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Oestrogen and progesterone receptor expression in subtypes of canine mammary tumours in intact and ovariectomised dogs

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ABSTRACT

The objective of this study was to investigate as a potential prognostic indicator the relationship between histological subtype of canine mammary tumours (CMTs) and oestrogen-α (ORα) and progesterone (PR) receptor expression. Using immunohistochemistry, receptor expression in neoplastic epithelial cells was assessed in 12 different subtypes in 113 CMTs (34 benign, 79 malignant) and 101 surrounding normal tissues. Sixty-eight and 45 CMTs were from intact and ovariectomised bitches, respectively.

Histological subtype strongly influenced ORα/PR expression: simple and complex adenomas as well as simple tubular carcinomas exhibited the greatest expression, whereas immunohistochemical labelling for these receptors was weakest in carcinoma and malignant myoepitheliomas, as well as in solid/anaplastic carcinomas and comedocarcinomas. Receptor expression was generally higher in benign relative to malignant neoplasms, and in the latter it was significantly lower in ovariectomised vs. intact bitches. Lymphatic invasion, mitotic index, nodule diameter, and tumour grade were significantly associated with ORα/PR expression. Although not found to be an independent prognostic indicator, tumours from dogs with <10% cells with ORα/PR expression had a poorer prognosis. Lymphatic invasion, the state of the margins of excision, and mitotic index were found to be independent prognostic indicators. Overall, the results suggest that differences in histological subtype and whether or not a bitch has been ovariectomised should be considered when evaluating the significance of ORα and PR expression in CMTs.

Introduction

Mammary tumours are the most frequent neoplasms diagnosed in both women and intact bitches (Jemal et al., 2011; Withrow and MacEwen, 2012). In humans, the immunohistochemical (IHC) assessment of oestrogen-α (ORα) and progesterone (PR) receptors has both predictive and prognostic significance, and decreased hormone receptor (HR) expression is associated with poorer clinical outcomes (Reis-Filho and Tutt, 2008; Allred, 2010). The prognostic value of HR expression in canine mammary tumours (CMTs) is much less clearly understood. Although CMTs, like human breast cancers, readily express HRs (MacEwen et al., 1982; Rutteman et al., 1988; Martin de las Mulas et al., 2005; Millanta et al., 2005; Chang et al., 2009), and ORα/PR levels are decreased in malignant neoplasms (Rutteman et al., 1988; Nieto et al., 2000; Millanta et al., 2005; Thuróczy et al., 2007; Chang et al., 2009), their expression has been shown to vary over a number of studies.

In addition to differences in methods of markers detection and 'scoring' (Peña et al., 2014), CMTs can also display very diverse histological features (Goldschmidt et al., 2011) with differing prognoses (Misdorp et al., 1999; Meuten, 2002). The role of ORα/PR in the development of specific histological subtypes remains unknown and requires further investigation. The variability with which CMT subtypes express different HRs within sample-cohorts may significantly influence the findings of studies, and these details are often missing. One study identified statistically significant greater expression of HRs in complex and mixed, compared with simple, CMTs (Martin de las Mulas et al., 2005), although information relating to simple tumours other than tubulopapillary and solid subtypes, is not currently available.

Since the production of sex hormones may influence the expression of HRs (Rutteman et al., 1988; Donnay et al., 1995; Nieto et al., 2000; Sorenmo et al., 2000; Chang et al., 2009), these should be assessed in CMTs in both intact and ovariectomised bitches. The objective of the present study was to investigate ORα/PR expression in the most prevalent CMT histological subtypes in both intact and ovariectomised animals (Goldschmidt et al., 2011). The prognostic value of HR assessment, as well as that of other clinical and histopathological features were also evaluated.

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Materials and methods

Sample selection, histopathological and 'follow-up' assessment

Samples of CMT from 113 female dogs were retrospectively selected from the archive of the Diagnostic Service of Veterinary Anatomical Pathology, Department of Comparative Biomedicine and Food Science, University of Padua, Italy. The samples had been submitted from veterinary practices between 2005 and 2009. Samples were selected so as to represent 12 different tumour subtypes (Goldschmidt et al., 2011) (Table 1) from similar numbers of ovariectomised and intact bitches, and where 'follow-up' clinical data were available for ≥2 years post-surgery. Where different subtypes of CMT were found in the same dog, the type with the highest histological grade was selected for analysis. The samples were classified by two board-certified veterinary pathologists according to a modified WHO classification and graded focusing exclusively on the neoplastic epithelial components of the tumours (Clemente et al., 2010; Goldschmidt et al., 2011). Identification of neoplastic myoepithelium was performed according to a previous study (Rasotto et al., 2012).

The following details were recorded for each case: breed, age at time of tumour diagnosis, number of histologically visible neoplastic foci (single/multiple), tumour diameter (post-fixation), the presence of neoplastic cells at the margins of surgical excision, mitotic index (number of mitotic figures in 10 high power magnification fields [hpfs] in the most proliferative areas), and lymphatic invasion. Where multiple foci of the same tumour subtype were found in the same dog, the largest focus was measured. Animals ovariectomised at the time of surgery to remove a mammary tumour were considered 'intact'. Surgical excision was the only treatment used in all cases. Two-year 'follow-up' clinical data were obtained through regular telephone interviews with referral veterinarians. Overall survival (OS) was calculated as the time from initial surgery to remove the mammary tumour until death. Death was considered related to the mammary tumour when animals died naturally or were euthanased with metastases identified by diagnostic imaging. Necropsy data were not available.

Immunohistochemical assessment

Immunohistochemistry was used to detect ORα/PR expression in all of the selected tumours. Antigen labelling was achieved using the ultraView Universal DAB Detection Kit (Ventana Medical Systems) in an automatic immunostainer (BenchMark Ventana Medical Systems). Monoclonal mouse anti-human ORα (code NCL-ERα-LH2, LH2 clone, Novacastra; 1:30 dilution), and PR (code IM-1546, 10A9 clone, Immunotech; 1:80 dilution) were diluted using a commercially available diluent (Ventana Medical Systems). In brief, the IHC protocol included high temperature antigen unmasking (1 h at 95 °C) and incubation for 1 h at room temperature for both antibodies. Canine ovarian and endometrial tissues were included as positive controls and negative controls involved replacing the primary antibodies with antibody diluent. Receptor labelling within nuclei was evaluated in tumour epithelial cells and in adjacent normal or hyperplastic mammary tissue.

The percentage of ORα- or PR-positive cells was calculated by counting ≥1000 cells within 10 distinct, randomly selected microscopic fields (40 × magnification). No positive cut-off was established and antigen labelling intensity (iORα and iPR) was scored between '1' and '5': 1, very low; 2, low; 3, moderate; 4, intense; and 5, very intense. The intensity of labelling of non-neoplastic mammary tissue was considered the reference scoring value of '5' in order to exclude any bias due to fixation. When a neoplastic lesion exhibited heterogeneous staining, the predominant intensity percentage was selected.

Statistical analysis

Frequency analyses based on Fischer's exact test were used to assess the significance of categorical variables association. Product-moment Pearson's correlations were estimated particularly between ORα and PR percentages and intensities of antigen expression, tumour diameter, and mitotic index. A general linear model (GLM) was used to investigate the relationship between ORα and PR expression and multiple explanatory variables: reproductive state (intact/ovariectomised female), type of tissue (neoplastic/non-neoplastic), type of tumour (benign/malignant), number of subpopulations of cells in tumour (simple/complex mixed), histological subtype, number of nodules (single/multiple), tumour diameter (≤1, >1 and ≤2, and >2 cm), mitotic index (<5, ≥5 and <10, ≥10 and <20, and ≥20 mitoses per hpf), lymphatic invasion (present/absent), grading, state of excision margins (clean/unclean).

When assessing the effect of the type of tissue (neoplastic/non-neoplastic), an 'animal effect' was included in the model to account for subject-related HR variation due to technical factors such as tissue fixation. The statistical significance of the investigated effects was based on F-tests. Survivor functions were estimated on the basis of the Kaplan–Meier non-parametric method. Cox's regression analysis was used to investigate associations between the risk of death and the explanatory variables. All analyses were conducted after normalisation of data with logarithmic transformation. Findings were considered significant where $P < 0.05$.

Results

Clinical and pathological findings and 'follow-up' assessment

The study population consisted of 68 intact and 45 ovariectomised dogs. In 16/45 (35.6%) the age at which ovariectomy was performed was known; only one animal was ovariectomised prior to 1-year-old. Age at surgery (107/113) ranged from 3 to 15 years (mean 9.4 ± 2.6 years). Most dogs (68/113, 60.2%) were purebreds (28 different breeds), 67/113 (59.3%) had a single neoplastic nodule, while 46/113 (40.7%) had multiple nodules. The results refer to the unique tumour subtype diagnosed or to the subtype of highest histological grade: of the 113 CMTs, 79 malignant and 34 benign tumours were selected (Table 1). In 101/113 (89.4%) cases, hyperplastic mammary tissue surrounded the tumour. Lymphatic invasion was present in 17/113 (15.0%) cases (15 grade III and two grade I carcinomas). Neoplastic cells were evident at excisional margins in 20/113 (17.7%) cases. Mean tumour diameter (108/113) was 1.5 ± 1.4 cm: in 56/113 (49.6%) cases the neoplastic nodule was ≤1 cm, in 89/113 (78.8%) the nodule was >1 but ≤2 cm, and in 19/113 (16.8%) they were >2 cm (Table 1).

Table 1
Details of histopathological subtype, ovariectomy status of bitch, lymphatic invasion, mean mitotic index, and tumour diameter of canine mammary tumours studied.

Histological subtype	Number of samples				Mean mitotic index (±SD)	Mean tumour diameter cm (±SD)
	Total [Grade I, II, III]	Ovariectomy [Grade I, II, III]	Intact [Grade I, II, III]	Lymphatic invasion		
Benign tumours	34	15	19		0.8 ± 0.7	0.7 ± 0.3
Simple adenoma	10	3	7		1 ± 0.8	1.4 ± 0.2
Complex adenoma	12	6	6		0.6 ± 0.7	1.7 ± 0.3
Benign mixed tumour	12	6	6		0.8 ± 0.6	1.5 ± 0.3
Malignant tumours	79 [47,12,20]	30 [18,3,9]	49 [29,9,11]	17	10.3 ± 9.6	1.8 ± 1.5
Solid carcinoma	6 [0,1,5]	3 [0,0,3]	3 [0,1,2]	3	23.7 ± 13.4	2.0 ± 0.9
Intraductal papillary carcinoma	6 [3,2,1]	2 [1,0,1]	4 [2,2,0]	1	9.5 ± 4.4	2.0 ± 1.3
Anaplastic carcinoma	6 [0,0,6]	1 [0,0,1]	5 [0,0,5]	6	12.8 ± 5.3	5.2 ± 4.2
Simple tubular carcinoma	7 [5,2,0]	3 [2,1,0]	4 [3,1,0]	0	6.6 ± 4.8	1.5 ± 0.7
Simple tubulopapillary carcinoma	9 [5,3,1]	3 [2,1,0]	6 [3,2,1]	1	8.7 ± 5.9	2.21 ± 0.6
Comedocarcinoma	9 [0,2,7]	4 [0,0,4]	5 [0,2,3]	4	24.6 ± 13.7	3.0 ± 1.1
Carcinoma and malignant myoepithelioma	11 [11,0,0]	4 [4,0,0]	7 [7,0,0]	2	4.0 ± 1.8	2.9 ± 1.1
Carcinoma arising in benign mixed tumour	12 [11,1,0]	5 [5,0,0]	7 [6,1,0]	0	5.9 ± 2.8	2.4 ± 0.7
Complex carcinoma	13 [12,1,0]	5 [4,1,0]	8 [8,0,0]	0	5.3 ± 2.0	2.3 ± 0.7