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Histopathological and Immunohistochemical Characterization of Canine Prostate Cancer

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BACKGROUND. In this study we try to identify the origin of canine prostate cancer (cPC) by classifying the tumors histological subtypes and relate these subtypes to their combined expressional characteristics of several tissue specific and differentiation markers.

METHODS. cPCs were examined histomorphologically and by immunohistochemical detection of the cytokeratin markers CK14, HMWCK, CK5, CK18, and CK7, and of the markers UPIII, PSA and PSMA.

RESULTS. Histopathologically, six growth patterns could be differentiated. The most frequent patterns were solid, cribriform and micropapillary growth patterns, while sarcomatoid, small acinar/ductal, and tubulo-papillary growth patterns were less frequent present. Solid growth patterns were significantly ($P = 0.027$) more often seen in castrated dogs. Immunohistochemically, about half of the cPC cases showed expression of PSA (8/20) and PSMA (10/20); 85% and 60% of the cPC expressed UPIII (17/20) and CK7 (12/20), while 13 and 12 cPC expressed CK5 and CK14, respectively; all cPC expressed CK18. CK14 was significantly more often and UPIII less frequent expressed in the solid growth patterns than in the micropapillary and cribriform patterns, respectively.

CONCLUSIONS. Canine prostate cancer appear to be more aggressive and of a less differentiated type than most common human prostate cancers. Comparing the expression patterns of the markers in cPC to those in normal canine prostate tissue, cPC most likely originates from the collecting ducts rather than from the peripheral acini. Given also the fact that canine prostate cancer is unresponsive to androgen withdrawal therapy, canine prostate cancer mostly resembles human, androgen refractory, poorly differentiated prostate cancer. *Prostate 68: 477–488, 2008.* © 2008 Wiley-Liss, Inc.

KEY WORDS: **PSMA; growth pattern; animal model**

INTRODUCTION

Apart from man the dog is the only species known to develop prostate cancer spontaneously. Interestingly, canine prostate cancer (cPC) shares several similarities with human prostate cancer (hPC). Both are most commonly found in the elderly patient [1], tumor growth outside the prostate is common and the distribution of distant metastases (bone and lung) is similar to that seen in humans [2,3]. In this respect, cPC may serve as a precious model for human prostate cancer, filling a gap between the rodent model studies and human clinical research. Characterization of cPC in relation to its human counterpart is therefore important, both in terms of morphologic features and in the identification of the cell of origin.

Canine PC has been reported to show heterogeneity in its histopathology. According to the classification of

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tumors of domestic animals made by the World Health Organization, two major types of cPC can be discerned, adenocarcinoma and poorly differentiated carcinoma [4]. Within the group of adenocarcinomas, intraalveolar and acinar subtypes are recognized. However, several independent studies reported the occurrence of additional histopathologic types of cPC including glandular, urothelial, squamoid, sarcomatoid, and discrete epithelial types [2,3,5]. A mixed morphology was often noticed, concurrently showing two or more different patterns [2,5]. None of these authors however, reported the actual distributions of these patterns and any possible relationships between them. Nor were these patterns compared to those commonly observed in hPC.

Another matter of debate is which type of cell is the origin of prostate cancer in the dog. In hPC studies cytokeratins (CK) have been used to characterize prostate epithelial cells as they express different CKs at different stages of epithelial development and differentiation [6–9]. Basal cells can be identified by the immunostaining with antibodies recognizing high molecular weight cytokeratins (HMWCK) including CK5 and 14, while luminal/secretory cells are stained by the antibodies to CK8 and 18. Both cell types have a low capacity of proliferation. A third group of cells, which co-express CK5 and 18, are regarded as intermediate cells. These intermediate cells have a short lifespan and a high proliferation rate [10–12].

In order to understand the pathogenesis of cPC it would be necessary to identify the cell of origin in this species. Mahapokai et al. [13] investigated the three different developmental stages of the prostate epithelium in the dog. In addition, in this study HMWCK positive basal cells were recognized as the major proliferative cell type in the neonatal and adult canine prostate. The acinar basal cells are highly proliferative, and disappear after castration, while the majority of ductal basal cells are seldom Ki67 positive, but remain present after castration [14]. As cPC is seen more often in castrated animals [15] these ductal HMWCK positive cells may be involved in the carcinogenesis of cPC [14]. Further attempts have been made to identify the cellular origin of cPC with the help of PSA and CK7, however without definite proof [16,17].

In the present study we try to identify the cellular origin of cPC by classifying the tumors by their histological subtypes and relating these subtypes to the expression patterns of different cytokeratins (HMWCK, CK14, CK5, CK18, CK7) and Uroplakin III, PSA and prostate specific membrane antigen (PSMA).

MATERIALS AND METHODS

Tissue Specimens

Formalin-fixed paraffin-embedded specimens from 20 dogs with spontaneous prostate tumors were collected from the archives of the Department of Pathobiology, Faculty of Veterinary Medicine, Utrecht University. These comprised tumors collected at necropsy from 11 castrated and 9 non-castrated dogs. The basis for inclusion of cases in the study were: (1) a histological diagnosis of prostate carcinoma in biopsy material or in prostatectomy material, and (2) availability of formalin-fixed, paraffin-embedded tissue blocks. Four micrometer sections were cut and stained with haematoxylin and eosin for histological examination. From each tissue block at least 10 consecutive sections of 3 μm were cut for immunohistochemistry.

Immunohistochemistry

Immunohistochemistry was performed using an indirect avidin–biotin–peroxidase staining procedure. The antibodies used are described in Table I. All incubations were performed at room temperature unless indicated otherwise. Following deparaffinization and rehydration of the sections, several antigen retrieval methods were used. For the antibody 34βE12, sections were incubated with 0.1% pronase (w/v in distilled water) (Roche Diagnostics, Almere, The Netherlands; catalog # 11459643001) at 37°C for

TABLE I. Monoclonal Antibodies Used in This Study

Antigens	Clone	Dilution	Source
Cytokeratins 1, 5, 10, 14 (HMWCK)	34βE12	1:50	DAKO, Capinteria
Cytokeratin 5	RCK103	1:5	Monosan, Uden, The Netherlands
Cytokeratin 7	OV-TL12/30	1:40	BioGenex, San Ramon
Cytokeratin 14	LL002	1:50	BioGenex
Cytokeratin 18	DC-04	1:200	Abcam, Cambridge, UK
Uroplakin III	AU-1	1:10	Progen, Heidelberg, Germany
PSA	polyclonal	1:150	DAKO, Carpinteria
PSMA	Y/PSMA1	1:40	Biodesign, Saco

10 min. For the antibodies recognizing PSMA, CK14, CK5 and CK18, antigen retrieval was achieved by submerging the sections in pre-heated 0.1 M sodium citrate (pH 6) and subsequent further heating in a microwave oven (700 W, near boiling) for 10 min and cooling for 20 min. For the antibodies UPIII and CK7, sections were incubated with ready-to-use proteinase K (DAKO Corporation, Carpinteria; catalog # S3020) at room temperature for 10 and 15 min, respectively. For the polyclonal antibody recognizing PSA, no antigen retrieval was necessary.

Then, endogenous peroxidase was neutralized by submersion of the slides in 0.3% H_2O_2 in 40% methanol-PBS for 30 min. After a short rinse with PBS the sections were pre-incubated with 10% normal goat serum (for anti-PSA) or normal horse serum (all other antibodies) for 15 min. Sections were incubated with the primary antibodies at 4°C overnight, using the antibody concentrations as indicated (Table I) in PBS or PBS with 10% normal goat serum (PSA staining). After washing the slides three times for 5 min in PBS/0.05% Tween, sections were incubated with biotinylated secondary antibody in PBS (for PSA: goat-anti-rabbit diluted 1:250, E0432, DAKO corporation; for all other antibodies: horse-anti-mouse diluted 1:125, BA-200, Vector Laboratories, Inc., Burlingame) for 30 min. Slides were washed three times for 5 min in PBS/0.05% Tween and incubated with peroxidase coupled AB complex (ABC Kit, Vector Laboratories) for 30 min as indicated by the manufacturer, and washed three times for 5 min in PBS. Peroxidase activity was then visualized by incubation with 3,3′diaminobenzidine (0.5 mg/ml in 0.05 M Tris (pH 7.6)/0.3% H_2O_2, Sigma–Aldrich Chemie B.V., Zwijndrecht, The Netherlands) for 10 min in the dark. Slides were then washed two times for 5 min in MilliQ, counterstained with haematoxylin, dehydrated, and mounted. As a negative control, primary antibodies were substituted with PBS. To evaluate the specificity of the antibodies, known positive tissues were used as controls. Canine skin was used as a positive control for the antibodies recognizing HMWCK, CK5 and CK14, while canine intestine was used as a positive control for CK18. For CK7 and UPIII, reactivity of normal urinary bladder epithelium was assessed, whereas human prostate tissues were used as positive controls for PSA and PSMA.

PSMA Expression

Isolation of total RNA and cDNA synthesis. As the expression of PSMA in the canine prostate has been questioned recently additional expression studies were undertaken [18]. Prostate tissue was collected from 10 control dogs, which had no disease related to the prostate, and from 11 dogs with prostate cancer. Tissues were snap frozen in liquid nitrogen and stored at −70°C until further use. Total RNA was isolated using the RNeasy kit (Qiagen, Leusden, The Netherlands) according to the manufacturers protocol. cDNA was synthesized from 0.5 μg of total RNA using the iSscript^{TM} cDNA synthesis kit (BioRad, Veenendaal, The Netherlands) according to the manufacturer's protocol.

Quantitative measurement of the PSMA mRNA levels. Dog-specific primers for PSMA were designed using the sequence of the canine folate hydrolase 1 isoform-1 (sFOLH1/PSMA; Gene ID: LOC476775), which is 89% homologous to human PSMA transcript. The sequences of the forward and reverse primers were 5′-GTGTTTGGTGGCATTGACC and 5′-TTCTG-CATCCCAGCTTGC, respectively.

QPCR reactions were performed in a total volume of 25 μl containing 12.5 μl 2× SYBR green super mix (BioRad), 1 μl of each primer at 400 nM, 1 μl cDNA and 9.5 μl RNase and DNase free water. The PCR was performed using a BioRad MyiQ detection system with SYBR green fluorophore according to a scheme of a primary denaturation (95°C, 2 min), followed by 40 cycles of amplification (95°C for 20 sec), annealing (58°C for 20 sec) and elongation (72°C for 20 sec). Specificity of the QPCR reactions was validated by performing a melting curve procedure. Canine HPRT and RPS5 were used as reference genes for normalization.

Statistics

Differences among groups were assessed by the chi-square or Fisher exact test. Differences in relative expression of PSMA mRNA in tumor and normal tissue were assessed using a Pair-wise fixed reallocation randomization test using the REST-XL software [19]. Differences were considered to be significant at $P < 0.05$.

RESULTS

Histopathology

Tumor growth throughout the whole prostate, extending from periurethral to (sub)capsular areas was seen in almost all cases. Capsular penetration was found in 8 of the 18 cases in which a complete prostate could be evaluated. In five of these cases tumor growth in tissues surrounding the prostate was found, including the tunica muscularis of the rectum. Vessel invasion by tumor cells was present in 8 cases.

We observed a remarkable variation in tumor growth patterns, both between and within individual cases of prostate cancer. In total, we were able to differentiate six different growth patterns:

(i) micropapillary, in which delicate papillary projections of neoplastic cells were formed within an extended duct (Fig. 1a);
(ii) cribriform, showing a duct completely extended by tumor cells with the formation of regular fenestrae (Fig. 1b), often accompanied with central necrosis ('comedonecrosis');
(iii) solid, a highly infiltrating, anaplastic undifferentiated carcinoma characterized by pleomorphic tumor cells arranged as solid nests or sometimes apparently individual cells within the stroma (Fig. 1c);
(iv) sarcomatoid, in which the tumor cells have a spindle like morphology with an irregular or fascicular orientation (Fig. 1d);
(v) small acinar/ductal, with variously sized microacini, arranged within a scirrhous looking fibromuscular stroma (Fig. 1e);
(vi) tubulo-papillary, dilated ducts comprising irregular, single to stratified layers of tall, columnar cells (Fig. 1f).

The distribution of the cPC patterns among the individual dogs is described in Table II. In short, the most common morphological patterns were solid, cribriform and micropapillary, which were observed in 13, 11, and 10 cases respectively. In addition, the mixed morphologies of cPC were mostly composed of these three patterns. In six prostates, solid, cribriform, and micropapillary patterns were concurrently present, another three cases showed mixed micropapillary and cribriform patterns and one case showed mixed micropapillary and solid patterns. Sarcomatoid, small acinar/ductal and tubular papillary patterns were seen less often, sometimes in combinations but often in combination with a solid growth pattern. In total, 15 out of the 20 cases (75%) of cPC showed mixed histological growth patterns of the tumor within the same cross-section.

Solid growth patterns were significantly ($P = 0.027$) more often observed in castrated dogs than in non-castrated dogs. No significant differences existed between the other histological types and castration status.

Apart from histologic growth patterns there was also a heterogeneity of cell types within these growth patterns. Mixtures of squamoid cells (Fig. 1g), mesenchymal cells and signet cells (Fig. 1h) could be found in all growth patterns.

Immunohistochemistry

A detailed description of the immunostaining results is presented in Tables II and III. In general, about half of the cPC cases showed expression of PSA (Fig. 2a) and PSMA (Fig. 2b), whereas 12 and 17 of the 20 cases showed expression of UPIII (Fig. 2c) and CK7 (Fig. 2d), respectively. Thirteen and 12 cases of cPC expressed CK5 (Fig. 2e) and CK14 (Fig. 2f) respectively, whereas all 20 cases of cPC expressed CK18 (Fig. 2g).

To better characterize cPC, we also analyzed the expression of the different markers per growth pattern. Starting with the most abundant growth pattern, 3 and 6 out of the 13 solid patterns expressed PSA and PSMA, respectively. All solid patterns expressed CK18, whereas seven out of the 13 solid patterns expressed CK14, CK5, or HMWCK (comprising of CK1, CK5, CK10, CK14; Fig. 2h). Nine and four of the 13 solid patterns expressed CK7 and UPIII, respectively. A comparable expression pattern was seen in the micropapillary and cribriform patterns, with the exception that there was a significant higher ($P = 0.03$) expression frequency of CK14 (7/11) in the solid pattern than in the cribriform pattern and a significant lower ($P = 0.007$ and $P = 0.01$) expression frequency of UPIII in the solid pattern than in the micropapillary and cribriform patterns, respectively. The other growth patterns were less abundantly present in our tissue sections, and no significant differences in expression of the markers could be detected. Remarkably, none of the sarcomatoid patterns expressed any of the investigated markers. The expression of the markers in the small acinar/ductal, and tubular papillary growth patterns grossly resembled those observed in the solid, micropapillary and cribriform patterns: PSA and PSMA were expressed in about half of the cases, CK18 expression was detected in all, and CK5, HMWCK and CK14 in decreasing numbers of the individual growth patterns. In addition, most small acinar/ductal, and tubular papillary growth patterns expressed CK7. None of the small acinar/ductal or tubular papillary growth patterns investigated expressed UPIII.

PSMA Expression

As the presence of PSMA in canine prostate tissues has been questioned recently [18], the PSMA expression was further investigated using quantitative RT-PCR. Expression analysis in a panel of 11 frozen prostate carcinomas and 10 control prostate samples revealed the presence of PSMA mRNA in all tissues with a mean threshold cycle (C_t) value of 28.84 for control tissues and 26.75 for carcinomas. After normalization to the house-keeping genes HPRT and RPS5 the expression in carcinomas was 5.2 ± 2.8 times higher in comparison to control tissue.

DISCUSSION

Canine cPC may serve as a precious spontaneous animal model for human prostate cancer. In order to understand the use and limitations of such a model

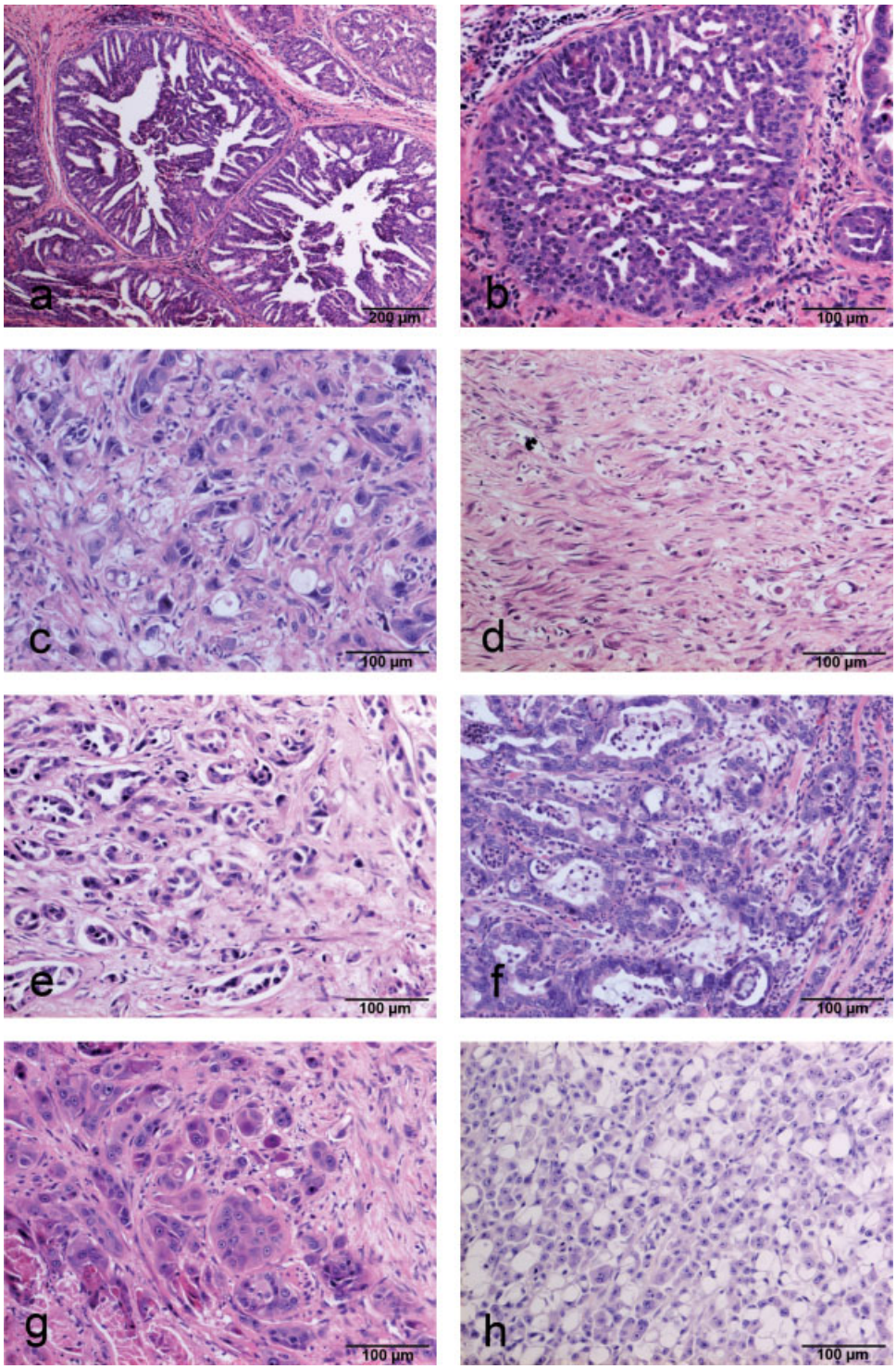

Fig. 1. Different histologic growth patterns found in canine prostate carcinoma. **a**: Micropapillary pattern, in which papillary projections are formed in duct-like structures (original objective: 4×, HE). **b**: Cribriform pattern, the tumor cells form regular fenestrae (original objective: 4×, HE). **c**: Solid pattern, pleomorphic tumor cells situated as solid nests or individual cells within the stroma (original objective: 10×, HE). **d**: Sarcomatoid pattern, tumor cells with a spindle shape like morphology (original objective: 10×, HE). **e**: Small acinar/ductal pattern, several micro-acini are arranged in fibromuscular stroma (original objective: 10×, HE). **f**: Tubulo-papillary pattern, some dilated ducts with single layer columnar cells, irregularly formed are present. In addition, an inflammatory reaction is present (original objective: 10×, HE). **g**: Squamous differentiation of tumor cells (original objective: 10×, HE). **h**: Signet cells with cytoplasmic vacuoles, suggesting urothelial differentiation (original objective: 10×, HE)

TABLE II. Details of Antigen Expression in Each Growth Pattern of Each Prostate Tumor

	Micropapillary	Cribriform	Solid	Sarcomatoid	Acinar/ductal	Tubular papillary
A (intact dogs)						
1	H, CK7, CK18, UPIII, PSMA	CK7, CK18, UPIII, PSMA	H, CK5, CK7, CK14, CK18, PSMA			
2					H, CK5, CK7, CK18	
3			CK7, CK14, CK18, PSMA	All negative	CK18, PSMA	
4	H, CK5, CK7, CK14, CK18, UPIII, PSMA	H, CK7, CK18, UPIII, PSMA				
5					CK5, CK7, CK14, CK18, PSA, PSMA	
6						CK7, CK18, (PSA*), (PSMA*)
7	H, CK5, CK7, CK18, UPIII	H, CK7, CK18, UPIII				
8	H, CK7, CK18, UPIII, PSA, PSMA	H, CK7, CK18, UPIII, PSA, PSMA	H, CK5, CK14, CK18			
9	CK5, CK7, CK18, UPIII, PSA, PSMA	CK7, CK18, UPIII, PSA, PSMA				
B (castrated dogs)						
1	CK5, CK7, CK14, CK18, UPIII, PSA	CK7, CK18, UPIII, PSA	H, CK5, CK7, CK14, CK18			
2	H, CK7, CK18, UPIII	CK7, CK18		All negative		
3	H, CK5, CK7, CK14, CK18, UPIII, PSA, PSMA	H, CK5, CK7, CK18, UPIII, PSA, PSMA	H, CK7, CK18, UPIII, PSA, PSMA			
4	CK5, CK7, CK18, UPIII, PSA		H, CK5, CK7, CK18, UPIII, PSMA			
5	CK5, CK7, CK14, CK18, UPIII	CK5, CK7, CK14, CK18, UPIII	CK5, CK7, CK18, UPIII			
6	CK7, CK18, UPIII	CK7, CK18, UPIII	H, CK5, CK7, CK14, CK18, PSA			
7			CK5, CK7, CK18, PSA, PSMA		CK7, CK18, PSA	CK14, CK18, PSA, PSMA
8			(CK14*), CK18			
9			(CK14*), CK18		CK18	
10			CK7, CK14, CK18, UPIII, PSMA		CK7, CK14, CK18, PSMA	
11			H, CK14, CK18			

H, HMWCK; (monoclonal*): missing value of that monoclonal.

TABLE III. Frequency of Expression of the Antigens in EachTumor and Each Growth Pattern

	Whole tumor (n = 20)	Micropapillary (n = 11)	Cribriform (n = 10)	Solid (n = 13)	Sarcomatoid (n = 2)	Acinar/ductal (n = 6)	Tubular papillary (n = 2)
HMWCK	11	6	4	7	0	1	0
CK5	13	7	2	7	0	2	0
CK14	12	4	1	7[b]	0	2	1
CK18	20	11	10	13	0	6	2
PSA	8	5	4	3	0	2	1[a]
PSMA	10	5	5	6	0	3	1[a]
CK7	17	11	10	9	0	4	1
UPIII	12	11	9	4	0	0	0

[a]One sample missing.
[b]Two samples missing.

however, it should first be thoroughly characterized and compared to human prostate cancer. In this study, we characterized 20 individual cPC cases in terms of histological growth patterns and their expression of several marker proteins.

Canine prostate tumors are very aggressive: the cancer lesion usually occupies the whole prostate, the tumor cells are often not confined within the natural boundary of the basal membrane, and we observed cancer cells invading the prostate capsule and surrounding tissues in a significant number of cPC cases. Although there is currently no Gleason like grading system to score the aggressiveness of canine prostate cancer, the majority of clinically diagnosed cPC cases would probably be graded highly malignant.

Previously, other authors have attempted to classify prostate cancer [2,4,5,20]. In the WHO classification, a distinction between adenocarcinoma and poorly differentiated tumors is made. In this classification, the adenocarcinomas are further subclassified as intraalveolar, which is the most common form, and acinar. Bell et al. [5] classified cPC in five groups, namely, intraalveolar, small acinar, syncitial, discrete epithelial and poorly differentiated. In his study, the small acinar type was the most frequent type in intact dogs, but in castrated dogs the only subtypes seen were intraalveolar and poorly differentiated. In the classification of Cornell et al. [2] tumors were classified as either adenocarcinoma, urethelial carcinoma, squamous-cell carcinoma or mixed morphology (including two or more types of differentiation: glandular, urothelial, squamoid, or sarcomatoid). In their study adenocarcinoma was the most common subtype (36%), although more than half of the cPC exhibited intratumoral heterogeneity. In many cases, primary tumors showed a mixed morphology, characterized by two or more growth patterns.

Our classification agrees with several of the findings of the above-mentioned authors in that we also observed alveolar and small acinar growth patterns. In our study however, the group of alveolar growth types is further refined and classified as a micropapillary, a cribriform and a tubular papillary growth pattern [21], based on differences in the histologic patterns and confirmed by differences in cytokeratin expression patterns. The previous studies in general simply categorized the rest of the morphological growth patterns as ''poorly differentiated'' or ''undifferentiated,'' which is insufficient to describe the distinct details and differences between these different growth patterns we observed. In addition, the classifications of the above mentioned authors all contain both descriptions of histological growth patterns and of cell morphologies. The presence of certain cell types, however, is not specific for the histological growth patterns. Mixtures of squamoid, mesenchymoid and signet cells were found within the solid growth patterns, but, for example, signet cells could sometimes also be observed in the other growth patterns. In short, our study (and that of Cornell et al. [2]) shows that canine prostate cancer (cPC) displays a high degree of morphologic heterogeneity, in terms of the number and combinations of growth patterns per tumor as well as of cellular morphology. Finally, castration leads to an increased appearance of less differentiated growth patterns in canine prostate cancer. In our study, solid growth patterns were significantly ($P = 0.027$) more often seen in castrated dogs than in non-castrated dogs, similar to Cornell et al. [2], who observed less adenocarcinomas in castrated dogs.

In humans, the majority (up to 95%) of prostate cancer is adenocarcinoma, mainly characterized by an acinar differentiation. Five to 10% of the histologic variants of prostate adenocarcinoma have distinctive

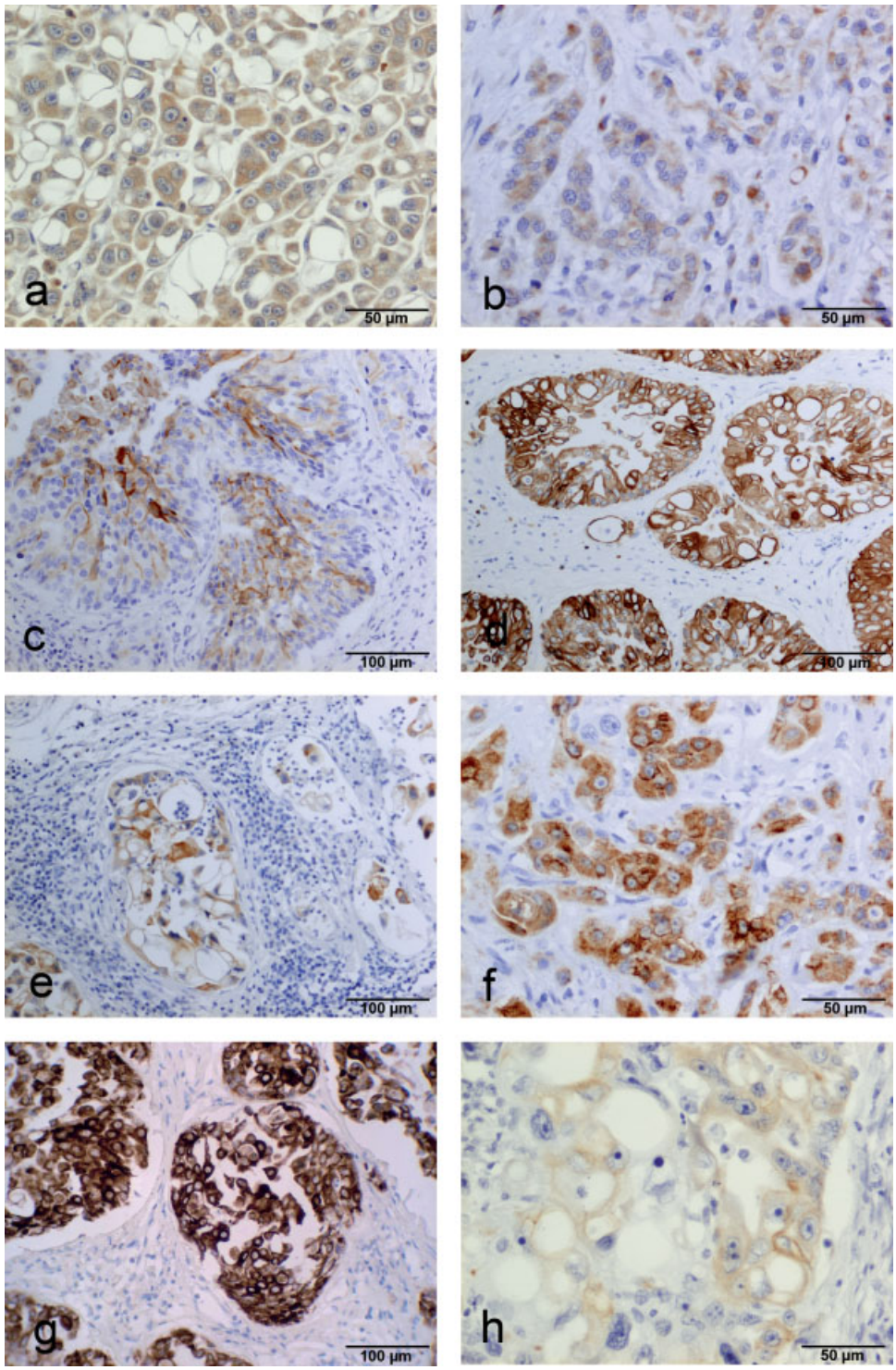

Fig. 2. Immunohistochemistry in canine prostate carcinoma. **a**: Clear PSA positivity in solid pattern of carcinoma with several signet cells (original objective: 20×). **b**: PSMA expression in small acinar/ductal pattern (original objective: 20×). **c**: UPIII expression in micropapillary tumor pattern (original objective: 10×). **d**: Strong CK7 expression in micropapillary growth pattern (original objective: 10×). **e**: CK5 expressed by tumor cells of small acinar/ductal pattern (original objective: 10×). **f**: The majority of the solid pattern tumor cells has a strong CK14 expression (original objective: 20×). **g**: Micropapillary tumor cells expressing CK18 (original objective: 10×). **h**: Irregular faint staining with HMWCK by tumor cells of solid pattern (original objective: 20×).

features, however [22]. Among these variants, Randolph et al. [22] differentiated sarcomatoid carcinoma, adenosquamous carcinoma, ductal carcinoma, and transitional cell carcinoma. Next to the adenocarcinomas, poorly differentiated carcinomas were recognized, of which the tumor cells are not confined to the acini. Differences in pathomorphological appearance of androgen-sensitive and androgen-refractory prostate cancer have also been reported in human prostate cancer. On basis of the WHO classification, Kondo et al. [23] classified the glandular (including large and/or small simple glands), micro-glandular, and cribriform growth patterns as androgen sensitive, while fused glands, medullary/solid and columnar/trabecular patterns were classified as androgen refractory components. Moreover, they reported that metastases tend to have androgen refractory components. In addition, Shah et al. [24] studied the characteristics of metastatic, hormone-refractory prostate cancer in terms of morphology, immunophenotype, and genotype. They concluded that androgen-independent prostate cancer is a heterogeneous group of diseases. The majority of cases showed a mixture of Gleason grades 4 and 5, including growth in solid sheets and nests with or without comedonecrosis, and confluent cribriform glandular patterns. This mixture of growth patterns seen in human androgen refractory prostate cancer remarkably resembles the mixture of growth pattern we have seen in canine prostate cancer.

About half of the investigated tumors expressed a positive reaction to PSA, although expression was only weak to mild. This is substantially more than what is found by others. McEntee et al. [25] reported a positive PSA reaction in 2 out of 31 canine prostate tumors, whereas Sorenmo et al. [17] found only 1 out of 58 cPCs to be positive for PSA expression. In humans, PSA has been used routinely in serum screening and immunohistochemical diagnosis of prostate disorders. In general, patients with prostate cancer show a higher PSA concentration in the serum, while PSA immunostaining intensity decreases in tumor cells with higher Gleason's grade [26–28]. Although several studies report immunoreactivity for human PSA in both the normal prostate and prostate cancer in the dog [17,25,29–31], so far no PSA could be found in the plasma of dogs with prostate cancer nor have PSA (hKLK3) orthologous genes been detected in the dog [31,32]. However, a gene encoding the prostatic arginine esterase has been identified as a canine ortholog of the related hKLK2 gene, and it carries the same conserved Androgen Responsive Elements directing prostate transcription as these genes [33,34]. Although the amino acid sequence homology between the canine arginine esterase and the human PSA is not very high (<60%), positive immunostaining with polyclonal human PSA antibodies may be attributed to conserved epitopes in both proteins [35]. In a previous study we found that in normal canine prostate strong positivity to human PSA is observed both in the acinar cells and in the ductal cells, even after castration, as well as a moderate expression in the urethral cells [36].

Besides PSA, PSMA is expressed at low levels in normal human prostatic epithelium but markedly increased in prostate cancer, and maintained in poorly differentiated tumors and prostate cancer metastases [37]. PSMA has therefore been used to identify cells of prostatic origin [38]. Although originally believed to be restricted to the prostate, recent studies have also demonstrated moderate PSMA expression in normal human urothelium and endothelial cells of tumor-associated neovasculature in several solid cancers [39].

In a comparative study of PSMA expression [18] it was stated that mice, monkeys and dogs do not express PSMA at all, making PSMA unique for humans, and these animals, including the dog, not suitable for investigating PSMA-activated intraprostatic prodrug therapies. However, in contrast to human PSA, a canine ortholog for human PSMA is clearly present in the canine genome. In our study, we found clear expression of PSMA transcripts in the canine prostate, that was enhanced in carcinomas by a factor 5. In addition, PSMA expression has also been shown by quantitative RT-PCR and western blot in several canine prostate cell lines [40].

The absence of a positive RT-PCR for PSMA in canine prostate tissue in the study of Aggarwal et al. [18] is most probably the result of the use of a reverse primer with a very low predicted melting temperature. They also only investigated normal canine prostate tissue in which the expression, as found by quantitative RT-PCR is some fivefold lower than in carcinomas. Immunohistochemically, PSMA could not be detected in the prostate of intact animals, and only weakly in the ductal and urothelial cells of castrated animals [36]. Half of the investigated cPC expressed detectable levels of PSMA protein, although this could not be associated to a histomorphological subtype or to the castration status of the dogs.

Seventeen (85%) and 12 (60%) out of the 20 prostate tumors expressed UPIII or CK7, respectively. A high percentage (79.3%) of CK7 expression in cPC has also been reported by Sorenmo et al. [17], but they did not associate the expression of CK7 with the histopathological growth patterns in the tumors. In our study we found a significant lower ($P = 0.007$ and $P = 0.01$) expression frequency of UPIII in the solid patterns compared to the micropapillary and cribriform patterns, respectively. In addition, we also observed some reduction of the CK7 staining frequency in these solid

patterns. This observation corresponds to previous studies concerning human urothelial carcinoma [41] and canine transitional cell carcinoma [42], in which it was mentioned that tumor cells of increasing invasiveness lose their expression of CK7 and UPIII. In human urogenital cancers, CK7 and UPIII have been used to identify the ductal and/or urothelial origin of tumor cells [43,44]. Mhawech et al. [44], showed that 27.5% of the human prostate tumors (and 86.6% of the urothelial carcinomas) shows CK7 positivity, while none of them expresses UPIII. In normal prostates of dogs, we [36] found that UPIII staining is restricted to the urethra, while CK7 is also expressed by the periurethral ductal cells. In normal prostates of castrated animals however, the vast amount of acinar cells disappear and the basal membranes of the remaining tubules are occupied by cells expressing CK7 and, to a lesser extend UPIII. Notably, castration increases the incidence of diagnosed prostate cancer [15]. Thus, our current and previous results taken together point to a ductal origin of canine prostate cancer, and underline an earlier suggestion by Leav et al. [14].

All canine prostate tumors, and more precisely, all growth patterns of cPC express CK18. This appears to indicate a predominance of differentiated cell types in the canine prostate tumors and parallels with observations in human prostate carcinoma [45]. CK5 and CK14 however, indicators for prostatic intermediate and basal cell types respectively, are also quite abundant in our canine tumors (65% and 60% of the tumors, respectively), and more often in the tumors of castrated animals. Since CK14 is also more abundantly expressed in solid growth patterns (compared to cribriform and micropapillary patterns) and solid growth patterns are seen more often in castrated animals compared to intact dogs, the conclusion may simply be that the less-differentiated growth patterns also contain more less-differentiated tumor cells. Markedly, in normal dog prostates, no CK14 expression was seen in intact dogs, while it could be observed in castrated animals [36]. This is in line with the notion that androgens are the major driver of differentiation of the prostatic epithelium [46]. Taken together, castration of dogs leads to a less differentiated prostatic epithelium [36], an increased risk of prostate cancer development [15], and an increase in less differentiated tumor types (this study), resulting in a higher content of stem cells and/or transiently amplifying cells. The induction of CK14 positive cells in the dog prostate may reflect a reduced drive towards differentiation of the epithelium.

Hormone refractory prostate cancer is the second leading cause of death due to cancer in western men. Most of these deaths are caused by hormone refractory prostate cancer for which an efficient treatment is urgently needed. A suitable animal model is important to develop an effective therapy. The aim of our study was to explore to what extend spontaneous prostate cancer of the dog provides such a model. In conclusion, we claim that our histomorphological classification is the most consistent and accurate presentation of the different subtypes of cPC to date, and that it provides an excellent basis for future cPC studies. In addition, canine prostate tumors appear to be more aggressive and of a less differentiated type than the most common, androgen withdrawal responsive, human prostate cancers. Canine prostate cancer is less differentiated in terms of histomorphology (solid, infiltrating growth patterns versus acinar type adenocarcinomas in humans) and cellular development (high percentage of CK14 and CK5 positive cells compared to hPC), and seems to originate from collecting ducts area rather than from peripheral acini. Given also the fact that canine prostate cancer is androgen withdrawal unresponsive [5], canine prostate cancer mostly resembles human, androgen refractory, poorly differentiated prostate cancer. Further studies may address genome wide expression changes involved in cPC in comparison to human (poorly differentiated) prostate cancer.

REFERENCES

1. Waters DJ, Patronek GJ, Bostwick DG, Glickman LT. Comparing the age at prostate cancer diagnosis in humans and dogs. J Natl Cancer Inst 1996;88(22):1686–1687.
2. Cornell KK, Bostwick DG, Cooley DM, Hall G, Harvey HJ, Hendrick MJ, Pauli BU, Render JA, Stoica G, Sweet DC, Waters DJ. Clinical and pathologic aspects of spontaneous canine prostate carcinoma: A retrospective analysis of 76 cases. Prostate 2000;45(2):173–183.
3. Waters DJ, Sakr WA, Hayden DW, Lang CM, McKinney L, Murphy GP, Radinsky R, Ramoner R, Richardson RC, Tindall DJ. Workgroup 4: Spontaneous prostate carcinoma in dogs and nonhuman primates. Prostate 1998;36(1):64–67.
4. Owen LN. TNM classification of tumors in domestic animals, Ed. 1 34 World Health Organization Geneva 1980.
5. Bell FW, Klausner JS, Hayden DW, Feeney DA, Johnston SD. Clinical and pathologic features of prostatic adenocarcinoma in sexually intact and castrated dogs: 31 cases (1970–1987). J Am Vet Med Assoc 1991;199(11):1623–1630.
6. Isaacs JT, Coffey DS. Etiology and disease process of benign prostatic hyperplasia. Prostate Suppl 1989;2:33–50.
7. Robinson EJ, Neal DE, Collins AT. Basal cells are progenitors of luminal cells in primary cultures of differentiating human prostatic epithelium. Prostate 1998;37(3):149–160.
8. Garraway LA, Lin D, Signoretti S, Waltregny D, Dilks J, Bhattacharya N, Loda M. Intermediate basal cells of the prostate: In vitro and in vivo characterization. Prostate 2003;55(3):206–218.
9. Schalken JA, van Leenders G. Cellular and molecular biology of the prostate: Stem cell biology. Urology 2003;62 (5 Suppl 1): 11–20.

10. van Leenders G, Dijkman H, Hulsbergen-van de Kaa C, Ruiter D, Schalken J. Demonstration of intermediate cells during human prostate epithelial differentiation in situ and in vitro using triple-staining confocal scanning microscopy. Lab Invest 2000;80(8): 1251–1258.
11. van Leenders GJ, Gage WR, Hicks JL, van Balken B, Aalders TW, Schalken JA, De Marzo AM. Intermediate cells in human prostate epithelium are enriched in proliferative inflammatory atrophy. Am J Pathol 2003;162(5):1529–1537.
12. van Leenders GJ, Schalken JA. Epithelial cell differentiation in the human prostate epithelium: Implications for the pathogenesis and therapy of prostate cancer. Crit Rev Oncol Hematol 2003;46 (Suppl):S3–S10.
13. Mahapokai W, Xue Y, van Garderen E, van Sluijs FJ, Mol JA, Schalken JA. Cell kinetics and differentiation after hormonal-induced prostatic hyperplasia in the dog. Prostate 2000;44(1): 40–48.
14. Leav I, Schelling KH, Adams JY, Merk FB, Alroy J. Role of canine basal cells in postnatal prostatic development, induction of hyperplasia, and sex hormone-stimulated growth; and the ductal origin of carcinoma. Prostate 2001;48(3):210–224.
15. Teske E, Naan EC, van Dijk EM, van Garderen E, Schalken JA. Canine prostate carcinoma: Epidemiological evidence of an increased risk in castrated dogs. Mol Cell Endocrinol 2002; 197(1–2):251–255.
16. LeRoy BE, Nadella MV, Toribio RE, Leav I, Rosol TJ. Canine prostate carcinomas express markers of urothelial and prostatic differentiation. Vet Pathol 2004;41(2):131–140.
17. Sorenmo KU, Goldschimdt M, Shofer F, Goldkamp C, Ferracone J. Immunohistochemical characterization of canine prostatic carcinoma and correlation with castration status and castration time. Vet Compar Oncol 2003;1(1):48–56.
18. Aggarwal S, Ricklis RM, Williams SA, Deanmeade SR. Comparative study of PSMA expression in the prostate of mouse, dog, monkey, and human. Prostate 2006;66(9):903–910.
19. Pfaffl MW, Horgan GW, Dempfle L. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res 2002;30(9):36.
20. Grieco V, Patton V, Romussi S, Finazzi M. Cytokeratin and vimentin expression in normal and neoplastic canine prostate. J Comp Pathol 2003;129(1):78–84.
21. Young RH, Srigley JR, Amin MB, Ulbright TM, Cubilla AL. Atlas of tumor pathology. Tumors of the prostate gland, seminal vesicles, male urethra, and penis. Armed Forces Institute of Pathology. Washington 2000; 69–288.
22. Randolph TL, Amin MB, Ro JY, Ayala AG. Histologic variants of adenocarcinoma and other carcinomas of prostate: Pathologic criteria and clinical significance. Mod Pathol 1997;10(6):612–629.
23. Kondo I, Miura T, Fujinami K, Satomi Y, Ida T, Ishizuka E, Uemura H, Noguchi S, Kubota Y, Hosaka M, Harada M. Comparative histological analysis of needle biopsy specimens, prostatectomized specimens and metastatic lymph nodes in prostatic adenocarcinoma—On the basis of the WHO histological classification. Hinyokika Kiyo 1997;43(2):97–101.
24. Shah RB, Mehra R, Chinnaiyan AM, Shen R, Ghosh D, Zhou M, Macvicar GR, Varambally S, Harwood J, Bismar TA, Kim R, Rubin MA, Pienta KJ. Androgen-independent prostate cancer is a heterogeneous group of diseases: Lessons from a rapid autopsy program. Cancer Res 2004;64(24):9209–9216.
25. McEntee M, Isaacs W, Smith C. Adenocarcinoma of the canine prostate: Immunohistochemical examination for secretory antigens. Prostate 1987;11(2):163–170.
26. Bjartell A, Paju A, Zhang WM, Gadaleanu V, Hansson J, Landberg G, Stenman UH. Expression of tumor-associated trypsinogens (TAT-1 and TAT-2) in prostate cancer. Prostate 2005;64(1):29–39.
27. Goldstein NS. Immunophenotypic characterization of 225 prostate adenocarcinomas with intermediate or high Gleason scores. Am J Clin Pathol 2002;117(3):471–477.
28. Xiao Q, Yin H, Lu Z, Meng K, Zhou X. Gleason histologic grading of prostate carcinoma in relation to serum PSA, PSA in situ and immunohistochemical expression of 34 beta E12 and P504S. Zhonghua Nan Ke Xue 2004;10(5):362–365.
29. Aumuller G, Seitz J, Lilja H, Abrahamsson PA, von der Kammer H, Scheit KH. Species- and organ-specificity of secretory proteins derived from human prostate and seminal vesicles. Prostate 1990;17(1):31–40.
30. Anidjar M, Villette JM, Devauchelle P, Delisle F, Cotard JP, Billotey C, Cochand-Priollet B, Copin H, Barnoux M, Triballeau S, Rain JD, Fiet J, Teillac P, Berthon P, Cussenot O. In vivo model mimicking natural history of dog prostate cancer using DPC-1, a new canine prostate carcinoma cell line. Prostate 2001;46(1): 2–10.
31. Bell FW, Klausner JS, Hayden DW, Lund EM, Liebenstein BB, Feeney DA, Johnston SD, Shivers JL, Ewing CM, Isaacs WB. Evaluation of serum and seminal plasma markers in the diagnosis of canine prostatic disorders. J Vet Intern Med 1995; 9(3):149–153.
32. Karr JF, Kantor JA, Hand PH, Eggensperger DL, Schlom J. The presence of prostate-specific antigen-related genes in primates and the expression of recombinant human prostate-specific antigen in a transfected murine cell line. Cancer Res 1995; 55(11):2455–2462.
33. Olsson AY, Lilja H, Lundwall A. Taxon-specific evolution of glandular kallikrein genes and identification of a progenitor of prostate-specific antigen. Genomics 2004;84(1):147–156.
34. Dube JY, Chapdelaine P, Guerin S, Leclerc S, Rennie PS, Matusik RJ, Tremblay RR. Search for androgen response elements in the proximal promoter of the canine prostate arginine esterase gene. J Androl 1995;16(4):304–311.
35. Chapdelaine P, Gauthier E, Ho-Kim MA, Bissonnette L, Tremblay RR, Dube JY. Characterization and expression of the prostatic arginine esterase gene, a canine glandular kallikrein. DNA Cell Biol 1991;10(1):49–59.
36. Lai CL, van den Ham R, van Leenders G, van der Lugt J, Teske E. Comparative characterization of the canine normal prostate in intact and castrated animals, Prostate (in press).
37. Horoszewicz JS, Kawinski E, Murphy GP. Monoclonal antibodies to a new antigenic marker in epithelial prostatic cells and serum of prostatic cancer patients. Anticancer Res 1987;7(5B): 927–935.
38. Fair WR, Israeli RS, Heston WD. Prostate-specific membrane antigen. Prostate 1997;32(2):140–148.
39. Gala JL, Loric S, Guiot Y, Denmeade SR, Gady A, Brasseur F, Heusterspreute M, Eschwege P, De Nayer P, Van Cangh P, Tombal B. Expression of prostate-specific membrane antigen in transitional cell carcinoma of the bladder: Prognostic value? Clin Cancer Res 2000;6(10):4049–4054.
40. van den Ham R, Lai C-L, van Wolferen ME, van Leenen D, Teske E, Mol JA. Canine and human prostate cancer derived cell lines share aberrant signaling pathways (submitted for publication).
41. Parker DC, Folpe AL, Bell J, Oliva E, Young RH, Cohen C, Amin MB. Potential utility of uroplakin III, thrombomodulin,

high molecular weight cytokeratin, and cytokeratin 20 in noninvasive, invasive, and metastatic urothelial (transitional cell) carcinomas. Am J Surg Pathol 2003;27(1): 1–10.

42. Ramos Vara JA, Miller MA, Boucher M, Roudabush A, Johnson GC. Immunohistochemical detection of uroplakin III, cytokeratin 7, and cytokeratin 20 in canine urothelial tumors. Vet Pathol 2003;40(1):55–62.
43. Mai KT, Ford JC, Morash C, Gerridzen R. Primary and secondary prostatic adenocarcinoma of the urinary bladder. Hum Pathol 2001;32(4):434–440.
44. Mhawech P, Uchida T, Pelte MF. Immunohistochemical profile of high-grade urothelial bladder carcinoma and prostate adenocarcinoma. Hum Pathol 2002;33(11):1136–1140.
45. Okada H, Tsubura A, Okamura A, Senzaki H, Naka Y, Komatz Y, Morii S. Keratin profiles in normal/hyperplastic prostates and prostate carcinoma. Virchows Arch A Pathol Anat Histopathol 1992;421(2):157–161.
46. van Leenders GJ, Aalders TW, Hulsbergen-van de Kaa CA, Ruiter DJ, Schalken JA. Expression of basal cell keratins in human prostate cancer metastases and cell lines. J Pathol 2001;195(5):563–570.