Veterinary Immunology and Immunopathology 198 (2018) 54–64

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Matrix metalloproteinases expression in spontaneous canine histiocytic sarcomas and its xenograft model

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ARTICLE INFO

Keywords:
Canine histiocytic sarcoma
MMP
Murine xenotransplantation model
TIMP
Tumor associated macrophages
Microvessel density

ABSTRACT

Canine histiocytic sarcoma (HS) represents a malignant neoplastic disorder often with a rapid and progressive clinical course. A better understanding of the interaction between tumor cells and the local microenvironment may provide new insights into mechanisms of tumor growth and metastasis. The influence of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) on tumor angiogenesis, invasion and metastasis has been detailed in previous studies. In addition, inflammatory cells infiltrating neoplasms especially tumor associated macrophages (TAM) may contribute significantly to tumor progression. Due to the high variability of spontaneously occurring canine HS, standardized models are highly required to investigate tumor progression and interaction with its microenvironment. Therefore, the present study comparatively characterized the intratumoral macrophage infiltration as well as the expression of MMP-2, MMP-9, MMP-14 and TIMP-1 in spontaneous canine HS and its murine model. In spontaneous canine HS, scattered MAC 387-positive macrophages were randomly found in tumor center and periphery, whereas tumor cells were negative for this marker. Interestingly, quantitative analysis revealed that MMPs and TIMP-1 were mainly expressed at the invasive front while tumor centers exhibited significantly reduced immunoreactivity. Similar findings were obtained in xenotransplanted HS. Interestingly, murine tumor associated macrophages (TAM), characterized by Mac3 expression (CD107b/LAMP2), which was not present in xenotransplanted histiocytic sarcoma cells, strongly express MMPs and TIMP-1. In addition, MMPs are known to regulate angiogenesis and a positive correlation between MMP-14 expression and microvessel density was demonstrated in xenotransplanted histiocytic sarcomas. Summarized similar findings with respect to MMP and TIMP distribution and the role of macrophages in spontaneously-occurring and xenotransplanted HS indicate the high suitability of this murine model to further investigate HS under standardized conditions. Moreover results indicate that MMP expression contributes to tumor progression and invasion and TAMs seem to be major players in the interaction between neoplastic cells, the microenvironment and vessel formation indicating that therapeutic approaches modulating TAM associated molecules might represent promising future treatment options.

1. Introduction

Canine proliferative histiocytic diseases represent a range of well-defined disorders with marked differences in their clinical behavior and pathologic features (Affolter and Moore, 2002; Moore, 2014). This group of diseases, which are common in dogs and less frequently observed in cats, include cutaneous histiocytoma, the histiocytic sarcoma (HS) complex, reactive histiocytoses (cutaneous and systemic forms) and hemophagocytic histiocytic sarcomas (Moore and Rosin, 1986; Moore et al., 1996; Affolter and Moore, 2000; Kraje et al., 2001; Affolter and Moore, 2002). The HS complex of dogs comprises two distinct types of malignant proliferative disorders, characterized by infiltration of neoplastic cells arising from interstitial dendritic cells (Fulmer and Mauldin, 2007). The localized HS most often involves a single tissue or organ, usually the skin, whereas the disseminated HS spreads beyond the local draining lymph nodes to distant sites, usually lungs, lymph nodes, liver, spleen and central nervous system (Constantino-Casas et al., 2011; Moore, 2014). HS are typically composed of poorly

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https://doi.org/10.1016/j.vetimm.2018.03.002
Received 24 October 2017; Received in revised form 28 February 2018; Accepted 1 March 2018

demarcated sheets of large, pleomorphic cells with one or multiple nuclei, marked cellular atypia and a high mitotic index with variable numbers of inflammatory cell infiltrates consisting mainly of T lymphocytes (Constantino-Casas et al., 2011; Moore, 2014; Pazdzior-Czapula et al., 2015). HS is a highly aggressive neoplasm in dogs with a rapid and progressive clinical course leading to a poor prognosis (Moore and Rosin, 1986).

Several studies have provided evidence that increased expression of matrix metalloproteinases (MMPs) is associated with invasion, metastasis and poor prognosis in numerous human and animal malignancies, including lung and oral neoplasms, breast carcinoma and esophageal squamous cell carcinoma (Kawano et al., 1997; Kawamata et al., 1998; Moses et al., 1998; Talvensaari-Mattila et al., 1998; Ohashi et al., 2000; Loukopoulos et al., 2003). An increased MMP expression is often accompanied by an enhanced invasion and metastasis rate and therefore a poor prognosis (Egeblad and Werb, 2002; Reis et al., 2012). However, little is known about the importance and distribution of MMPs and their inhibitors in HS.

MMPs are a family of zinc-dependent endoproteinases whose enzymatic activity is directed mainly against components of the extracellular matrix (ECM) (Fanjul-Fernandez et al., 2010). These proteinases are linked by a core of common domain structures and by their relationship to a family of proteinase inhibitors called tissue inhibitors of metalloproteinases (TIMPs) (Kleiner and Stetler-Stevenson, 1999). MMPs play important roles in tumor angiogenesis, invasion and metastasis through degradation of the stromal connective tissue and basement membrane components which permit the migration of tumor cells and secretion of growth factors, cytokines and vascular growth factors necessary for tumor development (Sternlicht and Werb, 2001; Visse and Nagase, 2003; Bourboulia and Stetler-Stevenson, 2010; Gialeli et al., 2011; Gong et al., 2014). In addition, MMPs are also able to influence the tumor microenvironment by regulating innate and acquired immunity through modulating the function of cytokines and chemokines and increase the infiltration of inflammatory cells (Parks et al., 2004; Manicone and McGuire, 2008). Notably, MMPs and their inhibitors are secreted by different cell types, including stromal fibroblasts in the vicinity of the neoplasm, tumor infiltrating macrophages or from the tumor cells themselves (Sunderkotter et al., 1991; Lewis et al., 1995; Hagemann et al., 2004; Alyahya et al., 2008). Inflammatory cells infiltrating tumors include macrophages, dendritic cells, myeloid-derived suppressor cells and T-cells which contribute either positively or negatively to tumor invasion, growth and metastasis (Talmadge et al., 2008). Analysis of cellular phenotypes of inflammatory cells infiltrating the neoplasm seems to be of critical importance for predicting the biological behavior of a tumor (Talmadge et al., 2008). Tumor associated macrophages (TAM) are considered as the major player of tumor-related inflammation and are one important source of MMPs (Chanmee et al., 2014; Galdiero et al., 2013). It's suggested that tumor cells use MMPs produced by TAMs, fibroblasts and other stromal cells for invasion and tumor progression, including metastasis, which is only possible through close interaction between neoplastic and stromal cells (Saad et al., 2002; Quail and Joyce, 2013). However, functional interactions between tumor and surrounding stromal cells are not completely understood and are often unpredictable in spontaneously arising neoplasms (Ruiter et al., 2002).

These difficulties highlight the importance of a well characterized, standardized model for detailed investigation of specific questions and analysis of the effectiveness of novel treatment options especially in relatively rare tumor types. To overcome the limitations of spontaneously-occurring HS with respect to detailed analysis of the tumor microenvironment without complicating treatment-related parameters, site of tumor localization and genetic variables of affected patients the aim of the present study was to comparatively analyze spontaneous canine HS and xenotransplanted neoplasms in a recently established and preliminary characterized murine model (Pfankuche et al., 2017) to substantiate the usefulness of this model for a detailed analysis of the role of TAMs. Therefore intratumoral macrophage infiltration in spontaneous canine HS and expression of selected MMPs and TIMPs was analyzed and obtained data were compared to xenotransplanted canine histiocytic sarcomas in this murine model.

Table 1
Breed, sex, age and location of histiocytic sarcoma of affected dogs.

Animal number	Breed	Sex	Age	Tumor location
1	Bernese mountain dog	f	3 y	Skin
2	Bernese mountain dog	f	4 y	Elbow joint
3	Bernese mountain dog	f	7 y	Lymph node
4	Bernese mountain dog	f	8 y	Lymph node
5	Bernese mountain dog	m	3 y	Skin
6	Bernese mountain dog	m	5 y	Skin
7	Bernese mountain dog	m	5 y	Muscle
8	Bernese mountain dog	m	5 y	Lung
9	Rottweiler	m	6 y	Skin
10	Bernese mountain dog	m	6 y	Spleen
11	Bernese mountain dog	m	6 y	Lung and liver
12	Bernese mountain dog	m	6 y	Skin
13	Rottweiler	m	8 y	Spleen
14	Bernese mountain dog	m	6 y	Lymph node
15	Bernese mountain dog	m	4 y	Knee joint
16	Bernese mountain dog	m	8 y	Spleen

m = male; f = female; y = years.

2. Materials and methods

2.1. Samples of spontaneous canine histiocytic sarcomas

Spontaneous histiocytic sarcomas were obtained from 16 dogs either by biopsy or necropsy as part of the diagnostic service of the Department of Pathology of the University of Veterinary Medicine. Breed, sex, age and investigated tumor location are detailed in Table 1. After excision of biopsies and necropsy, respectively, obtained samples were fixed in 10% neutral-buffered formalin, routinely embedded in paraffin wax and stained with hematoxylin and eosin (Fig. 1A).

2.2. Canine histiocytic sarcoma xenotransplantation model

All animal experiments were approved and authorized by university animal welfare officer and the local authorities (Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit (LAVES), Oldenburg, Germany, permission number: 33.9-42502-04-08/1515). All animal procedures were performed in accordance with the German regulations and legal requirements.

Canine histiocytic sarcoma cells (DH82 cells) were obtained from the European Collection of Authenticated Cell Cultures (ECACC no. 94062922). Prior to implantation DH82 cells were cultured in Eagle's minimal essential medium with Earle's salts (MEME; PAA, Cölbe, Germany) supplemented with 10% fetal calf serum (PAA, Cölbe, Germany), 1% penicillin/streptomycin (PAA, Cölbe, Germany) and 1% non-essential amino acids (Sigma Aldrich, Taufkirchen, Germany) as formerly described (Puff et al., 2009, Heinrich et al., 2015a,b).

DH82 cells were transplanted subcutaneously into severe combined immunodeficient mice (CB17/Icr-*Prkdc*^{scid}/IcrIcoCrl mice) as described previously (Pfankuche et al., 2017).

Briefly, mice were injected once subcutaneously into the left flank with a total of 3.0 million DH82 cells (passage 10). Subsequently tumor development was monitored every second to third day and calculated as [(shortest diameter^2 x longest diameter)/2] (Grote et al., 2001). Necropsies were performed and tumor tissue was harvested on day 7, 14, 21, 35 and 77 after transplantation. Animals were sacrificed ahead of schedule when tumors extended a volume of 1.7 cm^3 (equals a diameter of approx. 1.5 cm). Tumor sections were stained with hematoxylin and eosin (Fig. 1B)