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A.C.Camargo Cancer Center
Especializado em Vida

The expression of P-glycoprotein and Androgen Receptor under the influence of Androgen Hormone stimulation in Canine Prostate Cancer

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Introdução

Canine prostate cancer (PC) is an aggressive disease, and dogs can be considered comparative models for humans PC and canine PC has been shown to resemble human castration resistant prostate cancer. The influx and efflux of testosterone into the luminal cells of the prostate is regulated by P-glycoprotein (P-gp). Therefore, in human PC, expression of P-gp is generally absent while expression of androgen receptors (ARs) is preserved. However, this co-expression has not been studied in dogs.

Casuística e Métodos

This study was approved by the Institutional Ethics Committee for the Use of Animals (CEUA, # 107/2015) and followed national and international recommendations for the care and use of animals. Thirty-five prostate samples of paraffin-embedded tissue from dogs were used. Double immunofluorescence was performed based on a previously described technique for double immunohistochemistry with adaptations. Dual-color immunofluorescence staining was performed with P-glycoprotein and AR antibodies. Tissue samples were deparaffinized, antigen retrieval was performed, and unpacified binding proteins were blocked according to the laboratory standardized protocol. Tissue macro-dissection, mRNA extraction, cDNA synthesis, and qPCR were performed as described in the literature, as was pyrosequencing analysis. Statistical analysis was performed qualitatively and quantitatively using ANOVA.

Resultados

We identified AR/P-gp double immunofluorescence co-expression of both proteins in normal luminal cells. However, in canine PC, cells lack AR expression and exhibit increased P-gp expression. These results were confirmed by gene expression analyses.

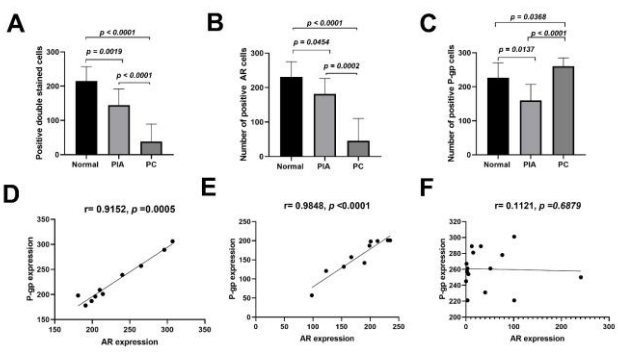

Figure 1. Expression of androgen receptor (AR), P-glycoprotein (P-gp) and doubled stained cells (AR/P-gp) in canine prostatic samples. A: Analysis of variance (ANOVA) revealing high doubled stained cells (AR/P-gp) in normal samples and decreased expression on proliferative inflammatory atrophy (PIA) and prostate cancer (PC) samples. B: High AR expression in normal samples compared to PIA and PC. C: ANOVA analysis revealing higher P-gp expression on PC samples, compared to normal and PIA. D: Positive correlation between the number of AR and P-gp positive stained cells in normal samples, indicating a dependency of both variables. Therefore, in normal prostate, increased AR expression also increase P-gp expression. E: Correlation analysis revealing that samples with higher AR expression also demonstrate higher P-gp expression in PIA samples. Similar to normal samples, in PIA samples, AR and P-gp expression presents dependency, indicating control of P-gp to androgen hormones influx. F: No correlation is found between AR and P-gp expression in PC samples. Thus, in canine PC, no dependency o relation is detected between AR and P-gp expression.

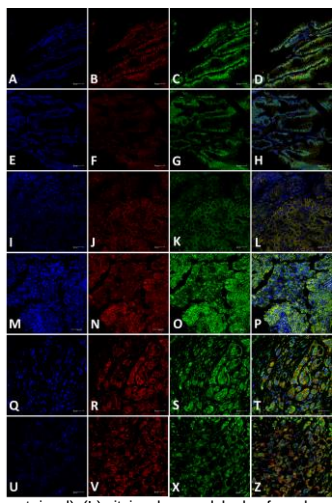

Figure 2. Immunofluorescence analysis of androgen receptor (AR) (green color) and P-glycoprotein (P-gp) (red color) in canine prostatic samples. A, B, C and D: expression of P-gp (B) and AR (C) in a normal prostate samples and merged image (D) demonstrating the doubled stained cells with AR expression in nucleus and P-gp in cell membrane. E, F, G and H: expression of P-gp (F), AR (G) and merged image (AR/P-gp) in PIA samples with surrounding normal tissue. The normal prostatic cells are showing positive P-gp expression in the membrane and nuclear AR expression (arrows). Conversely, PIA areas are showing no membranous P-gp and lack of AR expression (nucleus stained with DAPI – blue color) (arrowhead). I, J, K and L: canine PC with solid pattern showing cytoplasmic and membranous P-gp expression (J) and AR cytoplasmic expression (K). In the merged image (AR/P-gp double-stained) (L), it is observed lack of nuclear AR (cells stained with DAPI) and cell cytoplasm with yellowish stained (indicating a colocalization of AR and P-gp in cytoplasm). M, N, O and P: PC samples showing low P-gp expression and high AR cytoplasmic expression with no nuclear expression (P). Q, R, S, and T: PC samples showing P-gp membranous expression with AR stained in nucleus and cytoplasm (T). U, V, X, and Z: PC samples revealing membranous P-gp expression with AR cytoplasmic expression (Z). Immunofluorescence images, DAPI counterstaining.

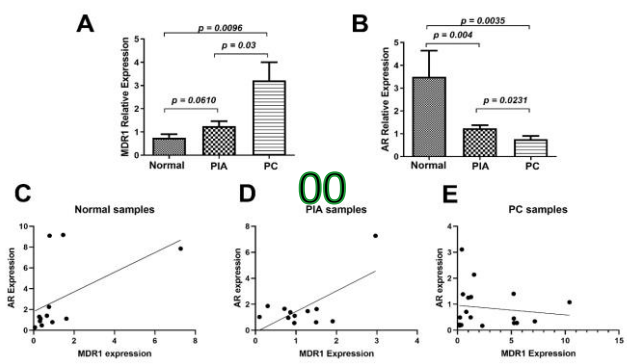

Figure 3. MDR1 and AR gene expression analysis in canine prostatic samples. A: MDR1 expression revealing higher transcript levels on canine prostate cancer (PC), compared to normal and proliferative inflammatory atrophy (PIA) samples. B: AR transcripts in canine prostatic samples. A higher AR expression on normal samples, compared to normal and PIA samples is observed. C: Linear regression analysis revealing a positive association between MDR1 and AR transcripts. D: Canine PC lacking association between MDR1 and AR transcripts. E: Linear regression revealing positive association between MDR1 and AR transcripts.

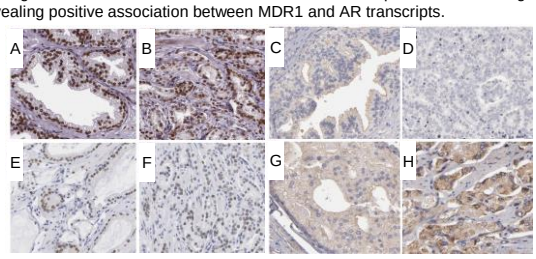

Figure 4 AR immunohistochemical expression human prostatic sample in: A: Normal prostate gland demonstrating strong nuclear AR expression in luminal cells. B: A high-grade prostate cancer (PC) showing strong nuclear AR expression. E: A low grade PC sample showing moderate AR nuclear expression. F: A high-grade PC showing moderate nuclear AR expression. P-glycoprotein (P-gp) immunohistochemical expression human prostatic samples in C: normal prostate gland showing weak membranous P-gp expression by luminal cells. D: A high-grade prostate cancer (PC) showing no P-gp expression. G: A PC representing moderate cytoplasmic and membranous P-gp expression. H: A high-grade PC showing strong membranous and cytoplasmic P-gp expression. Image credit of the IHC images: Human Protein Atlas, www.proteinatlas.org.

Conclusões

Overall, our results strongly suggest that testosterone influx in the normal canine prostate may be regulated by the expression of P-gp and that prostate cells lack expression of AR and overexpress P-gp during progression to PC. P-gp expression in the canine prostate PC may be related to the multiple drug resistance phenotype.

Contato

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