J. Comp. Path. 2007, Vol. 136, 197–201

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SHORT PAPER

Steroid Receptors in Canine and Human Female Genital Tract Tumours with Smooth Muscle Differentiation

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Summary

The expression of oestrogen receptor-α (ERα) and progesterone receptor (PR) was examined in 32 canine genital tract tumours diagnosed as smooth muscle tumours (benign or malignant, pure or mixed). The immunohistochemical expression of calponin was used to assess the smooth muscle differentiation of the tumours. Nineteen human uterine leiomyomas were also examined. Calponin expression was detected in 89.3% of canine and 100% of human genital tract tumours diagnosed as leiomyomas, as well as in the majority of other tumours examined (canine or human, genital or extragenital, benign or malignant) with the exception of canine negative control tumours (cutaneous fibroma and hepatoid gland adenoma). ERα was found in 56.3% of canine and 52.6% of human leiomyomas, while PR was found in 84.4% of canine and 94.7% of human tumours. These results indicate that calponin is a good marker for differentiating neoplasia of the canine genital system of uncertain origin, as in human patients. They also show that canine tumours with smooth muscle differentiation of the genital tract of the bitch express steroid hormone receptors, a finding that opens up the possibility of hormone therapy.

Keywords: calponin; dog; genital tract; hormone receptors; leiomyoma; man; tumour

The most common tumours in the genital tract of the bitch are benign smooth muscle tumours of the vagina and vulva, and the average age at diagnosis is 10.8 years (Klein, 2001). In the human female the predilection site of benign smooth muscle tumours is the uterus, where such tumours occur in as many as 30% of women over the age of 35 (Maruo *et al.*, 2004).

In the bitch, benign smooth muscle tumours of the genital tract are referred to as leiomyomas, fibroleiomyomas, fibromas or polyps, on the basis of the amount of connective tissue present (Klein, 2001; MacLachlan and Kennedy, 2002). The use of markers of smooth muscle differentiation (e.g., desmin, smooth muscle actin, calponin) would help to identify the precise participation of smooth muscle in the neoplastic growth (Frost *et al.*, 2003; Zhu *et al.*, 2004). Such identification is of importance because neoplastic smooth muscle cells, but not other types of cell, may contain steroid hormone receptors (McGinley *et al.*, 1997). Thus, oestrogen receptors (ERs) and progesterone receptors (PRs) are present in human and feline leiomyomas of the genital tract (Martín de las Mulas *et al.*, 2002; Bodner *et al.*, 2004; Zhu *et al.*, 2004). In the dog, however, there is no comparable information, although subjective data indicate that genital tract leiomyomas may be hormone-dependent (Klein, 2001). In the present study, the expression of ERα and PR was examined in 32 canine and 19 human genital tract tumours diagnosed as smooth muscle tumours after the precise identification of smooth muscle differentiation with calponin.

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0021-9975/$ - see front matter
doi:10.1016/j.jcpa.2007.01.004

The 32 canine tumours included 28 leiomyomas (four uterine, one cervical, 15 vaginal, six vulval, two perineal), three fibroleiomyomas (two vaginal, one vulval), and one vulval leiomyosarcoma. The 19 human tumours examined, all of which were uterine leiomyomas, included 10 usual leiomyomas and nine histological variants of leiomyoma (three cellular, two atypical, one haemorrhagic, three epithelioid). The paraffin wax blocks of these canine and human tumours had been stored for 2 years and 25 years, respectively. Histological diagnosis, according to the WHO classification of genital tract tumours of the canine (Kennedy *et al.*, 1998) and human (Tavassoli and Devilee, 2003) species, had been performed on haematoxylin and eosin (HE)-stained tissue sections of formalin-fixed, wax-embedded biopsy samples. Age, breed, ovariectomy status and concurrent disease status of the 32 dogs were collected from our records.

Immunohistochemistry (IHC) was performed on 3-μm sections by an avidin-biotin-peroxidase complex (ABC) technique (Vectastain, ABC kit Elite; Vector Corporation, Burlingame, CA, USA). Monoclonal mouse antibodies raised against human calponin (clone CALP; Dako S.A, Saint Just Desvern, Barcelona, Spain) diluted 1 in 400, human ER (clone 1D5; Dako) diluted 1 in 50, and human PR (clone PRA109; Immunotech, Marseilles, France) diluted 1 in 500 were used after high-temperature antigen retrieval by incubation with 0.01 M citrate buffer, pH 6.0, at 95 °C in a waterbath for 8 min (clone CALP) or 25 min (clone PRA109), or in a stainless steel pressure cooker for 3 min (clone 1D5). All further IHC labelling procedures were performed as previously described (Espinosa de los Monteros *et al.*, 2002; Martín de las Mulas *et al.*, 2002). Tissue sections were counterstained with Mayer's haematoxylin. Positive control tissues included formalin-fixed, wax-embedded samples of normal canine and human uterus. Replacement of the specific primary antibody by phosphate-buffered saline was used as a negative control in every assay. In addition, canine (one urinary bladder and one urethral leiomyoma, and one urinary bladder leiomyosarcoma) and human (three angioleiomyomas, three piloleiomyomas, three leiomyomas and one leiomyosarcoma of the skin and soft tissues) smooth muscle tumours located outside the genital tract, as well as one canine cutaneous fibroma and one canine adenoma of the hepatoid glands were all used as negative control tumours. Immunolabelling was assessed semi-quantitatively by examining the entire tumour present in each tissue section by the method of Allred *et al.* (1998); this method is based on a combination of the percentage of tumour cells showing positive labelling (proportion score, PS) and staining intensity (intensity score, IS), to obtain a total score (TS). "Blind" assessment of immunolabelling was made by two investigators.

The median age of the dogs was 11 years. There were 22 pure breed animals (two Yorkshire terriers, two poodles, three Pekingese, one Pyrenees mastiff, three cocker spaniels, one Staffordshire bull terrier, four boxers, three German shepherd dogs, two samoyeds and one chihuahua) and seven mixed-breed dogs. The breed was unknown in three cases. Two bitches (6.6%) had been spayed. Four dogs presented with cysts of the ovary, and two of them also had cystic endometrial hyperplasia. Three animals had mammary gland carcinomas. Histologically, the canine tumours that had been diagnosed as leiomyomas had similar features, regardless of location. They were composed of interwoven bundles of smooth muscle cells with occasional mitotic figures and scant fibrous connective tissue (Fig. 1). The vulval leiomyosarcomas were highly cellular, showing large nuclei and three mitotic figures per high power field, some atypical. Fibroleiomyomas of the vagina and vulva were composed of smooth muscle cells with a variable amount of fibrous tissue.

Leiomyomas of the human genital tract were composed of smooth muscle, with whorled, anastomosing fascicles of uniform, fusiform cells. In addition to 10 leiomyomas of the usual type, there were: three cellular leiomyomas, characterized by a significantly higher cellularity than that of the surrounding myometrium (Fig. 2); two atypical leiomyomas with focal cytological atypia; one haemorrhagic leiomyoma; and three epithelioid leiomyomas composed of epithelial-like cells, which were round and polygonal and arranged in clusters, with nuclei that were round, relatively large, and centrally positioned.

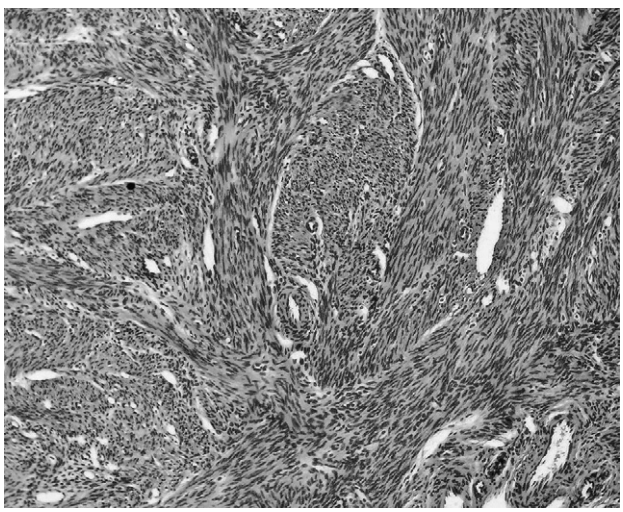

Fig. 1. Canine vaginal leiomyoma composed of interwoven bundles of smooth muscle cells with scant fibrous connective tissue between them. HE. × 100.