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Comparative Characterization of the Canine Normal Prostate in Intact and Castrated Animals

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BACKGROUND. Prostate diseases in the dog are generally regarded as representative for their human counterparts. We characterized the normal canine prostate in comparison to the normal human prostate.
METHODS. Prostates of dogs were examined histomorphologically and by immunohistochemical detection of the markers CK14, HMWCK, CK5, CK18, CK7, UPIII, PSA, and PSMA.
RESULTS. Histomorphologically, the canine prostate lacks the human zonal differentiation, has much more prominent acini, while comprising less stromal tissue. In general, the canine prostate epithelium displayed a highly differentiated character, with no cells expressing CK14, minimal amounts of cells expressing HMWCK/CK5 and the vast majority of cells expressing CK18 and PSA. After castration, the prostate epithelium regressed, and the remaining tubules were largely populated by cells showing a ductal phenotype (HMWCK+/CK5+/CK18+/CK7+).
CONCLUSIONS. The human and canine prostate are histologically differently organized. The general scheme of cellular differentiation of the prostate epithelium may however be applicable to both species. *Prostate 68: 498–507, 2008.* © 2008 Wiley-Liss, Inc.

KEY WORDS: **morphology; cytokeratins; uroplakin; PSA; PSMA; animal model**

INTRODUCTION

The dog is widely regarded as a spontaneous model for prostate diseases associated with aging, such as benign prostatic hyperplasia (BPH) and prostate cancer [1,2]. In fact, the dog is the only species other than man that naturally develops prostate cancer regularly [3–5]. Therefore, the dog has been used extensively as a model to study the biological behavior of these diseases as well as to develop effective treatments [1–3,6–10]. However, although the diseases in both species have several clinical aspects in common, not much is known about their comparative histology or the expression of markers like cytokeratins (CKs), prostate specific antigen (PSA), prostate specific membrane antigen (PSMA), and uroplakin III (UPIII) in the canine prostate. In order to make better use of the dog as a model for human prostate diseases, the similarities and differences between the normal prostates of the two species should first be investigated.

CKs are widely used to characterize and indicate several populations of cells within the human prostate. While basal cells are characterized by the expression of CK5 and CK14, the luminal epithelium highly expresses CK8 and CK18 [11,12]. Based on the expression pattern of these CKs, at least two intermediate cell populations have been identified. The first

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population is localized in the basal compartment and identified by the expression of CK5 in the absence of CK14. The second population is part of the luminal cell layer and expresses CK5 as well as CK8 and CK18 [12]. Besides the expression of CKs, luminal cells also show the presence of PSA and PSMA [13,14], while UPIII and CK7 have been used to differentiate between cancer of urothelial origin and prostatic origin [15,16].

In the dog, expression of CKs and PSA have mainly been analyzed with respect to prostatic disease [1,8,17–19]. As in man, Leav et al. [1] and Mahapokai et al. [8] used high molecular weight cytokeratin (HMWCK: CK1, CK5, CK10, CK14) stainings to identify the basal cells, and CK5 and CK18 to specify intermediate and secretory cells, respectively. Similarly, Landry et al. [20] used, among others, HMWCK expression to discriminate between the basal and secretory epithelial cells in hyperplastic dog prostates, whereas Grieco et al. [19] reported the expression of various CKs in the neoplastic canine prostate. LeRoy et al. [17] used CK7 expression to differentiate between an urothelial or prostatic origin of canine prostate carcinomas. Finally, Sorenmo et al. [21] used the expression of PSA to determine the prostatic origin of cells in canine prostate carcinoma, while Anidjar et al. [22] used PSMA to characterize the canine prostate cancer cell line DPC-1.

As indicated, the aforementioned studies discuss mainly the pathological conditions of the canine prostate and use normal dog prostate tissue only as a reference. This limits the use of these markers in the characterization of the canine prostatic pathologies as suitable models for their human equivalents, as it is not obvious whether the normal canine prostate is similar to the normal human one. Therefore, in this study we specifically examine the morphology as well as the expression of PSA, PSMA, UPIII, and the CKs CK5, CK7, CK14, and CK18 in normal prostate epithelia in sexually intact as well as castrated dogs, and emphasize the similarities and differences in comparison to the morphology and expression of these markers in the human prostate.

MATERIALS AND METHODS

Tissues

Prostate tissue was collected from eight intact (ages from 22 months to 9 years) and three castrated adult dogs (ages from 26 months to 12 years and 10 months), which were sacrificed for reasons not related to prostate disorders. Tissues were fixed in 10% formalin and processed through paraffin. Four micrometer sections were cut and stained with hematoxylin 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 specifically indicated. Following deparaffinization and rehydration of the sections, several antigen retrieval methods were used. For the antibody 34βE12 recognizing HMWCK, sections were incubated with 0.1% pronase (w/v in distilled water; Roche Diagnostics, Almere, The Netherlands; catalog # 11459643001) at 37°C for 10 min. For the antibodies recognizing PSMA, CK14, CK5, and CK18, antigen retrieval was achieved by submerging the sections in preheated 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. 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 preincubated

TABLE I. Monoclonal Antibodies Used to Stain the Canine Prostates

Antigens	Clone no.	Dilution	Manufacturer
Cytokeratins 1, 5, 10, 14 (HMWCK)	34βE12	1:50	DAKO Corporation
Cytokeratin 5	RCK103	1:5	Monosan, Uden, 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 Corporation
PSMA	Y/PSMA1	1:40	Biodesign, Saco

with 10% normal goat serum (for anti-PSA) or normal horse serum (all other antibodies) for 15 min. Sections were then incubated with the primary antibodies at 4°C overnight, using the antibody concentrations in PBS or PBS with 10% normal goat serum (PSA staining) as indicated (Table I). 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 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 hematoxylin, 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.

RESULTS

Intact Dogs

The canine prostate has a uniform morphology along the longitudinal axis and lacks a zonal differentiation based on glandular differentiation and epithelial cell morphology as in humans. Closely packed acini containing secretory epithelium constitute the major part of the canine prostate, from the periphery to the peri-urethral area. Here, several end ductal structures are embedded within the stroma (Fig. 1a). Although present, ductal structures can hardly be discerned in the periphery of the prostate when no specific staining is applied. The peri-urethral stroma extends dorsally and ventrally toward the outer boundary of the prostate and several less broad strings laterally, hereby forming several lobules of acinar epithelia. The urethra crosses the prostate just dorsal from the longitudinal central axis.

The secretory acini consist mainly of columnar epithelium with only few basally located stretched cells in the peripheral acini. The columnar cells of the prostatic acini gradually change to a single lining of cubic epithelial cells within the ductal structures. The cells of the urethra are variably sized, cubic, or columnar cells, arranged as simple or stratified epithelia.

In the acini, strong PSA positive staining was observed in the luminal cells of all eight animals. No expression of either PSMA or UPIII could be detected in acinar cells, whereas weak expression of CK7 in this area could be detected in the luminal cells of two animals. CK14 expression was not observed in the acini, whereas positive staining of HMWCK and CK5 was observed in scattered basal cells of a few acini in two and five prostates, respectively (Fig. 1b). CK18 expression was observed extensively in the luminal acinar cells of all eight prostates. Although ductal structures are hard to detect at the periphery of the prostate when no or other immunostaining procedures were used, prostatic ducts in this area could in half of the prostates be identified when stained with the CK5 antibody.

In the peri-urethral area, PSA positive ductal cells were present in six prostates whereas no expression of PSMA staining could be detected in the prostatic ducts of any animal. Staining for UPIII was observed in scattered cells of the peri-uretheral ducts in one prostate, which was also one of the four cases positive for CK7 in the peri-urethral ducts (Fig. 1c and d), and the only case positive for CK7 in the peripheral ducts. CK14 staining was never observed in the prostatic ducts. The antibody recognizing HMWCK stained the peri-urethral ductal cells of three prostates, while the CK5 immunostaining was seen in six out of eight animals. CK18 staining was detected in the ductal cells of all eight prostates.

The prostate markers PSA and PSMA could be observed in the luminal cells of the urethra in six and four out of the eight prostates of intact dogs, respectively (Fig. 1e and f). The urethral cells of all PSMA positive specimens were also positive for PSA. UPIII and CK7 staining was seen in the urethral luminal cells of eight and six out of eight prostates, respectively (Fig. 1g and h). Whereas CK7 staining was observed equally throughout these luminal cells, UPIII positivity was mainly restricted to the apical surface of the urethral luminal cells (Fig. 1g and h). CK14 expression was scarce and could only be detected in the basal cells of the urethra of one prostate. Positive staining for HMWCK and CK5 was present in the urethra of six and eight animals, respectively. Both markers intensively stained a continuous layer of basal cells. This intensity decreased toward the luminal cells of the urethra, and HMWCK was only expressed in the luminal cells of three of the eight prostates. Expression of CK18 was generally seen in the luminal urethral cells of seven prostates.

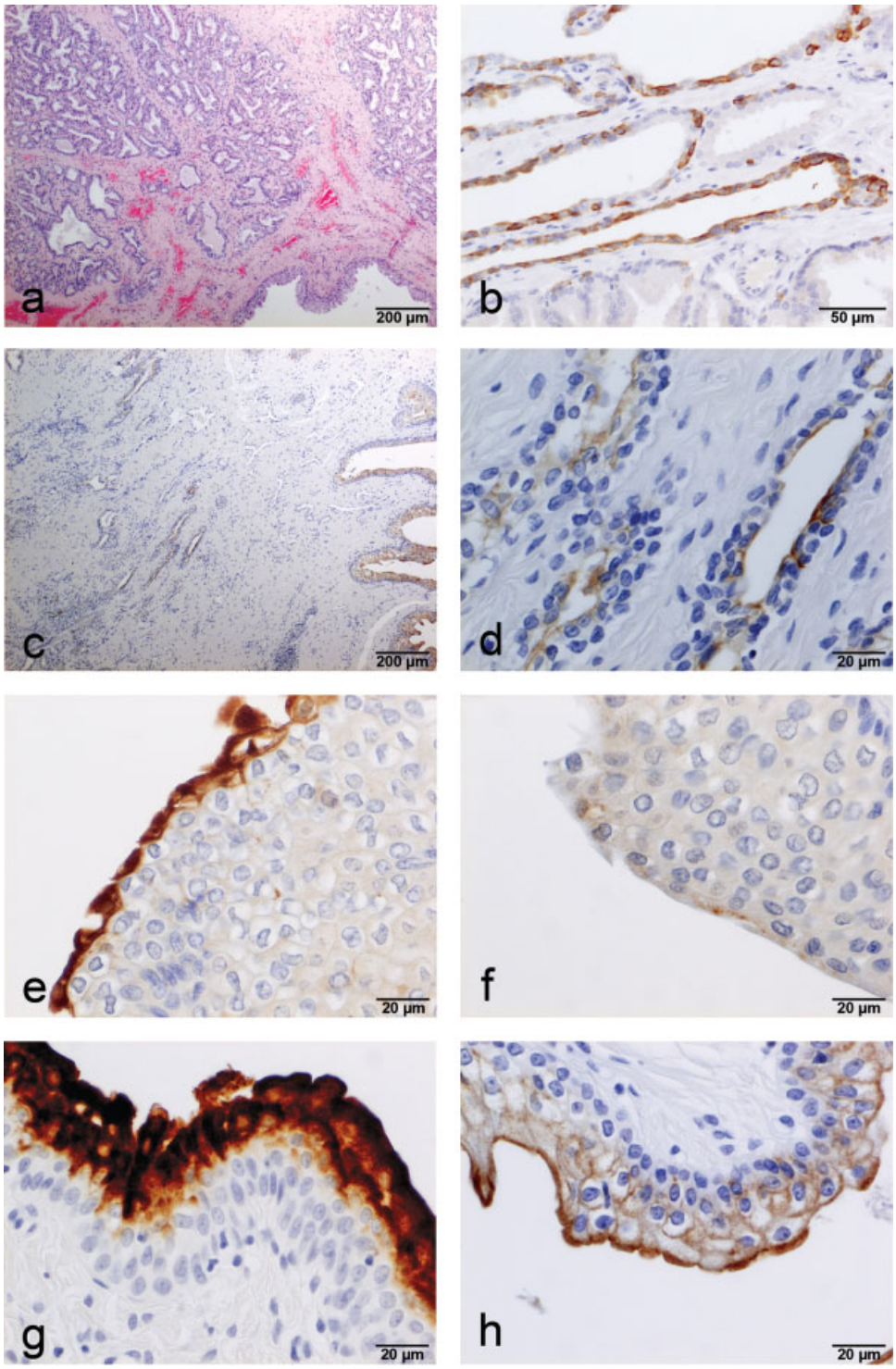

Fig. 1. **a**: Histology of a normal prostate of an intact dog (original objective: 4X, HE). **b**: CK5 stained the duct cells at the periphery of the gland (original objective: 20X). **c**: CK7 stained the cells of peri-urethral duct (original objective: 4X). **d**: High power view at the peri-urethral ducts of IC (original objective: 40X, HE). **e**: PSA stained the transitional cells of the prostatic urethra (original objective: 40X). **f**: PSMA stained scattered transitional cells of the prostatic urethra (original objective: 40X). **g**: UPIII stained the apical surface of the transitional cells (original objective: 40X). **h**: CK7 stained the transitional cells of the prostatic urethra (original objective: 40X).

TABLE II. Immunohistochemistry Results for Each Marker in the Prostate From Eight Intact Dogs

	Urethra		Peri-uretral duct	Peripheral duct	Acini	
	Basal	Luminal			Basal	Luminal
PSA	–	++(6/8)	++(6/8)	++(6/8)	–	++(8/8)
PSMA	–	+/−(4/8)	–	–	–	–
UPIII	–	++(8/8)	+(1/8)	–	–	–
CK7	–	+(6/8)	+(4/8)	+(1/8)	–	+/−[c](2/8)
CK14	+(1/8)	–	–	–	–	–
HMWCK	++(6/8)	+[a](3/8)	++(3/8)	–	+[b](2/8)	–
CK5	++(8/8)	+[a](8/8)	++(6/8)	+(4/8)	+(5/8)	–
CK18	–	+(7/8)	+(8/8)	+(7/8)	–	++(8/8)

Intensity: +/−, weak staining; +, positive; ++, strong staining.
[a]The staining intensity decrease gradually from the basal side toward the luminal side.
[b]Discontinuous basal layer.
[c]50% of acinar secretory cells revealed mild positive in one prostate.

The expression of the described markers in the normal prostate of intact dogs is summarized in Table II.

Castrated Dogs

After castration, the prostate gland shows atrophy characterized primarily by atrophy of the acini. In advanced atrophy only tubular structures with a single lining of epithelial cells remain in the prostate. Distinction between ductal structures and atrophic acini by light microscopy and morphological criteria of HE stained sections alone is then hardly possible (Fig. 2a). We will therefore further refer to these structures as ''tubules.'' The three prostates of castrated animals used in this study show an increasing degree of atrophy; whereas in the least atrophic prostate several acinar structures with multiple layers of cells could still be detected (Fig. 2b), only tubules with a single lining of epithelial cells remained in the two most atrophic prostates (Fig. 2c). However, the lobular structure of the prostates could still be recognized by the interstitial stroma. Unfortunately, one of the prostate samples from the castrated animals did not contain a urethra.

Staining for PSA was observed in the tubules of all three prostates, that is, in the single lining of tubular cells in the two most atrophic prostates and in the luminal lining of tubular cells in the least atrophic prostate. PSMA staining was present in the tubular cells of the two most atrophic prostates (Fig. 2d). UPIII staining was only found in the single lining of tubular cells in the peri-urethral area of two prostates. In the least atrophic prostate, CK7 positive staining was only observed in the tubules at the peri-urethral area, whereas in the two most atrophic prostates, CK7 staining was present in the majority of the tubular structures across the whole section (Fig. 2e). CK14 staining was found only in the scattered cells of the tubules at the periphery of the least atrophic prostate. HMWCK staining was seen in the tubules in the peri-urethral area in the least atrophic prostate, as well as in an estimated 20% and all tubules across the whole section of the two advanced atrophic prostates. CK5 positive staining was found scarcely in the basal lining of tubular cells in the least atrophic prostate and abundantly in the single lining of tubular cells in the other two prostates (Fig. 2f). In addition, the absolute number of HMWCK and CK5 positive cells per cross-section in the atrophied prostates appeared to be increased compared to their number in the intact dogs (Fig. 2g and h). CK18 was expressed in the single or luminal lining of tubular cells in all three prostates of the castrated dogs.

No morphological differences could be detected in the urethra of the castrated dogs when compared to those of the intact animals. Likewise, immunostainings of the urethra were generally similar to those observed in the intact animals. Staining of PSA, PSMA, UPIII, and CK7 appeared in the luminal cells of the urethra of the two dogs. CK14 expression could not be detected, whereas HMWCK was present in the basal layer of the urethra in one prostate and CK5 in the basal and luminal cells of the urethra in two prostates. CK18 expression was noticed in the luminal cells of both urethras.

DISCUSSION

Human and dogs share several similarities in their prostate disorders. The dog has therefore generally been regarded as a suitable animal model for the study of human prostate diseases. However, to better

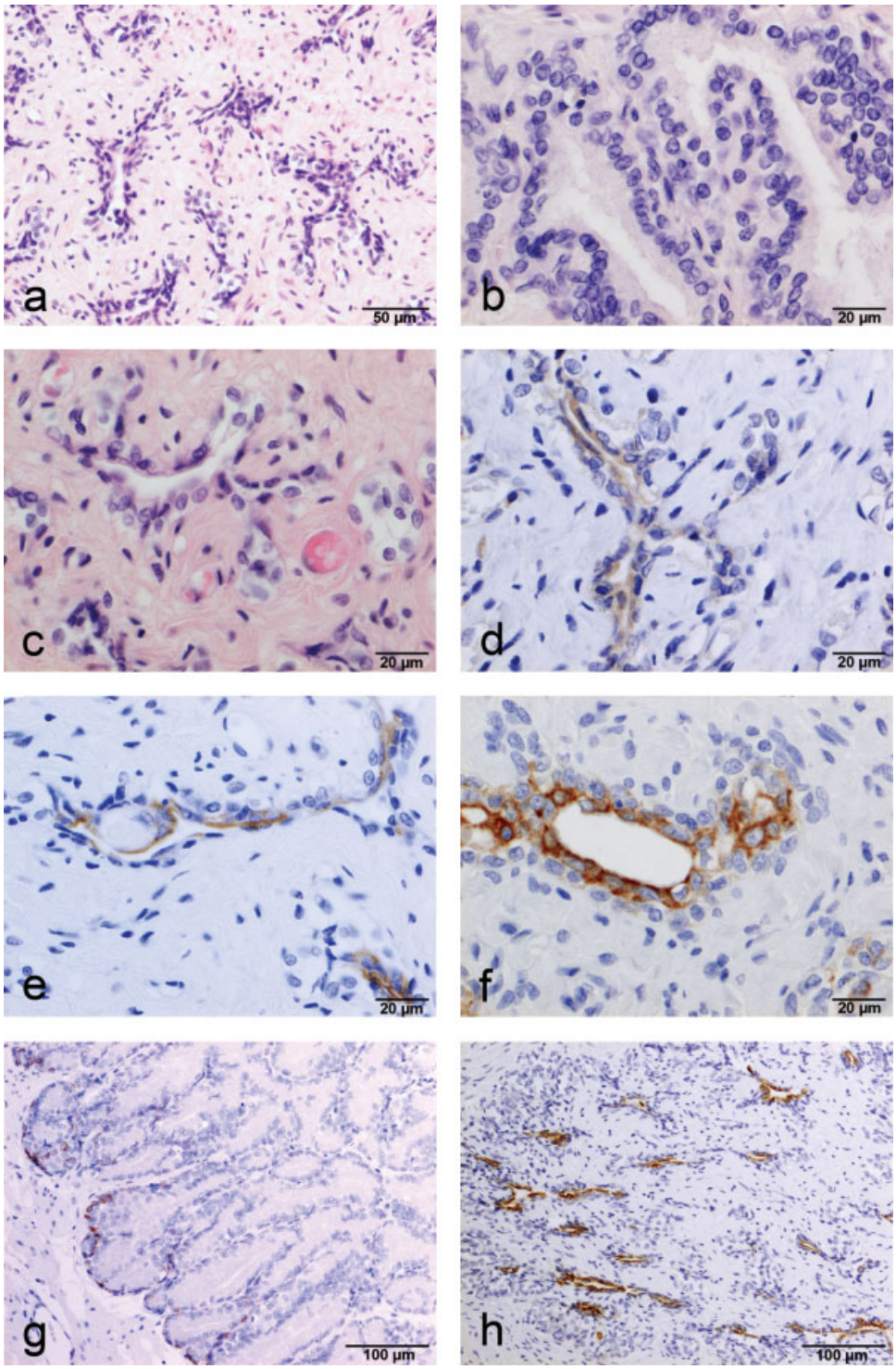

Fig. 2. **a**: Histology of the prostate of a castrated dog (original objective: 10X, HE). **b**: The least atrophic prostate kept multiple layers of epithelia (original objective: 40X, HE). **c**: The most atrophic prostate showed only single lining of the tubular structure (original objective: 40X, HE). **d**: PSMA stained the single lining of the tubule (original objective: 40X). **e**: CK7 stained the single lining of the tubule (original objective: 40X). **f**: CK5 stained the single lining of the tubule (original objective: 40X). **g**: CK5 stained scattered basal cells of the acini at the periphery of the gland from an intact dog (original objective: 10X). **h**: Increasing number of the CK5 positive stained cells in the prostate from a castrated dog (original objective: 10X).

understand the significance of the dog as a spontaneous animal model for prostate disease, detailed comparative characterization of the normal canine prostate in terms of histomorphology and immunohistochemistry is a prerequisite. Since androgen depletion, or castration, affects the initiation of prostate disorders of both men and dogs [23,24], additional characterization of the canine prostate after castration may further improve our understanding of the initiation of prostate diseases.

The human prostate is characterized by the presence of different zones (the peripheral zone, the transition zone, and the central zone), each of which has a characteristic normal histology and predisposition to developing a certain disease [25,26]. Glands of the peripheral zone have a simple, rounded shape, with gentle ripples of the luminal borders. Of the carcinomas, 70–75% arise in this zone. Glands of the transition zone are similar to that of peripheral zone, and about 15–20% of prostate carcinomas arise in this area, while it is the main source of BPH. Glands of the central zone are larger and are often arranged in lobules with luminal ridges and papillary foldings. The epithelium of the central zone usually has a granular cytoplasm. Only about 10% of carcinomas arise in the central zone [27].

The dog prostate lacks such zonal differences based on epithelial cell morphology, but rather displays a uniform morphology along the longitudinal axis. The canine prostate is organized with end ductal structures that surround the urethra from the peri-urethral area to the periphery of the gland, ending into secretory acini. The acinic structures in the dog are much more prominent in comparison to the human prostate, while there is less stromal tissue. As in humans, most dogs develop a certain degree of BPH with increasing age [28]. In contrast to man however, where BPH develops in a nodular fashion in the transition zone of the prostate, the condition in dogs affects the gland diffusely [29]. In addition, no preferential site of initiation of prostate cancer in the canine prostate has been mentioned in the literature.

The human prostate epithelium is morphologically composed of two cell layers. The basal layer consists of flat to triangular cells that are regarded as progenitors of the luminal epithelium. Luminal cells are cuboidal to columnar and secrete proteins such as PSA into the glandular lumina [30]. Basal cells are characterized by the expression of CK5 and CK14, while luminal epithelium highly expresses CK8 and CK18 [11,12]. Based on the expression pattern of these CKs, at least two intermediate cell populations have been identified. The first population is localized in the basal compartment and identified by the expression of CK5 in the absence of CK14. The second population is part of the luminal cell layer and expresses CK5 as well as CK8 and CK18 [12]. It is postulated that these gradual shifts in CK expression reflect physiological differentiation from basal cells (CK5/14) to terminally differentiated luminal cells (CK8/18) [9].

In the vast majority of canine prostates, we did not find any CK14 expression by the basal cells of either the ductal or the acinic structures of the canine prostate. Only one prostate, the least atrophic prostate from the group of castrated dogs, had scattered expression of CK14. Thus, although CK14 expressing cells do exist in the canine prostate, they are generally very scarce. This corresponds to the results of LeRoy et al. [17], but is in contrast to what is seen in the human prostate, where CK14 expressing cells are more prominent [11]. HMWCK and CK5 are, compared to CK14, somewhat more abundantly expressed in the canine prostate. Typically, we found expression of CK5 in scattered cells at the periphery of the acini. Leav et al. [1] investigated the role of the basal cells in the developing and the sexually mature dog. They found cell aggregations at the tips of canalizing ducts, radiating from the prostatic urethra in the developing prostate, that were destined to form the peripheral acini. In the mature prostate, they also reported the scattered basal cells in the acini at the periphery of the mature prostate that expressed HMWCK and the proliferation marker, KI-67. From this, they suggested that the HMWCK-stained basal cells constituted the major proliferative component of the canine prostate epithelium throughout life. Although the number of HMWCK positive cells in the human prostate is generally higher compared to that in the canine prostate, the proliferative characteristic of the intermediate/amplifying/HMWCK [8,9] positive cells seems similar. In general, the normal canine prostate has a more differentiated character compared to the human prostate, with less of the ''immature'' epithelial cells that finally differentiate to the CK8/18 expressing cells. The general scheme of prostate epithelial cell differentiation proposed by Isaacs and Coffey [31], however, seems also applicable to the canine prostate epithelium. The differentiated character of the epithelium, the dominance of acini and a relative lack of stromal tissue in the canine prostate compared to the human prostate account to the vast changes in the general morphology of the canine prostate seen after castration.

Several markers have been applied to differentiate the cellular origin of various tumors appearing in the prostate, such as PSA and PSMA to indicate prostate carcinomas [14,16], and UPIII or CK7 to identify transitional cell carcinomas [15,16]. Although PSMA is known as a prostate cancer marker in humans, its usefulness in canine specimens has never been examined. Immunoreactivity for human PSA has been reported in several studies in both normal prostate

and prostate cancer in the dog [32,33], but so far no genes have been detected in the dog that share a high homology with human PSA [34]. However, a gene encoding the prostatic arginine esterase has been identified as an ortholog to the progenitor of the PSA and hK2 genes, carrying the same conserved androgen responsive elements directing prostate transcription as these genes [35]. Positive immunostaining with human PSA antibodies may be attributed to conserved epitopes in both proteins. With regard to PSMA, immunoreactivity to PSMA has been reported before for a canine prostate carcinoma cell line (DPC-1) [36] and MDCK cells [37], although the gene for PSMA in the dog has not yet been published. In a recent study it was stated that dogs do not express PSMA at all [38]. However, in contrast to human PSA, a canine ortholog for human PSMA is clearly present in the canine genome. In a recent study using RT-PCR, we found clear expression of PSMA transcripts in the canine prostate, that was enhanced in carcinomas by a factor 5 [39]. In addition, PSMA expression has also been shown by quantitative RT-PCR and Western blot in several canine prostate cell lines [40].

As in humans [14,41], prostatic cells are positive to PSA, but negative to PSMA. In our experiments, however, both markers are moderately expressed in the urethral cells of the dog, disqualifying these two markers for the identification of a prostatic origin of tumors within the canine prostate. Although originally believed to be restricted to prostate, recent studies in humans have also demonstrated moderate PSMA expression in normal urothelium and endothelial cells of tumor-associated neovasculature [42]. The expression of the urothelial markers UPIII and CK7 was generally restricted to the urethra and the ducts in the peri-urethral area. Thus, UPIII and CK7 are, alone or in combination, good candidates to indicate a urethral or a ductal origin of canine prostate pathologies.

Castration status affects both the histomorphology and the expression of the investigated markers of the canine prostate. Prostate epithelia regress and the shape of acini changes to tubular formations with a single lining of epithelial cells, which makes it hardly possible to identify these tubules either to be regressed acini or pre-existing ducts. Androgen depletion causes mainly the depletion of CK18 expressing secretory cells from the acini [43]. This is also seen in our castrated animals. Apart of this, there was an increase in the number of HMWCK+ and CK5+ cells in the atrophic areas of the prostates of the castrated dogs when compared to their number in the intact dogs. This finding may either indicate a simple accumulation of intermediate/amplifying cells that are not capable of differentiating further to secretory cells, or an active regeneration of the epithelium, trying to restore its original number of secretory cells. Similar mechanisms of epithelial regeneration have been described for the dermis and the testis [44,45]. However, additional experiments will have to be performed to indicate such mechanisms in the canine atrophic prostate.

In addition to the increased number of HMWCK/CK5 expressing cells, the remaining tubules in the prostate of the castrated animals also showed the expression of CK7 across the whole prostate and UPIII in the tubules in the peri-urethral area. This indicates that these remaining atrophic tubules are not so much repopulated by cells from the pre-existing acini, but by cells with a ductal phenotype. The remaining tubules in the peri-urethral area are in turn populated by cells with a urethral phenotype. Whether these repopulation mechanisms involve active proliferation and/or migration of the ductal and urethral cells needs further investigation. The finding that the number of cells with a ductal phenotype appear to be increased in the prostate of castrated animals may connect the finding that castrated dogs are at greater risk of developing prostate cancer [23] and the postulation that canine prostate cancer originates from cells with a ductal phenotype [1].

CONCLUSIONS

In conclusion, the canine and human prostate differ to some extend in their histomorphology and the expression of several marker proteins. The canine prostate epithelium displays a much stronger differentiated phenotype compared to that in humans. The general scheme of prostate epithelial cell differentiation as proposed by Isaacs and Coffey [31] however, is most likely also applicable to the differentiation of the canine prostate epithelium. Finally, the finding that the remaining epithelial cells in the prostate of castrated animals display a ductal phenotype may have important implications to better understand the origin of canine prostate diseases.

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