Expression of Androgen, Oestrogen α and β, and Progesterone Receptors in the Canine Prostate: Differences between Normal, Inflamed, Hyperplastic and Neoplastic Glands

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Summary

The expression of receptor for androgen (AR), oestrogen α and β (ERα and ERβ) and progesterone (PR) was examined immunohistochemically in canine prostate specimens (normal, hyperplastic, inflamed [prostatitis] or neoplastic). AR immunolabelling was seen in 100% of epithelial cells of normal and hyperplastic tissue, the corresponding figures for inflamed and carcinomatous tissue being 74% and 65%, respectively. ERα labelling was seen in 85% of epithelial cells in normal prostate glands, the corresponding figures for hyperplastic, inflamed and neoplastic glands being 35%, 22% and 12%, respectively. ERβ labelling was seen in 85% of epithelial cells of normal glands and in about 70% of such cells in glands showing pathological changes. On the other hand, PR expression (weak) in normal glands was observed in fewer epithelial cells (44%) than in hyperplastic (70%), inflamed (62%) or neoplastic (64%) glands.

Introduction

Of all mammals, only human beings and dogs are susceptible to the spontaneous development of benign prostatic hyperplasia (BPH) and adenocarcinoma (McNeal, 1978; Berry and Isaacs, 1984; Berry et al., 1986; Strandberg and Berry, 1987; Lowseth et al., 1990; Osterling, 1991). The prevalence of BPH approaches 100% in dogs above 7–8 years of age (Berry et al., 1986; Strandberg and Berry, 1987; Lowseth et al., 1990). While prostate cancer is relatively rare in dogs (Krawiec and Hefflin, 1992; Ladds, 1993; Bell et al., 1995), it is one of the most frequent causes of death from cancer in older men (Boring et al., 1993).

Although the exact pathogenesis of prostatic disorders is not well understood, hormones undoubtedly play a role. Androgens play a role in the development and physiology of male accessory sex organs, as well as in the function of other organs and tissues (Carson-Jurica et al., 1990). By immunohistochemistry, androgen receptor (AR) has been detected in a variety of tissues in men and women, including the reproductive organs (Kimura et al., 1993; Ruizeveld de Winter et al., 1994). Similarly, in the rat and the dog AR has been detected in male and female reproductive tissues (Sar et al., 1990; Hirai et al., 1994; Tetsuka et al., 1995; Murakoshi et al., 2000; Pelletier, 2000; Vermeirsch et al., 2001).

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Oestrogen receptors (ERs) and progesterone receptor (PR) are important attributes of oestrogen target cells and play a pivotal role in the development of oestrogen-dependent tumours. Both androgens and oestrogens may play a role in the pathogenesis of prostatic adenocarcinoma. The recent discovery of ERα in pre-malignant lesions, as well as in metastatic and androgen-insensitive tumours, suggests that oestrogens can affect prostatic carcinogenesis and tumour progression through a receptor-mediated process (Bonkhofer et al., 1999). The presence of PR in a significant number of metastatic and androgen-insensitive prostatic adenocarcinomas suggests that these tumours harbour a functional ER, mediating the "downstream events" of oestrogen signalling. This supports the concept that prostate cancer cells can escape androgen deprivation by using oestrogens for their growth and maintenance (Bonkhofer et al., 2001).

ERα has been reported in the male and female reproductive system of dogs (Schulze and Barrack, 1987), mice (Krege et al., 1998; Rosenfeld et al., 1998), rats (Kuiper et al., 1996; Saunders et al., 1998; Pelletier, 2000), rabbits (Danzo and Eller, 1979; Danzo et al., 1983), roosters (Kwon et al., 1997), goats (Goyal et al., 1997, 1998), monkeys (Fisher et al., 1997) and human beings (Linja et al., 2003; Tsurusaki et al., 2003). The tissue distribution of ERβ and PR in the prostate has been described in detail in man (Papadimitriou et al., 1992; Hiramatsu et al., 1996; Pasquali et al., 2001); it has not, however, been studied in the dog.

The complex interactions of steroid hormones with the different cell subsets of the prostate are essential in the pathogenesis of prostate diseases, particularly hyperplasia and cancer. The aim of the present study was to examine the expression of AR, ERα, ERβ and PR in normal, inflamed, hyperplastic and neoplastic canine prostate glands.

Materials and Methods

Samples

Archival paraffin wax-embedded prostate specimens (n = 38) from the Veterinary Pathology Diagnostic Service of the Universitat Autònoma de Barcelona were used in this study. These tissues were obtained at necropsy or for biopsy, from mixed-breed, sexually mature dogs. In addition, prostate samples were obtained from five normal beagles aged 18–24 months, which had been used in an unrelated project. Samples were either from normal prostate glands (n = 8), hyperplasia (n = 11), prostatitis (n = 11) or carcinoma (n = 8). They were fixed in 10% neutral buffered formaldehyde, dehydrated, and embedded in paraffin wax. Sections (5 μm) were cut from each block and used for immunohistochemistry.

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Immunohistochemistry (IHC)

AR was detected with a polyclonal antibody (NCL-Arp; Novocastra, Newcastle upon Tyne, UK) at a 1 in 20 dilution, applied for 30 min. The antibody against ERα was a monoclonal mouse antibody (NCL-ER-LH2; Novocastra), used at a 1 in 50 dilution for 30 min. An ERβ polyclonal rabbit antibody (H-150: sc-8974; Santa Cruz Biotech, CA, USA) was used at a 1 in 200 dilution for 1 h and a PR monoclonal antibody (BioGenex, San Ramon, CA, USA) at a 1 in 20 dilution for 30 min.

Sections were placed on positively charged slides (Dako, Glostrup, Denmark) and air-dried for 90 min at 60°C. After dewaxing in xylene and rehydration, the sections were heated in antigen-retrieval citrate buffer solution (Dako) at pH 7.4 for 1 min at 121°C. After cooling in buffer for 30 min at room temperature, the slides were placed on DakoCytomation TechMate 500-plus and autostainer platforms. The immunolabelling was performed by the En Vision method (K 5007; Dako). The slides were then counterstained with haematoxylin, dehydrated, mounted in DPX, and examined with a light microscope at x400 magnification. In all immunolabelled batches, omission of the primary antibody and replacement with the diluting solution alone served as a negative control. Positive control consisted of cryostat sections of normal canine prostate gland and paraffin wax sections of normal human prostate. For ER and PR, canine ovary and uterus were also used as controls.

Scoring of IHC Results

For each dog, a tissue section was labelled for each steroid receptor. The results were assessed independently by two “blind” observers. Any scoring discrepancy was resolved by an additional examination made simultaneously by the two observers. An “intensity” and a “proportional” score were obtained in every case. The former reflected the intensity of the labelling in the cell nuclei or cytoplasm (0, none; 1+, weak; 2+, moderate; 3+, strong; 4+, very strong). The proportional score reflected the percentage of cell nuclei immunolabelled in the different cell aggregates. These two scores were obtained in areas containing similar amounts of glandular epithelium and stromal cells. At least 100 parenchymal and stromal cells were evaluated in each sample.

Fig. 2A–C. Distribution of AR in normal (A), inflamed (B) and neoplastic (C) canine prostate. (A) Strong cytoplasmic immunolabelling was evident in epithelial cells. Nuclear labelling was evident in stromal cells. (B) The intensity of nuclear labelling was less than in normal or hyperplastic glands. (C) AR labelling was absent in most epithelial cells. Nuclear labelling was also observed in stromal cells. IHC. (A) x 200; (B) x 400; (C) x 500.

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Results

The cellular location of AR, ERα, ERβ and PR expression in prostate tissue (normal, hyperplastic, inflamed [prostatitis] or cancerous) of adult dogs was examined immunohistochemically. The results (intensity score and proportional score) for the various receptors and disorders studied are shown in Fig. 1.

AR Expression

Positive labelling was observed in the nuclei of epithelial and stromal cells and also, to a lesser degree, in the cytoplasm of the epithelial cells. Labelling was absent in all negative controls. Results for AR were similar in normal (Fig. 2a) and hyperplastic epithelial cells, both in intensity (3+ to 4+) and percentage (≥95%). A decrease in the intensity of labelling and percentage of positive epithelial cells was observed in prostatitis (2+ and ca 74%, respectively) (Fig. 2b). The lowest percentage was observed in carcinoma (Fig. 2c), with 58% of positive cells. The labelling intensity of tumour tissue was heterogeneous, some areas showing high reactivity (3+) and others little (1+) or none. In all cases, a small percentage (10–20%) of positive stromal cells was observed.

ER Expression (α and β)

ERα and ERβ labelling was observed only in nuclei. No such labelling was observed in any of the negative controls. In both epithelial and stromal cells, ERα was confined to the nuclei. In normal glands, 85% of epithelial cells gave positive results, with a labelling intensity of 3+ (Fig. 3a). Lower percentages were noted in hyperplastic glands (35%; Fig. 3b), inflamed glands (22%), and neoplastic glands (13%; Fig. 3c). Nuclear ERβ labelling was uniformly present in prostatic epithelial cells but absent in stromal cells. No labelling was seen in negative controls. The highest percentage of ERβ-positive cells (85%) with a labelling intensity of 3+ was again seen in normal prostatic tissue. The corresponding figures in prostatic tissue showing the various pathological changes were lower (ca 70% and 1+ to 2+; Fig. 4). Of the 38 samples, eight failed to react for ERα and two for ERβ.

Progesterone Receptor Expression

PR positivity was restricted to the nuclei of epithelial prostatic cells, nuclei of stromal cells being completely negative. No labelling was shown by negative controls. The percentage of positive epithelial cells varied from

![Fig. 3A-C. Distribution of ERα in normal (A), hyperplastic (B) and neoplastic (C) canine prostate. (A) Strong nuclear labelling was observed in most epithelial cells and in 30% of stromal cells. (B) The intensity of nuclear labelling of epithelial cells varied from strong to weak. Nuclear labelling was also evident in 30% of stromal cells. (C) Strong nuclear labelling was observed in 12% of epithelial cells. Nuclear labelling was detected in a very low percentage of stromal cells. IHC. (A) × 250; (B) × 200; (C) × 400.](image-url)
43% in normal prostate tissue (Fig. 5a) to 70% in hyperplastic (Fig. 5b), and 62% in inflammatory tissue. PR labelling intensity was weak in 95% of the samples. In positive controls (canine ovary and uterus), labelling intensity varied from weak to strong. In carcinomas, the mean PR expression was ca 50%, but there were striking variations (10–90%) between different cases (Fig. 5c). Labelling intensity in this subset was weak, except in one case (a prostatic adenocarcinoma with solid and cribriform nodules), in which moderate labelling intensity was observed.

Discussion

In the present study, IHC was used on paraffin wax sections from prostatic tissue to investigate the distribution pattern of AR, ERα, ERβ and PR in healthy dogs, and in dogs with various prostate diseases. To the best of our knowledge, this report records expression of ERβ and PR for the first time in canine prostatic glands. The demonstration of most nuclear receptors in paraffin wax-embedded tissue sections by IHC is difficult and usually requires antigen-retrieval pre-treatment to unmask epitopes that become antigenically inert because of fixation and processing (Taylor et al., 1994).

ARs were located predominantly in the nucleus of the epithelial cells and to a lesser degree in the nucleus of the stromal cells. In normal and hyperplastic canine prostate samples, the percentage of positive epithelial cells was similar, while it was markedly reduced in the presence of other pathological changes. These results are similar to those reported in healthy dogs and those with BPH (Murakoshi et al., 2000) and rats (Pelletier, 2000), in which AR was detected in the nuclei of both glandular epithelial and fibro-muscular stromal cells. Similarly, in BPH, human prostate epithelial cells show uniformly intense nuclear labelling for AR (Sar et al., 1990).

Oestrogens play a role in the control of growth and cellular differentiation of the prostate in male animals and in the development of canine prostatic disorders. They are believed to be of critical importance in the pathogenesis of BPH. Treatment of castrated dogs with androgen alone is insufficient to induce BPH, in contrast to the simultaneous administration of oestradiol and androgen (oestradiol-17β plus 5α-dihydrotestosterone) (Winter et al., 1995; Winter and Liehr, 1996). To date, two isoforms of ER have been identified (ERα and ERβ), with several variants of each.
In the present study, immunolabelling for ER\textsubscript{a} was invariably detected mainly in the nuclei of both glandular epithelial and stromal cells. A striking feature of this receptor was its heterogeneous distribution within any given case, regardless of the percentage of positive cells and of the overall intensity of the reaction. Similar observations have been reported in human prostatic tissue (Papadimitriou et al., 1992).

Schulze and Barrack (1987) reported ER labelling confined to the nuclei of the canine prostatic stroma and the prostatic duct epithelium but, in contrast to our findings, detected no specific labelling in the acinar epithelium. This apparent discrepancy may have been related to limited specificity of the antibody used, an ineffective method of antigenic retrieval, differences in sample processing, or differences in the sensitivity of techniques (Pasquali et al., 2001).

In the canine prostate, ER\textsubscript{a} was highly expressed in the epithelial secretory cells, but none was detected in stromal cells. Similar findings have been reported in human beings (Enmark et al., 1997), monkeys, (Pelletier et al., 1999) and rats (Pelletier, 2000). In the present study, ER\textsubscript{a} was found to be reduced in diseased prostate glands; this accorded with recent experimental studies suggesting an antiproliferative effect of this receptor in prostatic epithelium (Krege et al., 1998; Weihua et al., 2002).

In the prostate tissues with pathological changes, a decrease in the percentages of ER\textsubscript{a}-positive epithelial prostate cells was observed. Oestradiol enhances androgen-induced glandular hyperplasia in the canine prostate. The lower expression of ER in BPH than in normal prostatic epithelial cells suggests that alternative mechanisms should be considered for the effect of oestrogen on the glandular epithelium, including an indirect effect through prostatic stroma (Cunha et al., 1983). Moreover, in the present study the observed reduction in AR expression as well as ER\textsubscript{a} and ER\textsubscript{b} expression, in malignant neoplastic epithelial cells supports the contention that the actions of oestrogens on the prostate are complex and suggests a dual role in the aetiology of prostate cancer (Bonkhoff et al., 1999).

In the canine prostate, ER\textsubscript{b} was highly expressed in the epithelial secretory cells, but none was detected in stromal cells. Similar findings have been reported in human beings (Enmark et al., 1997), monkeys, (Pelletier et al., 1999) and rats (Pelletier, 2000). In the present study, ER\textsubscript{b} was found to be reduced in diseased prostate glands; this accorded with recent experimental studies suggesting an antiproliferative effect of this receptor in prostatic epithelium (Krege et al., 1998; Weihua et al., 2002).

The presence of PR in prostatic tissue of dogs suggests a possible physiological and pathological role. PR has been demonstrated in prostatic hyperplasia and neoplasia in man (Hiramatsu et al., 1996) and, in the present study, in diseased canine prostate. The effect of progesterone on the prostate is worthy of further investigation (Hiramatsu et al., 1996), in both human beings and dogs. It is well established that transactivation of the PR gene is...
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