J. Comp. Path. 2014, Vol. 150, 184–193

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER
www.elsevier.com/locate/jcpa

NEOPLASTIC DISEASE

Lymphangiogenesis in Canine Mammary Tumours: A Morphometric and Prognostic Study

N. Sleeckx*, L. Van Brantegem†, G. Van den Eynden‡, E. Fransen§, C. Casteleyn*, S. Van Cruchten*, E. Veldhuis Kroeze¶ and C. Van Ginneken*

** Laboratory of Applied Veterinary Morphology, Department of Veterinary Sciences, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, † Department of Pathology, Bacteriology and Poultry Diseases, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, ‡ Department of Pathology and Cytology, GZA Hospitals, Oosterveldlaan 24, 2610 Wilrijk, § StatUa Center for Statistics, University of Antwerp, Prinsstraat 13, 2000 Antwerpen, Belgium and ¶ Viroscience Laboratory, Erasmus Medical Center, Dr. Molewaterplein 50, 3015 GE Rotterdam, The Netherlands*

Summary

Canine mammary tumours (CMTs) are the most common tumours of entire female dogs and represent a promising model for human breast cancer. Little is known about the presence and prognostic value of lymphangiogenesis in CMTs. The aims of the present study were to analyze selected characteristics of lymphatic vessels in CMTs, to evaluate their prognostic significance and to compare these results with studies of human breast cancer. Fifty-six benign CMTs, 55 malignant CMTs and 13 control samples of normal canine mammary gland tissue were studied. Serial immunohistochemical labelling with the lymphatic marker prox-1 and the proliferation marker Ki67 was performed. In intratumoural (IT) and peritumoural (PT) regions, the lymphatic vessel density (LVD), mean lymphatic vessel perimeter (LVP) and relative area occupied by lymphatic vessels (LVA) were analyzed. Lymphatic endothelial cell proliferation (LECP) and tumour cell proliferation (TCP) were also measured. Lymphatic vessels were identified in IT and PT regions and lymphangiogenesis was present in both regions. The IT lymphatic vessels were smaller, less numerous and occupied a smaller relative area compared with those of the PT region. Although no differences in lymphatic vessel parameters were observed between benign and malignant tumours, control tissue differed significantly from neoplastic tissue. None of the lymphatic vessel parameters showed a prognostic value, except for LECP in PT regions of benign tumours. The findings were in accordance with results of investigations into human breast cancer, which supports the use of dogs with spontaneously occurring CMTs as an animal model in comparative oncology trials.
© 2013 Elsevier Ltd. All rights reserved.

Keywords: dog; immunohistochemistry; lymphatic vessels; mammary tumour

Introduction

Mammary tumours are the most common form of neoplasia in women and bitches. More than 40% of all tumours of entire female dogs are canine mammary tumours (CMTs) and approximately 50% are malignant (MacEwen and Withrow, 1996; Sleeckx *et al.*, 2011). Almost half of affected dogs die or are humanely destroyed within 1 year of surgery because of tumour recurrence or metastasis (Graham and Myers, 1999). Survival can vary significantly depending on different tumour and host characteristics, yet the prognosis for CMTs remains difficult to predict (Perez Alenza *et al.*, 1997; Sleeckx *et al.*, 2011; Santos *et al.*, 2013). Therefore, there is a need to define additional features that can predict the biological behaviour of CMTs.

Tumour growth induces both angiogenesis and lymphangiogenesis. The formation of these new blood and lymphatic vessels originating from the pre-existing vascular network is essential for tumour growth, invasion and metastasis (Folkman, 1986;

Correspondence to: N. Sleeckx (e-mail: nathalie.sleeckx@uantwerpen.be).

0021-9975/$ - see front matter
http://dx.doi.org/10.1016/j.jcpa.2013.09.006

© 2013 Elsevier Ltd. All rights reserved.

Fox *et al*., 1996; Saaristo *et al*., 2000; Al-Rawi *et al*., 2005; Sleeman *et al*., 2009; Holopainen *et al*., 2011). In human medicine, angiogenesis has been studied in great detail in breast cancer (Weidner *et al*., 1991; Uzzan *et al*., 2004; Van der Auwera *et al*., 2004). During the last two decades, some research on this topic has also been performed on different canine tumour types including CMTs (Griffey *et al*., 1998; Graham and Myers, 1999; Restucci *et al*., 2000, 2003; Maiolino *et al*., 2001; Luong *et al*., 2006; Lavalle *et al*., 2009; Im *et al*., 2011). However, to our knowledge, information on lymphangiogenesis in and around CMTs and its association with clinical features is still lacking. A previous study evaluated immunohistochemical blood and lymphatic vessel markers in normal and neoplastic canine mammary tissue. The homeodomain protein prospero-related homeobox I (prox-1) was shown to be the most sensitive and specific lymphatic marker in all tissue types (Sleeckx *et al*., 2013). Active lymphangiogenesis can be assessed by evaluating prox-1 expression in combination with that of the proliferation marker Ki67, and CD31 labelling allows discrimination between lymphatic and blood vessels (Van den Eynden *et al*., 2006).

As CMTs show similarities to human breast cancer (Khanna and Hunter, 2005; Porrello *et al*., 2006; Lavalle *et al*., 2009; Uva *et al*., 2009; Klopfleisch *et al*., 2011; Queiroga *et al*., 2011; Casteleyn *et al*., 2013), pet dogs with naturally occurring CMTs could be used as an animal model in comparative oncology trials. The dog could be used to evaluate anti-angiogenic and anti-lymphangiogenic therapies, which have generated great interest in human breast cancer studies (Ferrara *et al*., 2005; Thiele and Sleeman, 2006; Stacker and Achen, 2008; Potente *et al*., 2011; Witte *et al*., 2011). More information about lymphatic vessels in CMTs is required in order to establish a comparative oncology model for anti-lymphangiogenic therapy. Therefore, the aims of this study were (1) to investigate the characteristics of lymphatic vessels in intratumoural (IT) and peritumoural (PT) regions, both in benign and malignant CMTs, (2) to assess the proliferative status of tumour-associated lymphatics as an indicator of ongoing lymphangiogenesis and (3) to examine the prognostic value of these lymphatic vessel characteristics.

Materials and Methods

Samples

One hundred and twenty-four samples of mammary gland were collected. Healthy canine mammary gland ($n = 13$) was collected during necropsy examination of bitches with normal non-neoplastic mammary glands. These dogs ranged in age from 6 to 16 years (mean age 10.75 years) and different breeds were represented. The CMTs ($n = 111$) were surgically removed from female dogs with a mean age of 10 years (5–17 years) and were submitted to the Laboratory of Applied Veterinary Morphology of the University of Antwerp, Belgium. The CMTs were classified according to the World Health Organization criteria (Misdorp *et al*., 1999). Grading of the malignant tumours was performed according to the Elston and Ellis method adapted to CMTs (Clemente *et al*., 2010; Pena *et al*., 2013). Clinical follow-up data (i.e. recurrence, metastases and survival) were obtained from the case records or from client and veterinarian follow-up for a minimum of 12 months.

Immunohistochemistry

Samples were fixed in 4% neutral buffered formalin, processed routinely and embedded in paraffin wax. Serial sections were stained by haematoxylin and eosin (HE) and labelled immunohistochemically for expression of prox-1 (RELIATech, Wolfenbüttel, Germany), Ki67 (Dako, Glostrup, Denmark) or CD31 (Dako). A summary of the immunohistochemistry (IHC) protocols can be found in Table 1. Three washes with Dako wash buffer were performed between each step of the procedure. Reactions were 'visualized' using 3,3′-diaminobenzidine (DAB; Dako) and counterstaining with haematoxylin was performed. Positive controls included a section of canine haemangioma and lymph node for CD31 and prox-1, respectively. Blood and lymphatic vessels in normal mammary tissue and in non-neoplastic areas of the tumour tissue samples served as additional internal controls. Epidermis on the sections was used as internal positive control for Ki67 because of the physiological presence of proliferating keratinocytes in the stratum basale. For negative controls, the primary antibody was replaced with 0.05 M Tris buffered saline (TBS) containing 0.3% Triton X-100 (Sigma Aldrich, St Louis, Missouri, USA) and 1% bovine serum albumin (Sigma Aldrich).

Assessment of Morphological Characteristics

All slides (four from each sample, $n = 496$) were evaluated using an Olympus BX61 microscope (Olympus, Aartselaar, Belgium) equipped with an Olympus DP50 digital camera connected to a computer system running the Olympus software programme Analysis Pro™. Image analysis was performed without knowledge of clinicopathological

Download English Version:

https://daneshyari.com/en/article/8500711

Download Persian Version:

https://daneshyari.com/article/8500711

Daneshyari.com