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Expression of Oestrogen Receptor, Progesterone Receptor and Akt in Canine Circumanal Gland Tumours

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Summary

We investigated the expression of oestrogen receptor alpha (OR-α), progesterone receptor (PR) and Akt in canine circumanal gland tumours. Immunohistochemistry was conducted on seven normal circumanal glands, 30 circumanal gland adenomas and 40 circumanal gland carcinomas. The expression of OR-α and PR was significantly lower in circumanal gland carcinomas than in circumanal gland adenomas. In contrast, the expression of Akt was markedly higher in circumanal gland carcinomas than in circumanal gland adenomas. These results indicate that the progression of canine circumanal gland tumours is influenced by changes in the expression levels of OR-α, PR and Akt. Identifying the molecular mechanisms of canine circumanal gland tumours requires further study.

Keywords: circumanal gland tumours; dog; immunohistochemistry; sex steroid hormone receptor

Introduction

The circumanal glands, also known as the hepatoid or perianal glands, are modified sebaceous glands unique to dogs and marsupials (Maita and Ishida, 1975). They are located up to 2 cm from the anus in a uniform circle and are scattered over areas on the prepuce, tail, hindlimbs and trunk (Maita and Ishida, 1975). Each individual circumanal gland lobe is supported by an incomplete layer of fibroblasts that produce connective tissue fibres, interdigitated with basaloid reserve cells (Baker, 1967). Basaloid reserve cells are cuboidal, have little cytoplasm with a basophilic nucleus and are situated at the periphery of the circumanal gland lobes (Baker, 1967). The main mass of the lobe is composed of polyhedral eosinophilic cells that resemble hepatic cells (Baker, 1967).

Circumanal gland adenomas have been found to be sex hormone-dependent, while circumanal gland carcinomas are not (Withrow *et al.*, 2012). Castration without excision of the adenomas has been successful in promoting regression with no recurrence (Wilson and Hayes, 1979). The progression of circumanal gland adenomas is likely stimulated by androgenic hormones and suppressed by oestrogenic hormone therapies (Nielsen and Attosmis, 1964; Chaisiri and Pierrepoint, 1979; Withrow *et al.*, 2012). Older, intact males are at a higher risk of developing adenomas and adenomas in females appear to occur almost solely in ovariohysterectomized animals, probably because their low levels of oestrogen cannot suppress tumour progression (Nielsen and Attosmis, 1964; Wilson and Hayes, 1977; Berrocal *et al.*, 1989; Withrow *et al.*, 2012). Androgen receptor (AR) expression was found to be significantly higher in hyperplastic circumanal glands than in normal circumanal glands, while circumanal gland adenomas and carcinomas only

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showed a slight increase in AR expression levels compared with that in normal circumanal glands (Pisani *et al.*, 2006).

A previous study revealed the expression of growth hormone (GH) in circumanal gland tumours (Petterino *et al.*, 2004). Progesterone has been revealed to stimulate the expression of growth hormone in normal canine mammary glands and may induce hyperplastic or neoplastic lesion in the canine mammary gland (van Garderen *et al.*, 1997). Therefore, Petterino *et al.* (2004) implied that GH in canine circumanal gland tumours may induced by progesterone and contribute to the occurrence of the tumours.

Akt, the essential element of the PI3K/Akt signalling pathway, is a protein kinase with roles in various cellular processes including cell proliferation, survival and apoptosis. Overexpression of Akt is a common feature in early and advanced tumours (Mundi *et al.*, 2016). In man, Akt gene amplification has been described in breast, ovarian, gastric, pancreatic, colonic and thyroid cancers (Mundi *et al.*, 2016). Similarly, upregulation of Akt and its associated factors was demonstrated in canine cancer cell lines and tumour tissues (Chen *et al.*, 2012; Campos *et al.*, 2014). In addition, interactions between Akt and oestrogen receptor (OR) and between Akt and androgen receptor (AR) have been discovered in breast cancer and prostate cancer (Tsai *et al.*, 2001; Wang *et al.*, 2007; Bhat Nakshatri *et al.*, 2008; Mikhailova *et al.*, 2008).

Circumanal gland tumours are common in dogs (Pakhrin *et al.*, 2007; Goldschmidt and Goldschmidt, 2016); however, the molecular aspects underlying the involvement of sex hormones and receptors in the development of these tumours are unclear, even though it is widely accepted that circumanal gland tumours and sex hormones have an obvious correlation. The aim of the present study was to characterize the expression of two sex steroid hormone receptors, OR-α and PR, as well as Akt, in normal canine circumanal glands, circumanal gland adenomas and carcinomas.

Materials and Methods

Samples and Histological Classification

Formalin-fixed and paraffin wax-embedded tissues, previously diagnosed as normal perianal skin or circumanal gland tumours, were retrieved from the archives of the Konkuk University Veterinary Medical Diagnostic Laboratory (Small Animal Tumour Diagnostic Centre) between 2011 and 2017. Sections (4 μm) were stained with haematoxylin and eosin (HE). In total, sections from 77 cases were examined independently by two pathologists (S.-H. Kim and B.-J. Seung). Histological analysis was conducted in accordance with the World Health Organization (WHO) classification (Goldschmidt *et al.*, 1998). Samples included normal circumanal glands ($n = 7$), adenomas ($n = 30$) and carcinomas ($n = 40$).

Immunohistochemistry

The molecules were detected by immunohistochemistry (IHC) using primary antibodies specific for OR-α (diluted 1 in 60, mouse monoclonal; Biogenex, Fremont, California, USA), PR (diluted 1 in 500, mouse monoclonal; Beckman Coulter, Brea, California, USA) and pan-Akt (diluted 1 in 200, rabbit monoclonal; Abcam, Cambridge, UK). Sections (4 μm) were prepared on silane-coated slides. For dewaxing, the slides were immersed into two series of xylene baths for 5 min each, hydrated in graded ethanols and washed three times, each for 3 min, in phosphate buffered saline (PBS). Endogenous peroxidase activity was blocked by incubating the tissue sections in 3% H_2O_2 for 20 min at room temperature (RT). Sections were then washed in PBS three times, for 3 min each. Heat-induced antigen retrieval for primary antibodies was carried out by boiling in Tris-EDTA buffer for 15 min (pH 9.0; for OR-α and PR) and in citric acid buffer for 20 min (pH 6.0; for Akt) using a microwave oven (650 W). Sections were cooled in cold water and washed in PBS three times, for 3 min each. To reduce non-specific background labelling, sections were incubated with 5% normal goat serum for 30 min at RT, before being incubated with primary antibodies overnight at 4°C.

After incubation, the sections were washed in PBS to remove any unbound primary antibodies. Secondary antibodies were applied using a ready-to-use, peroxidase-based kit (Dako, Glostrup, Denmark) for 40 min at RT. Horseradish peroxidase and 3, 3′-diaminobenzidine were used for visualization (Dako); thereafter, sections were counterstained with Gill's haematoxylin, dehydrated in graded ethanols and coverslipped.

In order to confirm the reactivity of the primary antibodies, the following isotype controls were used: mouse IgG1 for OR-α, mouse $IgG2_a$ for PR and monoclonal rabbit IgG for Akt. To assess the suitability of the protocols, normal mammary gland tissues were used as positive controls for OR-α and PR. Akt antibody was applied to sections of a mast cell tumour as a positive control (Rodriguez *et al.*, 2012).