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COX-2 and TGF-β expression in proliferative disorders of canine prostate

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Abstract

COX-2 and TGF-β expression was determined in order to correlate non-neoplastic lesions, preneoplastic lesions and carcinoma in the prostate of dogs. The results show that neoplastic and preneoplastic lesions express more COX-2 and TGF-β when compared to carcinomas, which suggests these proteins may cooperate in the process of prostate tumorigenesis.

Key Words: carcinoma prostate, COX-2, dog, preneoplastic lesions, TGF-β.

Introduction

Prostatic disorders frequently represent a problem in adult and elderly dogs (5,12,17). The most common prostatic lesions in dogs include benign prostatic hyperplasia (BPH), prostatic inflammation, cysts and adenocarcinoma(9), all of which result in prostatomegaly (2).

The stromal component alters epithelial cell differentiation (11) contributing to the pathogenesis of benign prostatic hyperplasia (BPH). Stromal growth factors, such as transforming growth factor-β (TGF-β) and fibroblast growth factor (FGF) accumulate and contribute to BPH progression (16).

Canine prostate cancer is similar to that observed in humans with respect to age at diagnosis, its association with high-grade prostatic intraepithelial neoplasia (PIN) and its propensity for skeletal metastasis (1). Canine PIN is considered a preneoplasic lesion, and was described by Waters & Bostwick in 1997 and its diagnosis analogous with humans (14)

Significant alterations in the expression of oncogenes, growth factors and growth factor receptors occur in the transition of benign epithelial tissue to PIN, highlighting the fact that this lesion represents an important stage in the process of carcinogenesis (21)

Another lesion that has more recently been under investigation in men is prostatic glandular atrophy associated with inflammatory infiltration. This disorder is also considered a prostatic adenocarcinoma precursor, and occurs in association with chronic inflammation. For this reason it was designated as "proliferative inflammatory atrophy" (PIA) by De Marzo et al.(3). In men, this lesion occurs in areas adjacent to high-grade PIN (HGPIN) and/or cancer and presents with similar genetic abnormalities (19). PIA can development into HGPIN and, subsequently, prostatic carcinoma. Investigations regarding the frequency, distribution, proliferative index and genetic alterations in PIA suggest a relation with HGPIN and prostatic cancer (20). This lesion has not been described in dogs to date.

COX-2 promotes tumor development by a variety of mechanisms, including induction of angiogenesis, possibly by generating free radicals, and carcinogenesis (13). Studies using immunohistochemistry have confirmed high levels of COX-2 expression in human prostate cancer tissue and high-grade prostate intraepithelial neoplasia (15).

TGF-β expression has been observed in several neoplasias. Immunohistochemical studies revealed intense staining in prostate tumors compared to normal prostate epithelial tissue, suggesting that neoplastic epithelial cells produce and secrete TGF-β (22).

The objective of this study was to compare COX-2 and TGF-β immunolabeling in prostate

hyperplasic, preneoplastic (PIA and PIN) and neoplastic lesions in dogs.

Materials and methods

Five cases of each of the following diagnoses were obtained from Veterinary Pathology Service archive material, Veterinary Medicine Faculty (FMVZ), São Paulo State University (UNESP), Botucatu, São Paulo, Brazil: epithelial hyperplasia, stromal hyperplasia, PIA and prostatic carcinoma, and three cases of high grade PIN from canine prostate. Five normal prostates were also collected as control tissue.

Each diagnosis was confirmed blindly by two pathologists (MMPR and RLA) through review of the H&E stained slides. Epithelial and stromal hyperplasia and prostatic carcinoma diagnoses were made according to Foster and Ladds(6). PIN according Waters & Bostwick(21) and PIA according to De Marzo et al.(4).

New sections (3 µm) were generated from the selected paraffin-embedded tissue for immunohistochemical analysis. Sections were subjected to deparaffinization and rehydration. Sections were preheated in a water bath at 96ºC for 25 minutes prior to antibody staining. For antigen retrieval: 10 mM citrate solution, pH=6.0 was used for the COX-2 primary antibody, clone CX-294 (Dako Corp., Carpinteria, CA) and 10 mM EDTA solution, pH=8.0, for TGFβ antibody, clone 17 (Novocastra, Norwell, MA). Endogenous peroxidase activity was quenched using 10 volumes H2O2 for 20 minutes. Each primary antibody was diluted 1:50 in 1% BSA, and slides were incubated in a humidity chamber for 18 hours at 4°C. The slides were then washed with TRIS and incubated with secondary antibody and streptavidin enzyme complex (LSAB) (Dako Corp., Carpinteria, CA) for 30 minutes, each reagent, in the case of TGF-β antibody and with Envision (Dako Corp., Carpinteria, CA) for 30 min for COX-2 primary antibody. Color was developed for 3 minutes at room temperature with a freshly prepared solution of 3,3' diaminobenzidine (Dako Corp., Carpinteria, CA) and the sections were then lightly counterstained with haematoxylin, dehydrated and mounted. Negative control slides were generated by incubating with mouse immunoglobulins (Dako Corp., Carpinteria, CA).

COX-2 and TGF-β immunohistochemical analyses were performed by evaluating and scoring the staining intensity (no staining, weak, moderate and strong) and the percentage of stained cells (no staining; 1 - 25%; 26 - 50%; 51 - 75% and >76%), adapted from Heller et al. (7), throughout the entire slide at 40x magnification with the assistance of image analysis software (QWin v3.0, Leica, Wetzlar, Germany) and a digital camera mounted on the microscope.

Results

COX-2 immunolabeling was cytoplasmic with intensity differences observed between the diagnostic groups (Fig. 1A, C, E, G, I). In the normal prostate tissue no immunolabeling was seen. However, in epithelial hyperplasia the epithelial cells were weakly positive. Positive staining was observed in the stromal cells in stromal hyperplasia, whereas the epithelial compartment was negative for COX-2 expression.

Immunohistochemical staining in the PIA and PIN samples demonstrated that the dysplastic epithelial cells were positive for COX-2, with higher intensity and more positive cells in PIA than in PIN samples (Table 1). However, the prostate carcinoma group exhibited the most intense staining and the most number of positive COX-2 cells. These data suggest that COX-2 expression is correlated with prostate carcinoma progression.

Epithelial cells of epithelial hyperplasia, PIA, PIN and carcinoma were positive for cytoplasmic TGF-β staining (Fig. 1B, D, F, H and Table 2). In contrast, in normal canine prostate and stromal hyperplasia samples only the stromal cells showed positive staining. PIA and PIN groups showed a similar pattern of TGF-β and COX-2 expression. However, prostate carcinoma samples exhibited lower intensity and a lower percentage of cells for TGF-β staining than for COX-2 in these samples.

The less differentiated neoplasias and metastastic samples had the greatest staining intensity and number of positive cells, for both markers, showing the potential involvement of TGF-β and COX-2 in prostate cancer progression.

Table 1. Distribution of prostate glands by diagnosis (Epithelial hyperplasia, stromal hyperplasia, PIN, PIA and prostatic carcinoma) regarding the intensity and percentage of COX-2 positive cells.

Diagnosis	Intensity				Percentage of COX-2 positive cells				
	-	+	++	+++	0	1-25%	26-50%	51-75%	>75%
Normal (n=5)	2	3	0	0	2	3	0	0	0
Epithelial Hyperplasia (n=5)	4	1	0	0	4	1	0	0	0
Stromal Hyperplasia (n=5)	1	2	2	0	1	2	2	0	0
PIA (n=5)	0	0	4	1	0	0	3	1	1
PIN (n=3)	0	1	2	0	0	3	0	0	0
Carcinoma (n=5)	0	1	2	2	0	1	2	0	2

Table 2. Distribution of prostate glands by diagnosis (Epithelial hyperplasia, Stromal hyperplasia, PIN, PIA and prostatic carcinoma) regarding the intensity and percentge of TGF-β positive cells.

Diagnosis	Intensity				Percentage of TGF-β positive cells				
	-	+	++	+++	0	1-25%	26-50%	51-75%	>75%
Normal (n=5)	2	2	1	0	2	2	1	0	0
Epithelial Hyperplasia (n=5)	2	2	1	0	2	3	0	0	0
Stromal Hyperplasia (n=5)	0	2	3	0	0	3	2	0	0
PIA (n=5)	0	1	2	2	0	1	1	2	1
PIN (n=3)	0	2	1	0	0	2	1	0	0
Carcinoma (n=5)	0	2	3	0	0	1	3	1	0

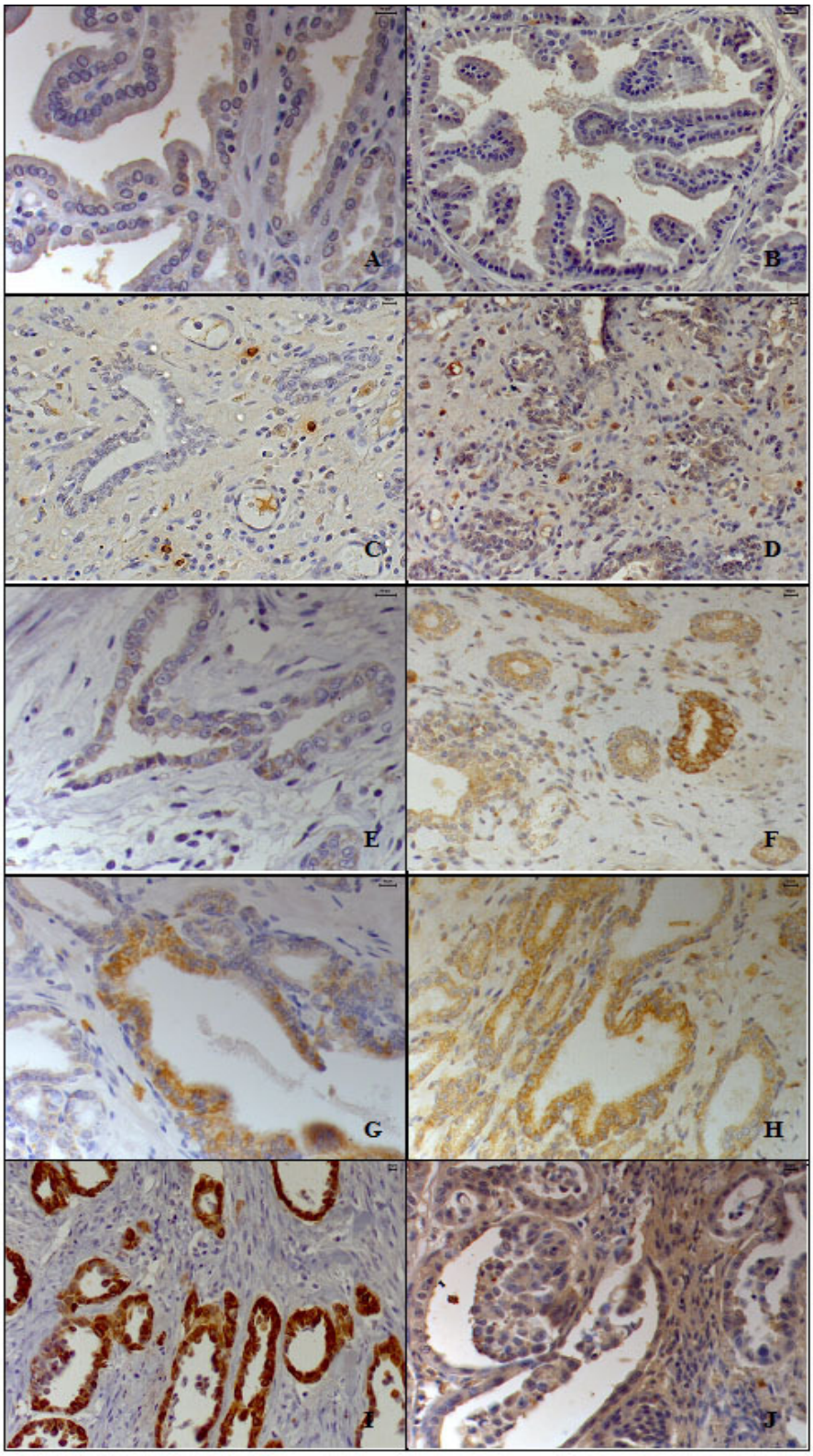

Figure 1. (A) Prostatic epithelial hyperplasia, COX-2, 640x. (B) Prostatic epithelial hyperplasia, TGFβ, 400x. (C) Prostatic stromal hyperplasia, COX-2, 400x. (D) Prostatic stromal hyperplasia, TGFβ, 400x. (E) Proliferative inflammatory atrophy, COX-2, 500x. (F) Proliferative inflammatory atrophy, TGFβ, 400x. (G) Prostatic intraepithelial cell neoplasia, COX-2, 500x. (D) Prostatic intraepithelial cell neoplasia, TGFβ, 400x. (I) Prostatic carcinoma, COX-2, 400x. (J) Prostate carcinoma, TGFβ, 400x Scale bar = 10 µM. Immunohistochemistry, Envision, DAB, Hematoxilin counter staining.

Discussion

It was possible possible to make PIA diagnosis in dogs using the same criteria as described for humans by De Marzo et al (4). Results from this study show that epithelial cells in PIA had a greater intensity and number of positive cells than PIN for COX-2 staining, however, this was lower than for canine prostate carcinomas. These results are in accordance with what has been demonstrated for human PIA, as described by Sugar et al (19), where immunostaining for COX-2 expression in PIA samples showed a high percentage of positive cells. In this group, staining was observed in atypical cells, suggesting a fundamental role for this protein in the progression of preneoplasic lesions toward neoplastic, as described by Patel et al. (18)

In our study, the carcinoma group demonstrated variation regarding the percentage of COX-2 positive cells (one sample scored 1, two scored 2 and another two scored 4). These results could be related to the degree of histological differentiation of the neoplasia (i.e., undifferentiated or metastatic tumors presented positive expression in greater than 75% of cells).

Our results further demonstrated that epithelial cells in normal prostate tissue exhibited COX-2 immunostaining at low intensity. In contrast, Kirschenbaum et al. (10) reported COX-2 expression in smooth muscle and basal cells in normal prostate tissue samples. Luminal cells in these glands do not express COX-2, although they are positive when found in areas presenting inflammatory foci.

In agreement with Hussaim et al. (8), COX-2 expression was observed in adenocarcinoma and PIN, though this was more evident in areas of PIA. Therefore, we believe that high COX-2 expression in atrophic lesions is associated with a chronic inflammatory process implying that inflammation participates in neoplasia progression, which has been described by Sugar et al. (19).

TGF-β and COX-2 perform opposing tasks in the processes of cellular proliferation and apoptotic inhibition, thus in lesions presenting high COX-2 staining intensity, low TGF-β levels might be expected. However, observations in this study showed a high percentage of positive cells for both TGF-β and COX-2 in neoplasias. The role that this increased number of TGF-β and COX-2 positive cells in prostate adenocarcinoma should be investigated in canine and human prostate lesions.

In glands presenting stromal hyperplasia, higher TGF-β expression was noted compared to epithelial hyperplasia. These results were expected due to the role of the stroma in altering epithelial cell differentiation in the development of this lesion (11). TGF-β accumulates in the stromal cells, contributing to BHP progression (16).

Canine carcinoma samples exhibited a higher percentage of TGF-β positive cells than those normal prostate and epithelial hyperplasia samples. These data demonstrate that TGF-β expression is higher in neoplasic than in hyperplasic lesions, as confirmed by Wolff et al. (22).

COX-2 is likely an important component in prostatic cancer development, since its expression was higher in the epithelial cells of PIA, PIN and carcinoma, than in hyperplastic and normal tissue. TGF-β expression was higher in the stromal compartment in PIA and PIN, which may contribute to the carcinogenic process through TGF-β's role in angiogenesis.

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