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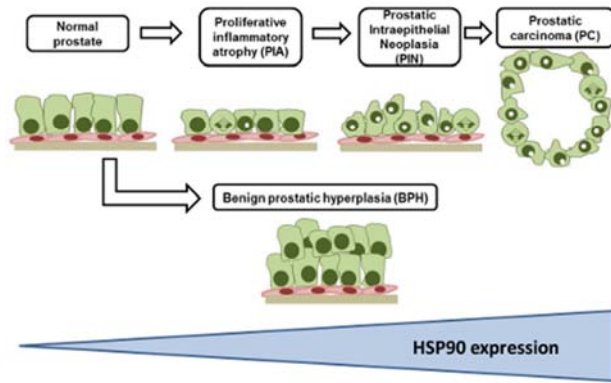
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Short Title: HSP90 Expression in Canine Prostate

**Heat Shock Protein 90 is Associated with Hyperplasia and Neoplastic Transformation of
Canine Prostatic Epithelial Cells**

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Summary

Heat shock protein 90 (HSP90) is a molecular chaperone that regulates critical signalling proteins of cancer development and progression. Abnormal levels of HSP90 have been observed in human prostatic carcinoma (PC), with prognostic and therapeutic implications. Since spontaneously arising canine PC is a valuable model for the human disease, the aim of this study was to evaluate the immunohistochemical expression of HSP90 in two normal canine prostates, 17 canine prostates with benign prostatic hyperplasia (BPH) and five canine prostates with PC. HSP90 was expressed in the cytoplasm of epithelial cells in all samples; with a significant increase in labelled cells in PCs. Nuclear labelling was observed occasionally in normal tissue, but was increased in BPH and PC. HSP90 immunoreactivity in preneoplastic lesions (proliferative inflammatory atrophy and prostatic intraepithelial neoplasia) was similar to that in PCs. Increased HSP90 expression in canine PCs suggests the involvement of this molecule in carcinogenesis and tumour progression, supporting HSP90 as a potential target for therapeutic intervention.

Keywords: dog; prostate; HSP90; immunohistochemistry

Prostatic carcinoma (PC) is a significant cause of morbidity and mortality in the western world (Jemal *et al.*, 2010) and, despite an excellent initial response to the androgen deprivation therapy, the failure rate is high with the emergence of the aggressive castration-resistant PC (CRPC). Controlling CRPC presents a challenge that requires new treatment strategies. In addition to man, the dog is the only mammal that spontaneously develops invasive PC (Waters and Bostwick, 1997), with a disease process similar to that of man (Rosol *et al.*, 2003). PC in dogs is typically late stage and androgen independent, therefore

representing a useful model to explore the mechanisms of cancer progression in both species (Leroy and Northrup, 2009).

Heat shock proteins (HSPs) are highly conserved molecular chaperones involved in a diverse range of normal intracellular activities and in different pathogenic processes (Morimoto *et al.*, 1990). Up-regulation of HSPs is observed commonly in cancer cells and is believed to support malignant transformation (Sreedhar *et al.*, 2004). HSP90 is the most abundant HSP within cells and is therefore considered an anti-cancer target (Scaltriti *et al.*, 2012). Inhibition of HSP90 is of particular significance for PC since HSP90 is overexpressed in prostate cancer cells with a stage- and malignancy-dependent expression (Cornford *et al.*, 2000; Cardillo and Ippoliti, 2006). In-vitro, the androgen receptor (AR)-negative prostate cancer cell line PC-3, corresponding to a more advanced stage of PC development, has higher HSP90 expression than the LNCaP cell line, which is androgen-sensitive and AR-positive and represents an early stage of PC (Stope *et al.*, 2012). The AR is one of the HSP90 'client proteins' as it binds HSP90 as part of a multimolecular complex. The AR drives the growth of CRPC through a number of mechanisms that intimately rely on HSP90 for cell survival, especially AR overexpression and gain-of-function AR gene mutations (Waltering *et al.*, 2012). Many preclinical studies have shown the potential utility of HSP90 inhibitors in prostate cancer (Eskew *et al.*, 2011; O'Malley *et al.*, 2012) and new-generation HSP90 inhibitors with improved physical and pharmacological properties may achieve more durable suppression of CRPC growth and minimize drug resistance (Centenera *et al.*, 2013). Importantly, some HSP90 inhibitors (e.g. Ganetespib[®]; Synta Pharmaceuticals Corporation, Lexington, Massachusetts, USA) appear to be effective in cells expressing splice variants of the AR that are devoid of the ligand binding domain and therefore resistant to conventional AR antagonists (He *et al.*, 2013). HSP90 inhibitors have been used in a phase I study of a canine model of spontaneously arising cancer (London *et al.*, 2011) and HSP90 expression

has been shown in a few primary tumours of the dog, including mammary cancer (Romanucci *et al.*, 2006) and osteosarcoma (Romanucci *et al.*, 2012), with an expression pattern paralleling the human counterparts. All of these findings strongly support the potential use of the canine model for testing new HSP90-targeted cancer therapy and studying human carcinogenesis. The aim of the present study was to evaluate immunohistochemically the expression of HSP90 in normal, hyperplastic, preneoplastic and carcinomatous canine prostatic epithelial cells.

Formalin-fixed, paraffin wax-embedded samples of canine prostate were selected from the departmental archive, unfortunately without follow-up data. Tissue samples included two normal prostates (dogs aged 5 and 6 years), 17 cases of benign prostatic hyperplasia (BPH) and five PCs. BPH and PC samples were from dogs aged 5–11 years (mean 7 years) and 8–11 years (mean 9 years), respectively. Sections (5 μ m) were stained with haematoxylin and eosin (HE). PCs were classified according to Lai *et al.* (2008).

Immunohistochemistry (IHC) was performed using an avidin–biotin–peroxidase system (Vectastain Standard Elite; Vector Laboratories, Burlingame, California, USA) as described by Romanucci *et al.* (2012). A murine monoclonal anti-human HSP90 (StressGen/Assay Designs, Inc., Ann Arbor, Michigan, USA; 1 in 1,800 dilution) was used as primary antibody. The primary antibody was omitted from negative control sections, which were incubated with phosphate buffered saline. Sections of normal canine testes were used as positive controls.

The intensity (i.e. weak, moderate or strong) and distribution (i.e. nuclear, perinuclear or diffuse cytoplasmic) of antigen labelling in normal, hyperplastic and neoplastic cells was recorded. The percentage of labelled cells was calculated semiquantitatively in 10 high-power fields ($\times 400$) as follows: (1) percentage of positively labelled cells/total cells (positive score), (2) percentage of cells with nuclear labelling/total positive cells (nuclear-positive

score), (3) percentage of cells with perinuclear labelling/total positive cells (perinuclear-positive score), and (4) percentage of cells with diffuse cytoplasmic labelling/total positive cells (diffuse cytoplasmic-positive score). Both assessments were made independently by two pathologists. HSP90 expression was classified as: grade I, no labelled cells; grade II, up to 10% of cells labelled; grade III, 10–50% of cells labelled; grade IV, >50% of cells labelled. Results were expressed as percentage of cases of each grade out of the total number of cases in each category (i.e. normal, BPH and PC). To compare the IHC scores, differences in percentages were calculated with the Chi-square test. $P < 0.01$ was considered significant.

Epithelial hyperplasia, mild stromal fibrosis and cystic acini were observed in hyperplastic lesions. PCs had different histological patterns: (1) small acinar/ductal ($n = 2$), (2) cribriform with multifocal central necrosis ($n = 2$), or (3) undifferentiated ($n = 1$). In two of the five cases of PC, neoplastic emboli were detected within blood vessels. Metastases to the lung and lymph nodes were observed in one case of PC. Preneoplastic lesions, such as proliferative inflammatory atrophy (PIA) and prostatic intraepithelial neoplasia (PIN) were observed in five samples. Multifocal areas of PIA (Supplementary Fig. 1) were observed in four cases of BPH and one case of undifferentiated adenocarcinoma; multifocal epithelial PIN-like proliferations were observed admixed with PIA lesions in one case of BPH.

Moderate to strong HSP90 expression was demonstrated in samples of BPH and PC, and in PIN and PIA lesions, while mild to moderate labelling was present in normal prostatic cells. Significantly fewer labelled cells were present in normal prostate (20–30% of cells were positive, grade III) (Fig. 1) than in BPH (Fig. 2) and PC. In all PCs and most cases of BPH (82.3%), the labelled cells comprised >50% of the entire cell population (grade IV), while 17.7% of samples of BPH contained 10–50% labelled cells (Table 1). The cytoplasmic distribution of HSP90 was predominantly perinuclear in normal cells, both perinuclear and diffuse in BPH, and mainly diffuse in PC, PIN and PIA. Nuclear labelling was observed

occasionally in normal tissue, with progressively more nuclear expression in BPH and PC (Fig. 3). A significant difference in nuclear score was observed between normal prostate and BPH (grade II, III, IV; $P < 0.01$), normal prostate and PC (grade II, III, IV; $P < 0.01$) and BPH and PC (grade II; $P < 0.01$). The metastases observed in one case of PC had labelling that was similar in distribution and score to that of the primary tumour (Supplementary Fig. 2). In PIA and PIN lesions, a high number of cells had nuclear labelling (Supplementary Fig. 3). The mean scores are summarized in Table 1.

The results of the present study have therefore demonstrated increased immunohistochemical labelling for HSP90 in canine PC cells compared with normal and hyperplastic prostates, suggesting a role for this protein in prostatic carcinogenesis and neoplastic progression, as in man (Bubendorf *et al.*, 1999; Cornford *et al.*, 2000; Akalin *et al.*, 2001; Zellweger *et al.*, 2005; Kurahashi *et al.*, 2007). High HSP90 expression has been reported in advanced human PC with high Gleason grade (Isaacs *et al.*, 2003; Lebret *et al.*, 2003; Solit *et al.*, 2003; Cardillo and Ippoliti, 2006); however, in the present cases HSP90 expression was not associated with any specific histological type of PC and the lack of follow-up information has hindered any correlation with biological behaviour and prognosis.

HSP90 is involved in regulation of the cell cycle (Helmbrecht *et al.*, 2000) and it can initiate cellular proliferation by affecting proteins necessary for cell growth (Pechan, 1991). This would account for the increased expression of the molecule in canine prostatic hyperplasia, as occurs in human prostate (Thomas *et al.*, 1996; Akalin *et al.*, 2001). Studies of mammary gland (Zagouri *et al.*, 2010) and endometrium (Wataba *et al.*, 2001) support the involvement of this protein in the pathogenesis of benign proliferative lesions, as HSP90 expression follows the continuum of epithelial cell transformation from precursors through pre-invasive to carcinomatous lesions in the mammary gland and from the proliferative phase of the menstrual cycle in the endometrium to pathological endometrial hyperplasia.

The high expression of this chaperone protein in PIA and PIN is similar to that observed in PC, suggesting that increased HSP90 expression is a relatively early event during prostatic carcinogenesis. Zellweger *et al.* (2005) and Elmore *et al.* (2008) have shown that HSP90 is differentially expressed in PIN compared with BPH and therefore HSP90 immunolabelling could minimize the likelihood that a small focus of intraepithelial neoplasia is overlooked in a microscopical field of hyperplastic tissue. The characterization of preneoplastic lesions in the dog is still under investigation, as demonstrated by the sparse literature on PIA (Rodrigues *et al.*, 2010; Toledo *et al.*, 2010) and PIN (Aquilina *et al.*, 1998; Leroy and Northrup, 2009). However, the occurrence of PIA and PIN in normal and hyperplastic tissues should be carefully considered when examining canine prostate excisional biopsies, even if the tissue is normal or the lesion benign.

In the present study, the neoplastic epithelial cells and cells in PIA and PIN lesions were characterized by increased nuclear immunoreactivity compared with normal and hyperplastic prostate. Approximately 3% of the intracellular HSP90 pool is found in the nucleus (Csermely *et al.*, 1998) and this chaperone protein can regulate several nuclear events, contributing to tumorigenesis (Trepel *et al.*, 2010). Among these, HSP90 regulates the activity of the heat shock transcription factor (HSF)-1 involved in cell survival under stressful conditions (Trepel *et al.*, 2010). In the nucleus, HSP90 is known to bind to BCL-6 with this complex suppressing the transcription of several tumour suppressor genes (Cerchietti *et al.*, 2009), and to regulate telomerase assembly and function (Holt *et al.*, 1999). Akalin *et al.* (2001) observed specific nuclear expression of HSP90 in human PC cells, suggesting that the protein remains associated with the functional telomerase enzyme (Forsythe *et al.*, 2001). Gebhard *et al.* (1999) found a positive correlation between HSP90 nuclear labelling and high expression of class I molecules of the major histocompatibility complex (MHC) in human breast cancer, with lack of HSP90 nuclear localization in tumours

with reduced MHC class I expression. Tumour cells with high MHC class I expression and susceptibility to cytotoxic T lymphocytes may escape apoptosis by a mechanism involving increased expression of nuclear HSP90 (Gebhard *et al.*, 1999). However, the most important nuclear event for prostate carcinogenesis regulated by HSP90 is the activity of steroid hormone receptors (SHRs), including AR. HSP90 is crucial for modulating SHR cellular location, protein stability, ability to bind ligand and transcriptional activity (Echeverria *et al.*, 2009). Androgens drive prostate tumour growth; hence many therapies aim to reduce the synthesis of circulating androgens and/or inhibit the AR itself (Jenster, 1999). In androgen-refractory prostate cancer, AR remains active despite androgen ablative conditions (Zegarra-Moro *et al.*, 2002). The mechanism of the apparent loss of hormone dependence is poorly understood; several models have been proposed, including changes in the intracellular trafficking of AR leading to ligand-independent nuclear importation or impairment of nuclear export (Feldman and Feldman, 2001). The ligand-dependent nuclear translocation of AR can be inhibited by the HSP90 inhibitor geldanamycin, suggesting a role for HSP90 in the nuclear importation of AR (Georget *et al.*, 2002). Saporita *et al.* (2007) have demonstrated that a geldanamycin derivative, 17-AAG, can prevent the ligand-independent nuclear localization of AR in C4-2 cells, a model for androgen-refractory prostate cancer cells, partially restoring androgen-dependent regulation of AR and thus potentially providing an improved efficacy of androgen ablation therapy.

In conclusion, we have demonstrated that HSP90 is consistently up-regulated in malignant canine prostatic epithelial cells, strongly supporting the canine model for further testing of HSP90-targeted cancer therapy. We believe that increased expression of this chaperone protein may have prognostic value in predicting prostate cancer or tumorigenic/metastatic potential in less aggressive disease. Further studies into tumour grade

and biological behaviour will be critical to understanding the role of this chaperone protein during prostate cancer progression.

References

- Akalin A, Elmore LW, Forsythe HL, Amaker BA, McCollum ED *et al.* (2001) A novel mechanism for chaperone-mediated telomerase regulation during prostate cancer progression. *Cancer Research*, **61**, 4791–4796.
- Aquilina JW, McKinney L, Pacelli A, Richman LK, Waters DJ *et al.* (1998) High grade prostatic intraepithelial neoplasia in military working dogs with and without prostate cancer. *Prostate*, **36**, 189–193.
- Bubendorf L, Kononen J, Koivisto P, Schrami P, Moch H *et al.* (1999) Survey of gene amplifications during prostate cancer progression by high-throughput fluorescence in situ hybridization on tissue microarrays. *Cancer Research*, **59**, 803–806.
- Cardillo MR, Ippoliti F (2006) IL-6, IL-10 and HSP-90 expression in tissue microarrays from human prostate cancer assessed by computer-assisted image analysis. *Anticancer Research*, **26**, 3409–3416.
- Centenera MM, Fitzpatrick AK, Tilley WD, Butler LM (2013) HSP90 still a viable target in prostate cancer. *Biochimica et Biophysica Acta*, **1835**, 211–218.

- Cerchietti LC, Lopes EC, Yang SN, Hatzi K, Bunting KL *et al.* (2009) A purine scaffold HSP90 inhibitor destabilizes BCL-6 and has specific antitumor activity in BCL-6-dependent B cell lymphoma. *Nature Medicine*, **15**, 1369–1376.
- Cornford PA, Dodson AR, Parsons KF, Desmond AD, Woolfenden A *et al.* (2000) Heat shock protein expression independently predicts clinical outcome in prostate cancer. *Cancer Research*, **60**, 7099–7105.
- Csermely P, Schnaider T, Soti C, Prohaska Z, Nardai G (1998) The 90-kDa molecular chaperone family: structure, function and clinical applications. A comprehensive review. *Pharmacology and Therapeutics*, **79**, 129–168.
- Echeverria PC, Mazaira G, Erlejman A, Gomez-Sanchez C, Piwien Pilipuk G *et al.* (2009) Nuclear import of the glucocorticoid receptor-HSP90 complex through the nuclear pore complex is mediated by its interaction with Nup62 and importin beta. *Molecular Cell Biology*, **29**, 4788–4797.
- Elmore LW, Forsythe R, Forsythe H, Bright AT, Nasim S *et al.* (2008) Overexpression of telomerase-associated chaperone proteins in prostatic intraepithelial neoplasia and carcinomas. *Oncology Reports*, **20**, 613–617.
- Eskew JD, Sadikot T, Morales P, Duren A, Dunwiddie I *et al.* (2011) Development and characterization of a novel C-terminal inhibitor of HSP90 in androgen dependent and independent prostate cancer cells. *BMC Cancer*, **11**, 468.

- Feldman BJ, Feldman D (2001) The development of androgen-independent prostate cancer. *Nature Reviews Cancer*, **1**, 23–45.
- Forsythe HL, Jarvis JL, Turner JW, Elmore LW, Holt SE (2001) Stable association of HSP90 and p23, but not HSP70, with active human telomerase. *Journal of Biological Chemistry*, **276**, 15571–15574.
- Gebhard B, Schutz G, Ecker RC, Steiner GE, Rudas M *et al.* (1999) MHC-class-I expression in human breast cancer correlates with nuclear localization of the 90 kDa heat-shock-protein. *Anticancer Research*, **19**, 5293–5297.
- Georget V, Terouanne B, Nicolas JC, Sultan C (2002) Mechanism of antiandrogen action: key role of HSP90 in conformational change and transcriptional activity of the androgen receptor. *Biochemistry*, **41**, 11824–11831.
- He S, Zhang C, Shafi AA, Sequeira M, Acquaviva J *et al.* (2013) Potential activity of the HSP90 inhibitor ganetespib in prostate cancer cells irrespective of androgen receptor status or variant receptor expression. *International Journal of Oncology*, **42**, 35–43.
- Helmbrecht K, Zeise E, Rensing L (2000) Chaperones in cell cycle regulation and mitogenic signal transduction: a review. *Cell Proliferation*, **33**, 341–365.
- Holt SE, Aisner DL, Baur J, Tesmer VM, Dy M *et al.* (1999) Functional requirement of p23 and HSP90 in telomerase complexes. *Genes and Development*, **13**, 817–826.

Isaacs JS, Xu W, Neckers L (2003) Heat shock protein 90 as a molecular target for cancer therapeutics. *Cancer Cell*, **3**, 213–217.

Jemal A, Siegel R, Xu J, Ward E (2010) Cancer statistics, 2010. *CA: a Cancer Journal for Clinicians*, **60**, 277–300.

Jenster G (1999) The role of the androgen receptor in the development and progression of prostate cancer. *Seminars in Oncology*, **26**, 407–421.

Kurahashi T, Miyake H, Hara I, Fujisawa M (2007) Expression of major heat shock proteins in prostate cancer: correlation with clinicopathological outcomes in patients undergoing radical prostatectomy. *Journal of Urology*, **177**, 757–761.

Lai CL, L'Eplatteneier H, Van den Ham R, Verseijden F, Jagtenberg A *et al.* (2008) Androgen receptor CAG repeat polymorphism in canine prostate cancer. *Journal of Veterinary Internal Medicine*, **22**, 1380–1384.

Lebret T, Watson RW, Molinie V, O'Neill A, Gabriel C *et al.* (2003) Heat shock proteins HSP27, HSP60, HSP70, and HSP90: expression in bladder carcinoma. *Cancer*, **98**, 970–977.

Leroy BE, Northrup N (2009) Prostate cancer in dogs: comparative and clinical aspects. *Veterinary Journal*, **180**, 149–162.

- London CA, Bear MD, McCleese J, Foley KP, Paalangara R *et al.* (2011) Phase I evaluation of STA-1474, a prodrug of the novel HSP90 inhibitor ganetespib, in dogs with spontaneous cancer. *PLoS One*, **6**, e27018.
- Morimoto RI, Tissieres A, Georgopoulos C (1990) *Stress Proteins in Biology and Medicine*. Cold Spring Harbor Laboratory Press, New York.
- O' Malley KJ, Langmann G, Ai J, Ramos-Garcia R, Vessella RL *et al.* (2012) HSP90 inhibitor 17-AAG inhibits progression of LuCaP35 xenograft prostate tumors to castration resistance. *Prostate*, **72**, 1117–1123.
- Pechan PM (1991) Heat shock proteins and cell proliferation. *FEBS Letters*, **280**, 1–4.
- Rodrigues MM, Rema A, Gartner F, Soares FA, Rogatto SR *et al.* (2010) Overexpression of vimentin in canine prostatic carcinoma. *Journal of Comparative Pathology*, **144**, 308–311.
- Romanucci M, Marinelli A, Sarli G, Della Salda (2006) Heat shock protein expression in canine malignant mammary tumours. *BMC cancer*, **6**, 171.
- Romanucci M, D'Amato G, Malatesta D, Bongiovanni L, Palmieri C *et al.* (2012) Heat shock protein expression in canine osteosarcoma. *Cell Stress and Chaperones*, **17**, 131–138.
- Rosol TJ, Tannehill-Gregg SH, LeRoy Be, Mandi S, Contag CH (2003) Animal models of bone metastasis. *Cancer*, **97 (Suppl. 3)**, 748–757.

- Saporita AJ, Ai J, Wang Z (2007) The HSP90 inhibitor, 17-AAG, prevents the ligand-independent nuclear localization of androgen receptor in refractory prostate cancer cells. *Prostate*, **67**, 509–520.
- Scaltriti M, Dawood S, Cortes J (2012) Molecular pathways: targeting HSP90 – who benefits and who does not. *Clinical Cancer Research*, **18**, 4508–4513.
- Solit DB, Scher Hi, Rosen N (2003) HSP90 as a therapeutic target in prostate cancer. *Seminars in Oncology*, **30**, 709–716.
- Sreedhar AS, Kalmar E, Csermely P, Shen YF (2004) HSP90 isoforms: functions, expression and clinical importance. *FEBS Letters*, **562**, 11–15.
- Stope MB, Sauermann A, Ronnau C, Zimmermann U, Walther R *et al.* (2012) Androgen receptor and heat shock proteins in progression of prostate cancer cells. *International Journal of Clinical Pharmacology and Therapeutics*, **50**, 65–67.
- Thomas SA, Brown IL, Holins GW, Hocken A, Kirk D *et al.* (1996) Detection and distribution of heat shock proteins 27 and 90 in human benign and malignant prostatic tissue. *British Journal of Urology*, **77**, 367–372.
- Toledo DC, Rodrigues Faleiro MB, Marcondes Pinto Rodrigues M, Di Santis GW, Laufer Amorim R *et al.* (2010) Caracterização histomorfológica da atrofia inflamatória proliferativa na próstata canina. *Ciência Rural, Santa Maria*, **40**, 1372–1377.

- Trepel J, Mollapour M, Giaccone G, Neckers L (2010) Targeting the dynamic HSP90 complex in cancer. *Nature Reviews Cancer*, **10**, 537–549.
- Waltering KK, Urbanucci A, Visakorpi T (2012) Androgen receptor (AR) aberrations in castration-resistant prostate cancer. *Molecular and Cellular Endocrinology*, **360**, 38–43.
- Wataba K, Saito T, Fukunaka K, Ashihara K, Nishimura M *et al.* (2001) Overexpression of heat shock proteins in carcinogenic endometrium. *International Journal of Cancer*, **91**, 448–456.
- Waters DJ, Bostwick DG (1997) The canine prostate is a spontaneous model of intraepithelial neoplasia and prostate cancer progression. *Anticancer Research*, **17**, 1467–1470.
- Zagouri F, Sergentis TN, Nonni A, Papadimitriou CA, Michalopoulos NV *et al.* (2010) HSP90 in the continuum of breast ductal carcinogenesis: evaluation in precursors, preinvasive and ductal carcinoma lesions. *BMC Cancer*, **10**, 353.
- Zegarra-Moro OL, Schmidt LJ, Huang H, Tindall DJ (2002) Disruption of androgen receptor function inhibits proliferation of androgen-refractory prostate cancer cells. *Cancer Research*, **62**, 1008–1013.

Zellweger T, Ninck C, Bloch M, Mirlacher M, Koivisto PA *et al* (2005) Expression patterns of potential therapeutic targets in prostate cancer. *International Journal of Cancer*, **113**, 619.

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Figure Legends

Fig. 1. Multifocal perinuclear HSP90 expression in normal canine prostate. IHC. ×600.

Fig. 2. Benign prostatic hyperplasia showing diffuse HSP90 expression. IHC. ×100.

Fig. 3. Strong and diffuse nuclear and cytoplasmic HSP90 labelling of prostate cancer cells. IHC. ×600.

Supplementary Figure Legends

Fig. 1. Preneoplastic lesions (proliferative inflammatory atrophy) randomly scattered in the canine prostate (arrows). Note the inflammatory cells infiltrating the surrounding tissue. HE. ×100.

Fig. 2. Prostate cancer metastatic to the lung with a high number of HSP90-positive cells. IHC. ×100.

Fig. 3. PIA foci showing epithelial cells with strong nuclear and cytoplasmic HSP90 expression. IHC. ×600.

Legend to Graphical Abstract

HSP90 expression increases as epithelium transforms from preinvasive to carcinomatous lesions in the canine prostate.

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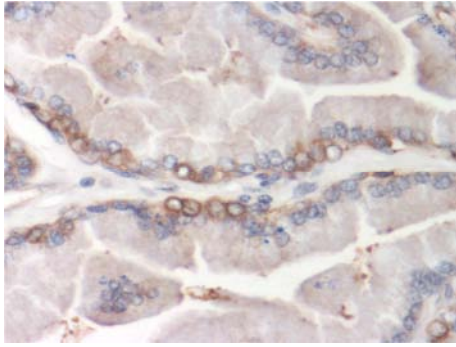
Table 1**HSP90 expression in normal, hyperplastic and neoplastic canine prostate**

	<i>Positive score</i>				<i>Nuclear-positive score</i>				<i>Perinuclear-</i>	<i>Diffuse</i>
	<i>(% of positive cells/total cells)</i>				<i>(% of cells with nuclear labelling/total positive cells)</i>				<i>positive score</i>	<i>cytoplasmic-positive score</i>
Grade	I	II	III	IV	I	II	III	IV	<i>(% cases)</i>	<i>(% cases)</i>
Normal prostate*	0	0	100	0	0	100	0	0	100	0
BPH*	0	0	17.7	82.3	5.9	23.5	35.3	35.3	23.5	76.5
PC*	0	0	0	100	0	0	40	60	0	100

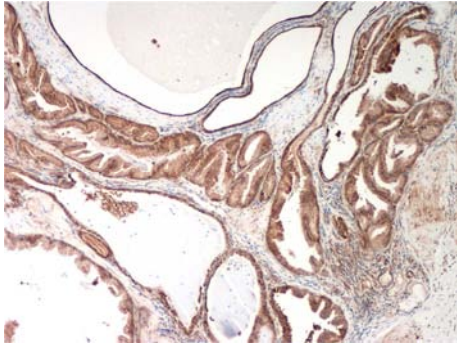
Grade: I, no labelling; II, up to 10% of cells labelled; III, 10–50% of cells labelled; IV, >50% of cells labelled.

*Percentage of cases/total cases

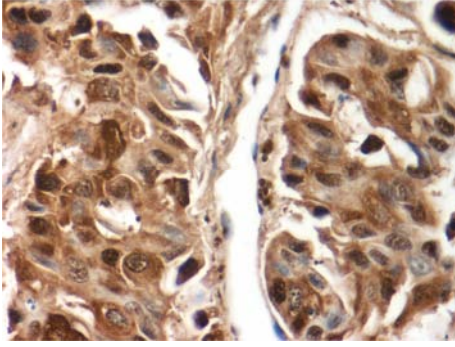
BPH, benign prostatic hyperplasia; PC, prostatic carcinoma



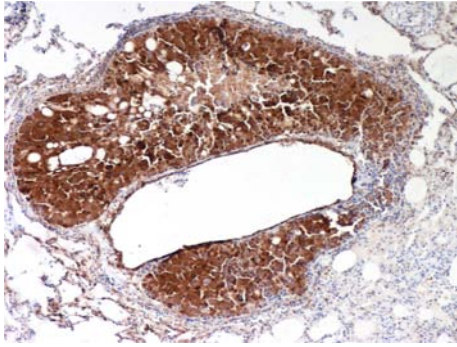
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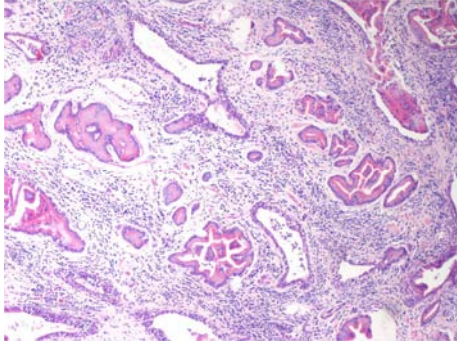
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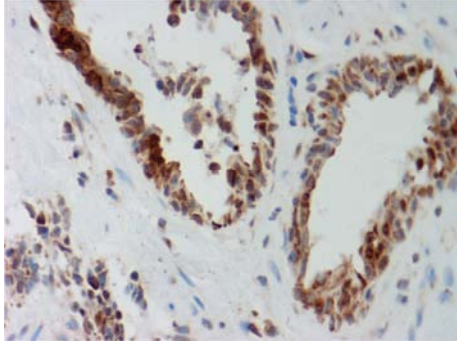
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