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Original Article

Human urogenital sinus mesenchyme is an inducer of prostatic epithelial development

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Abstract: Objective: To determine whether human fetal urogenital sinus mesenchyme (UGM) can induce prostatic development in a responsive mouse epithelium. Method: Male and female human fetal UGM was combined with mouse urinary bladder epithelium (BLE), and the resultant human UGM + mouse BLE tissue recombinants were grown under renal capsules of male athymic mice. Human male and female UGM was derived from reproductive tracts 9 and 14 weeks of gestation obtained following elective termination of pregnancy. At these ages prostatic ducts had already emerged from the urogenital sinus epithelium, and the human UGM remained contaminated with human prostatic epithelium. This unavoidable problem was tolerated because the induced mouse prostatic epithelium could be distinguished from contaminating human prostatic epithelium. Results: The simple columnar epithelium induced from mouse bladder epithelium by human male and female UGM resembled mouse prostatic epithelium by: (a) histology, (b) the pattern of basal cell distribution, (c) Hoechst dye nuclear staining, (d) expression of NKX3.1, (e) the pattern of androgen receptor expression and (f) the expression of probasin, a mouse prostatic secretory protein. Summary/Interpretation: These findings provide validation for mouse as a model of human prostatic development as the molecular dialogue involved in mesenchymal-epithelial interactions are sufficiently conserved that human UGM can induce mouse bladder epithelium to undergo prostatic development.

Keywords: Urogenital sinus, urogenital sinus mesenchyme, urogenital sinus epithelium, bladder epithelium, androgen receptor, dihydrotestosterone, prostate

Introduction

Prostatic development is dependent upon androgens irrespective of genetic sex of the urogenital sinus (UGS): (a) embryonic and neonatal female mouse UGS forms prostate when exposed to androgens [1], (b) prostate develops in female patients with congenital adrenal hyperplasia [2, 3], and (c) mutations abrogating AR signaling prevent prostatic development [4, 5]. In rodents, urogenital sinus mesenchyme (UGM) induces prostatic epithelial development, based primarily upon analysis of tissue recombinants composed of UGM plus urinary bladder epithelium (UGM + BLE) [6]. Surprisingly, rodent UGM can induce prostatic development from both embryonic and adult BLE [6].

The relevance of the animal studies to human prostatic development has been pursued over many decades. Four observations are in accord with the animal studies: (a) The absence of prostatic development in patients with complete androgen resistance [5, 7]. (b) Prostatic development in female patients with congenital adrenal hyperplasia [2, 3]. (c) Experimental DHT induction of prostatic development in human fetal female urethra (UGS) [8]. (d) Tissue recombinants composed of rodent UGM + human embryonic or adult BLE formed ductal structures that expressed PSA [6, 9], confirming that human bladder epithelium is responsive to prostatic induction from rodent UGM. The remaining idea to be addressed is to determine whether human UGM (like that of the rodent) can induce prostatic development, which is the subject of this report.

Materials and methods

Human fetal prostatic rudiments immediately below the bladder were dissected from human male urogenital tracts: two 9-week, one 10-week and one 14-week. The urethra immedi-

Table 1. Antibodies used in this study

Antibody	Source	Catalogue #	Concentration
Keratin 14	BioGenex	LL002	1/100
TP63	Santa Cruz Biotechnology	Sc-8343	1/100
Androgen receptor	Genetex	GTX62599	1/100
NKX3.1	Santa Cruz Biotechnology	SC-393190	1/100
Probasin	Santa Cruz Biotechnology	SC-17124	1/100

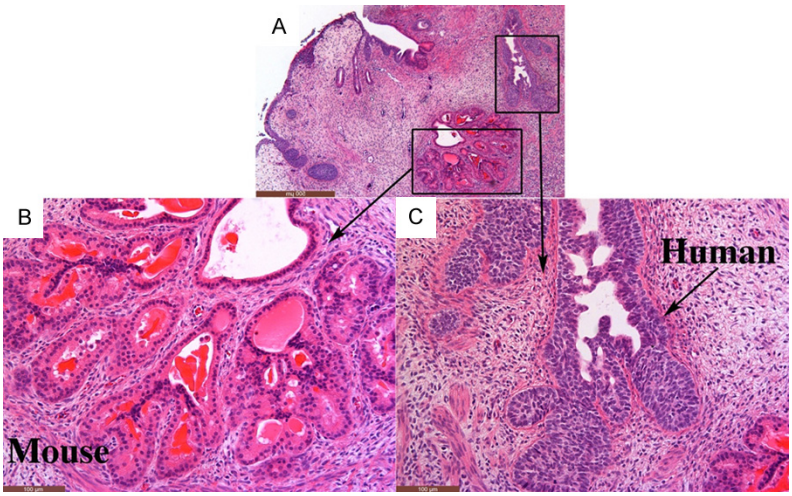

Figure 1. A human UGM + mouse BLE tissue recombinant grown in a DHT-treated athymic male host. The low magnification overview (A) shows two distinctive epithelia: (B) well-differentiated prostatic ducts containing secretion and (C) contaminating immature human prostatic ducts, partially canalized.

ately below the bladder of a 10-week female was also dissected (Committee on Human Research at UCSF, IRB# 12-08813). Gestational age of the human fetal specimens was estimated using heel-toe length [10]. Urinary bladder epithelium was derived from 3-day-old neonatal CD-1 male mice (Charles River Laboratories, Wilmington, MA). Human UGM + mouse BLE tissue recombinants were prepared with UGM from the human fetal prostatic rudiments or female urethra plus mouse urinary bladder epithelium as described [11]. Mesenchyme of the female urethra is also considered UGM. The resultant human UGM + mouse BLE tissue recombinants were grafted under the renal capsule of intact male athymic mice (Charles River, Hollister, CA) treated subcutaneously with a 20 mg DHT pellet. After 6 weeks of in vivo growth the tissue recombinants were harvested, fixed in 10% buffered formalin, embedded in paraffin and serially sectioned at 7 μm. Every 20th section was stained with H&E to assess histology. Intervening paraffin sections were immunostained (**Table 1**) as described previously [8]. For negative controls the primary antibodies were omitted.

For technical reasons, the isolated human fetal UGM was contaminated with residual urogenital sinus epithelium (UGE). This problem could be tolerated because the contaminating human UGE could be distinguished from prostatic ducts of mouse BLE origin induced by human fetal UGM through use of histology, Hoechst dye staining (Millipore-Sigma, St. Louis, MO, USA) [12] and (c) immunohistochemistry. By this method, species origin of epithelia within human UGM + mouse BLE tissue recombinants showed that male and female human UGM can induce prostatic epithelial differentiation.

Results

Human UGM + mouse BLE tissue recombinants grown for 6 weeks in athymic male hosts contained two types of epithelia identified histologically as contaminating human fetal prostatic epithelium and mouse prostatic epithelium (**Figure 1**). Contaminating human fetal UGE formed branched solid ductal structures (some canalized) that resembled human fetal prostate [8, 13]. The canalized human fetal prostatic ducts were lined by immature prostatic epithelium [8, 13] (**Figure 1C**). The other epithelium in human UGM + mouse BLE tissue recombinants resembled adult mouse prostatic epithelium consisting of ducts of simple columnar epithelium containing secretion (**Figure 1B**). Tissue recombinants composed of female UGM + mouse BLE exhibited a similar epithelial profile (not illustrated). Hoechst dye staining confirmed provisional epithelial identification in human UGM + mouse BLE tissue recombinants: (a) putative mouse

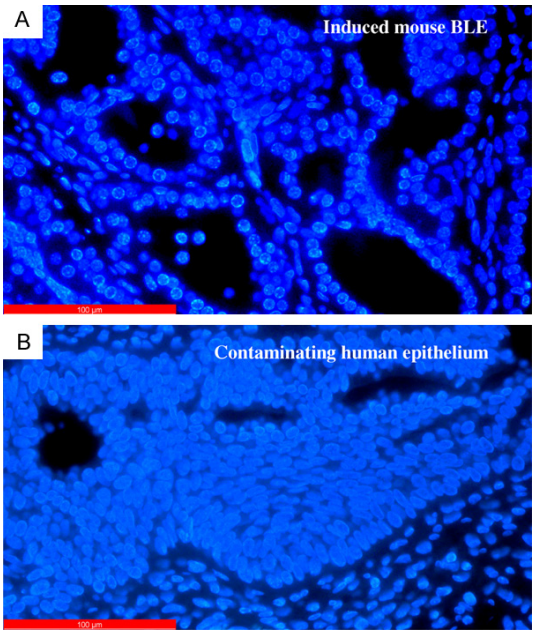

Figure 2. Human UGM + mouse BLE tissue recombinant stained with Hoechst dye. A. Region of the tissue recombinant; putative induced mouse prostatic epithelium exhibits epithelial nuclei with bright chromatin clumps indicative of mouse nuclei, while the stromal nuclei stain homogeneously, indicative of human nuclei. B. Is an area of contaminating human fetal prostatic epithelium (See **Figure 1C**) whose nuclei stain homogeneously, a pattern characteristic of human nuclei.

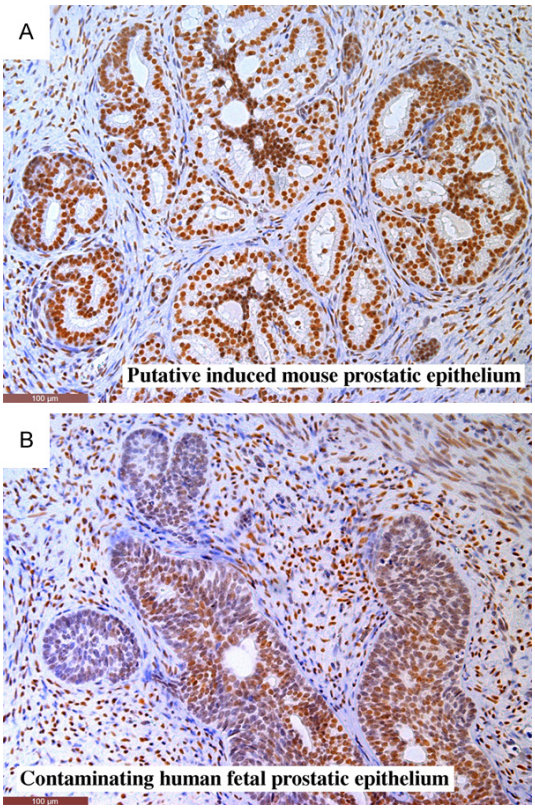

Figure 3. AR immunohistochemistry of human UGM + mouse BLE tissue recombinants showing putative induced mouse prostatic epithelium which uniformly expresses AR. In contrast, the contaminating human fetal prostatic epithelium expresses AR in regions of canalization, but solid ducts are mostly AR-negative as reported previously [8, 13].

prostatic epithelium exhibited the chromatin pattern characteristic of mouse nuclei, namely bright coarse chromatin clumps (**Figure 2A**); provisionally identified human prostatic epithelium exhibited a fine diffuse chromatin pattern characteristic of human nuclei [12] (**Figure 2B**).

AR expression is vastly different in mature mouse versus contaminating human fetal prostatic epithelium [13, 14]. The induced mouse prostatic glands are simple columnar and are uniformly AR-positive (**Figure 3A**), indicative of mouse prostatic epithelium [14]. Contaminating human prostatic epithelium was mostly AR-negative as "solid ducts" and contained AR-positive luminal cells when canalized, the pattern characteristic of human fetal prostate [8, 13] (**Figure 3B**).

TP63 and KRT14 immunostaining distinguishes epithelia of the human fetal prostate versus mature mouse prostate. Human fetal prostate has a continuous layer of TP63- and KRT14-positive basal epithelial cells [13], whereas the basal epithelial cell layer of mature mouse prostate is discontinuous [15]. These 2 distinctive patterns of TP63 were observed in human UGM + mouse BLE tissue recombinants (**Figure 4**) (KRT14, not illustrated), thus providing further verification of the species origin of the two epithelia.

NKX3.1, a marker of adult mouse prostatic epithelium, and probasin, a mouse prostatic secretory protein, are expressed in induced mouse prostatic epithelium (**Figure 5**) of human UGM + mouse BLE tissue recombinants. In summary, analysis of human UGM + mouse BLE tissue recombinants demonstrate that male and female human UGM can induce prostatic development in a responsive mouse epithelium (BLE).

Discussion

Prostatic development is dependent upon requisite mesenchymal-epithelial interactions.

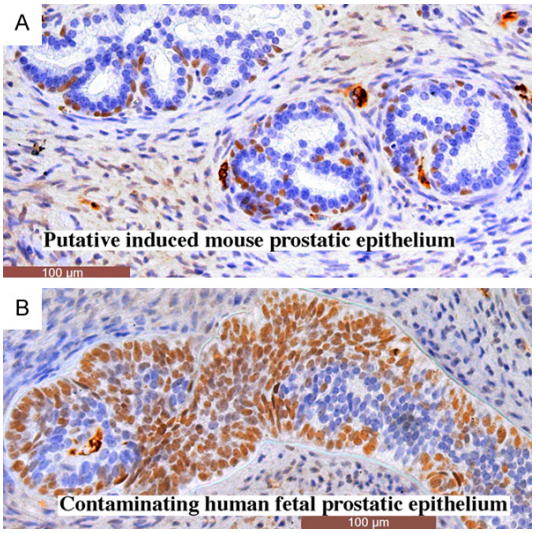

Figure 4. TP63 immunostaining of putative induced mouse prostatic epithelium (A) and contaminating human fetal prostate (B) in a human UGM + mouse BLE tissue recombinant. TP63-positive basal cells formed a discontinuous basal layer (A) in the putative induced mouse prostatic epithelium. In the contaminating human fetal prostatic epithelium TP63-positive cells were organized into a multi-layered continuous basal layer (B).

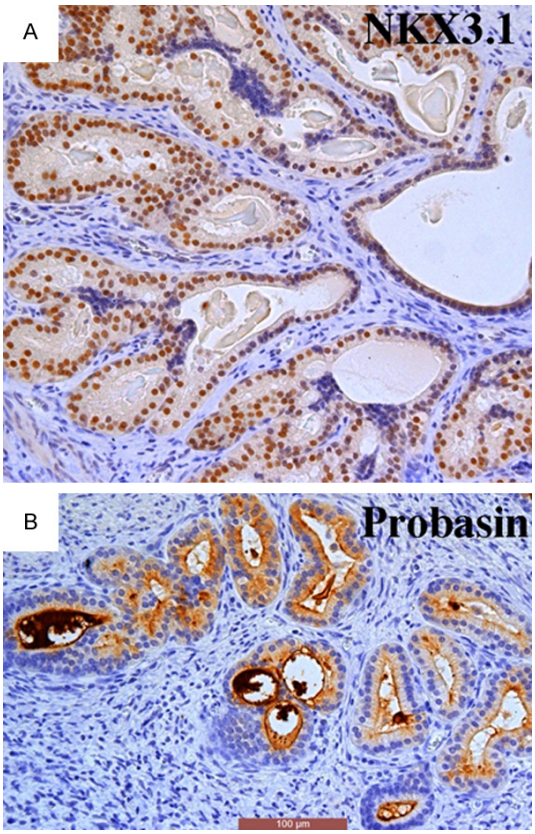

Figure 5. NKX3.1 and probasin immunostaining of putative induced mouse prostatic epithelium in a human UGM + mouse BLE tissue recombinant.

The prostatic mesenchymal-epithelial interactions require (a) an inductive influence from UGM, (b) an epithelium responsive to prostatic induction and (c) androgen action. Both rodent and human UGE and BLE are responsive to prostatic induction by UGM [6, 16, 17]. The present study verifies that human male and female UGM is a prostatic epithelial inducer and consequently that the molecular mediators of these mesenchymal-epithelial interactions are sufficiently conserved that (a) rodent epithelium is responsive to prostatic induction from human UGM (current study) and (b) human epithelium is responsive to prostatic induction from rodent UGM as summarized above. Such conserved human/rodent developmental mechanisms validate rodent prostatic development as a model for the molecular regulation of human prostatic development. The ability of human female UGM to function as a prostatic induced in the presence of androgens is consistent with our recent report of prostatic development in xenografts of human fetal female urethra grown in DHT-treated hosts [8].

Given the difficultly of isolating human UGM free of contaminating human UGE, we anticipated that human UGM + mouse BLE tissue recombinants used in this study would contain: (a) contaminating immature human fetal prostatic epithelium and (b) newly induced mouse prostatic epithelium. This expectation was born out, necessitating the need to unequivocally distinguish these two epithelia (**Table 2**). Expression of NKX3.1 demonstrates the prostatic nature of the induced mouse BLE, and the expression of probasin is indicative of *mouse* prostatic epithelium [18]. The possibility that the mouse prostatic epithelium was derived from the host's kidney or to host stem cells is highly unlikely because: (a) historic subrenal capsule grafts of rodent UGM from previous studies never contained mature prostatic epithelium, and (b) serial sections of human UGM + mouse BLE tissue recombinants failed to reveal connections between epithelium within tissue recombinants and the hosts renal parenchyma.

The prostate gland is a distinctive anatomic denominator of mammals present in nearly "all

Table 2. Features distinguishing the two epithelia within human UGM + mouse BLE tissue recombinants

	Human	Mouse
Histology	Solid “ducts” and immature canalized ducts w/o secretion	Well differentiated ductal epithelium containing secretion
Hoechst dye staining	Diffuse homogeneous nuclear staining	Bright coarse chromatin clumps in nuclei
Basal cell status (KRT14 & TP63)	Continuous KRT14- and TP63-positive basal cell layer	Discontinuous KRT14- and TP63-positive basal cell layer
NKX3.1	Absent	Present
Probasin	Absent	Present
AR pattern	Solid “ducts” = negative for AR, canalized ducts = luminal cells AR-positive	AR confined to the simple columnar luminal layer

of the thousands of male mammals" [19], while the prostate is absent in reptiles from which mammals evolved about 359 million to 299 million years ago [20]. Coffey asserted that "the prostate and breast appeared at the same time 65 million years ago" [19], but did not give a cogent explanation for this statement. Rodents (a) originated 65 to 74 million years ago, (b) diversified throughout the Eocene (55-34 million years ago), and (c) today's recognizable rodent families emerged by 20 million years ago [21]. The common ancestor of rodents and humans is thought to have existed 145 million years ago [22]. When the prostate actually evolved as an anatomic entity in mammals is unknown but could be 100 to 200 millions years ago during early mammalian evolution, which is vastly earlier than the emergence of Homo sapiens in Africa around 300,000 years ago [23]. Given the conserved molecular dialogue in human-rodent prostatic tissue recombinants that serve as the basis of (a) the ability of mouse, rat and human UGM to induce human prostatic development, we can infer that the molecular underpinning of prostatic development is written in genes that have been present and conserved in the mammalian lineage for millions of years prior to the appearance of homo sapiens. Consequently, our study suggests that human paracrine prostatic mesenchymal mediators can regulate rodent epithelial genes that have been present in the rodent lineage for millions of years. While our mesenchymal-epithelial studies provide confirmation of common prostatic developmental pathways in mouse and human, the striking differences in prostatic pathogenesis (prostate cancer and BPH) in mice versus humans remains to be determined.

The standard method for preparation of tissue recombinants in the study mesenchymal-epithelial interactions is to use organ rudiments at a developmental stage in which mesenchyme and epithelium can be isolated cleanly. Unfortunately, this is not the case for the specimens utilized in this study, and thus contaminating human fetal prostatic epithelium was present in all human UGM + mouse BLE tissue recombinants examined. This problem could be tolerated since the newly induced mouse prostatic epithelium was distinguished from the contaminating human fetal prostatic epithelium by multiple confirmatory observations (**Table 2**).

In summary, it is now demonstrated that human UGM (like that of rodent UGM) is a potent prostatic epithelial inducer. We presume that the requisite androgenic effects in human UGM + mouse BLE tissue recombinants are mediated via AR within the human UGM, an expectation that will await the future analysis of human UGM + X^{Tfm}/Y mouse BLE tissue recombinants.

Remembrance

I start this paper with a remembrance and a thank you to my friend and collaborator, Leland W. K Chung, who had an important influence on my scientific development. I initially became acquainted with Leland Chung thru his intriguing papers on neonatal imprinting in prostate and seminal vesicle development that stemmed from his postdoctoral studies with Don Coffey. After I moved to the University of Colorado Medical School in Denver in 1976, I was surprised one day when Leland walked into my office and introduced himself. Leland had recently taken a position in the School of Pharmacy at the University of Colorado at Boulder, and thus we were only 30 miles apart. We had a long engaging chat that day which resulted in many years of productive collaboration. Several months later I remember with great fondness watching the scintillation counter with Leland as confirmatory data emerged for our first joint paper, a cherished moment. I introduced Leland to stromal-epithelial interactions and Leland introduced me to adult prostatic biology and prostatic hyperplasia (BPH, a topic rather distant for a developmental biologist). Our association led to two collaborative NIH grants on BPH. Another memorable occasion was a party at Leland's home in which he introduced me to Don Coffey, another pivotal event in my scientific life. Over the course of 5 years, Leland and I shared a postdoctoral fellow (Blake Neubauer) and a graduate student (Tim Thompson) and co-authored 11 papers. After those memorable years in Colorado, we both moved to other Universities, but saw each other frequently at meetings even though our respective research moved in different directions. I last saw Leland at a prostate cancer symposium in Tokyo. How

far our research had both come to a common ground over all those years from rather disparate directions. Thinking back on that fateful day when Leland walked into my office, that single event was a particularly important episode in my scientific development, and one that also affected the direction of Leland's research. This paper concerns a topic that Leland and I discussed many years ago.

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Disclosure of conflict of interest

None.

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