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Molecular characterization of the genital organizer: Gene expression profile of the mouse urethral plate epithelium

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Abstract

Purpose—Lower urinary tract malformations are among the most common congenital anomalies in humans. Molecular genetic studies of mouse external genital development have begun to identify mechanisms that pattern the genital tubercle and orchestrate urethral tubulogenesis. The urethral plate epithelium is an endodermal signaling region that plays an essential role in external genital development; however, little is known about the molecular identity of this cell population or the genes that regulate its activity.

Material and Methods—We used microarray analysis to characterize differences in gene expression between urethral plate epithelium and surrounding tissue in mouse genital tubercles. *In situ* hybridizations were performed to map gene expression patterns and ToppCluster was used to analyze gene associations.

Results—Here we report on 84 genes enriched at least 20-fold in urethral plate epithelium relative to surrounding tissue. We show that the majority of these genes are expressed throughout the urethral plate in males and females at E12.5, when the urethral plate is known to signal. Functional analysis using ToppCluster revealed genetic pathways with known functions in other organ systems but unknown roles in external genital development. Additionally, a 3D molecular atlas of genes enriched in urethral plate epithelium has been generated and deposited at the GenitoUrinary Development Molecular Anatomy Project website (GUDMAP.org).

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Conclusions—This study identifies dozens of genes previously unknown to be expressed in the urethral plate epithelium at a crucial developmental period, and provides a novel panel of genes for analysis in animal models and in humans with external genital anomalies.

Keywords

Genital tubercle; Urethral epithelium; Hypospadias; Urethral tube

Introduction

External genitalia are complex organs that perform excretory and reproductive functions. Congenital malformations of the penis are among the most common human birth defects, with hypospadias — an incomplete formation of the urethral tube that is characterized by ectopic, multiple or oversized urethral meatus — affecting approximately 1 in 250 live male births[1]. Although the causes of hypospadias remain poorly understood, progress in mouse developmental genetics has identified a small number of genes with essential roles in urethral tubulogenesis[2–7]. Nonetheless, the details of the gene regulatory networks involved in genital development remain largely uncharacterized[3,8].

In the mouse, external genital development begins at embryonic day 10.5 (E10.5) with the initiation of paired genital swellings on either side of the cloacal membrane[3,9]. These swelling merge medially to form a single genital tubercle and endoderm from the ventral side of the cloaca extends with the tubercle to form the bilaminar urethral plate. In mouse, the lumen of the penile urethra develops when the two sides of the urethral plate epithelium delaminate from proximal to distal[10]. The urethral plate separates from ventral surface ectoderm by apoptosis, and the adjacent mesenchyme fills the space previously occupied by the ventral urethral plate to internalize the urethral tube[9,11,12]. The human urethra also forms from cloacal endoderm, but urethral tubulogenesis differs from mouse. Morphogenesis of the human urethral tube resembles neurulation (closure of the neural tube), in that the lateral edges of the urethral plate are brought together medially to transform the plate into a tube[13,14]. Although the processes involved in urethral lumen formation in mouse and human are becoming clearer, the underlying mechanisms are poorly understood and few candidate genes are available for screening patients with external genital defects.

In addition to giving rise to the lining of the urethra, the urethral plate epithelium functions as a signaling center to control outgrowth and patterning of the genital tubercle[3,15]. The signaling activity of the urethral plate epithelium is mediated by a secreted signaling molecule encoded by the *Sonic hedgehog* (*Shh*) gene. Shh signals to surrounding mesenchymal tissue, where it regulates the molecular pattern of the genital tubercle and controls outgrowth and urethral tubulogenesis via control of cell cycle kinetics[11]. Early deletion of *Shh* causes agenesis of the genital tubercle[2,3] and, at later stages, *Shh* is required for urethral tube formation and sexual differentiation of the phallus[16–18].

A specialized transitional epithelium, known as urothelium, lines the bladder and functions as a barrier to contain urine. Urothelial development has been well characterized[19]; however, the molecular control of cell type differentiation in the urethra is not well understood. Progress in identifying the mechanisms of urethral cell signaling and differentiation has been

hindered by the paucity of molecular markers for urethral epithelial cells. To better characterize the transcriptome of early urethral epithelium, we used fluorescence activated cell sorting (FACS) to purify genetically labeled urethral plate epithelial cells from mouse genital tubercles at E12.5, when these cells have been shown to have signaling activity[3], for microarray analysis. Here we report on the identification of 84 genes that are enriched in the urethral plate endoderm of the developing mouse genital tubercle.

Materials and Methods

Mice

Shh is expressed in endodermal cells that give rise to urethral plate epithelium of the genital tubercle[2,3,10], which allowed us to label urethral plate epithelial cells using a GFP-Cre fusion cassette knocked into the *Shh* locus (*Shh*^{GFPCre})[20]. Endodermally-derived cells were genetically labeled by crossing males heterozygous for *Shh*^{GFPCre} with females homozygous for the *R26R*^{eYFP} allele. *Shh*$^{GFPCre/+}$; *R26R*^{eYFP} embryos develop normally and all *Shh*-expressing cells and their descendants are positive for eYFP[10,20], which resulted in labeling of the entire urethral plate epithelium (fig. 1A, B). The morning a vaginal plug was detected was considered E0.5. Pregnant dams were euthanized by cervical dislocation followed by thoracotomy and the embryos were harvested in ice-cold phosphate buffer saline (PBS). For cell purification experiments, embryos were harvested at embryonic day E12.5 and viewed under a stereomicroscope with epifluorescence in order to detect GFP/eYFP activity. In all embryos confirmed by PCR to carry the *Shh*^{GFPCre} allele and at least one copy of R26R^{eYFP}, we detected bright yellow-green fluorescence throughout the urethral plate epithelium of the genital tubercle. The genital tubercle was dissected at the base of the genital tubercle and placed in 0.05% trypsin at 37 degrees for 5 minutes. A single cell suspension was achieved by trituration using glass pasture pipette. These cells were centrifuged at 1800 RPM for 2 minutes and re-suspended in 0.5umM EDTA containing 0.5% BSA in PBS for sorting. We refer to the endodermal cell population within the dissected genital tubercle as “urethral plate epithelium”, although proximally (at the base of the genital tubercle), the urogenital and anorectal sinuses have not completely separated and, therefore, this cell population includes a small proportion of endodermal cells that line the cloacal membrane and the urorectal septum (fig. 1A, B).

FACS and preparation of RNA

Cells that were GFP/eYFP-positive (urethral) and negative (rest of the genital tubercle) were purified by FACS (Vantage SE TurboSort; BD Biosciences, San Jose, CA; fig. 1C) and re-analysis of the recovered sample confirmed >94% enrichment in GFP/YFP-positive cells. A total of three cell sorts were performed to produce three independent pools of GFP/eYFP-positive (urethral epithelial cells) and GFP/eYFP-negative (cells outside the urethra) cells. Total RNA was then extracted from each FACS-sorted populations using the RNeasy Mini Kit (QIAGEN, Inc., Valencia, CA) according to the manufacturer’s protocol. Sample integrity was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA) to compare the relative amounts of 18S and 28S rRNA and to verify that each sample yielded at least 0.1μg of RNA.

Microarray and gene function analysis

For each sample, 50 ng of total RNA was used as template in the Affymetrix Two-Cycle cDNA Synthesis kit according to the Affymetrix Eukaryotic Target Preparation Manual (Affymetrix, Inc., Santa Clara CA). Following cleanup, biotin-labeled cRNA was synthesized using the Affymetrix GeneChip IVT Labeling Kit. For each sample, 15 ug of labeled cRNA was fragmented and hybridized to an Affymetrix Mouse Genome 430 2.0 GeneChip at 45°C for 16 hours. The GeneChips were washed and stained with a GeneChip Fluidics Station 450 according to the Expression Analysis Technical Manual. A GeneChip Scanner 3000 was used to collect data into the GeneChip Operating Software using default parameters and global scaling as the normalization protocol. The trimmed mean target intensity was arbitrarily set at 500 for each GeneChip. Expression data from all 6 chips was collated into a single class comparison analysis using BRB ArrayTools (developed by Drs. R. Simon and A. Lam) to identify genes expressed at elevated levels in the urethral epithelium. In order to identify cell-specific markers of urethral plate epithelium that are effectively absent from the adjacent mesenchyme, we set a differential expression threshold of 20-fold, which allows a presence/absence call. The complete gene expression dataset is available at on the GUDMAP website (http://www.gudmap.org/gudmap/pages/focus_insitu_browse.html?batchId=368). Gene enrichment analysis was performed using ToppCluster, which classifies genes by known molecular functions, biological processes, pathways, co-expression, and mouse phenotypes. Co-expression analysis was performed by comparing our data to previously reported transcriptomic data on the GUDMAP website. Cytoscape was used to annotate the figures.

Probe construction and *in situ* hybridization

Probes were made either from I.M.A.G.E. clone plasmids (Open Biosystems) containing the known full-length cDNA clones, or by PCR amplification from cDNA. Sense control and antisense probes were synthesized in transcription reactions containing Dig-UTP. *In situ* hybridization was conducted on E12.5 embryos as described previously[3] with the following adjustments: embryos were digested in 10μg/ml proteinase K for 10 minutes, probe concentration was 0.1μg/ml–0.5μg/ml, and seven KTBT washes of 1 hour each were performed immediately before the color reaction.

Results

Using a minimum threshold of twenty-fold (see Methods for rationale), we identified 89 genes that are differentially expressed between the urethral and surrounding tissue, 84 of which were expressed at least 20-fold higher in the urethral endoderm (complete dataset is available at http://www.gudmap.org/gudmap/pages/focus_insitu_browse.html?batchId=368 and Table 1 [JU staff: please insert URL]). Only five genes were expressed at least 20-fold higher in the surrounding mesenchyme/ectoderm than in the urethral plate epithelium.

Given that the screen was designed to identify novel markers of the urethral plate epithelium by identifying genes with enriched expression in the GFP/eYFP-positive cells, we expected that *Shh* would be detected at higher levels in the GFP/eYFP-positive cells. We found that *Shh* transcripts were >20 times higher in GFP/eYFP-positive cells than in unlabeled

mesenchyme and ectoderm, consistent with our objective to purify urethral epithelial cells (Table 1, Journal of Urology URL to be inserted by journal staff).

We next performed *in situ* hybridizations to validate that the genes recovered from our array screen are detectable in the urethral endoderm, and to map their spatial domains in the embryo. Expression domains were mapped for a subset of 54 genes, of which 45 showed strong expression in the urethral plate. These domains were homogenous throughout the developing urethral plate epithelium and lacked any obvious regionalization along the developing urethra (selected genes shown in fig. 2; complete dataset can be accessed at http://www.gudmap.org/gudmap/pages/focus_insitu_browse.html?batchId=368). Two genes (*Sez6ld* and *Cab39l*) showed no expression in the genital tubercle, although specific staining was seen in other areas of the embryo (e.g., neural tube and limb). Seven genes showed expression in the genital tubercle mesenchyme. For example, the expression patterns of *Mpeg1* and *C1qa* were detected in mesenchyme at the distal tip of the genital tubercle, on either side of the distal urethral epithelium (fig. 3A) and *Ano9* and *Dmd* was observed surrounding the urethral plate (fig. 3B). A few genes such as *Aldoa*, *Smc2* (fig. 3C), and *Pcdhl2* were expressed in urethral and mesenchymal cells.

Gonad differentiation in mice begins at E11.0[21]. To determine whether early markers of sexual differentiation can be detected in the urethral plate at E12.5, we looked for sexually dimorphic expression patterns in staged matched male and female embryos that were genotyped for sex. Analysis of 54 genes by *in situ* hybridization revealed no obvious differences in expression patterns between male and female genital tubercles at E12.5 (fig. 2 & fig. 3; http://www.gudmap.org/gudmap/pages/focus_insitu_browse.html?batchId=368).

To predict functions and potential interactions among genes enriched in the urethral epithelium, we used ToppCluster to annotate their gene ontology and their connectivity (fig. 4; the complete ToppCluster dataset, can be accessed at http://www.gudmap.org/gudmap/page to be provided by GUDMAP editorial staff). Results of the ToppCluster analysis revealed that many of the enriched genes have known biological processes in development of other epithelial organs, such as epithelial tube branching in the lung, epithelial-mesenchymal signaling in the prostate gland, regulation of cell division, and cell-cell adhesion (fig. 4). We found that structural molecules were the most represented functional class and FoxA genes were the most represented pathway. We also compared the list of genes enriched in urethral epithelium of E12.5 mice to the set of genes previously found to be enriched in the urothelium of the developing bladder (E12.5, E13.5, E17.5, E18.5 and P2) and in the urethral epithelium (E13.5) and identified 31 genes unique to the E12.5 urethral epithelium (Table 2).

Discussion

To date, very few genes (e.g., *Shh* and *Fgf8*) have been found to specifically label the urethral plate epithelium of the genital tubercle. A lack of genetic markers for these cells has hampered progress in understanding (a) how this signaling center regulates patterning of the external genitalia, (b) how the urethral epithelium undergoes tubulogenesis and differentiation, and (c) how mutations and copy number variants in humans may relate to

abnormal external genital development. The gene expression profiling experiment reported here has identified 84 novel markers of the urethral plate epithelium at E12.5, the stage at which transplantation studies have shown polarizing activity[3]. Based on this analysis, our data suggest that some of the genes identified in the urethral plate may be involved in its organizing function, and some could have roles in the initation of urethral tube development. Forty-five genes that the microarrays showed to be enriched in the urethral plate epithelium were found by *in situ* hybridization to be selective markers of these cells (i.e., expression was undetectable in the adjcent mesenchyme). By producing a large set of novel markers of the early urethral plate epithelium, our analysis of differential gene expression between cell types in the E12.5 genital tubercle extends previous work that profiled gene expression in later stage, intact genital tubercles[22]. We anticipate that mutations in some of these genes could disrupt external genital development and, thus, this panel may provide new candidate genes for use in genetic screening of patients affected by genitourinary anomalies.

Although, synthesis of sex hormones by the gonads is initiated at the developmental stage examined here (E12.5), sexually dimorphic expression of genes located on sex chromosomes and autosomes can be regulated independently of hormones by genetic and epigenetic mechanisms[2324]. Therefore, we compared gene expression patterns in male and female genital tubercles by *in situ* hybridization; however, we found no obvious expression differences between the sexes at E12.5. These findings are consistent with previous reports that sexual differentation of the genital tubercle does not begin until approximatley E15 in mouse[10].

It is noteworthy that for some genes, including *Ano9* and *Dmd*, the microarrays indicated that they were enriched in the urethral epithlium, whereas the *in situ* expression patterns revealed staining in the mesenchyme as well as the epithelium (fig. 3). While we cannot offer a definitive explanation for this, they could represent type I errors in the array dataset. More interesting is the expression pattern of C1qa and Mpeg, each of which localized to a pair of focal populations of cells on either side of the distal urethral epithelium. Delineation of these molecular subdomains of the distal genital tubercle provides a new opportunity to interrogate the identity and role of these cells, and highlights the ability of gene expression profiling to identify previously uncharacterized cell populations.

Using ToppCluster we identified pathway interactions involving the Forkhead box A (FoxA) family of transcription factors (*FoxA1, FoxA2*) *and Shh* (Sup Table 1; http://www.gudmap.org/gudmap/page to be provided by GUDMAP editorial staff). Interestingly, *FoxA1, FoxA2* and *Shh* were among the most enriched genes in the urethral epithelium (Table 1, Journal of Urology URL to be inserted by journal staff). *FoxA* proteins have been labeled pioneer transcription factors based on their ability to remodel the chromatin environment at their binding sites and to facilitate the recruitment of other transcription factors[25]. FoxA1 and FoxA2 play important roles in differentiation and function of a number of different cell types, and deletions of the *FoxA* genes have been shown to cause malformations of the pancreas, vertebrae, and liver[25,26].

Moreover, FoxA1 and FoxA2 have been implicated in prostate and liver cancer, where they can interact directly with androgen receptor (AR), modulate expression of AR- and estrogen

receptor-regulated genes, and regulate sexually dimorphic disease[27,28]. Although the functions of FoxA1 and FoxA2 in the urethra are unknown, it is tempting to speculate that they may have roles in hormonally mediated sexual differentiation of the external genitalia. Furthermore, FoxA proteins can regulate *Shh* in other organ systems[29]. Although Shh is required for external genital development[2,3,10,16–18], its regulation in the genital tubercle is not well understood. If *FoxA1* and *FoxA2* act upstream of *Shh* in the urethra, as they do in other organs, then deletion of both genes in the urethral plate could recapitulate the phenotype of $Shh^{-/-}$ mutants.

Genes identified by ToppCluster as having structural functions include proteins with known roles in tissue maturation and maintenance, including *Cldn3*, *Cldn6*, *Cldn7*, *Crygc*, *Dmd*, *Krt19*, *Krt6a*, *Krt7*, *Krt8*, *Upk1b* and *Wwc2* (fig. 2; http://www.gudmap.org/gudmap/pages/focus_insitu_browse.html?batchId=368). During urethral development, structural proteins in epithelial cells establish cell polarity and control cell permeability[30]. In later stages of bladder development, many of these structural proteins contribute to development of the urothelium, which functions as a barrier to urine[19]. Discovery of these factors in the urethral plate of the genital tubercle raises the possibility that lower urethral epithelial cells may share some properties with the bladder urothelium.

Some of the genes enriched in the urethral endoderm at E12.5, including *Upk1b, Cldn7, Krt8* and *Sox2*, continue to be expressed at later stages of lower genitourinary development. This suggests that they might function at multiple stages, such as during early patterning of the genital tubercle and during later morphogenesis of the urethral tube (Table 2). In addition, we identified 31 genes (*Fgf4, Sox7, Gpr1*, etc) that are enriched in the developing urethra but have not been reported to be enriched in other epithelial populations in the lower urinary tract (Table 2, genes highlighted green). This raises the possibility that these genes may have specific roles in early development of the urethral epithelium. Future studies of their functions will be needed to determine if and how they contribute to urethra development.

Conclusions

Taken together, the data presented here identify dozens of new markers of the urethral plate epithelium, most of which have unknown functions in external genital development, and contributes to a molecular atlas of gene expression in the genital tubercle (all data are publically available at http://www.gudmap.org/gudmap/pages/focus_insitu_browse.html?batchId=368). Our analysis focused on a developmental stage (E12.5) when the urethral epithelium funcitons as a signaling center -- organizing gene expression and patterning in the adjacent mesenchyme -- but before tubulogenesis has been initiated. Previous studies have examined later embryonic stages, when urethral tubulogenesis is underway (E13.5–E17)[8]. Together these data sets provide a weath of new tools for investigations into the molecular mechanisms of external genital development and dysmorphogenesis. We anticipate that the genes identified here will serve as useful molecular markers for urethral epithelial cell types, drive new research aimed at discovering their developmental functions, and.provide new candidate genes for analysis in patients with structural defects of the external genitalia.

Acknowledgments

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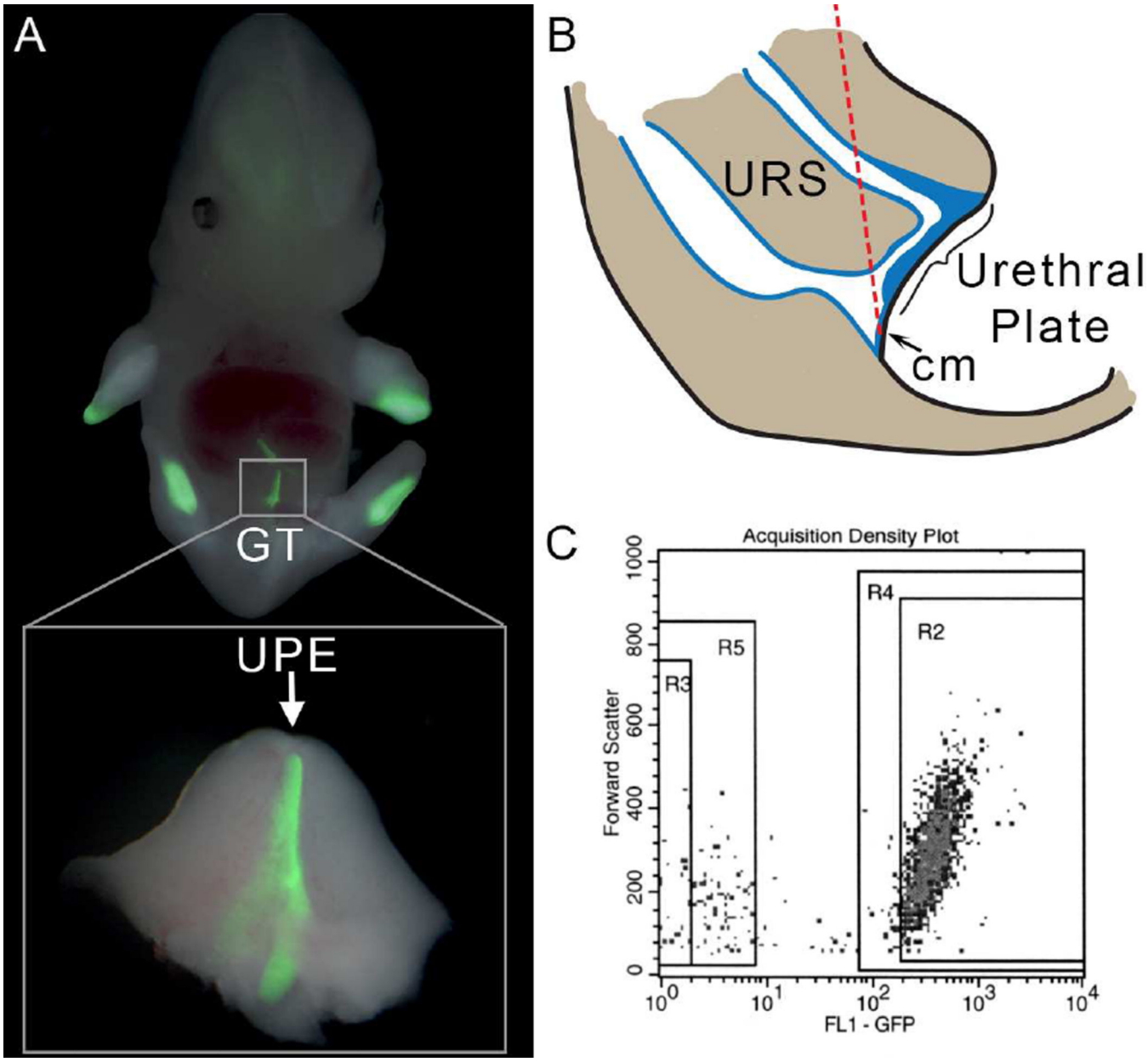

Figure 1.

A. Whole mount *Shh*^{GFPCre};*R26R*^{eYFP} mouse at E12.5. Insert shows high magnification image of the genital tubercle. B. Schematic of a sagittal section through an E12.5 mouse genital tubercle showing the *Shh*-expressing epithelial tissue (blue) and the plane of dissection (dashed red line)[10]. C. Cell distribution according to amount of fluorescence emitted during FACS sorting. GT- Genital tubercle, cm - Cloacal membrane, UPE- Urethral plate epithelium, UGS- Urogenital sinus, URS- Urorectal Septum.

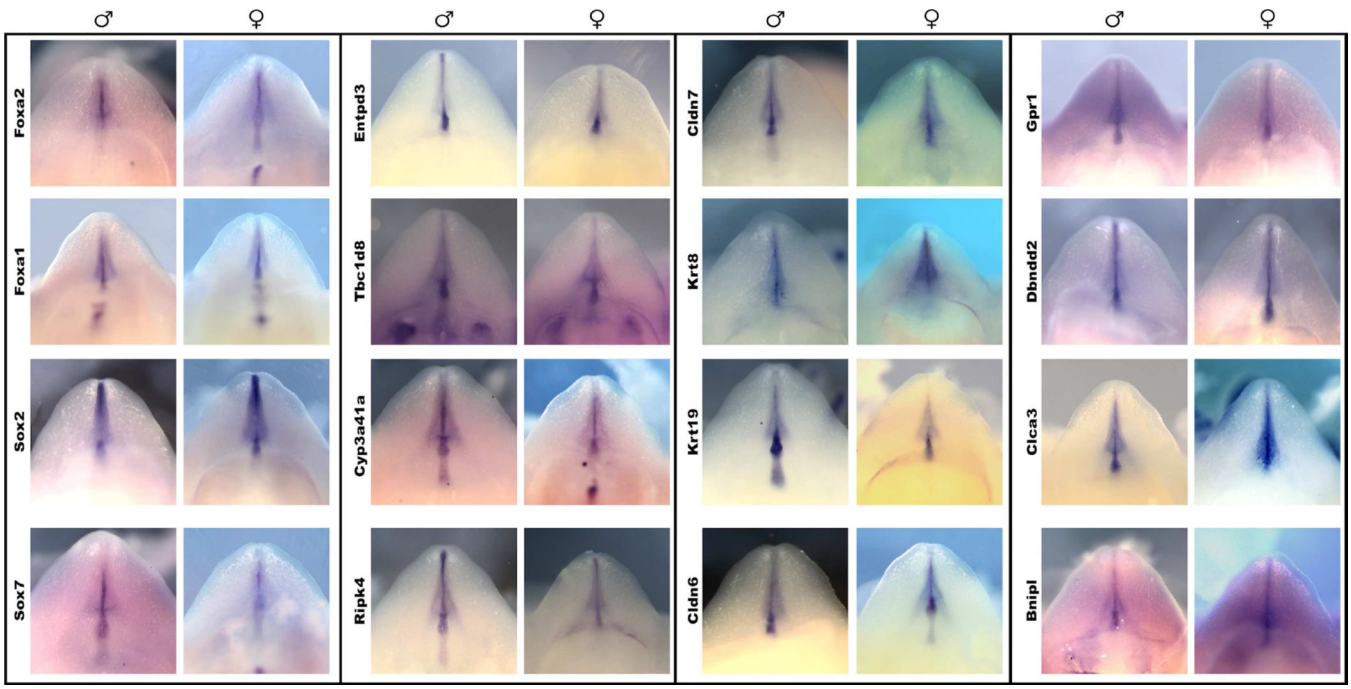

Figure 2.

In situ hybridizations of selected genes. Note that patterns show little spatial regionalization along the urethral plate and there are no obvious differences between male and female expression patterns.

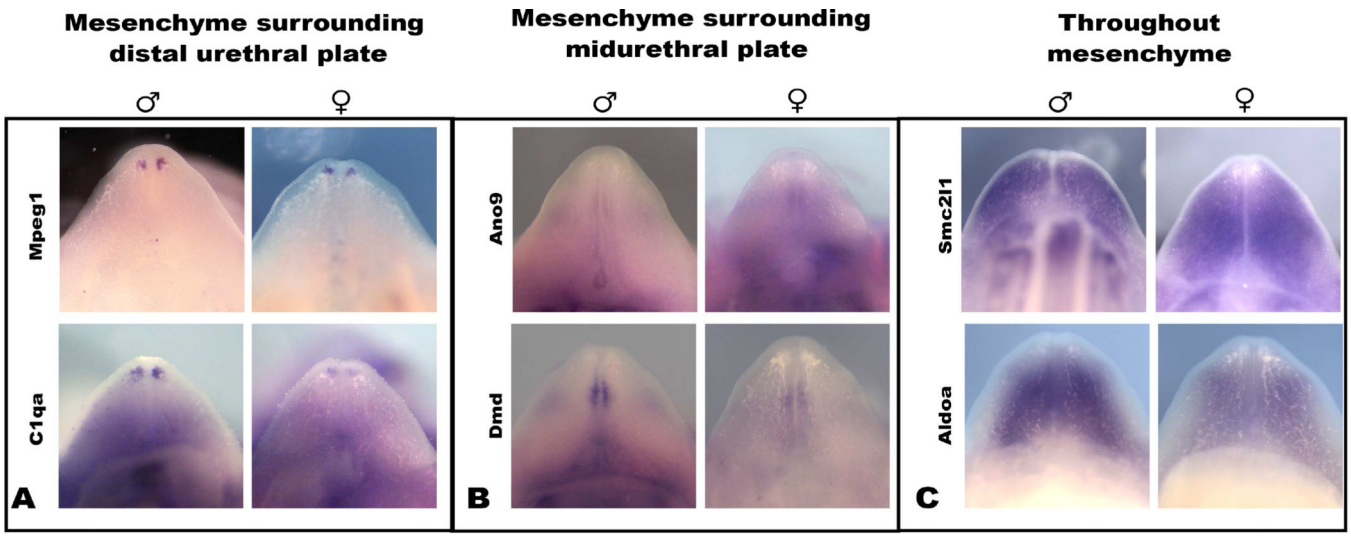

Figure 3.

In situ hybridization showing expression patterns of genes not found in the urethral plate. A. Gene expressed in the mesenchyme surrounding the distal urethral plate. B. Genes expressed in the mesenchyme surrounding the midurethral plate. C. Genes expressed throughout the mesenchyme.

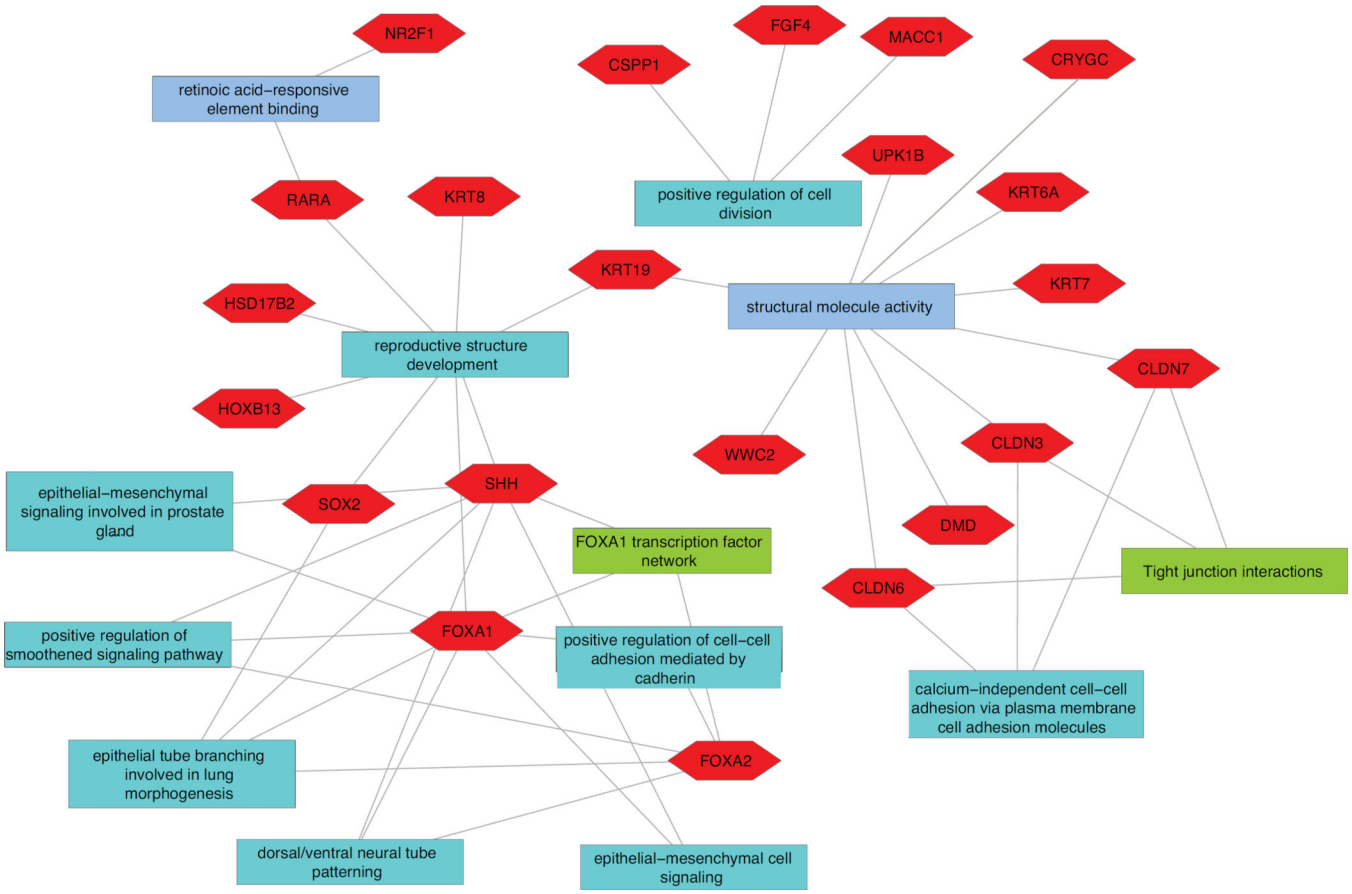

Figure 4.

ToppCluster analysis of genes enriched in the urethral epithelium associated with molecular function (light blue), pathways (green), and biological process (teal).

Table 1

Genes with >20 fold expression differences in the endoderm vs surrounding tissue of the genital tubercle at E12.5.

Gene symbol	Gene Name	Molecular Function	t-value	Fold Change
Foxa2	Forkhead box A2	Transcription factor	6.85	156.3
Krt8	Keratin complex 2, basic, gene 8	Structural molecule	6.928	154.83
Shh	Sonic hedgehog	Signaling	6.694	142.07
Clca3	Chloride channel calcium activated 3	Signaling	6.158	140.34
Mal2	Mal, T-cell differentiation protein 2	Signaling	6.786	127.77
Foxa1	Forkhead box A1	Transcription factor	6.397	116.6
Gstt3	Glutathione S-transferase, theta 3	Catalytic activity	6.64	113.67
Ripk4	Receptor-interacting serine-threonine kinase 4	Catalytic activity	6.135	84.24
Cldn3	Claudin 3	Structural molecule	6.01	83.58
Bnipl	BCL2/adenovirus E1B 19kD interacting protein like	Signaling	6.058	77.35
Cxcl15	Chemokine (C-X-C motif) ligand 15	Signaling	5.252	72.22
	CDNA clone MGC:58416 IMAGE:6707555, complete cds	Unknown	5.813	61.57
Cyp3a41a	Cytochrome P450, family 3, subfamily a, polypeptide 41a	Catalytic activity	5.479	61.11
Krt6a	Keratin complex 2, basic, gene 6a	Structural molecule	5.652	54.67
Anxa8	Annexin A8	Signaling	5.252	52.14
Ces3	Carboxylesterase 3	Catalytic activity	5.593	51.31
Aldoa	Aldolase 1 A	Catalytic activity	5.324	50.35
Cacna1b	Calcium channel, voltage-dependent, N type, alpha 1B subunit	Signaling	5.235	49.47
C1qa	Complement component 1, q subcomponent, alpha polypeptide	Signaling	5.423	45.42
Arhgef33	Rho guanine nucleotide exchange factor (GEF) 33	Signaling	4.777	45.21
Il17re	Interleukin 17 receptor E	Signaling	4.665	45.13
Dennd1c	DENN/MADD domain containing 1C	Catalytic activity	5.129	42.07
Cldn7	Claudin 7	Structural molecule	4.848	41.67
Upk1b	Uroplakin 1B	Signaling	4.946	41.21
Hoxb13	Homeo box B13	Transcription factor	4.651	40.43
Crygc	Crystallin, gamma C	Structural molecule	4.906	39.1
Fabp1	Fatty acid binding protein 1, liver	Catalytic activity	5.108	38.68
Krt19	Keratin complex 1, acidic, gene 19	Structural molecule	4.332	38.53
Akap10	A kinase (PRKA) anchor protein 10	Signaling	4.99	38.3
3110037L02Rik	RIKEN cDNA 3110037L02 gene	Unknown	5.075	38.21
Dbndd2	Dysbindin (dystrobrevin binding protein 1) domain containing 2	Signaling	5.082	37.49
	clone:A330061J11	Unknown	4.909	35.56
S100a6	S100 calcium binding protein A6 (calcyclin)	Signaling	4.459	35.24

Gene symbol	Gene Name	Molecular Function	t-value	Fold Change
2610528J11Rik	RIKEN cDNA 2610528J11 gene	Unknown	4.59	34.53
Sez6l2	Seizure related 6 homolog (mouse)-like 2	Signaling	4.987	34.51
	Transcribed locus	Unknown	4.84	32.96
Entpd3	Ectonucleoside triphosphate diphosphohydrolase 3	Catalytic activity	4.742	32.71
Ano9	Anoctamin 9	Signaling	4.619	32.32
Fgf4	Fiberblast growth factor 4	Signaling	4.563	32.16
Sox7	SRY-box containing gene 7	Transcription factor	4.875	32.15
Ccl2	Chemokine (C-C motif) ligand 2	Signaling	4.431	31.24
Macc1	Metastasis associated in colon cancer 1	Signaling	4.832	31.12
Gls2	Glutaminase 2 (liver, mitochondrial)	Catalytic activity	4.796	30.88
	Transcribed locus	Unknown	4.798	30.82
Eps8l3	ESP8-like 3	Signaling	4.349	30.72
Gpr1	G protein-coupled receptor 1	Signaling	4.605	30.68
Cab39l	Calcium binding protein 39-like	Signaling	4.707	30.58
Pcdh17	Protocadherin 17	Structural Molecule	4.477	30.48
H2-D1	Histocompatibility 2, D region locus 1	Signaling	4.767	30.32
4930403O15Rik	RIKEN cDNA 4930403O15 gene	Unknown	4.596	30.11
Slc16a12	Solute carrier family 16 (monocarboxylic acid transporters), member 12	Signaling	4.461	30.06
Fbxw2	F-box and WD-40 domain protein 2	Signaling	4.378	29.93
Drd1a	Dopamine receptor D1A	Signaling	4.413	29.57
Spata16	Spermatogenesis associated 16	Signaling	4.364	29.43
		Unknown	4.751	28.9
Il16	Interleukin 16	Signaling	4.655	28.62
Dmd	Dystrophin, muscular dystrophy	Structural molecule	4.501	28.21
	Transcribed locus	Unknown	4.478	27.69
Klrd1	Killer cell lectin-like receptor, subfamily D, member 1	Signaling	4.424	27.5
Cpa6	Carboxypeptidase A6	Catalytic activity	4.708	27.31
	Transcribed locus	Unknown	4.533	26.96
Smc2	SMC2 structural maintenance of chromosomes 2-like 1 (yeast)	Catalytic activity	4.641	26.81
Plxnb3	Plexin B3		4.54	26.77
Hsd17b2	Hydroxysteroid (17-beta) dehydrogenase 2	Catalytic activity	4.563	26.46
Cspp1	Centrosome and spindle pole associated protein 1	Structural Molecule	4.327	26.45
8030498B09Rik	RIKEN cDNA 8030498B09 gene	Unknown	4.459	26.34
Sox2	SRY-box containing gene 2	Transcription factor	4.465	26.13
Acpp	Acid phosphatase, prostate	Catalytic activity	4.511	26.12
A630095E13Rik	RIKEN cDNA A630095E13	Unknown	4.599	25.64

Gene symbol	Gene Name	Molecular Function	t-value	Fold Change
2310045N14Rik	RIKEN cDNA 2310045N14 gene	Unknown	4.565	25.08
Elavl3	ELAV (embryonic lethal, abnormal vision, Drosophila)-like 3 (Hu antigen C)	Signaling	4.382	24.75
Mpeg1	Macrophage expressed gene 1	Signaling	4.341	24.2
R3hdm1	R3H domain (binds single-stranded nucleic acids)	Signaling	4.331	23.75
Tbc1d8	TBC1 domain family, member 8	Catalytic activity	4.422	23.35
Asb14	Ankyrin repeat and SOCS box-containing protein 14	Signaling	4.419	23.03
Arhgef19	Rho guanine nucleotide exchange factor (GEF) 19	Signaling	4.396	23.02
C030048H21Rik	RIKEN cDNA C030048H21	Unknown	4.392	23.02
Krt7	Keratin complex 2, basic, gene 7	Structural molecule	4.361	22.49
Wwc2	WW, C2 and coiled-coil domain containing 2	Signaling	4.371	22.41
4930558F17Rik	RIKEN cDNA 4930558F17	Unknown	4.383	22.3
	Transcribed locus	Unknown	4.419	22.27
Rara	Retinoic acid receptor, alpha	Transcription factor	4.36	22.01
Ear3	Eosinophil-associated, ribonuclease A family, member 3	Catalytic activity	4.347	21.79
Cldn6	Claudin 6	Structural molecule	4.331	21.63
Fam46c	Family with sequence similarity 46, member C		−4.966	−34.48
Hbb-b1/ Hbb-b2	Hemoglobin, beta adult major chain /// hemoglobin, beta adult minor chain		−4.96	−35.71
Slc4a1	Solute carrier family 4 (anion exchanger), member 1		−5.08	−38.46
Hbb-y	Hemoglobin Y, beta-like embryonic chain		−4.518	−90.90
Hbb-bh1	Hemoglobin Z, beta-like embryonic chain		−5.002	−104.16

Table 2

Comparison of genes found in E12.5 urethral plate epithelium to genes reported to be in urogenital sinus epithelium during different developmental stages according to ToppCluster.

	E12.5 urothelium	E13.5 bladder urothelium	E13.5 bladder neck and urethra	E17.5 bladder urothelium	E18.5 bladder urothelium	P2 urothelium		E12.5 urothelium	E13.5 bladder urothelium	E13.5 bladder neck and urethra	E17.5 bladder urothelium	E18.5 bladder urothelium	P2 urothelium
2610528j11rik	■	■		■	■	■	FGF4						
ACPP	■	■	■	■	■	■	FOXA1	■	■		■	■	■
AKAP1							FOXA2	■	■	■			
ALDOA				■	■		GLS2						
ANO9	■	■		■	■	■	GPR1						
ANXA8	■			■	■		H2-D1						
ARHGEF19	■			■	■		HOXB13	■	■	■	■	■	
ARHGEF33							HSD17B2		■		■	■	
ASB14							IL16						
BNIPL	■			■	■		IL17RE	■	■	■	■	■	■
C1QA							KLRD1						
CAB39L							KRT6A	■	■	■	■	■	
CACNA1B		■	■				KRT7	■	■	■	■	■	■
CCL2							KRT8	■	■	■	■	■	■
CES3	■	■	■				KRT19	■	■	■	■	■	■
CLCA3							MACC1	■	■	■	■	■	■
CLDN3	■	■	■	■	■		MAL2	■	■	■	■	■	■
CLDN6	■	■	■	■	■		MPEG1						
CLDN7	■	■	■	■	■	■	PCDH17						
CRYGC							PLXNB3						
CSPP1							R3HDM1						
CPA6	■	■		■	■	■	RARA						
CXCL15							RIPK4	■	■	■	■	■	■
CYP3A41A							S100A6	■	■				
DBNDD2				■	■	■	SEZ6L2						
DENND1C	■			■			SHH	■	■	■	■	■	■
DMD			■				SLC16A12						■
DRD1A							SMC2						
EAR3							SOX2	■	■	■	■		
ELAVL3							SOX7						
ENTPD3	■	■	■	■	■	■	SPATA16						
EPS8L3							TBC1D8	■			■	■	
FABP1		■					UPK1B	■	■	■	■	■	■
FBXW2			■				WWC2						

Genes in green boxes are only reported in E12.5 urethral plate epithelium and blue boxes indicate that the genes have been reported in E12.5 urethral plate and other urogenital sinus epithelium.