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A Preliminary Study of the Baboon Prostate Pathophysiology

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BACKGROUND. Prostate cancer, benign prostatic hyperplasia, and prostatitis frequently affect men worldwide. At present there are no suitable animal models for these diseases. This study explores the potential use of the baboon as a model for prostatic diseases.
METHODS. Prostates of 48 baboons of different ages were studied. Prostate specific antigen (PSA) and alpha-methyl-acyl-CoA racemase (AMACR) were localized in the different lobes of the prostate by Western blotting and immunohistochemistry. PSA in baboon serum was demonstrated by radioimmunoassay and western blotting. Baboon AMACR cDNA was cloned and its expression assayed in baboon tissues.
RESULTS. The baboon prostate is anatomically and histologically similar to its human counterpart, with cranial and caudal lobes corresponding to central and peripheral zones of the human prostate. We found lymphocytic infiltration (91%), and sclerosing/atrophic lesions (34%). PSA tissue immunostaining intensity and alpha-methyl-acyl-CoA racemase (AMACR) gene expression levels differed between the cranial and caudal lobes of the prostate. The cloned baboon AMACR cDNA showed 96% homology with its human counterpart. Anti-human AMACR, PSA and basal keratin antibodies stained intracellular and basement membrane structures in the baboon prostate. The sclerosing/atrophic lesions were comparable to their human counterparts.
CONCLUSIONS. The similarity of baboon prostate to its human counterpart and the fact that human antibodies (AMACR, PSA, basal keratin) are reactive to baboon prostatic proteins indicates that the baboon is a promising model for human prostatic diseases. *Prostate 67: 1421–1431, 2007.* © 2007 Wiley-Liss, Inc.

KEY WORDS: **prostate cancer; PSA; BPH; AMACR; baboon**

INTRODUCTION

Common human prostatic diseases include prostate cancer, benign prostatic hyperplasia (BPH) and prostatitis. Prostate cancer is the most common cancer in American men, with 232,090 cases diagnosed annually resulting in 30,350 deaths [1]. BPH is a cause of nocturia and dysuria in men. This disease occurs in 8% of men between 31 and 40 years of age and approximates 90% by the age 90 [2,3]. Prostatitis is a common clinical disease in men. Its worldwide prevalence is between 2% and 10% [4]. In the U.S. it is estimated that 2 million

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outpatient visits per year are for prostatitis [5]. At present, there are three clinically important proteins used in the diagnosis and screening for prostatic diseases: prostate-specific antigen (PSA), alpha-methyl-acyl-CoA racemase (AMACR) and basal cell keratin.

Prostate cancer mortality has decreased in the U.S., probably as a result of widespread screening for PSA. However, there are problems with PSA as a screen for prostate cancer. It lacks sensitivity, with only 25–30% of men with an elevated PSA (>4 ng/ml) having prostate cancer on biopsy [6]. In addition, up to 15% of men with PSA <4 ng/ml will have prostate cancer if they get a biopsy [7].

AMACR is a mitochondrial and peroxisomal enzyme that is overexpressed in prostate cancer and is presently used as an immunohistochemical auxiliary test for prostate cancer [8]. Basal cell keratin antibodies stain positive in benign acinar lesions of the prostate and stain negative in adenocarcinoma. Basal cell keratin antibodies are therefore useful as an adjunct in the identification of prostate carcinoma because positive staining identifies a questionable focus as benign whereas negative staining helps to substantiate the diagnosis of carcinoma [9].

Presently there are no suitable animal models for either prostate cancer and/or BPH. Nonhuman primates are closer phylogenetically to humans than are other laboratory animals and therefore are considered to be suitable models for human prostatic diseases. Baboons (*Papio hamadryas*) are Old World monkeys. Only the anthropoid apes are more closely related to humans than are Old World monkeys. Baboons seem to be a good model for human prostatic diseases, not only because of their close evolutionary relationship but also due to their large size and habitual posture. Baboons spend long hours in a sitting position and occasionally practice bipedalism.

There are very few published reports of prostate cancer in nonhuman primates [10–12], although other spontaneous neoplasms have been reported in baboons and other nonhuman primates [13]. The relatively scant data may be due to the fact that most necropsies performed on nonhuman primates do not examine the prostate systematically. In regard to BPH, studies have indicated that baboons can be induced to develop BPH with the use of hormones [14] and that chimpanzees sometimes develop BPH spontaneously [15].

Southwest Foundation for Biomedical Research in San Antonio, Texas, has the world's largest colony of baboons for use in biomedical research, including a pedigree that has more than 2,000 living individuals. Some prostatic diseases have been reported to be familial in nature [16]; therefore a pedigreed colony is useful in the search for a suitable animal model.

The present study reports our initial findings on the baboon prostate. We discuss the anatomy, histology, pathology, and immunohistochemical study of the baboon prostate and also report on the molecular studies of AMACR and PSA, two genes that are of clinical importance to both prostate cancer and BPH.

MATERIALS AND METHODS

Tissue Collection

Baboon prostate tissue samples were collected from animals maintained at the Southwest National Primate Research Center, Southwest Foundation for Biomedical Research, in San Antonio, Texas. A total of 48 male baboons (*Papio hamadryas anubis)* were divided into four groups according to their developmental ages as follows: <1 year, 5–10 years, 10–20 years, and over 20 years. Baboons reach sexual maturity between 6 and 7 years of age [17]. At the Southwest Foundation for Biomedical Research (SFBR) male baboons are considered to be prime adult age males from about 9–15 and older adults from about 15–25 years of age. Infant mortality in all primates is high but if a baboon survives to age 5, then the average age at death is 11.10 ± 0.30 years. Only a small proportion of males (less than 5%) survive past 20 years of age [18]. After the animals had been humanely euthanized, the prostate was harvested and the cranial and caudal lobes separated from each other along a groove that is visible grossly and each was weighed. Caudal and cranial lobes of the prostate were cut in the coronal plane and samples fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned at 5 μm. The sections were stained with hematoxylin and eosin and evaluated by light microscopy. Some of the prostates were frozen at -70°C for further biochemical and molecular assays.

Immunohistochemistry

Immunohistochemical staining on formalin-fixed paraffin embedded prostate tissue sections was done in the Department of Pathology at the University of Texas Health Science Center at San Antonio, Texas (UTHSCSA) using an automatic immunostaining system (Ventana Medical Systems, Inc., Tucson, AZ). The following antibodies were used: PSA (monoclonal mouse anti-human PSA, clone ER-PR8, DakoCytomation, Carpenteria, CA), AMACR (rabbit anti-P504S antibody, Biocare Medical, Concord, CA) and Basal cell keratin (Keratin-903® clone 34βE12, Enzo Life Sciences, Inc., Farmingdale, NY).

Cloning of AMACR cDNA

Total RNA was extracted from thawed prostate tissue using Trizol reagent (Invitrogen, Carlsbad, CA).

RNA (1 μg) was reverse-transcribed at 42°C with Moloney murine leukemia virus (MMLV) reverse transcriptase and random decamers following the supplier's protocol (RETROscript kit, Ambion, Austin, TX). cDNA was stored at −20 C until required.

The cDNA of baboon AMACR was prepared by two rounds of touchdown polymerase chain reaction [19] (PCR) using Platinum® *Taq* DNA Polymerase High Fidelity (Invitrogen) and nested oligonucleotide primers shown in Table I. The primer sequences were based on the published rhesus monkey (*Macaca mulatta*) sequence. PCR products were characterized by agarose gel electrophoresis and cloned into the pCR2.1-TOPO vector (Invitrogen). Positive clones were identified by restriction enzyme analysis and nucleotide sequencing was carried out by the Advanced Nucleic Acid Core Facility at UTHSCSA.

Real-Time PCR for the AMACR mRNA

Quantitative reverse transcription (RT) PCR was carried out using TaqMan technology. Total RNA (1 μg) from baboon tissues was treated with RNase-free DNase-1 (DNA-*free*™, Ambion) to remove contaminating genomic DNA, and reverse-transcribed as above. Primers and probes for the quantitation of AMACR were designed using the Primer Express software (Applied Biosystems, Foster City, CA) and are shown in Table I. TaqMan 18*S* ribosomal RNA probe and primers (Applied Biosystems) were used as the internal control. Standard curves for 18*S* RNA and AMACR were prepared using a mixed sample of RNA from pooled tissues. For the assay, 5 μl of diluted cDNA samples or standards was added to wells of optical reaction plates containing the TaqMan Universal PCR 2X master mix (Applied Biosystems). Appropriate primers and probes (Table I) were added and the final volume was adjusted to 20 μl. All samples and standards were analyzed in triplicate. Real-time fluorescence-based PCR was conducted using ABI Prism 7900 real-time PCR thermal cycler under vendor-specified conditions. The values of the unknown samples were determined from the standard curves prepared in each assay, and the concentration of the AMACR transcript normalized to that of 18*S* RNA.

Immunoblotting

Baboon tissue samples were homogenized in sample buffer, separated by 12% SDS–PAGE, transferred to nitrocellulose membranes, and probed using standard conditions. Membranes were probed at room temperature for 1 hr for detection of AMACR with either a monoclonal mouse antibody to residues 139–229 of AMACR (cat no. 612082; BD Transduction Laboratories, San Jose, CA), or P504S AMACR antibody (Zeta Corporation, Sierra Madre, CA). A monoclonal mouse anti-human PSA, clone ER-PR8, (DakoCytomation) was used for detection of PSA. Secondary antibodies linked to horseradish peroxidase (Pierce, Rockford, IL) were added to the blots at a dilution of 1:5,000 and incubated for 1 hr at room temperature. Bound antibodies were detected by chemiluminescence (Supersignal West Pico, Pierce Biotechnology).

Serum PSA Assay

Serum was collected from baboons of different ages and submitted to either the University Health System Laboratory (San Antonio, TX) for the routine PSA test, ARUP Laboratories (Salt Lake City, UT) for the prostate specific antigen ultrasensitive test or AniLytics, Inc., (Gaithersburg, MD) for radioimmunoassays. Serum samples for assays were submitted within 6 months of collection and had been maintained at −20°C.

Serum PSA Immunoprecipitation

In order to establish presence of PSA in baboon serum, an immunoprecipitation method was used.

TABLE I. Oligonucleotides Primer Sequences

Primer	Exon	Sequence
AMACR cDNA cloning primers		
Outer forward		5′ GGCGTACTGAGGAGCGCCA3′
Outer reverse		5′ CTGTGGGCCTGGAAGTTAGA3′
Inner forward		5′ AGGAGCGCCATGGCACTGCA3′
Outer reverse		5′ GCCTGGAAGTTAGAGGCTAGCT3′
Demonstration of splice variant primer		
2E4FP	2	5′TGGCCACGATATCAACTATTTGG3′
4E5RP	5	5′GCTCATCTGATTGGGAAGTTCAT3′
AMACR real-time PCR and probe primer sequences		
AMACR forward		5′AAAATTGGCAGAAATGGTGAGAA
AMACR reverse		5′CAGCAAAATCAGCCAGGAGAT
AMACR probe		6FAM CCGTATGCCCCGCTGMGBNFQ

Monoclonal mouse anti-human PSA antibody was dialyzed against 0.1 M phosphate-buffered saline and thereafter used to immunoprecipitate PSA from baboon serum using the Seize® Primary Immunoprecipitation Kit (Pierce). The immunoprecipitated protein was then eluted using elution buffer and SDS–PAGE run followed by immunoblotting.

RESULTS

Anatomy and Histology

The baboon prostate is located distal to the urinary bladder and seminal vesicles but does not completely surround the urethra on the ventral side. It can be divided into two lobes along a groove that is visible grossly. The cranial lobe is closely associated with the seminal vesicles while the caudal lobe lies below the cranial prostate and is divided into two parts by the urethra. The texture of the cranial prostate is more nodular and resembles the seminal vesicle while the caudal prostate is smoother (Fig. 1). Histologically, the two lobes of the baboon prostate are distinctive. The cranial lobe is composed of larger irregularly shaped tortuous acini. The glands are lined by high columnar cells with nuclei located at different levels in the cells (Fig. 2). The caudal lobe of the prostate is composed of small regularly shaped acini. The glands are lined by simple cuboidal cells with basal oval shaped nuclei and are more widely separated by stroma (Fig. 2).

In this study, prostate weight correlated with body weight. Body weight increased steadily from birth to 10 years old and thereafter stabilized. Prostate weight also increased with age, although a plateau was not reached until the animals were 20 years old, and even after age 20 there were slight increases in prostate weight. The caudal prostate constituted 58%, 65%, 68%, and 66%; while the cranial prostate constituted 37%, 35% 32%, and 32% of the weight of the whole prostate in each of the age groups, respectively. Most of the increases in prostatic weight that took place as animals aged occurred in the caudal lobe (Table II, Fig. 3). This is similar to previous observations by others [14].

PSA Immunohistochemistry and Serum PSA Assay

Immunohistochemistry for PSA was done using a monoclonal mouse anti-human PSA clone ER-PR8 (DakoCytomation). In the caudal lobe of the prostate, strong PSA immunoreaction was detected in all prostate epithelial cells with no staining by stromal cells. However, in the cranial lobe PSA staining was very light and uneven in the epithelial cells (Fig. 4). PSA could not be detected in serum using the human-based diagnostic laboratory methods (ARUP or University Health System). However, PSA

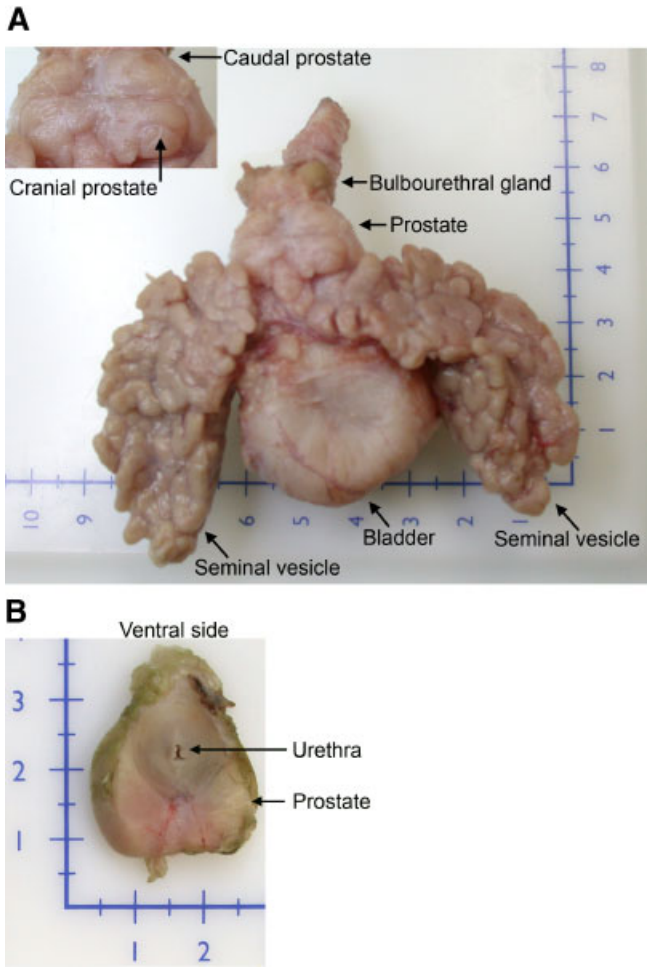

Fig. 1. **A**: Dorsal view of the baboon prostate showing the relationship of the prostate to the other urinogenital organs. Insert shows a closeup picture of the caudal and dorsal prostate. **B**: Gross section showing the position of the urethra in relation to the prostate in 5-year-old baboon. The tissue was fixed in 10% neutral buffered formalin and sectioned in the transverse plane. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

was detected in baboon serum using immunoprecipitation followed by Western blotting (Fig. 5). The presence of PSA in serum was further confirmed by the radioimmunoassay done by AniLytics, Inc. (Table III). Using a Western blotting method, PSA was abundant in the caudal prostate but barely detected in the cranial prostate (Fig. 5). In the caudal prostate, PSA was detected as two differently sized bands, an abundant one of approximately 36 kDa and faint one of a mass greater than 50 kDa. The PSA detected in serum had a higher molecular mass than that reported for free PSA (33 kDa). This can be attributed to the fact that PSA in serum circulates as a complex with other proteins (Fig. 5). The reason why PSA in serum was only detected by radioimmunoassay

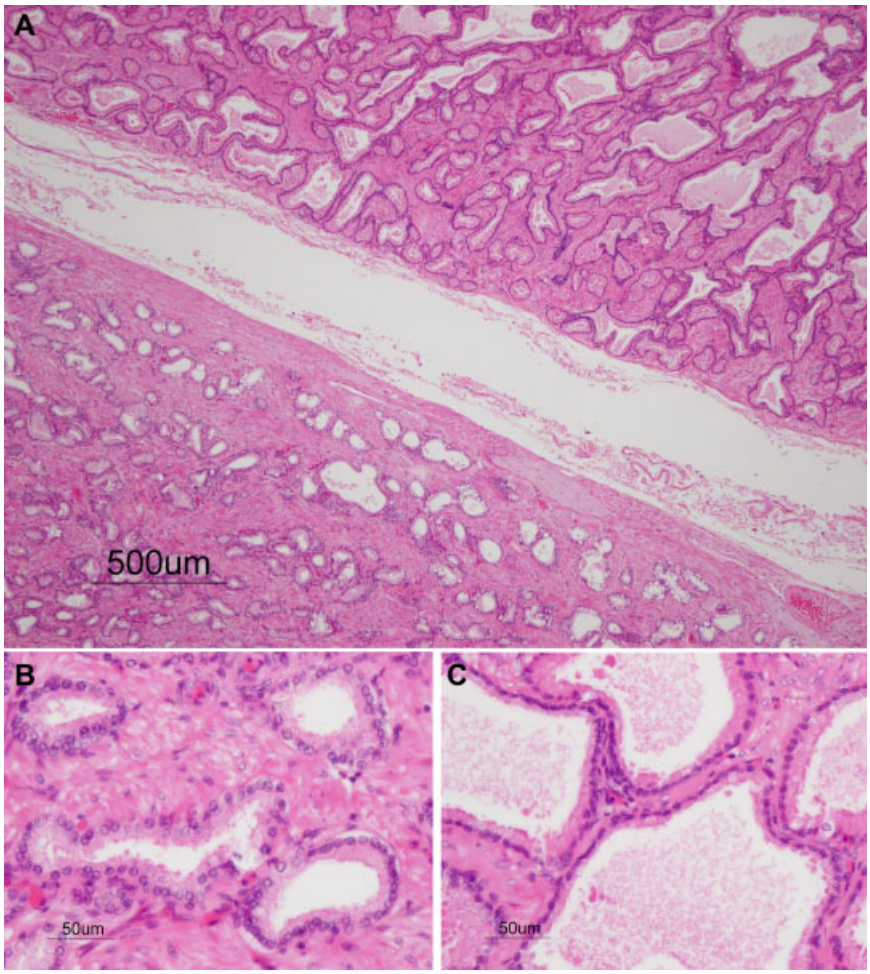

Fig. 2. **Panel A**: shows an H&E stained section taken at the junction of the caudal (upper) and cranial (lower) prostate 40×. **Panels B** and **C** show high power magnification of caudal and cranial prostate respectively 400×. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

and immunoprecipitation could be due to the very low levels of PSA in baboon serum. However, the possibility of the anti-human PSA antibody having lower affinity to baboon PSA cannot be ruled out.

Cloning of AMACR cDNA

In this study we cloned baboon AMACR cDNA transcript from the caudal prostate, liver, and kidney. The primers used were based on the rhesus monkey sequence. After two rounds of PCR, agarose gel electrophoresis indicated the presence of only one distinct band (≈1,200 bp) from both the kidney and liver samples (Fig. 6, Panel A). Cloning and nucleotide sequencing indicated that this product is alpha-methyl-acyl-CoA racemase (AMACR). The cloned baboon AMACR cDNA transcript is 96% identical to the reported human AMACR [20] and to

TABLE II. Body and Prostate Weight (Mean ± SD) of Baboons at Different Ages

	Age (years)			
	<1 (N = 13)	5–10 (N = 22)	10–20 (N = 7)	>20 (N = 6)
Body weight (kg)	1.16 ± 0.62	26.60 ± 4.10	28.47 ± 6.77	25.51 ± 3.07
Total prostate weight (g)	0.19 ± 0.05	7.37 ± 2.155	10.15 ± 2.87	10.40 ± 3.98
Cranial prostate weight (g)	0.07 ± 0.02	2.59 ± 0.92	3.22 ± 1.191	3.36 ± 2.82
Caudal prostate weight (g)	0.11 ± 0.05	4.78 ± 1.44	6.93 ± 1.76	6.82 ± 1.98

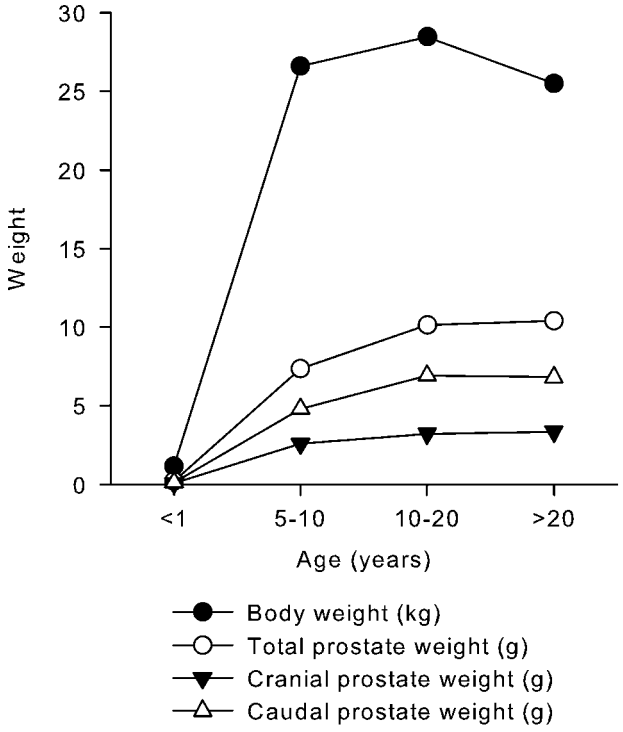

Fig. 3. Relationship between age and body weight, total prostate weight, caudal prostate weight, and cranial prostate weight.

the recently published rhesus monkey AMACR transcript variant 4 (GenBank accession number XM_001088403). The predicted baboon product is a 382-amino acid protein with molecular mass of 42 kDa and a pI of 6.36. The predicted protein is 93% homologous to its human counterpart (Fig. 7). The nucleotide sequence of the cloned baboon AMACR cDNA reported in this study is novel and has been deposited in GenBank under accession number EF405862. An attempt was made to determine whether the reported alternatively spliced variants of AMACR [21,22] are expressed in baboons. The human AMACR gene is made up of 5 exons. Because of alternative splicing of this gene, if primers are designed to span the whole cDNA, multiple bands of varying sizes will be seen on a gel. In the baboon sample, two bands were visualized on the gel (Fig. 6, Panel B). These results indicate that in addition to the main form of AMACR, at least one splice variant is expressed in baboons, albeit at very low levels. On ethidium bromide stained gels, this splice variant was only visualized in the kidney, and to some extent the colon. It was not found in other tissues, including the prostate (Fig. 6, Panel B).

Western Blot of AMACR

Two commercially available antibodies were used for immunoblot detection of AMACR in baboon

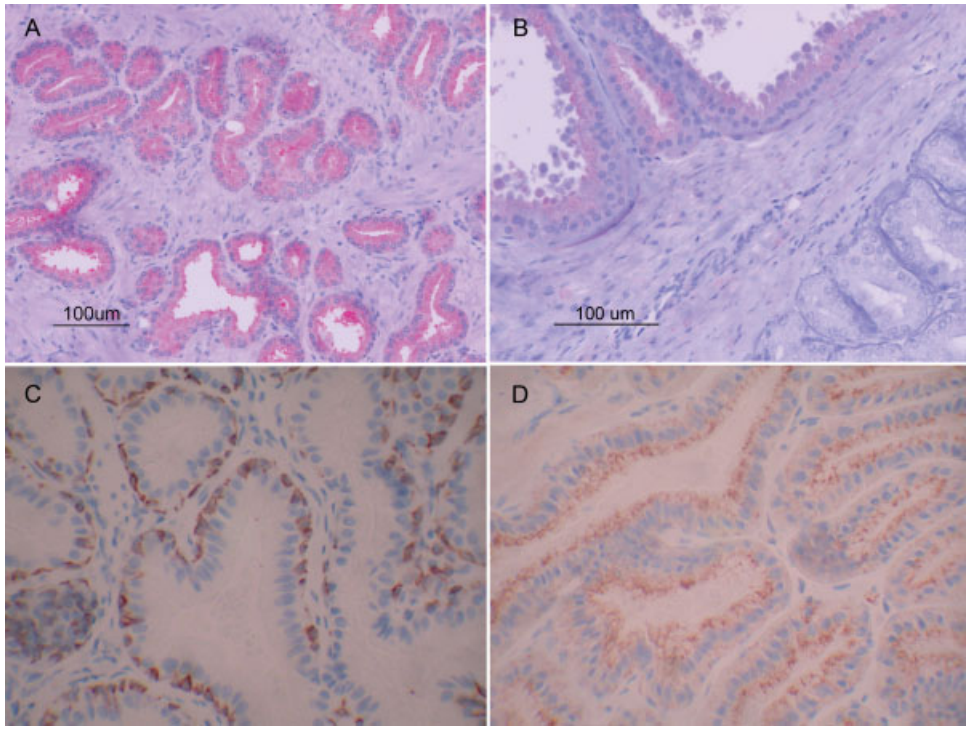

Fig. 4. Immunohistochemical staining on baboon prostate tissue sections using prostate specific antigen antibody (DakoCytomation), basal keratin, 343EI2 (Enzo Life Sciences) and AMACR P504S (Biocare Medical). PSA differential staining in the two lobes can be seen (**A** = Caudal prostate, and **B** = cranial prostate). Staining of basal cells is shown in (**C**) and AMACR staining is shown in (**D**) 400×.

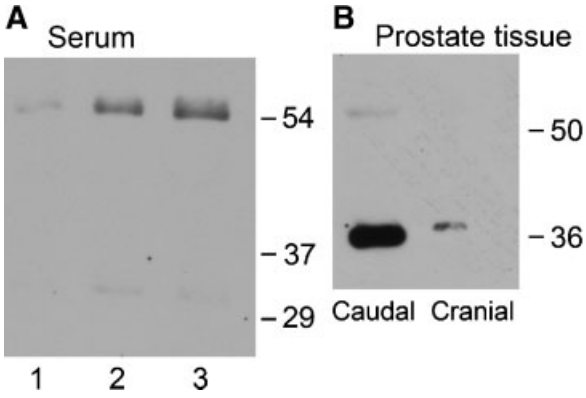

Fig. 5. PSA immunoblot of baboon serum and prostate tissue. **A**: Baboon serum was immunoprecipitated with PSA antibody (clone ER-PR8, DakoCytomation). The eluted proteins were then subjected to Western blot analysis with PSA antibody. **Lanes 1**, **2**, and **3** are separate elutes from an immunoprecipitation column. **B**: Prostate tissues were homogenized in sample buffer and equal amounts of protein loaded on SDS–PAGE gel followed by Western blotting. Differential protein expression is observed (B).

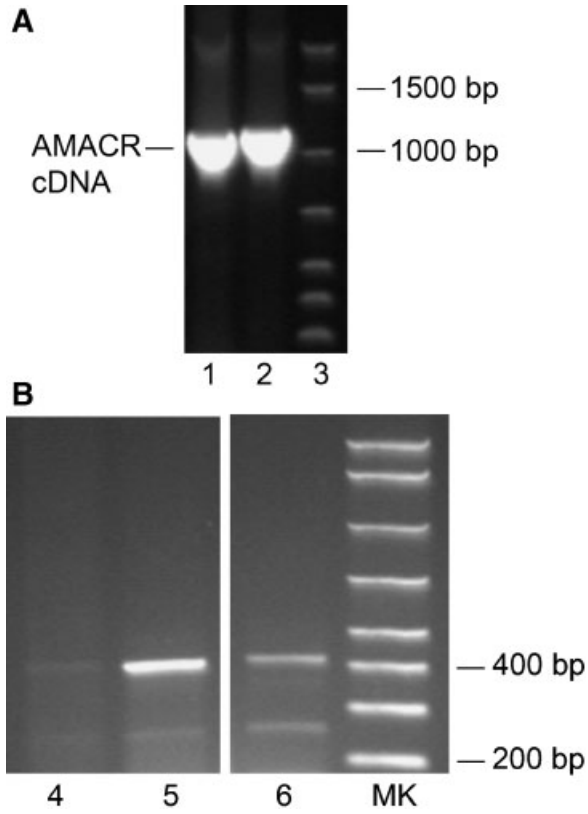

Fig. 6. **A**: Cloning of baboon AMACR cDNA. Ethidium bromide stained gel of PCR products from baboon liver (**lane 1**) and kidney (**lane 2**) after two rounds of touchdown PCR using primers that amplify the whole baboon AMACR cDNA. **B**: Ethidium bromide stained agarose gel demonstrates presence of an alternate splice variant of AMACR in baboon samples. Primers (2E4FP and 4E5RP) located in the exons 2 and 5 of the AMACR gene were used. **Lane 4**, baboon colon; **lane 5**, baboon kidney; **lane 6**, human prostate.

tissues. Antibody P504S is presently used clinically to aid in the diagnosis of prostate cancer, while the BD Transduction Laboratory antibody is used for research purposes only. Antibody P504S did recognize AMACR in human prostate cancer tissue but not in either normal baboon prostate or kidney. The antibody from BD Transduction Laboratories not only reacted to AMACR from human prostate cancer tissue, but also to AMACR from baboon kidney (Fig. 8).

Real-Time PCR

In order to study the expression of AMACR in baboon tissues, real-time PCR analysis was carried out. The results indicate that AMACR is mainly expressed in the colon, kidney, caudal prostate, and liver. While AMACR is barely expressed in the cranial prostate (Fig. 9), it is abundantly expressed in the caudal prostate.

TABLE III. Serum Prostate-Specific Antigen Values (ng/ml) Detected by Radioimmunoassay

Sample	Age (years)	PSA (ng/ml)
Baboon 1	23	0.30
Baboon 2	22	0.68
Baboon 3	26	0.63
Baboon 4	22	0.28
Baboon 5	22	0.24
Baboon 6	25	0.28
Baboon 7	26	0.3
Baboon 8	30	0.56
Baboon 9	24	0.59
Human	55	1.50

Pathology Lesions Found in Baboon Prostates

A total of 48 baboon prostates were studied. Most of the lesions were found in the caudal lobe. The most common lesions in the adult (over 6 years old) baboon caudal prostate were multifocal lymphocytic infiltration (91%), sclerosing/atrophic lesions (34%) and corpora amylacea (31%). The sclerosing/atrophic lesions consist of small glands, irregularly shaped, lined by cells with minimal cytoplasm lacking atypia and surrounded by dense stroma (Fig. 10). These sclerosing/atrophic lesions are likely comparable to those described as sclerosing/atrophic lesions in humans [23–25]. Basal cell keratin (keratin-903) studied by immunohistochemistry on the sclerosing/

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CLUSTAL W (1.82) multiple sequence alignment


Baboon AMACR    MALQGILVVELAGLAPGPFCAMVLADFGARVVRVERPGSHYDVSRLGRGKRSLALDLKQP
Human AMACR     MALQGISVVELSGLAPGPFCAMVLADFGARVVRVDRPGSRYDVSRLGRGKRSLVLDLKQP
                ****** ****:**********************:****:*************.******

Baboon AMACR    RGAAVLRRLCARSDVLLEPFRSGVMEKLQLGPEILQRDNPRLIYARLTGFGQSGSFSRLA
Human AMACR     RGAAVLRRLCKRSDVLLEPFRRGVMEKLQLGPEILQRENPRLIYARLSGFGQSGSFCRLA
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Baboon AMACR    GHDINYLALSGVLPKIGRNGENPYAPLNLLADFAGGGLMCVLGIMMALFERTRSGKGQVI
Human AMACR     GHDINYLALSGVLSKIGRSGENPYAPLNLLADFAGGGLMCALGIIMALFDRTRTGKGQVI
                *************.****.**********************.***:****:***:******

Baboon AMACR    DANMVEGTAYLSSFLWKTQKSSLWEAPRGQNILDGGAPFYTTYRTADGEFMAVGAIEPQF
Human AMACR     DANMVEGTAYLSSFLWKTQKSSLWEAPRGQNMLDGGAPFYTTYRTADGEFMAVGAIEPQF
                ********************************:****************************

Baboon AMACR    YELLIKGLGLKSDELPNQMSMDDWPEMKKKFAAVFAKKTKAEWCQIFDGTDACVTPVLTL
Human AMACR     YELLIKGLGLKSDELPNQMSMDDWPEMKKKFADVFAKKTKAEWCQIFDGTDACVTPVLTF
                ******************************** *************************:

Baboon AMACR    EEVVHHDHSKERGSFITNEEQSMSPRPAPLLSNTPAIPSFKRDPFVGEHTEEILDEFGFS
Human AMACR     EEVVHHDHNKERGSFITSEEQDVSPRPAPLLLNTPAIPSFKRDPFIGEHTEEILEEFGFS
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Baboon AMACR    REEIDQLKSDKIIESNKVKASL
Human AMACR     REEIYQLNSDKIIESNKVKASL
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Fig. 7. Protein alignment of predicted amino acid sequences of baboon and human AMACR. The alignment was carried out using the CLUSTAL W program [32]. Baboon AMACR amino acid sequence is 93% homologous to its human counterpart.

atrophic lesions showed uniform staining of the basal epithelial cells with greater intensity than was seen in the surrounding unaffected prostate. AMACR staining on the same sections showed small acini which over-expressed AMACR, but these glands lacked the morphological features of cancer such as prominent nucleoli. Periurethral glands also showed increased AMACR expression.

DISCUSSION

In this preliminary study we report data from the baboon prostate designed to establish the suitability of this animal as a model for prostatic diseases. Three prostatic diseases, namely prostate cancer, benign prostatic hyperplasia and prostatitis, are very common in the male population worldwide [1–4]. In fact, it is commonly said that if a man lives long enough he will have a prostate problem [26]. One main hindrance to our understanding of the pathogenesis of these diseases is a lack of a suitable animal model.

Our study shows that the baboon prostate is divided into two lobes which are easily identified grossly. These two lobes are labeled cranial and caudal depending on their proximity to the bladder and seminal vesicles. Histological studies have indicated that these two lobes are similar to the peripheral and central zones of the human prostate as described by McNeal [27]. In the human, the peripheral zone constitutes over 70% of the glandular prostate; a similar distribution (65–68%) was found in the adult baboon groups in our study (Table II).

In this study we report marked anatomic, histological and gene expression differences between the two lobes of the baboon prostate. Staining for PSA was robust in the caudal prostate while in the cranial prostate it was light and uneven. Alpha-methylacyl-CoA racemase (AMACR) mRNA expression was not detectable in the cranial prostate while it was abundantly expressed in the caudal part. Differential protein expression between different zones of the human prostate has also been reported [28,29]. Our report is the first one to document differential AMACR protein expression in different areas of the prostate. The clinical significance of AMACR differential expression is not clear. Results from AMACR cloning and Western blotting studies indicated that

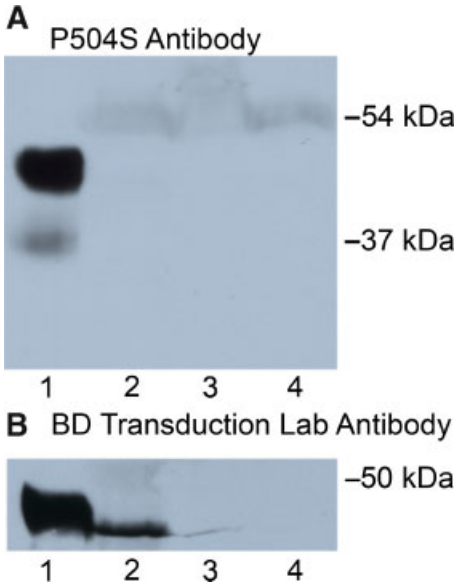

Fig. 8. Western blot analysis of baboon and human tissue extracts using AMACR antibodies P504S (**panel A**) and BD Transduction Laboratory (**panel B**). **Lane 1**, human prostate cancer tissue; **lane 2**, baboon kidney; **lane 3**, baboon caudal prostate; **lane 4**, baboon cranial prostate. P504S antibody is reactive only to prostate cancer tissue (panel A), while the BD Transduction Laboratory antibody is reactive to epitopes found in both prostate cancer and normal baboon kidney (panel B). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

baboon AMACR has a high homology to its human counterpart and that the commercially available human AMACR antibodies can be used successfully on baboon samples.

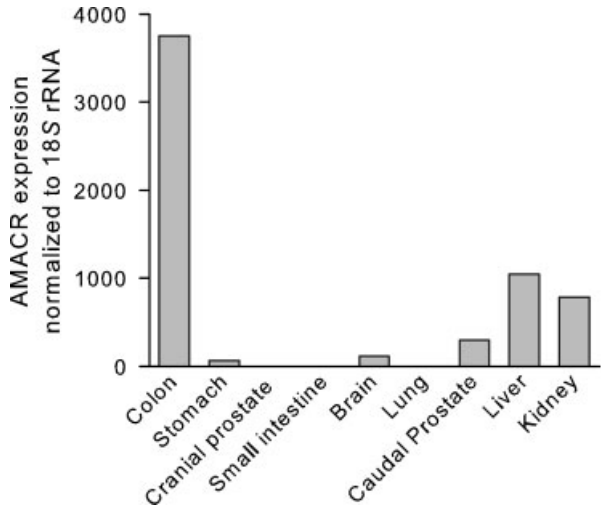

Fig. 9. Real-time PCR for AMACR mRNA performed on baboons tissues. Individual tissue samples were reverse-transcribed and AMACR mRNA quantitated using primers and probes described in Table I. Relative levels of AMACR were normalized to the 18S RNA levels in the same samples.

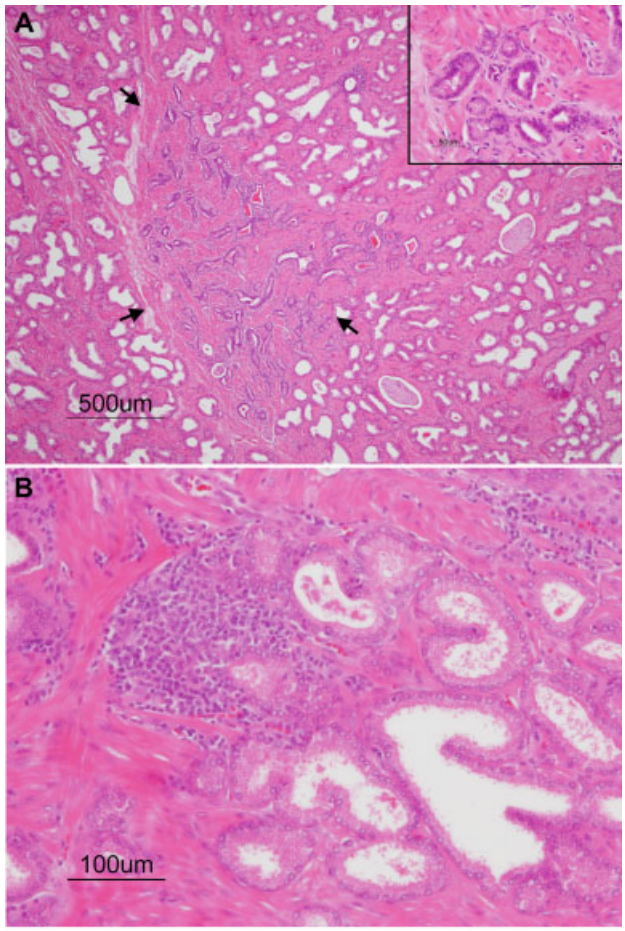

Fig. 10. Lesions commonly found in adult baboon prostates. **Panel A**: H–E stain of a sclerosing/atrophic lesion (arrows) in a 15-year-old baboon (100×). The insert in panel A shows a high magnification of the lesion. **B**: H–E stain showing lymphocytic infiltration in 5-year-old baboon (200×). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Our data also indicate that an alternatively spliced variant of AMACR is present in the baboon, albeit at very low levels. We could not demonstrate an alternatively spliced variant in the baboon prostate; however, a splice variant was shown in the colon and kidney samples of the baboon. This is in contrast to human samples in which they are readily amplified in the prostate and other tissues [21,22].

Prostate-specific antigen was detected in baboon serum, albeit at low levels as compared to humans. This, to the best of our knowledge, is the second study to report detection of PSA in baboon serum. PSA detection in baboons was first done by Williams et al. [30]. The reason why hospital-based diagnostic laboratories could not detect PSA in baboon serum could be that the amount of PSA in baboon serum is well below the detection levels of their assays. A study that examined PSA levels in chimpanzees also found

low serum PSA values [15]. Another possible explanation could be that the antibodies used in these human assays do not recognize baboon PSA epitopes. More studies involving other nonhuman primates are warranted. The fact that an antibody raised against human PSA was successfully used to detect PSA in baboon prostate and serum indicates the close homology between human PSA and baboon PSA. Studies are underway in our laboratory to use the radioimmunoassay method for large scale screening of our baboon colony and thereby establish the normal PSA range for baboons.

The lesions found in this study included multifocal lymphocytic infiltration, corpora amylacea to sclerosing/atrophic lesions, but not neoplasia. The high prevalence of lympocytic infiltration (91%) in this study warrants further investigation. Sclerosing/atrophic lesions were also common in baboons in our study; these lesions are of clinical importance in the medical field because of their resemblance to adenocarcinoma on histologic examination [23–25]. Because of the natural occurrence of sclerosing/atrophic lesions in the baboon, this animal might be an ideal model for the study of these adenocarcinoma mimicking lesions.

In the literature there are few reports of prostate cancer in nonhuman primates. Some authors suggest that nonhuman primates do not appear to be suitable models for spontaneous prostatic carcinoma [31]. Due to the small number of late age baboons in our study, we cannot draw conclusions concerning whether baboons do develop spontaneous prostate cancer.

In our study, the initial rapid increase in prostate weight was followed by a slow, consistent increase to the point that even after 20 years of age there were still slight increases in weight. The significance of continued increase in prostate weight even in late adulthood is not clear. Further studies with a large number of geriatric baboons are needed. Although the results presented in this study are preliminary; they indicate that the baboon has the potential to be developed into a useful model for prostatic diseases since (i) the baboon is a large animal with a prostate that is palpable by rectal digital exam and biopsies can be taken easily, (ii) biomarkers (PSA, AMACR, and basal cell keratins) that are presently used in diagnosis and screening of patients for prostatic diseases can be used in the baboon, and (iii) there are strikingly close similarities in the anatomy, histology, and biochemistry of the human and baboon prostates. Another additional advantage is that the diet and environment of baboons can be easily controlled and large pedigrees are available for genetic studies. Additional studies are in progress in our laboratory to establish if baboons do develop naturally occurring prostate cancer and benign prostatic hyperplasia.

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