ORIGINAL RESEARCH—ANATOMY/PHYSIOLOGY

The Human Female Prostate—Immunohistochemical Study with Prostate-Specific Antigen, Prostate-Specific Alkaline Phosphatase, and Androgen Receptor and 3-D Remodeling

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ABSTRACT

Introduction. The constitution of glands surrounding the human female urethra has been under debate; especially regarding as to what extent they equal the male prostate. Defining their composition may help to understand the development of neoplasms arising from this tissue.

Aims. The aim of this study was to define the existence, structure, and arrangement of a possible human female prostate.

Methods. Urethras of 25 women were investigated by immunohistochemistry and stained with specific monoclonal antibodies against prostate-specific antigen (PSA, mono- and polyclonal antibody), prostate specific alkaline phosphatase (PSAP), and androgen receptor (AR). From two urethras, which underwent a totally serial work up with PSA-staining, a three-dimensional model of the urethra and the prostatic glands was created to enable 3D-perception of the results.

Main Outcome Measure. The main outcome measures used in this study were identifying glandular structures in hematoxylin-eosin-staining, positive staining with the respective antibodies, and 3-D orientation of described glands.

Results. Fourteen of 25 patients had glandular structures encircling the urethra. Twelve of 14 showed positive staining for PSA, PSAP, and AR in gland acini, while the excretory ducts, the urethra, and the surrounding stroma did not express those proteins. The strongest PSA and PSAP expression was found in apical cytoplasm of the glandular cells, and AR was confined to cell nuclei. Prostatic glands were located laterally to the distal half of the urethra.

Conclusion. A female prostate was found in every second woman in this study and can be discriminated from other urethral caverns and immature paraurethral ducts. Possible neoplasms of this source tissue expressing the prostate-specific markers may therefore be denominated as female prostate tumors. **Dietrich W, Susani M, Stifter L, and Haitel A. The human female prostate—immunohistochemical study with prostate-specific antigen, prostate-specific alkaline phosphatase, and androgen receptor and 3-D remodeling. J Sex Med 2011;8:2816–2821.**

Key Words. Female Prostate; Glandular Structures Surrounding the Female Urethra; PSA; PSAP; Androgen Receptor; Immunohistochemistry

Introduction

The nature, constitution, and function of glandular structures surrounding the human female urethra has been under debate since De Graaf's first description in 1672 and Skene's report of the anatomy of two spacious glands, opening aside from the urethral orifice [1,2]. The major part of these structures is located inside the fibromuscular urethral wall, thus impeding delimitation as particular organ [3]. How far these glands or parts of them are equivalent or comparable with the male prostate has been tried to be revealed in several histological or imaging studies [4–8].

Because prostate-specific antigen (PSA) was discovered and the first polyclonal antibodies against

PSA became available, positively stained cells were found in female periurethral glands [9]. PSAP and especially PSA are known from male prostate as exclusive markers of mature secretory epithelium [10]. PSA is equal to human kallikrein 3, a trypsin-like serine protease that contributes to seminal clot liquefaction after ejaculation [11]. However, the reliability of those results was questioned because of the high intraindividual variations and ambiguous staining quality of the used antibodies, resulting in higher unspecific background staining and yielding positive results in nonprostatic tissue, e.g. breast and skin [12,13].

Repeated case reports presenting adenomyomatous hyperplasia or malignancies, e.g. metastasing adenocarcinomas, emamating from female periurethral ducts or prostatic tissue, showed structural and immunohistochemical similarities with male prostatic neoplasms, were largely positive for carcinoembyonic antigen (CEA), but varied in their PSA expression [14–18]. From the PSA-positive ones, the source tissue was denominated as Skene's gland and the tumors as Skene's gland adenocarcinoma resembling prostate (SARP), rather than female prostate. In other species, e.g. the gerbil, extensive studies and 3-D modeling of adult female prostate have been performed describing this organ as distinct and partially secretory active [19].

To terminate the confusion, combine the strengths of histochemistry and imaging, and further define the existence, structure, and arrangement of a human female prostate, we performed this immunohistochemical study using highly specific, validated mono- and polyclonal antibodies against PSA, prostate specific alkaline phosphatase (PSAP), and androgen receptor (AR) and generated a 3-D model of this thus far obscure organ.

Materials and Methods

Patients

Urethra specimens of 25 consecutive female patients (age 60–75 years), suffering from bladder carcinoma or rhabdomyosarcoma of the bladder in one case, were investigated. The urethras were removed in total adhering to the urinary bladder during standard radical cystectomy operations with consecutive construction of ileal conduits in a tertiary care facility without any treatment modification due to the study and were completely embedded in paraffin.

Expedited review board approval was obtained because of the terms of the local Ethics committee.

Immunohistochemistry

From the 25 urethras, the whole organ was longitudinally cut in the middle of the urethral lumen and embedded in paraffin. Those blocks were further cut into 4-μm slices in the same direction, so each section contains the urethral wall as well as the surrounding glands and stroma in the whole length from the bladder neck to the outer urethral resection margin. From these sections, serial slides at three levels underwent standard hematoxylin-eosin (HE) staining to identify glandular structures and immunohistochemistry according to the following protocol. For immunohistochemical analysis, the paraffin sections were dewaxed, rehydrated, and immersed in methanol with 0.6% hydrogen peroxide for 15 minutes. For the staining with the monoclonal antibody, the slides were heated one time for 20 minutes at 120W and three times for 5 minutes at 450W in a microwave oven, for polyclonal PSA antibody and PSAP staining no pretreatment was necessary. Then, the sections were incubated with the primary antibodies: against PSA with the mouse monoclonal antibody M0750 (DAKO, Glostrup, Denmark; dilution 1:50), and the rabbit polyclonal antibody A562 (DAKO; dilution 1:2,000); against PSAP with the mouse monoclonal antibody Ab-1 (Thermo Fisher Scientific, Fremont, CA, USA; dilution 1:2,000); and against AR with the mouse monoclonal antibody AR441 (DAKO; 1:100) at room temperature for 1 hour. Bound antibody was detected using the avidin-biotin-complex peroxidase method (ABC Kit, Vector, Burlingame, CA, USA) and 3,3′ diaminobenzidine.

The specificity of the antibodies used in this study was shown previously [20–22].

For 3-D modeling, the total urethra specimens of two female patients, dying at age of 21 years by suicide and 18 years because of pulmonal emboli, were embedded completely in paraffin and serially cut in 4-μm thick paraffin sections with a distance of 160 μm between the sections. Four slices (the first, the last, and two in between) were HE stained, the others were stained immunohistochemically with the polyclonal PSA antibody to detect PSA positive glands.

3-D Remodelling and Visualisation

The 3-D reconstruction was performed according to the work of Santos et al. with some modifications [23]:

The PSA-stained serial sections were scanned in color at 7,200 dpi by a CanoScan N650U system. Image capture and processing were carried

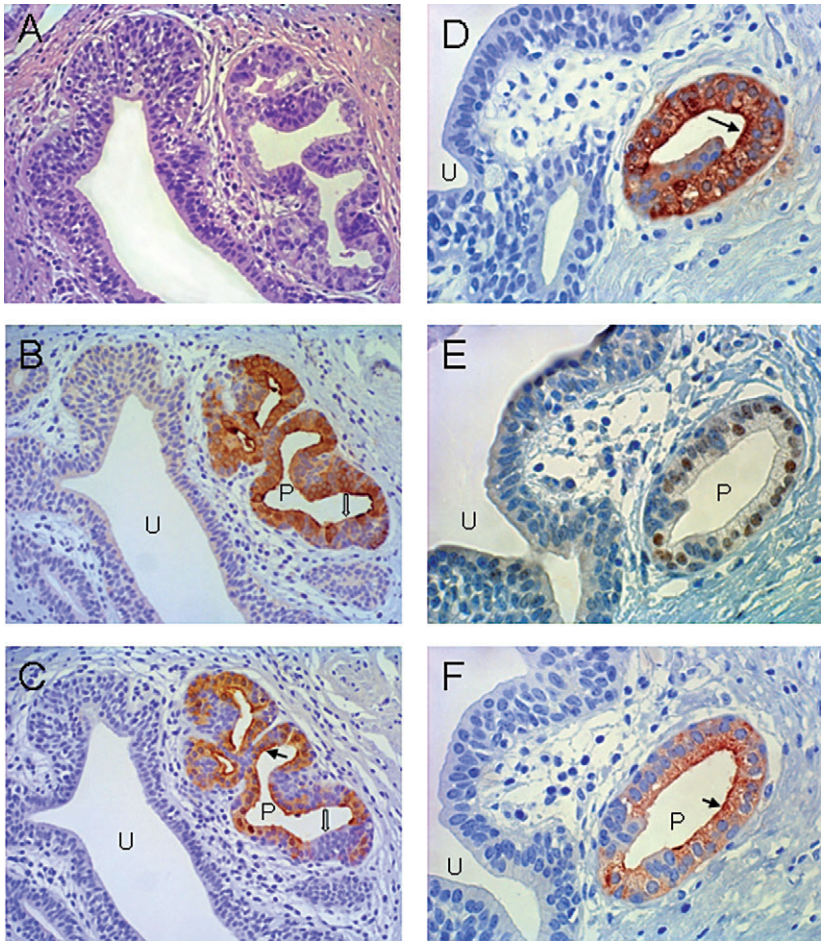

Figure 1 Immunohistochemistry on serial sections of human female prostate. (A) Hematoxylin-eosin staining of urethra and glandular structure suspected to be female prostate. (B) Polyclonal prostate-specific antigen (PSA) staining (brown color), light arrow indicates islet of urothelium with weak PSA expression. (C) and (F) monoclonal PSA staining (brown color), thick arrow indicates strong apical cytoplasmic accumulation of PSA, light arrow indicates islet of urothelium with weak PSA expression. (D) Monoclonal prostate specific alkaline phosphatase (PSAP) staining (brown color), showing strong apical cytoplasmic accumulation of PSAP (thick arrow). (E) Monoclonal androgen receptor (AR) staining (brown color), showing strong to moderate nuclear AR expression and AR negative cytoplasm. (A–C) Serial sections of the same prostatic gland. (D–F) Serial sections of the same prostatic gland. U = urethra; P = prostatic gland. All pictures at 100× magnification.

out using the AutoCAD-software. The contours of the brown-stained PSA-positive areas as well as the urethral urothelial lining were marked as 2-D spline in these high-resolution 2-D pictures. The vaginal epithelium was marked in blue color to create an area of reference to enable image alignment. The 2-D contours were imported to 3dsmax software, extruded with the section distance of 160 μm and fit together in the right sequence. To maintain the natural accuracy of the 3-D model, we omitted any smoothening of the processed picture data. For better illustration of the 3-D model, we have created an interactive camera in the visualization software Quest3D.

Results

Of the 25 urethral specimens screened representatively from the cystectomy-specimens, 14 showed glandular structures consisting of a basal cell layer covered by a singular layer of epithelium, 12 of them showed PSA positivity. The other 11 specimens showed only gland-like structures covered by a singular layer of urothelium. Each gland or gland-like structure had one, urothelial lined, excretory duct leading to the urethra. The prostatic gland acini were located in close contact to the urethral wall, mainly lateral and dorsolateral to the urethral lumen, and were confined to the distal half of the urethra.

Polyclonal PSA, monoclonal PSA, PSAP, and AR expression could be found in exactly the same areas of the specimens (Figure 1). The immunocytochemical reaction was not uniform, with the strongest PSA and PSAP staining in the apical cytoplasm and negative staining in the nuclei. Although the staining intensity of PSAP and polyclonal PSA reaction was slightly stronger than those of the monoclonal PSA antibody, there were no differences in staining distension or pattern. The glandular cells showed strong to moderate nuclear AR expression, without staining of the cytoplasm. The intersperced urothelial cells, as well as the urethra stained consistently negative for all markers.

One of both urethras, which underwent a total serial immunohistochemical work-up, showed

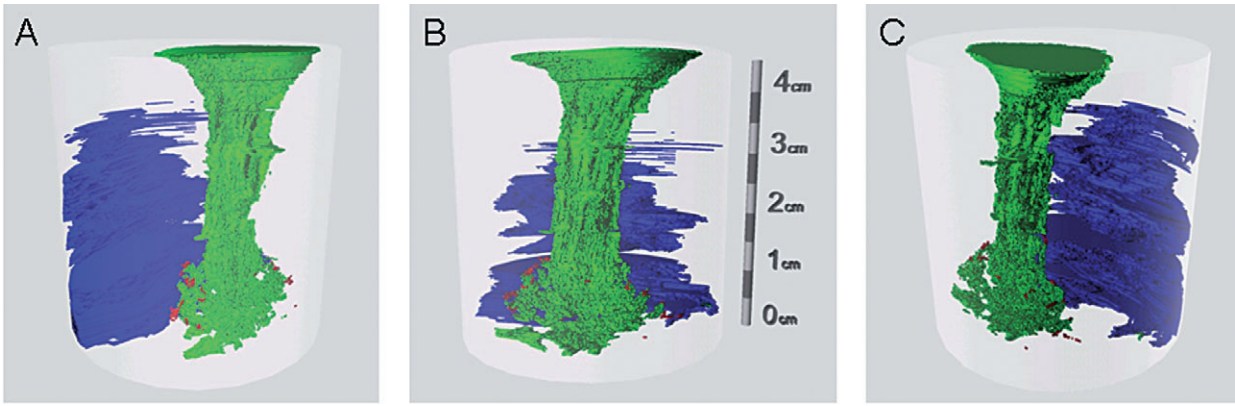

Figure 2 Total 3-D remodeling of a human female urethra and prostate. Each layer has a gauge of 160 μm. Green indicates urethra and urethral caverns represented by 2-D splines of urothelium. Blue indicates vaginal wall represented by 2-D splines of vaginal epithelium, equal to dorsal/caudal orientation. Red indicates female prostate, represented by 2-D splines of polyclonal prostate-specific antigen-staining. (A) View from right front side. (B) View from front with ruler (1 bar represents 5 mm). (C) View from left side.

developed secretory glandular structures. In the terminal parts of these glands, PSA-positive lining cells were found. Urethral caverns and ducts covered by a single layer of urothelium, were seen around the urethral lumen (Figure 2). The caverns and ducts around the second investigated urethra were lined by one layer of a typical urothelial epithelium only without ripe acini, hence, being consistent PSA negative (not shown).

Discussion

This study demonstrates the existence of a developed human female prostate in every second of the investigated women. Their glands consist of tubulo-alveolar acini at the end of one excretory duct, respectively, leading to the urethral lumen and are distributed mainly lateral or dorsolateral of the urethral axis. The finding of a more distal arrangement of the prostatic glands is in accordance with the reports of Huffman and Wernert, who described glands located around the distal half or two-thirds of the female urethra [5,24]. Other authors, in contrast, described a more proximal location extending to the neck of the urinary bladder, created models with up to six different types of glandular distribution or totally denied the existence of female urethral ducts and designated them as vestigial glands [6,24–27]. Moreover, those glandular structures may be encroached by the widely used mid-urethral slings, which are implanted to treat stress urinary incontinence and which are surpassing the urethra dorsal and lateral, exactly where the prostatic glands are located [28]. The differential location of prostatic glands being in males proximal to the pelvic diaphragm while being in females below thereof is still an open issue.

The proximal parts of the urethral wall were also found structured with some caverns in our study, which are comparable with those described by Sesterhenn et al. [25]. In our patients, these caverns were covered by urothelium only and were consistently PSA and PSAP negative, so that a mature prostatic differentiation can be excluded. Furthermore, urothelial caverns and ducts were also found scattered around the urethras of the other half of women without developed secretory portions, and those ducts, like the urethra itself, lacked any evidence of PSA-positive cells. These caverns and ducts show high interindividual variation in size, location, and length and represent immature prostatic ducts [5]. This high variability is known from previous anatomic studies and has lead to the development of anatomic models with up to six types of glandular distribution [5,6]. However, the immature ducts have now to be clearly discriminated from developed prostatic glands because of their histological and histochemical differences. This determination may also help to understand the fact that only a minority of female para-urethral adenocarcinomas express PSA, so only the PSA-positive ones may originate from developed secretory portions [14,18]. This point of view has already been stated before [29].

The cellular constitution of human female prostatic gland acini shows similarities to human early

fetal male prostate and adult female prostate from other species, e.g. the gerbil (*Meriones unguiculatus*) [30,31]. There, analog, cuboidal basal cells as well as more frequent columnar secretory cells were found. In contrast to immature male prostate, where the epithelial differentiation to the secretory columnar epithelium has not yet been driven by androgenic signaling, the human female prostatic glands produce the exclusive markers of mature secretory prostatic epithelium, PSAP, and PSA [10,32]. In the gerbil female prostate, the gland consists of at least two distinct secretory cell lines, a typical prostatic secretory cell, and a clear secretory cell [31]. These groups of "clear cells" could not be found in our study of human female prostate. The groups of PSA and PSAP negative cells been intersperced in human female prostatic acini were urothelial cells.

A peculiar exocrine function of human female paraurethral glands has been stated because acid phosphatase activity was found to be several times higher in female urethral expulsions than in the urine [33]. Further, acid phosphatase protein has been detected in the glandular epithelial lining, which could be confirmed by our study [34]. Those data have lead to distinct implications in forensic medicine, where the acid phosphatase test, therefore, lost its forensic relevance in identifying sperm spots that contain no spermatozoa [35].

The female urethral expulsions, that have been described as female ejaculation during orgasm, may now be seen as the fluid volume normally taken up the main urethral channel, as well as the (possibly spacious) PSA negative urethral caverns and ducts, as well as the developed prostatic glands, all of them located inside the muscular urethral wall, that gets expelled by smooth muscular contractions [36,37]. So, this volume is only in minor extent a prostatic secret. Hence, urethral caverns can be found around all female urethras, but only about 50% of women have developed prostatic glands, if those expulsions may be possible to be achieved in women, PSA detection therein may succeed only in a part [33].

Conclusion

Developed prostatic glands, defined by specific acinic expression of PSA, PSAP, and AR can be found in every second woman among the urethras studied. Those glands are located mainly lateral to the distal half of the urethra and are secreting their content into the urethral channel. A literal female prostate, albeit being a small organ, has to be clearly discriminated from other urethral caverns or immature prostatic ducts, because of its unique histochemical and secretory properties. So, the rare neoplasms, located around the female urethra and staining positive for the respective markers, might be correctly denominated as female prostatic carcinoma, instead of SARP.

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Conflict of Interest: All authors have nothing to declare.

Statement of Authorship

Category 1

(a) Conception and Design
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Category 2

(a) Drafting the Article
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(b) Revising It for Intellectual Content
Wolf Dietrich; Martin Susani; Lukas Stifter; Andrea Haitel

Category 3

(a) Final Approval of the Completed Article
Wolf Dietrich; Martin Susani; Lukas Stifter; Andrea Haitel

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