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Research Notes

Histopathological Characterization and Expression of Vitamin D Receptor in the Prostate of Healthy Dogs and Dogs with Prostatic Carcinoma

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Abstract: Vitamin D Receptor (VDR) expression is implicated in human Prostatic Carcinoma (PC), but its role in canine PC is unclear. To investigate how VDR expression is affected by age and castration in healthy dogs and how it changes with PC, we evaluated prostates from 8-month-old (n = 5) or 6-year-old (n = 8) intact males, 1-8-year-old healthy castrated males (n = 4) and 8-15-year-old castrated males (n = 7) with PC, by performing histopathology, immunohistochemistry, and ELISA. The results showed that VDR expression in canine prostate increased in an age-dependent manner and decreased after castration compared with intact dogs at 6 years of age. Castrated dogs with PC showed increased VDR expression compared with healthy castrated dogs and VDR expression in PC differed according to the pattern of tumor proliferation. The findings suggest that prostatic VDR expression may be a useful prognostic marker and therapeutic target for canine PC.

Keywords: Cancer, Castration, Dog, Prostate, Vitamin D Receptor

Introduction

The canine prostate anatomically and functionally resembles its human counterpart and Prostatic Carcinoma (PC) shows similar disease progression in dogs and humans, metastasizing to the lungs, bones, and other sites (Waters *et al*., 1996; LeRoy and Northrup, 2009). Considering PC from an endocrinological perspective, the human condition is initially androgen-sensitive, but recurs as a hormone-resistant tumor with progressive androgen depletion, while the canine condition develops independently of androgen, with castration potentially elevating the risk of onset (Bell *et al*., 1991; Kawakami *et al*., 2014; Teske *et al*., 2002). The pathogenesis of canine PC is thus regarded as mimicking that of hormone-resistant tumors in humans (Lai *et al*., 2009).

Vitamin D Receptors (VDR) mediate the principal response of cells to 1,25-dihydroxyvitamin. In people, VDR expression is found not only in normal tissues but also in cancer tissues (Christakos *et al*., 2016). Previous studies have suggested a role for VDRs in human PC (Gallagher and Fleshner, 1998; Batai and Kittles, 2016); however, the links between these receptors and PC in dogs are unclear. In this study, we aimed to elucidate the effects of age and castration on VDR expression in healthy dogs as well as the change in VDR in PC.

Materials and Methods

Prostate tissues were obtained from 8-month-old intact beagles (n = 5), in which the prostate had not yet fully matured and 6-year-old intact beagles (n = 8) with a mature prostate (Dorso *et al*., 2008). In addition, prostate biopsies were collected from 1-8-year-old healthy mixed-breed dogs (n = 4) at 6-8 months after castration as well as from 8-15-year-old castrated dogs diagnosed with PC at the veterinary medical teaching hospital of Nippon veterinary and life Science university (n = 7: Miniature dachshund n = 3, mixed breed n = 2, Maltese n = 1, Lhasa Apso n = 1) after total prostatectomy. A portion of each excised tissue sample was frozen at -40°C and used for Enzyme Linked Immunosorbent Assay (ELISA) evaluation and the remainder was fixed with 10% phosphate buffered formalin and kept for subsequent histopathological examination, which involved microscopy of Hematoxylin and Eosin (HE) stained sections, as well as immunohistochemical evaluation of serial sections. Subtypes of PC were classified histologically based on the system proposed by Lai *et al*. (2008), while VDR expression was evaluated according to the pattern of growth in prostatic adenocarcinoma. The VDR

immunohistochemical evaluation was performed with a streptavidin biotin method using the VECTASTAIN universal quick kit (vector laboratories, Burlingame, CA). Sections were treated with the primary antibody for VDR (Polyclonal 1:100, LS-C407668; Life Span BioSciences, Inc., Seattle, WA) and incubated overnight at 4°C. Sections were treated with biotin labeled secondary antibody and streptavidin/peroxidase complex, processed with H_2O_2 and 3,3′-diaminobenzidine for 2 min, and then subjected to nuclear staining with hematoxylin. The negative control was treated with PBS in place of the primary antibody. Specimens were observed in order to determine the expression sites. In the immunohistochemical VDR evaluation, image intensity was observed to determine positive staining reactions. VDR expression was graded qualitatively (-, no immunoreactivity; ±, slight intensity; +, mild intensity; ++, moderate intensity).

Additionally, VDR expression in frozen prostatic tissue samples was quantified using ELISA in all intact dogs (age: 8 months or 6 years), one healthy dog at 6 months post castration, one healthy dog at 8 months post castration and three dogs with PC, from among those targeted for histopathological examination. Samples were homogenized with protease inhibitor (complete ULTRA tablets; Roche, Basel, Switzerland) and centrifuged at 15,000 rpm for 15 min to extract proteins. Total protein concentration was determined by the Lowry method, using the BCA protein assay Kit (Thermo Fisher Scientific, Waltham, MA). VDR expression was quantified using the canine Vitamin D receptor ELISA kit (MyBioSource, San Diego, CA) in accordance with the manufacturer's instructions and the results were corrected for total protein concentration.

Results

Histopathology revealed spontaneous Benign Prostatic Hyperplasia (BPH) in three of the eight 6-year-old intact dogs. Within the BPH tissue, regions of glandular epithelial hyperplasia and cystic lumina were observed, with inflammatory cell infiltration evident in some areas. The prostates from the 8-month-old and 6-year-old intact dogs were all normal. In the castrated dogs, the glandular epithelium was greatly reduced and only connective tissue was observed, which indicated atrophy of the prostate gland (Fig. 1a-c). In the dogs with PC (Fig. 2a-e) all of which had been castrated the cancerous tissue extended across the entire prostate. Mixed growth patterns were observed in six of the seven dogs with PC. Micropapillary and cribriform patterns were observed in three dogs, small acinar/ductal and micropapillary patterns were observed in one dog, tubule papillary and small acinar/ductal patterns were observed in two dogs and a solid pattern was observed in one dog.

VDR expression was immunohistochemically observed in the glandular epithelium but not in the peripheral duct. For the five 8-month-old intact dogs, a positive reaction was observed in only one dog, which showed a slight positive reaction in the nuclei and cytoplasm of acinar cells. Positive reactions were observed in the nuclei and/or cytoplasm in all eight 6-year-old intact dogs, with the reaction reaching a mild intensity in one dog. In the four healthy castrated dogs, a slight positive reaction was observed in a few remaining prostatic acinar cells (Fig. 1d-f). All seven dogs with PC showed VDR-positive reactions in the nuclei and cytoplasm. In particular, the reaction intensified in the two PC cases that showed a small acinar/ductal cell growth pattern, for which the reaction intensity was graded as mild to moderate for the nuclei and cytoplasm (Fig. 2f-j).

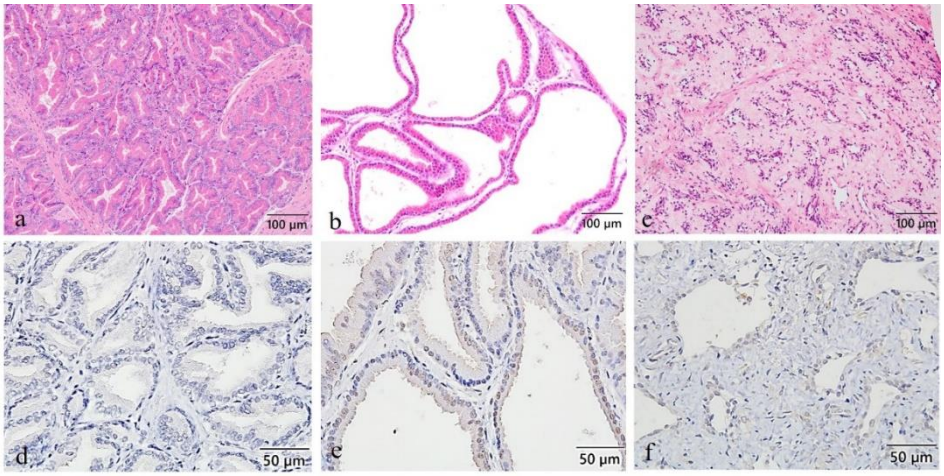

Fig. 1: HE stain in normal canine prostate. (a) 8 months of age. (b) 6 years of age. (c) 6 months after castration. Immunohistochemical localization of VDR in normal canine prostate. (d) 8 months of age (nuclear: -, cytoplasmic: -). (e) 6 years of age (nuclear: +, cytoplasmic: ±). (f) 6 months after castration (nuclear: ±, cytoplasmic: ±)

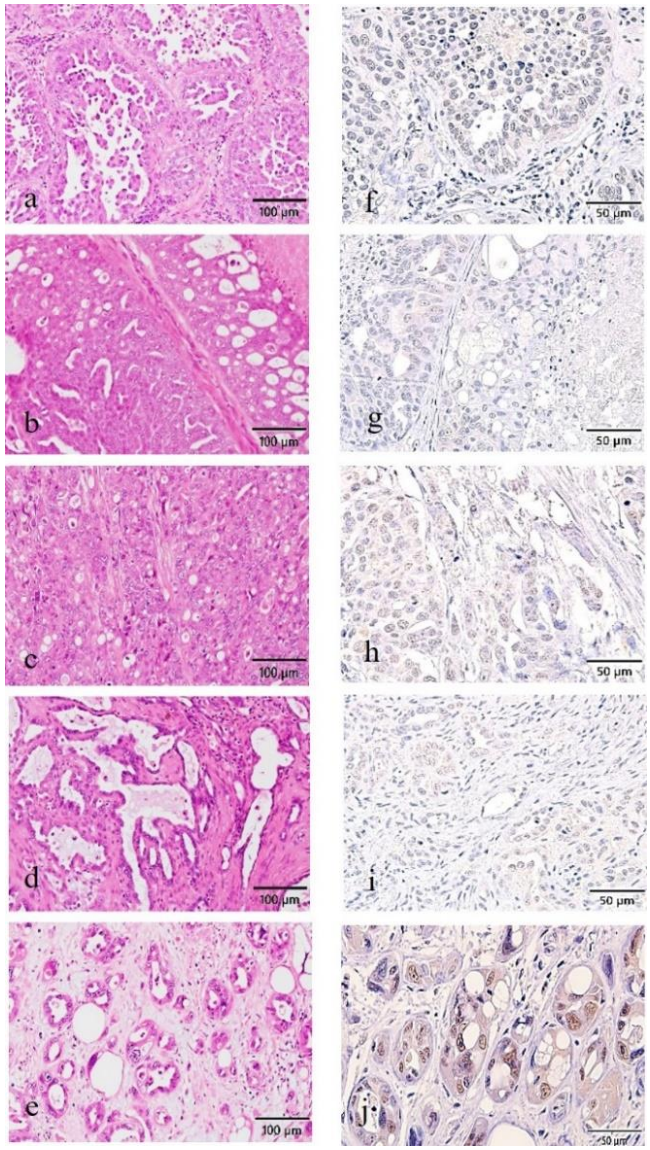

Fig. 2: Different growth patterns in canine prostate carcinoma. HE stain (a-e) and Immunohistochemical localization of VDR (f-j) in each growth pattern. (a) and (f): Micropapillary pattern, (b), and (g): Cribriform pattern, (c), and (h): Solid pattern, (d) and (i): Tubulo-papillary pattern, (e), and (f): Small acinar/ductal pattern. (a): Micropapillary pattern, in which papillary projections are formed in duct-like structures. (b): Cribriform pattern, the tumor cells with the formation of fenestrae. (c): Solid pattern, anaplastic undifferentiated carcinoma characterized by pleomorphic tumor cells arranged as a solid nest. (d): Tubulo-papillary pattern, some dilated ducts with single layer columnar cells, irregularly formed are present. (e): Small acinar/ductal pattern, variously sized micro-acini, arranged within a scirrhous-looking fibromuscular stroma. (f): Micropapillary pattern (nuclear: ±, cytoplasmic: ±). (g): Cribriform pattern (nuclear: ±, cytoplasmic: ±). (h): solid pattern (nuclear: ±, cytoplasmic: ±). (i): Tubulo-papillary pattern (nuclear: ±, cytoplasmic: ±). (j): Small acinar/ductal pattern (nuclear: ++, cytoplasmic: ++)

Table 1: ELISA results for VDR in the prostate of dogs

	Intact dog				Castrated/PC		
Group/patterns	8 months old	6 years old	Castrated		Micropapillary Cribriform	Micropapillary Small acinar/ductal	Solid
No. of dogs	5	8	1	1	1	1	1
mean ng/µg protein	11.3±3.0	28.7±7.7	1.3	ND	7.9	21.6	12.7

ND: Not Detected

Tissue VDR expression was quantified by ELISA. For the healthy intact dogs, mean VDR values were 11.3±3.0 ng/µg protein for the 8-month-old dogs and 28.7±7.7 ng/µg protein for the 6-year-old dogs, indicating an increase with age (Table 1). Among the healthy castrated dogs, VDR was undetectable in one case and the measured value was 1.3 ng/µg protein in the other dog evaluated, which was lower than the values in either the 8-month-old or 6-year-old intact dogs. Among the dogs with PC, the VDR values were 7.9 ng/µg protein for the mixed micropapillary and cribriform cell growth patterns, 21.6 ng/µg protein for the small acinar/ductal cell and mixed micropapillary growth patterns and 12.7 ng/µg protein for the solid cell growth pattern. Although these values showed numerical differences, the values in the PC dogs were all higher than those in the healthy castrated dogs.

Discussion

VDR expression in the canine prostate increases in an age-dependent manner and is decreased after castration compared with intact dogs at 6 years of age. Similar age-related increases in prostatic VDR levels have been found in humans (Krill *et al*., 2001) and rats (Campolina-Silva *et al*., 2018), consistent with our findings. VDRs and androgen receptors cooperate to regulate the gene expression of mRNAs involved in calcium homeostasis, thereby mediating various cellular processes such as cell proliferation, cell death, and cell motility, which influence hormone-responsive human prostate cancer progression. Meanwhile, an age dependent decrease in both 1,25 $(OH)_2D_3$ and testosterone leads to the de-differentiation of prostate cancer cells (Wang *et al*., 2011). In this study, castration led to tissue atrophy and a decrease in VDR expression. Thus, atrophy of prostatic tissue and decreased expression of VDR due to androgen depletion as a result of castration may influence the development of prostate cancer in dogs.

In the immunohistochemistry and ELISA analyses, castrated dogs with PC showed increased VDR expression compared with healthy castrated dogs and VDR expression in PC differed according to the pattern of tumor proliferation. Immunohistochemically, all dogs with PC showed VDR positive nuclei and cytoplasm. In particular, the three dogs with cancers that exhibited a small acinar/ductal cell growth pattern showed mild to moderate intensity in the nuclei and cytoplasm. The ELISA results for these dogs were consistent with those from immunohistochemistry. In human colorectal cancer, VDR expression is reported to increase in proliferative lesions such as polyps and aberrant crypt foci relative to the normal epithelium, with a greater increase in the well-differentiated proliferative lesions, but with a return to lower levels in poorly differentiated proliferative lesions as the malignant tumor progresses (Matusiak *et al*., 2005). Furthermore, an association between higher VDR expression levels and survival has been reported in human breast cancer (Thanasitthichai *et al*., 2015; Welsh, 2018; Xu *et al*., 2020) and bladder cancer (Jóźwicki *et al*., 2015), suggesting that VDRs represent a potential prognostic marker. As a therapeutic for human PC, the vitamin D compound DN-101 achieved a superior PSA response rate in combination with docetaxel to the docetaxel placebo combination (Trump and Aragon-Ching, 2018), suggesting that vitamin D has potential utility as a therapeutic target for this condition. Similar trends for VDR expression in canine PC tissue were observed in immunohistochemistry and ELISA, but further studies are needed to elucidate the relationship between VDR expression level and histological type in canine PC.

Conclusion

The results of this study indicate that VDR expression in the canine prostate increases with age and is reduced after castration compared with intact dogs at 6 years of age. In immunohistochemistry and ELISA analyses, castrated dogs with PC showed increased VDR expression compared with healthy castrated dogs, with this expression differing according to the pattern of tumor proliferation. Based on the cell growth pattern dependent differences in prostatic VDR expression in this study, we consider that VDRs have the potential as a prognostic marker and a therapeutic target for canine PC. We also consider that associations between VDR expression and survival and the relevant therapeutic potential should be investigated further.

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Author's Contributions

Akiko Ikeda: Designed and coordinated the experiment performed the experiment analyzed drafted the manuscript.

Masanori Kobayashi, Masaki Michishita and Eiichi Kawakami: Analyzed revised the manuscript and approved the final version.

Tatsuya Hori and Masato Kobayashi: Revised the manuscript and approved the final version.

Ethics

This article is original and has not been published previously. The corresponding author has confirmed that all the authors involved in this study have read and agreed with the contents of this article and that there are no ethical issues involved.

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