Travelling down the tube: developmental biology of the ureter and urinary bladder

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Who are they and what is it?
The Nobel Prize in Physics 2010
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University of Manchester, UK

Discovered graphene…
a new class of material…
….2D atomic crystals
Topics Covered in this Lecture

- Formation of smooth muscle in lower renal tract
- Urinary tract epithelial differentiation
- Neuro-muscular differentiation of the urinary bladder
End-Stage Renal Failure (ESRF) in Children

- Of the 800 children in the United Kingdom with ESRF, about half were born with renal tract malformations.
- As a cause of childhood ESRF, renal tract malformations are x6 more common than any of the following:
  - The congenital nephrotic syndromes;
  - Metabolic diseases (cystinosis, primary hyperoxaluria);
  - The nephronophthisis spectrum of disorders.
- North American Pediatric Renal Trials and Collaborative Studies database confirms that renal tract malformations are the commonest causes of chronic renal failure in children.
The Spectrum of Human Kidney Malformations

1. **Agenesis** (= the kidney never formed)
2. **Dysplasia** (= undifferentiated kidneys which may contain cysts)
3. **Hypoplasia** (= too few nephrons per kidney)
Spectrum of Kidney Malformations

Anomalies of the Ureter often Accompany Kidney Malformations

Multicystic Dysplastic Kidney with Atretic Ureter

Pelvi-Ureteric Junction Obstruction

Vesico-Ureteric Reflux
Metanephric Cell Lineages

Five weeks human gestation = embryonic day 10.5 mouse
Harmonised Kidney and Lower Renal Tract Development

Metanephric kidney

Fetal urinary bladder
Fetal Ureteric Peristalsis

1. Relaxed

2. Contraction begins

3. Wave half way down

4. Wave nears bladder

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Teashirt Transcription Factors

*teashirt* is implicated in the development of the *Drosophila* kidney where it is expressed in mesenchymal stellate cells precursors intercalating between principal cells.

Stellate cells express *teashirt* (yellow)

Data from Helen Skaer and Barry Denholm, Cambridge, UK
Developing Urinary Tract Muscle Expresses Teashirt-3 (Tshz3)

Teashirt-3 Expression at the Beginning of Renal Tract Development

Congenital Hydronephrosis in *Teashirt*-3 Homozygous Null Mutant Mice (Phenotype Apparent from Embryonic Day 16)

Wild-type  Null Mutant

There is no Anatomical Block to Urine Flow in Tshz3 Mutant Mice

Wild-type

Null Mutant

In Late Gestation, Proximal Ureters of *Tshz3* Mutants Lack Smooth Muscle

Organ Culture of E15 Ureters for Six Days - The Top of the Tshz3 Mutant Ureter Fails to Undergo Peristalsis

Failure to Initiate Expression of Smooth Muscle Contractile Proteins In the Tshz3 Mutant Mouse Proximal Ureter

Intact SHH and BMP4 Signals in Tshz3 Null Mutant Ureters

Yu J et al. Development 129:5301-5312

Teashirt-3 and Myocardin are downstream to BMP4 in smooth muscle differentiation

Myocardin

Tshz3 and Myocardin mRNA levels pre and post BMP4

Ureter Myogenesis: Putting Teashirt-3 into Context

Sonic Hedgehog growth factor

TSHZ3 and Human Renal Malformations?

- TSHZ3 is expressed in normal human embryos in the proximal ureter. 

- Translocations and a chromosomal deletion disrupting the 19q12 region where TSHZ3 resides reported in patients with hydonephrosis and multicystic dysplastic kidney

Groenen PM et al Genomics 38:141-148, 1996
In 48 children with pelvi-ureteric junction obstruction, we found no mutations of *TSHZ3*.

Non-significant increased frequency of missense (E469G) polymorphism versus 633 controls.

Glutamic acid, a negatively charged and hydrophilic molecule, is changed to glycine, a nonpolar and hydrophobic residue.

Jenkins D *et al* Nephrol Dial Transplant 2010;25:54-60
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Mature Urothelium *In Vivo*

Superficial urothelial “umbrella” cells are coated by plaques of Asymmetric Unit Membrane (AUM) which limit transcellular permeability.

Jenny Southgate, University of York, UK
Uroplakins are Major Urothelial Proteins

Fetal ureters

Uroplakin

$\alpha$-Smooth muscle actin

Uroplakins

- Asymmetric unit membrane
- Family of uroplakin proteins
Uroplakin III Null Mutant Mice Have Malformed Ureters and Bladders

Wildtype

Mutant

Vesico-ureteric junctions

Urothelial surfaces

Kidneys

Kong XT et al J Cell Biol 167:1195-1204, 2004
Uroplakin III Protein Expression in the Human Fetus

- Seven week bladder (urogenital sinus)
- Thirteen week ureter
- Thirteen week renal pelvis

De Novo UPIIIa Missense Mutations Cause Human Renal Tract Malformations

Xenopus Uroplakin III Triggers Frog Development

- In unfertilized frog eggs, xUPIII is protein is localised to the lipid rafts on the egg cell surface.
- After fertilization, xUPIII is phosphorylated on tyrosine residue-249 in the carboxyl-terminal cytoplasmic tail.
- An antibody against xUPIII blocks sperm-egg interaction and blocks egg activation.

Sakakibara K et al J Biol Chem 280: 15029-15037, 2005
Embryonic Urothelial Cells Express both *Uroplakins* and *SHH*

Jenkins D *et al* *J Anat* 211:620-629, 2007
HARMONISED URINARY TRACT DEVELOPMENT

Onset of urine production by fetal kidney

▼

Uroplakins sense a (yet-to-be defined) component of fetal urine

▼

Urothelia differentiate and establish a paracrine signalling centre

▼

Urothelialial growth factors pattern adjacent smooth muscle

▼

Ureteric peristalsis propels urine to the bladder
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Bernardo Ochoa Defined the Urofacial (Ochoa) Syndrome

• “During the last 40 years over 100 patients have been reported with a dysfunctional lower urinary tract associated with a peculiar distortion of the facial expression.
• Genetic studies have demonstrated that this condition is inherited as an autosomal recessive trait, mapped to chromosome 10q23-q24.
• Patients with this syndrome as well as those with subclinical neurological bladder, occult neuropathic bladder, non-neurogenic neurogenic bladder (or Hinman syndrome)…may be affected by the same congenital disorder of neurological origin”.

Individuals with Urofacial (Ochoa) Syndrome Who are Smiling

Cystograms Showing a Spectrum of Dysmorphology in Uro-facial Syndrome

Hyper-reflexic, incompletely emptying, trabeculated bladders with vesicoureteric reflux

Whole genome SNP mapping in index case defined an autozygous region of 16Mb on chromosome 10q23-24.

Homozygous mutations of HPSE2 found in this and then in several other families.

HPSE2 encodes a “haparanase-like” protein.

In health, HPSE2 is expressed in both brain and the urinary tract.
What Does Heparanase Do?

• Heparanase activity cleaves heparan sulphate, thus modifying side chains of proteoglycans.

• Heparanase activity is implicated in organogenesis, angiogenesis, inflammation and tumour metastasis.

• Heparanase expression is induced in many hematological and solid tumors, associated with poor prognosis.
Heparanase 2 (▲) Interacts with Heparan Sulfate with High Affinity and Inhibits Heparanase 1 (●) Activity

Immunolocalisation of heparanase-2 in fetal human urinary bladder

Stuart HM, Roberts N et al submitted
Innervation of the Urinary Bladder

*Hpse2* null mutant mice have megabladders

Wild type  Homozygous mutant

Woolf AS & Roberts N, unpublished data
Experimental downregulation of *hpse2* in *Xenopus tropicalis*

Roberts N, Hilton E et al. unpublished data
Experimental downregulation of *hpse2*
In *Xenopus tropicalis*
Impaired neurogenesis
Summary

- Urinary tract epithelial differentiation
  ... Uroplakins
- Formation of smooth muscle in lower renal tract.... Teashirt-3
- Neuro-muscular differentiation of the urinary bladder.... Heparanase-2
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