Comparative Pathology of Canine Soft Tissue Sarcomas: Possible Models of Human Non-rhabdomyosarcoma Soft Tissue Sarcomas

Citation	Milovancev, M., Hauck, M., Keller, C., Stranahan, L. W., Mansoor, A., & Malarkey, D. E. (2015). Comparative Pathology of Canine Soft Tissue Sarcomas: Possible Models of Human Non-rhabdomyosarcoma Soft Tissue Sarcomas. Journal of Comparative Pathology, 152(1), 22-27. doi:10.1016/j.jcpa.2014.09.005
DOI	10.1016/j.jcpa.2014.09.005
Publisher	Elsevier
Version	Version of Record
Terms of Use	http://cdss.library.oregonstate.edu/sa-termsofuse

J. Comp. Path. 2015, Vol. 152, 22–27

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER
www.elsevier.com/locate/jcpa

NEOPLASTIC DISEASE

Comparative Pathology of Canine Soft Tissue Sarcomas: Possible Models of Human Non-rhabdomyosarcoma Soft Tissue Sarcomas

M. Milovancev*, M. Hauck†, C. Keller‡,¶, L. W. Stranahan†, A. Mansoor§ and D. E. Malarkey‖

** Department of Clinical Sciences, Oregon State University, Corvallis, Oregon, † Department of Clinical Sciences, North Carolina State University, Raleigh, North Carolina, ‡ Pediatric Cancer Biology Program, Papé Family Pediatric Research Institute, Department of Pediatrics, Oregon Health & Science University, Portland, Oregon, § Department of Pathology, Oregon Health & Science University, Portland, Oregon, ‖ Cellular and Molecular Pathology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina and ¶ Children's Cancer Therapy Development Institute (cc-TDI), Fort Collins, CO 80524, USA*

Summary

Comparative analyses of canine and human soft tissue sarcomas (STSs) are lacking. This study compared the histological and immunohistochemical (labelling for desmin, smooth muscle actin [SMA], CD31, pancytokeratin, S100 and CD34) appearance of 32 archived, formalin-fixed, paraffin wax-embedded canine STS tumour specimens by board-certified veterinary and medical pathologists, both blinded to the other's interpretations. Comparison between the veterinary and human diagnoses revealed a generally consistent pattern of interpretation with few notable variations. Most tumours (13/32) were judged to display similar histomorphological appearance to human low-grade spindle cell sarcomas, appearing non-distinctive and morphologically of a fibroblastic/myofibroblastic type. Five canine cases resembled human liposarcoma, but with atypical desmin-positive epithelioid cells present. Five canine cases resembled human spindle cell sarcoma with myxoid features and two additional cases resembled human myxofibrosarcoma. Seven canine cases were noted to resemble human undifferentiated sarcoma. Findings in the present study demonstrate that canine STSs display histological and immunohistochemical features similar to their human equivalents. Because of these cross-species similarities, a particular opportunity exists to understand the biology and treatment of human STS by potentially including dogs as clinical models.

Keywords: comparative pathology; dog; man; soft tissue sarcoma

Pet dogs affected with spontaneously arising tumours are increasingly recognized as a clinically relevant model for studying similar tumours in man (Hahn *et al.*, 1994; Paoloni and Khanna, 2008; Pang and Argyle, 2009; Gordon and Khanna, 2010; Rankin *et al.*, 2012; Dickerson *et al.*, 2013). In contrast to people, soft tissue sarcomas (STSs) in dogs are considered relatively common and have been estimated to account for 8–15% of all cutaneous and subcutaneous tumours in the dog, with a standardized annual incidence rate of 122 cases per 100,000 dogs (Dobson *et al.*, 2002). As in man, canine STS represents a number of mesenchymal neoplasms with similar histological features and expected biological behaviours (Dobson *et al.*, 2002; Liptak and Forrest, 2007). Despite these recognized clinical similarities between human and canine STS, comparative studies focussing on this disease entity have been lacking in the peer-reviewed literature. This may be due to the complex and evolving nature of STS subtype nomenclature, which often relies on

Correspondence to: M. Milovancev (e-mail: milan.milovancev@oregonstate.edu).

0021-9975/$ - see front matter
http://dx.doi.org/10.1016/j.jcpa.2014.09.005

immunohistochemical, molecular and/or genetic tests, which are not available routinely in veterinary medicine (Folpe and Cooper, 2007; Thway, 2009; Dennis *et al.*, 2011; Taylor *et al.*, 2011). Therefore, a comparative analysis of human and canine STS should necessarily begin with standard histological techniques to attempt identification of canine STS subtypes that approximate their human counterparts. Therefore, the aim of the present study was to compare the histological and immunohistochemical characteristics of canine STS with their human STS correlates. Our hypothesis was that canine STSs would display histological and immunohistochemical features similar to their human equivalents.

Medical records from dogs seen at the Oregon State University Lois Bates Acheson Veterinary Teaching Hospital between 2007 and 2012 were searched to identify cases of histologically confirmed canine STS. All original histological diagnoses were derived from histopathology reports generated by the board-certified veterinary pathologist at the time of the original surgery or biopsy procedure. As per current veterinary diagnostic convention, specific STS subtypes included for the present study were fibrosarcoma, myxosarcoma, liposarcoma, malignant fibrous histiocytoma, peripheral nerve sheath tumours (neurofibroma, neurofibrosarcoma and schwannoma), spindle cell tumour and vascular wall tumours such as haemangiopericytoma (HPC). The following subtypes were specifically excluded: histiocytic sarcoma, lymphangiosarcoma, haemangiosarcoma, synovial cell sarcoma, leiomyosarcoma, osteosarcoma, rhabdomyosarcoma, sarcomas involving the oral cavity and brachial plexus nerve sheath tumours (Hendrick *et al.*, 1998; Dennis *et al.*, 2011). Each tumour was chemotherapy naïve, radiation naïve and arose spontaneously within an individual client-owned dog, with no dog contributing more than a single tumour to the study.

Routine haematoxylin and eosin (HE)-stained slides were prepared from paraffin wax-embedded, formalin-fixed tumour biopsy samples. Additional positively charged slides were prepared for immunohistochemical analysis of expression of desmin, smooth muscle actin (SMA), CD31, pancytokeratin (CK), S100 and CD34. Details of the immunohistochemistry (IHC) methodology are given in Table 1.

Board-certified human (AM) and veterinary (DEM) pathologists reviewed the resultant slides independently without knowledge of the original pathology reports. Pathologists reviewed HE sections for each case, excluding cases not consistent with the canine STS subtypes listed above. Mitotic index was determined by DEM and LS who counted mitotic figures in 10 arbitrary ×400 microscopical fields for viable areas within each neoplasm. Review of the IHC slides was performed following completion of the initial histomorphological review.

Grading of immunohistochemical labelling was approximated by light microscopy performed using a five-point scale: 0, no immunoreactivity within neoplastic cells; 1, minimal immunoreactivity with 5−10% of neoplastic cells displaying moderate to marked positive labelling; 2, mild immunoreactivity with 10−25% of neoplastic cells displaying moderate to marked positive labelling; 3, moderate immunoreactivity with 25−75% of neoplastic cells displaying moderate to marked positive labelling; 4, marked immunoreactivity with 75−100% of neoplastic cells displaying moderate to marked positive labelling. Control tissues for each antibody were strongly positive: desmin and SMA on muscle tissue, CD31 in and CD34 on vascular endothelium and S100 on nerve tissue.

The electronic medical records search and histopathological review identified 32 cases of canine STS, with the following subtype distribution: spindle cell tumour or STS ($n = 14$), fibrosarcoma ($n = 4$), myxosarcoma ($n = 5$), liposarcoma ($n = 4$) and HPC ($n = 5$). There were no predispositions in gender (16 neutered males, 13 neutered females, two entire males and one entire female), age (median 10.5 years, range 2−16 years) or breed (15 mixed breed dogs, four Labrador retrievers, three German shepherd

Table 1
Summary of immunohistochemical methodology

Marker	*Manufacturer*	*Antibody identification*	*Dilution*	*HTAR*
Desmin	Dako, Carpinteria, California, USA	M0724	1 in 50	+
Smooth muscle actin	Dako	M0851	1 in 30	−
CD31	Dako	M0823	1 in 50	+
Pancytokeratin	Dako	Z0622	1 in 500	−
S100	Dako	Z0311	1 in 400	+
CD34	Santa Cruz Biotechnology, Dallas, Texas, USA	SC-7045	1 in 80	+

HTAR, high temperature antigen retrieval at pH 6.0.

dogs, two Chihuahuas, two dachshunds and one each of Alaskan malamute, Cavalier King Charles spaniel, doberman pinscher, Great Pyrenees, miniature schnauzer, standard poodle and wheaten terrier). All tumours were from cutaneous or subcutaneous locations, affecting the limbs ($n = 23$), body trunk ($n = 6$), head ($n = 2$) and neck ($n = 1$).

A summary of the final veterinary and human pathology histological diagnoses together with the immunohistochemical features of each case are summarized in Table 2.

Seven canine tumours were noted to have a similar histomorphological appearance to human undifferentiated sarcoma ($n = 3$; Supplementary Fig. 1). The final veterinary diagnoses in these cases included HPC ($n = 3$), neurofibrosarcoma ($n = 2$), fibrosarcoma ($n = 1$) and malignant fibrous histiocytoma ($n = 1$). Six of these seven tumours were considered high grade (based on cellular morphology, high mitotic index and foci of necrosis), with the remaining tumour given an intermediate grade (two out of three). This group of tumours included those with the highest mitotic index from the study cohort, with a median of 12 and a range of 4–28. A representative photomicrograph of one of these seven canine tumours alongside an example of human undifferentiated sarcoma, high grade, is provided in Supplementary Figs. 1 and 2.

Five canine tumours were noted to have a similar histomorphological appearance to human spindle

Table 2
Histological diagnoses and immunohistochemical features of 32 canine STSs

Case	*Veterinary pathological diagnosis*	*Human pathological diagnosis*	*MI*	*Necrosis*	*Desmin*	*SMA*	*CD31*	*CK*	*S100*	*CD34*
1	HPC	Undifferentiated sarcoma, intermediate grade (2 of 3)	5	N	0	0	0	0	0	2
2	HPC	Undifferentiated sarcoma, high grade	4	Y	0	0	0	0	0	2
3	Malignant fibrous histiocytoma	Undifferentiated sarcoma, high grade	19	Y	0	0	0	0	0	1
4	NeuroFSA	Undifferentiated sarcoma, high grade	28	N	0	0	0	0	0	1
5	NeuroFSA	Undifferentiated sarcoma, high grade	25	Y	0	1	0	0	0	0
6	FSA	Undifferentiated sarcoma, spindle cell type, high grade	12	Y	0	0	0	0	0	1
7	HPC	Undifferentiated sarcoma, spindle to epithelioid type, high grade	4	Y	0	0	0	0	0	3
8	Myxosarcoma	Spindle cell sarcoma, low grade with myxoid features	0	N	0	0	0	0	0	2
9	Myxosarcoma	Spindle cell sarcoma, low grade with myxoid features	0	N	0	0	0	0	0	2
10	Myxosarcoma	Spindle cell sarcoma, low grade with myxoid features	0	N	0	0	0	0	0	2
11	Myxosarcoma	Spindle cell sarcoma, low grade with myxoid features	0	N	0	0	0	0	0	2
12	Myxosarcoma	Spindle cell tumor, myxoid (very hypocellular, not clear if this is a sarcoma)	0	N	0	1	0	0	0	2
13	Myxosarcoma	Resembles myxofibrosarcoma, low grade	0	N	0	0	0	0	0	3
14	Fibrosarcoma	Resembles myxofibrosarcoma, high grade	3	Y	0	0	0	0	0	1
15	NeuroFSA	Liposarcoma, high grade (mixed pleomorphic, myxoid type)	7	Y	0	0	0	0	0	1
16	Liposarcoma	Liposarcoma, high grade with epithelioid cells	8	Y	0	0	0	0	0	1
17	Liposarcoma	Liposarcoma, low grade (unusual: contains desmin-positive epithelioid cells)	0	N	3	0	0	0	0	2
18	Liposarcoma	Liposarcoma, low grade (unusual: contains desmin-positive epithelioid cells)	0	N	3	0	0	0	0	2
19	Liposarcoma	Liposarcoma, low grade (unusual: contains desmin-positive epithelioid cells)	0	N	2	0	0	0	0	2

Table 2 (*continued*)

Case	*Veterinary pathological diagnosis*	*Human pathological diagnosis*	*MI*	*Necrosis*	*Desmin*	*SMA*	*CD31*	*CK*	*S100*	*CD34*
20	Neurofibroma	Unclassified spindle cell sarcoma, low grade	0	N	0	1	0	0	0	2
21	HPC	Unclassified spindle cell sarcoma, low grade	3	N	0	0	0	0	0	3
22	HPC	Unclassified spindle cell sarcoma, low grade	0	N	0	0	0	0	0	0
23	HPC	Unclassified spindle cell sarcoma, low grade	0	N	0	0	0	0	0	2
24	HPC	Unclassified spindle cell sarcoma, low grade	0	N	0	0	0	0	0	2
25	Neurofibroma	Unclassified spindle cell sarcoma, low grade	0	N	0	0	0	0	0	2
26	HPC	Unclassified spindle cell sarcoma, low grade	0	N	0	0	0	0	0	2
27	HPC	Unclassified spindle cell sarcoma, low grade	0	N	0	0	0	0	0	2
28	FSA	Spindle cell neoplasm, fibroblastic/myofibroblastic type, low grade (resembles fibromatosis)	0	N	0	2	0	0	0	1
29	HPC	Spindle cell sarcoma, low grade, fibroblastic/myofibroblastic type	3	N	0	3	0	0	0	0
30	FSA	Spindle cell sarcoma, low grade, fibroblastic/myofibroblastic type	0	N	0	2	0	0	0	1
31	HPC	Spindle cell neoplasm, low grade, fibroblastic, whorled pattern (difficult to classify)	0	N	0	0	0	0	0	3
32	HPC	Low-grade tumour with pronounced inflammation (difficult to classify)	0	N	0	0	0	0	3	2

CK, cytokeratin; HPC, haemangiopericytoma; FSA, fibrosarcoma; Y, present in tumour sample; N, not present in tumour sample; MI, mitotic index; SMA, smooth muscle actin; refer to text for description of immunohistochemical grading on 0–4 scale.

cell sarcoma with myxoid features. All five of these were considered low-grade tumours. The final veterinary diagnoses in these cases were myxosarcoma ($n = 5$). A representative photomicrograph of one of these five canine tumours alongside an example of human spindle cell sarcoma with myxoid features is provided in Supplementary Fig. 3.

Two additional tumours were noted to have a similar histomorphological appearance to human myxofibrosarcoma. One of these tumours received a final veterinary diagnosis of myxosarcoma and the other fibrosarcoma.

Five canine tumours were noted to have a similar histomorphological appearance to human liposarcoma. Three of these tumours were considered low grade and contained desmin-positive epithelioid cells, which are not identified typically in human or canine liposarcomas. These three tumours received a final veterinary diagnosis on central review of liposarcoma. The other two tumours were considered high grade based on cellular morphology of large nuclei, prominent nucleoli, mitotic index and foci of necrosis. These two tumours received a final veterinary diagnosis of liposarcoma and neurofibrosarcoma. A representative photomicrograph of one of these five canine tumors alongside an example of human liposarcoma is provided in Supplementary Fig. 4.

The remaining 13 tumours were noted to have a similar histomorphological appearance to human spindle cell sarcomas, low grade. Ten of these 13 tumours appeared similar to tumours in people that would be labelled ‘unclassified spindle cell sarcomas’ and three displayed focal immunoreactivity for desmin and were therefore considered to be a fibroblastic/myofibroblastic subtype. Final veterinary diagnoses in this group of tumours were HPC ($n = 9$), fibrosarcoma ($n = 2$) and neurofibroma ($n = 2$). A representative photomicrograph of one of these 13 canine tumours alongside an example of human unclassified spindle cell sarcoma, low grade, is provided in Supplementary Fig. 5.

The specific aim of the present study was to compare the histological and immunohistochemical characteristics of canine STSs with their human correlates. The findings partially support our hypothesis, as certain canine STSs were found to display histological and immunohistochemical features similar to their human equivalents. Moreover, canine STSs

are classified in a similar fashion to human STSs, based on cellular differentiation, as seen by cellular morphology and IHC.

Differences in terminology and diagnostic schema between human and veterinary pathologists exist, but the two systems are often notably compatible. One example of a difference is identification and subclassification of undifferentiated or unclassified sarcomas. According to the 2013 World Health Organization classification scheme, this category has several subcategories including pleomorphic type, spindle cell type, epithelioid type or round cell type (Bosman *et al.*, 2013). Some tumours are recognized as a combination of the above. This group of tumours is heterogeneous, shows no identifiable specific cellular differentiation and is a diagnosis of exclusion.

To aid with categorization of the evaluated cases of canine STS, an IHC panel was performed in order to facilitate exclusion of other neoplasms. The markers were chosen due to their routine use with STS biopsy samples from people (Goldblum *et al.*, 2013). Most of the canine STSs in this study were judged to be non-distinctive and morphologically fibroblastic/myofibroblastic type (both are cells of the same lineage) on review by the participating human pathologist. Pragmatically, these might be termed fibrosarcoma. However, in people, the definition for fibrosarcoma follows strict criteria: monomorphic spindle cell sarcomas with long sweeping intersecting cellular fascicles (Bosman *et al.*, 2013). Except for one, the canine cases did not show this morphology and therefore these fibroblastic sarcomas were not considered to mirror human fibrosarcomas. Based on their histological morphology and IHC labelling patterns, a subset of five of the canine tumours appeared very similar to high-grade undifferentiated sarcoma in man. These were noted to be fibroblastic/myofibroblastic tumours with spindle-shaped to pleomorphic and bizarre cells. Another subgroup of canine STSs that displayed similar features to their human counterparts were those receiving a veterinary diagnosis of myxosarcoma. In human pathology, the term myxosarcoma is not used. Some clinicopathologically distinctive human fibroblastic sarcomas with myxoid features may be called myxofibrosarcoma and low-grade fibromyxoid sarcoma.

In human pathology, HPC is now termed as solitary fibrous tumour/HPC. The reason for this is that HPC was a non-specific category comprised of a variety of tumours with prominent branching vessels. Currently, the belief is that many of these tumours are fibroblastic, hence the term solitary fibrous tumour. The canine cases included in this series with a diagnosis of HPC do not correlate morphologically with what is described as solitary fibrous tumour in man. While the canine tumours with a veterinary diagnosis of HPC in the present study were recognized to contain a perivascular whorling pattern, in human pathology this is not considered equal to pericytic cell differentiation or HPC. Such tumours in man would be diagnosed as spindle cell sarcoma, fibroblastic/myofibroblastic type. Furthermore, in veterinary pathology, at least one report suggests that canine HPC may be a non-specific diagnosis indiscriminately applied to several neoplasms that are actually of different origin and proposes the use of a diagnosis of 'perivascular wall tumour' in the absence of specific immunohistochemical characteristics (Avallone *et al.*, 2007).

The canine STSs originally diagnosed as liposarcomas were noted to be somewhat different in morphological appearance and IHC labelling pattern from human liposarcoma and did not completely fit into human liposarcoma categories: well-differentiated liposarcoma, dedifferentiated liposarcoma, myxoid liposarcoma or pleomorphic liposarcoma. The primary difference was that the canine cases had a mixture of adipocytic cells and epithelioid cells, some of which were positive for desmin, which are not usually seen in human liposarcoma (Goldblum *et al.*, 2013).

In conclusion, the findings in the present comparative study demonstrate that canine STSs display histological and immunohistochemical features similar to their human equivalents. Because of these cross-species similarities, a particular opportunity exists to understand the biology and treatment of fibroblastic/myofibroblastic tumours such as (1) undifferentiated sarcoma and (2) spindle cell sarcoma with myxoid features, potentially using the dog as a clinical model. In man, the outcome for unresectable high-grade undifferentiated sarcoma is representatively poor (Spunt *et al.*, 2008; Ferrari *et al.*, 2011). The biology and clinical course of undifferentiated sarcoma in pet dogs is arguably similar (Dennis *et al.*, 2011). Furthermore, canine clinical trials might lend insight not only into the efficacy of potential therapies, but also resistance mechanisms, thus informing human trials.

Acknowledgements

The final two authors contributed equally to this manuscript.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jcpa.2014.09.005.

References

Avallone G, Helmbold P, Caniatti M, Stefanello D, Nayak RC *et al.* (2007) The spectrum of canine

cutaneous perivascular wall tumors: morphologic, phenotypic and clinical characterization. *Veterinary Pathology*, **44**, 607–620.

Bosman FT, Jaffe ES, Lakhani SR, Ohgaki H (2013) *WHO Classification of Tumours of Soft Tissue and Bone*. *International Agency for Research on Cancer*. Lyon, France.

Dennis MM, McSporran KD, Bacon NJ, Schulman FY, Foster RA *et al*. (2011) Prognostic factors for cutaneous and subcutaneous soft tissue sarcomas in dogs. *Veterinary Pathology*, **48**, 73–84.

Dickerson EB, Marley K, Edris W, Tyner JW, Schalk V *et al*. (2013) Imatinib and dasatinib inhibit hemangiosarcoma and implicate PDGFR-beta and Src in tumor growth. *Translational Oncology*, **6**, 158–168.

Dobson JM, Samuel S, Milstein H, Rogers K, Wood JL (2002) Canine neoplasia in the UK: estimates of incidence rates from a population of insured dogs. *Journal of Small Animal Practice*, **43**, 240–246.

Ferrari A, Miceli R, Rey A, Oberlin O, Orbach D *et al*. (2011) Non-metastatic unresected paediatric non-rhabdomyosarcoma soft tissue sarcomas: results of a pooled analysis from United States and European groups. *European Journal of Cancer*, **47**, 724–731.

Folpe AL, Cooper K (2007) Best practices in diagnostic immunohistochemistry: pleomorphic cutaneous spindle cell tumors. *Archives of Pathology and Laboratory Medicine*, **131**, 1517–1524.

Goldblum JR, Weiss SW, Folpe AL (2013) *Enzinger and Weiss's Soft Tissue Tumors*. Elsevier Saunders, Philadelphia. pp. 137–187; 484–523.

Gordon IK, Khanna C (2010) Modeling opportunities in comparative oncology for drug development. *Institute for Laboratory Animal Resarch Journal*, **51**, 214–220.

Hahn KA, Bravo L, Adams WH, Frazier DL (1994) Naturally occurring tumors in dogs as comparative models for cancer therapy research. *In Vivo*, **8**, 133–143.

Hendrick MJ, Mahaffey EA, Moore FM, Vos JH, Walder EJ (1998) *Histological Classification of Mesenchymal Tumors of Skin and Soft Tissues of Domestic Animals*. Armed Forces Institute of Pathology in cooperation with the American Registry of Pathology and The World Health Organization Collaborating Center for Worldwide Reference on Comparative Oncology, Washington, DC.

Liptak JM, Forrest LJ (2007) Soft tissue sarcomas. In: *Small Animal Clinical Oncology*, 4th Edit., Vol. 1, SJ Withrow, DM Vail, Eds., Saunders Elsevier, St Louis, pp. 425–454.

Pang LY, Argyle DJ (2009) Using naturally occurring tumours in dogs and cats to study telomerase and cancer stem cell biology. *Biochimica et Biophysica Acta*, **1792**, 380–391.

Paoloni M, Khanna C (2008) Translation of new cancer treatments from pet dogs to humans. *Nature Reviews Cancer*, **8**, 147–156.

Rankin KS, Starkey M, Lunec J, Gerrand CH, Murphy S *et al*. (2012) Of dogs and men: comparative biology as a tool for the discovery of novel biomarkers and drug development targets in osteosarcoma. *Pediatric Blood and Cancer*, **58**, 327–333.

Spunt SL, Skapek SX, Coffin CM (2008) Pediatric non-rhabdomyosarcoma soft tissue sarcomas. *Oncologist*, **13**, 668–678.

Taylor BS, Barretina J, Maki RG, Antonescu CR, Singer S *et al*. (2011) Advances in sarcoma genomics and new therapeutic targets. *Nature Reviews Cancer*, **11**, 541–557.

Thway K (2009) Pathology of soft tissue sarcomas. *Clinical Oncology*, **21**, 695–705.

[*Received, September 3rd, 2014*
Accepted, September 30th, 2014]