of tumoural angiogenesis, decreased tumoural apoptosis, increased invasion and metastasis, immune suppression and tumour associated inflammation (Ghosh et al., 2010). Various studies indicate a link between high Cox-2 expression and malignancy in both human (Balan et al., 2011; Costa et al., 2002; Lee et al., 2011) and canine tumours (Queiroga et al., 2007; Queiroga et al., 2010). In the dog, Cox-2 ''up-regulation'' has been investigated in different tumours, including prostate (L'Eplattenier et al., 2007), ovarian (Borzacchiello et al., 2007), bladder (Khan et al., 2000), intestinal (McEntee et al., 2002), mammary (Queiroga et al., 2005; Queiroga et al., 2007; Dias Pereira et al., 2009), nasal carcinomas (Impellizeri & Esplin, 2008) and sarcomas, including osteosarcoma and oral fibrosarcoma (Heller et al., 2005; Mullins et al., 2004). In human cancer, Cox-2 expression has been detected in a considerable number of epithelial tumours as breast, lung, colon, prostate, head and neck, gastric, ovary, among many others (Ghosh et al., 2010; Menczer, 2009; Wu et al., 2010).

Concerning malignant melanoma, in humans it was recently reported that changes in Cox-2 expression levels were correlated with development and progression of human melanoma (Goulet et al., 2003; Kuzbicki et al., 2006). COX-2 expression arises as a potential immunohistochemical marker for distinguishing human cutaneous melanomas from benign melanocytic lesions (Minami et al., 2011; Pires et al., 2010). Additionally, Cox-2 expression appears as a useful prognostic marker being related with histological and clinical malignant melanoma aggressiveness (Becker et al., 2009).

In canine melanocytic tumours, Cox-2 expression was recently described (Paglia et al., 2009; Pires et al., 2010). Cox-2 over-expression was related with a tumoural malignant behavior (Pires et al., 2010) and with a poor overall survival (Martínez et al., 2011). In the present work, Cox-2 expression was associated with ulceration (p=0,005), necrosis (p=0,003), mitotic index (p=0,029), nuclear grade (p=0,035) and degree of pigmentation (p=0,001). These associations could represent the higher aggressiveness of Cox-2 positive melanocytic tumours. Curiously, these associations lost their statistical significance when we consider for statistical analysis only the group of malignant melanomas. However, an association with mitotic index is observed, that suggests that Cox-2 is associated with a higher cellular proliferation. The real significance of these results needs to be clarified. Clinical studies, with follow-up information and proliferation markers will be necessary to clarify if Cox-2 could be more important in early phases of carcinogenesis or also in tumour progression.

4.3 Relationship between TAMs and Cox-2 expression in canine melanocytic tumours

Tumour associated macrophages and high COX-2 expression have been both associated with malignancy in canine melanocytic tumours, but their potential interdependence has not yet been evaluated. The present study showed that there is a close relationship between TAMs and Cox-2 expression in canine melanocytic lesions. This is the first time, to our best knowledge, that a similar relationship was investigated in tumours of domestic animals. This crosstalk is referred in human tumours, as colorectal carcinoma (Naghshvar et al., 2009), urotelial carcinoma (Chen et al., 2009), basal cell carcinoma (Tjiu et al., 2009) and in breast cancer cells (Hou et al., 2011). The pathways involved in this relationship are diverse, and remain to be completed elucidated. In human basal cell carcinoma, macrophages induced COX-2-dependent release of matrix metalloproteinase-9 and subsequent increased invasion and induced COX-2-dependent secretion of basic fibroblast growth factor and

vascular endothelial growth factor-A, and increased angiogenesis (Tjiu et al., 2009). Macrophage-mediated induction of COX-2 in breast cancer cells is a consequence of IL-1β-mediated stimulation of ROS→Src→MAP kinase→AP-1 signaling. IL-1β-dependent induction of COX-2 in breast cancer cells provides a mechanism whereby macrophages contribute to tumour progression (Hou et al., 2011). Another mechanism proposed could involve Cox-2 induced angiogenesis through increasing TAM infiltration or hypoxia-inducible factor-1alpha by hypoxia (Chen et al., 2009).

Considering only malignant melanomas, the association was not significant that could suggested that this cross-talk (between TAMs and Cox-2 positive tumoural cells) could be decisive in carcinogenesis but not in tumour progression. Only more studies could help to investigate the meaning of these findings. In turn, in human oral squamous cell carcinoma, Cox-2 and TAMs are not related (Boas et al., 2010).

The development of therapeutic targeting of cancer promoting inflammatory reactions is crucially dependent on defining the underlying cellular and molecular mechanisms in relevant systems (Allavena et al., 2008). By inhibiting the release of prostaglandins from the tumour and by blocking COX activity in immune effector cells, NSAIDs may also bias the function of immune cells towards a more tumouricidal phenotype (Lang et al., 2006).

5. Conclusion

In conclusion, this study presents a close relationship of TAM and Cox-2 expression in canine melanocytic lesions. It is possible that proinflammatory cytokines released by intratumoural macrophages, up-regulate Cox-2 tumour cells expression stimulating tumour progression. Additionally, the differences observed between benign and malignant melanocytic lesions may suggest that TAM and Cox-2 are implicated in the progression of melanocytic precursor lesions to malignant melanoma.

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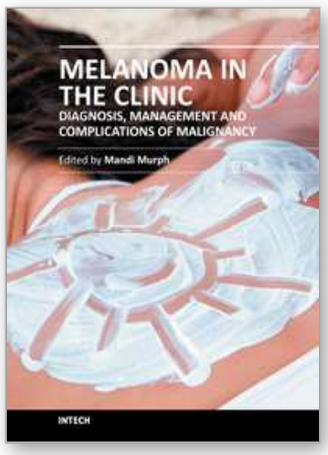

Melanoma in the Clinic - Diagnosis, Management and Complications of Malignancy

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This book provides an excellent overview of how melanoma is treated in the clinic. Since oncologists and clinicians across the globe contributed to this book, each area also explores the unique burdens that geographical areas experience from melanoma subtypes and how these are treated in different settings. It also includes several chapters that illustrate novel methods for diagnosing melanoma in the clinic using new technologies, which are likely to significantly improve outcomes. Several chapters cover surgical techniques and other present very rare or challenging clinical cases of melanoma and how these were treated. The book is geared towards informing clinicians and even patients how melanoma arises, what tools are available and which decisions need to be made by patients and their families in order to treat this devastating disease.

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