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NEOPLASTIC DISEASE

Regression of Canine Cutaneous Histiocytoma is Associated with an Orchestrated Expression of Matrix Metalloproteinases

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Summary

Canine cutaneous histiocytoma (CCH) is a common benign skin tumour originating from epidermal Langerhans cells. These tumours often display spontaneous regression and therefore represent a valuable animal model for investigation of tumour regression. Based on previous studies it was hypothesized that up-regulation of cytokines during CCH regression leads to up-regulation of matrix metalloproteinases (MMPs) favouring infiltration of lymphocytes and enhanced tumour regression. The expression of MMPs and their inhibitors (tissue inhibitors of metalloproteinases, TIMPs) was investigated immunohistochemically in 27 CCHs. The tumours were classified into four groups defined as having no regression (group 1), early regression (group 2), intermediate regression (group 3) or late regression (group 4). The distribution and expression intensity of MMP-1, -2, -3, -7, -9, -13 and -14 and TIMP-1 and -2 were determined in peripheral and central areas of each tumour. Group 3 and 4 CCHs showed up-regulation of expression of MMP-1, -9 and -14 at the periphery. Variable expression of MMP-2 and -3 was observed. Expression of the remaining MMPs and TIMPs showed no group-specific changes. Most MMPs and TIMPs displayed significantly higher expression at the tumour periphery compared with the centre, independently of the stage of regression and indicating more pronounced proteolysis in the peripheral areas. The results are consistent with cytokine-enhanced MMP expression, particularly of MMP-9, leading to enhanced lymphocyte recruitment in combination with elevated cleavage of extracellular matrix and basement membranes.

Keywords: canine cutaneous histiocytoma; matrix metalloproteinase; regression; tissue inhibitors of metalloproteinases

Introduction

Canine cutaneous histiocytoma (CCH) represents a common, benign skin tumour originating from epidermal Langerhans cells (Moore *et al.*, 1996). The Langerhans cell origin has been demonstrated immunohistochemically by positive labelling of the tumour cells with antibodies directed against major histocompatibility complex (MHC) class II, CD1 and CD11c molecules (Moore *et al.*, 1996; Kipar *et al.*, 1998). CCHs most commonly occur in young dogs as solitary or multiple lesions, predominantly on the head, pinnae and limbs (Taylor *et al.*, 1969; Kaim *et al.*, 2006; Fulmer and Mauldin, 2007). Grossly, CCHs are well circumscribed, often dome-shaped lesions of diameter 0.2–4 cm (Taylor *et al.*, 1969). Microscopically, the tumours are characterized by non-encapsulated growth of histiocytic cells, often with invasion of the overlying epidermis and with secondary epidermal ulceration (Taylor *et al.*, 1969). Initially, the tumours grow rapidly as indicated by multiple mitotic figures in histological sections and they then show spontaneous regression in the majority of cases (Taylor *et al.*, 1969; Cockerell and Slauson, 1979; Guvenc *et al.*, 2002). Therefore, CCH represents one of the few naturally occurring neoplasms that display spontaneous regression.

Microscopically, the regression is characterized by infiltration of mature lymphocytes, beginning at the tumour periphery and extending subsequently to the centre of the tumour (Cockerell and Slauson, 1979). This reaction is interpreted as a sign of the

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host immune response (Cockerell and Slauson, 1979; Moore *et al*., 1996). Immunophenotyping reveals that the infiltrate, especially in late stages, consists mainly of $CD8^+$ T lymphocytes and contains only scant numbers of $CD4^+$ T lymphocytes and B lymphocytes (Moore *et al*., 1996; Kaim *et al*., 2006). Furthermore, regression is correlated with up-regulation of interleukin (IL)-2, tumour necrosis factor (TNF)-α, interferon (IFN)-γ and inducible nitric oxide synthase (iNOS) (Kaim *et al*., 2006). Additionally, increasing membrane expression of MHC class II molecules was detected in tumours undergoing regression (Kipar *et al*., 1998).

Since the infiltration of inflammatory cells requires both degradation of the extracellular matrix and chemotactic stimuli, it was hypothesized that the increased expression of ILs and MHC class II leads to an increased expression of matrix metalloproteinases (MMPs). MMPs comprise a family of zinc-dependent endopeptidases that are capable of degrading nearly all components of the extracellular matrix. They have been shown to play a major role in tumour angiogenesis, invasion and metastasis as well as in many other pathological processes (Freije *et al*., 2003; Miao *et al*., 2003; Björklund and Koivunen, 2005; Puff *et al*., 2009). On the other hand, MMPs have important roles in embryo development, morphogenesis and tissue remodelling (Nagase, 1997). In addition to their ability to degrade the extracellular matrix, MMPs are able to cleave and modify the activities of cytokines, chemokines, growth factors and proteinase inhibitors (Opdenakker *et al*., 2001; Parks *et al*., 2004). MMPs and their inhibitors (tissue inhibitors of metalloproteinases, TIMPs) are secreted by a multitude of different cell types, including dendritic cells, macrophages and lymphocytes (Goetzl *et al*., 1996). T lymphocytes, activated by any of several mechanisms, stimulate macrophages to produce increased amounts of MMP-1 and MMP-9 (Miltenburg *et al*., 1995). These increased protease concentrations accelerate the degradation of the extracellular matrix and therefore facilitate further inflammatory cell infiltration, thus creating a self-perpetuating process. The interaction between neoplastic cells and the immune system includes a multitude of different cell types and mediators. Therefore, a detailed understanding of the mechanism of spontaneous regression in a naturally occurring tumour may provide important information regarding antitumour immunity. To gain further insight into these mechanisms, the aim of the present study was to investigate the expression intensity of different MMPs and their inhibitors, as well as the distribution pattern of these molecules, in different stages of regression of CCHs.

Materials and Methods

Tissue Samples

In total, 27 CCHs, excised for medical reasons at the owner's request, were evaluated in this study. The animals' history, including breed, age and sex, was provided by the attending veterinarian (Table 1). After surgery, tumours were immediately fixed in 10% non-buffered formalin and sent for histopathological evaluation. After embedding in paraffin wax, sections (2–4 μm) were stained with haematoxylin and eosin (HE). Serial sections were prepared for immunohistochemistry (IHC). CCHs were classified into four groups according to the degree and distribution of lymphocytic infiltration (Cockerell and Slauson, 1979; Kaim *et al*., 2006). The four groups represented different stages of tumour regression, including no regression (group 1), early regression (group 2), intermediate regression (group 3) and late regression (group 4). In group 1 tumours, lymphocytic infiltration was minimal to absent and limited to the periphery of the tumour. Group 2 tumours were characterized by moderate lymphocytic infiltration with diffuse distribution in the centre of the tumour and nodular infiltration at the periphery. In group

Table 1
Breed, sex and age of affected dogs and tumour group

Animal number	*Breed*	*Sex*	*Age*	*Group*
1	German shepherd dog	F	2 y	1
2	NA	M	2 y	1
3	Malinois	M	5 m	1
4	Bernese mountain dog	M	5 m	1
5	Rottweiler	M	8 m	1
6	NA	NA	NA	1
7	Bernese mountain dog	NA	NA	1
8	Bernese mountain dog	M	3 y	2
9	NA	M	1 y	2
10	NA	M	1 y	2
11	Italian greyhound	MN	9 y	2
12	NA	F	6 y	2
13	NA	M	NA	2
14	NA	NA	NA	3
15	NA	NA	NA	3
16	NA	M	7 y	3
17	Münsterländer	M	NA	3
18	NA	M	7 m	3
19	NA	M	1 y	3
20	NA	M	7 y	3
21	Great Dane	M	4 m	4
22	Bernese mountain dog	M	2 y	4
23	Dachshund	M	4 y	4
24	Bernese mountain dog	M	5 y	4
25	Malinois	M	1 y	4
26	NA	F	2 y	4
27	Bernese mountain dog	F	NA	4

NA, not available; M, male; MN, neutered male; F, female; y, years; m, months.