Ureter Myogenesis: Putting Teashirt into Context

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ABSTRACT

After the basic shape of the mammalian ureter is established, its epithelia mature and a coat of smooth muscle cells differentiate around nascent urothelia. The ureter actively propels tubular fluid from the renal pelvis to the bladder, and this peristalsis, which starts in the fetal period, requires coordinated smooth muscle contraction. Teashirt-3 (Tshz3) is expressed in smooth muscle cell precursors that form the wall of the forming mammalian ureter. The Teashirt gene family was first identified in Drosophila where Teashirt (Tsh) protein acts as a transcription factor directing embryonic anterior-posterior patterning and leg and eye development. In fly embryonic renal tubules, Tsh is expressed in mesodermally derived stellate cells intercalating between principal cells, and a paralogue, tipstop, is expressed in forming tubules. Teashirt is a component of several gene networks in flies and it is notable that similar networks control mammalian renal tract development. Null mutation of Tshz3 in mice leads to failure of functional muscularization in the top of the ureter and this is followed by congenital hydronephrosis. A signaling pathway can be envisaged, starting with sonic hedgehog secreted by the nascent ureteric urothelium and ending with ureteric smooth muscle cell differentiation, with Tshz3 downstream of bone morphogenetic protein 4 and upstream of myocardin and smooth muscle cell contractile protein synthesis. The phenotype of Tshz3 mutant mice resembles that of human congenital pelviureteric junction obstruction, and we suggest these individuals may have mutations of genes encoding molecules in the differentiation pathway mediated by Tshz3.

The initiation of the metanephros, the precursor of the mature kidney, occurs between 10 and 11 days (d) of gestation in the mouse, anatomically equivalent to 24 to 30 d gestation in humans.¹ The ureter originates as an epithelial outgrowth of the mesonephric duct, and this ureteric bud (UB) then induces a subset of intermediate mesodermal cells to form nephrons. Hence, the severest form of ureter malformation is agenesis (failure to initiate), and this is necessarily accompanied by kidney agenesis. UB growth is stimulated by glial-cell-line-derived neurotrophic factor,² the renal mesenchymal expression of which is controlled by transcription factors³ and modified by matrix molecules.⁴ The paired box-2 (PAX2) transcription factor upregulates expression of RET, the main glial-cell-line-derived neurotrophic factor receptor on the UB.⁵ On the basis of examination of over 700,000 livebirths, stillbirths, and induced abortions, Wiesel et al.⁶ reported unilateral renal tract agenesis in 0.008% of these individuals, with bilateral renal agenesis and/or severely malformed kidneys in 0.013%. Human RET mutations are associated with renal agenesis,⁷ as are mutations of FRASI and KAL1, genes encoding for basement-membrane-associated UB proteins.⁸⁻¹⁰

A too cranial or caudal UB origin causes incomplete interaction with renal mesenchyme, respectively resulting in an obstructed kidney associated with an ectopic ureter inserted into the urethra or a kidney attached to a ureter affected by vesicoureteric reflux (VUR).¹¹,¹² Despite numerous molecules that regulate UB positioning,¹³⁻¹⁶ anomalies associated with ectopic/exuberant budding are common, with VUR occurring in at least 1% to 2% of children¹⁷ and ureteric duplication in approximately 2% of the population.¹⁸ The lower part of the UB stalk must integrate with the nascent urinary bladder, a process mediated by RET.¹⁹ Variants of RET²⁰ and ROBO2 (a gene regulating UB position)¹⁶ and PAX2 mutations²¹ are associated with VUR, and ROBO2 mutations are also reported in humans with duplex ureters.¹⁶

EPITHELIAL DIFFERENTIATION OF THE URETER

After the basic shape of the ureter has

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been established, its epithelia mature and the epithelial tube acquires a coat of smooth muscle cells (SMCs; Figure 1). UB stalk epithelia differentiate to become a watertight barrier, a property conferred by uroplakin (Upk) proteins, which form heterodimers on the apical epithelial surface. Null mutations of UpkIIIA or UpkII in mice lead to malformed ureters, which are either refluxing or obstructed by epithelial overgrowth. Of note, between 28 to 35 d of normal human gestation (anatomically equivalent to days 10.5 to 12 in mice), the ureteric lumen becomes obstructed and then re-canalizes: the reason is not established but it may be related to epithelial maturation changes. Human UpkIIIA mutations are described in patients with anomalies including VUR, hydronephrosis (dilated renal pelvis), and multicystic dysplastic kidney, the latter a malformation associated with a ureter that lacks a patent lumen.

THE DIFFERENTIATION OF URETERIC MUSCLE

The mature ureter actively propels tubular fluid from the renal pelvis to the bladder, and this peristalsis requires coordinated smooth muscle (SM) contraction and relaxation. SM bundle arrangement varies along the length of the mature ureter. In mice, SM formation begins in the proximal ureter nearest to the kidney at embryonic day 15 and proceeds distally: it is characterized by upregulation of the characteristic muscle proteins smooth muscle α actin (SMAA), SM myosin heavy chain, and a 22-kD SM protein. Similarly, in humans, muscularization begins in the proximal ureter, with spiral SM bundles detected at 12 weeks (wk) gestation. From the 17th week, longitudinal bundles are seen in the distal ureter wall, and from 22 wk a muscle coat forms around the portion of the ureter traversing the bladder wall.

The renal pelvis and proximal ureter contain sites of activity that initiate peristaltic waves: the pacemakers themselves may be “atypical” SMCs (akin to gut interstitial cells of Cajal), which have few contractile filaments and sparse immunoreactivity to SMAA. Cells expressing the Kit receptor tyrosine kinase, thought to derive from a periaortic region, surround the nascent ureter and may be involved in pacemaker activity and/or propagation of the contraction wave because antibodies to Kit block embryonic ureteric peristalsis. Interestingly, embryonic ureteric proximal to distal peristalsis occurs when isolated organs are maintained in organ culture, thus peristalsis can occur without innervation; however, in vivo sensory afferents are thought to modulate peristalsis.

TEASHIRT-3 IN THE GENETIC CONTROL OF URETERIC SM FORMATION

We recently reported that null mutation of the Teashirt-3 (Tshz3) gene in mice causes congenital hydronephrosis, with 100% penetrance on a CD1 background. The teashirt gene family was first identified in Drosophila where Teashirt (Tsh) protein acts as a transcription factor directing embryonic anterior-posterior patterning and leg and eye development. Note that the name “Teashirt” arose as a corruption of “T-shirt” and that the expression of tsh in fly ectoderm recalled the pattern of this item of clothing. In fly embryonic renal (Malpighian) tubules, tsh is expressed in mesodermally derived stellate cells intercalating between principal cells, and a paralog, tiptop, is expressed in tubules and may be required for their morphogenesis. tsh is a component of several gene networks in flies and it is notable that similar networks control mammalian urinary tract development: these networks contain homeobox and paired-box transcription factors and molecules in Wnt and hedgehog pathways. Further, Wnt products are implicated in Wnt signaling, a pathway deregulated in rats with hereditary congenital obstructive uropathy. Three Tshz orthologues have been described in mice and each can substitute for Drosophila tsh in genetic rescue experiments.

It had already been noted that null mutation of Tshz1 in mice caused a spectrum of anomalies including middle ear and soft palate malformations and homoeotic transformations of the cervical
and thoracic vertebrae. Although Tshz1 is expressed in the somites, spinal cord, limb buds, and branchial arches we failed to find significant expression of this gene in the developing ureter (L. Fasano and X. Caubit, unpublished observations). By contrast, Tshz3 is expressed in undifferentiated mesenchymal cells, which surround the nascent urothelium of the ureteric stalk, and in SMCs, which form in the wall of the ureter. In Tshz3 null mutant mice, we found that although SMC precursors proliferate and aggregate around the epithelium in the proximal ureter as normal, they fail to differentiate into SMCs (Figure 1) as assessed by absence of SM cytoskeletal markers. Mitchell et al. previously noted that developing ureters upregulate myocardin, and we found that Tshz3 mutant ureters failed to express this transcriptional coactivator, which is implicated in differentiation of smooth muscles in other locations. Despite the lack of SMCs, the urothelium differentiates, as assessed by Upk expression (Figure 1). We also showed that when Tshz3 mutant embryonic ureters were explanted in organ culture before the onset of hydronephrosis, they failed to undergo peristalsis, as occurs in wild types. Thus the hydronephrosis seen in vivo is a direct result of functional urinary flow impairment, and the failure of SMC formation is a primary event rather than being secondary to mechanical disruption after hydronephrosis.

**PLACING TSH IN A MOLECULAR PATHWAY OF SMC DIFFERENTIATION**

Yu et al. note that sonic hedgehog (Shh) is expressed in fetal mouse urothelium and that targeted downregulation leads to dilation of the ureter along its whole length associated with a significant delay in ureteric SMC formation. In normal development, the ureteric epithelium acts as a signaling center, releasing Shh and signaling to periproximal mesenchymal cells through the patched homologue 1 (Ptc1) receptor. In vitro, it induces these cells to proliferate and, at first glance paradoxically, prevents their differentiation into SMCs. However, the situation is more complex in vivo because Shh is also necessary for the expression of bone morphogenetic factor 4 (Bmp4) and in vivo experiments show that Bmp4 is necessary for ureteric SMC formation. In vitro, the addition of Bmp4 to embryonic renal tract explants increases SM differentiation in ureteric cells, at least as assessed by SMAA expression. In Tshz3 null mutant ureters, Shh transcripts are expressed as normal by the urothelium, whereas transcripts for Ptc1 (encoding the Shh receptor) and Bmp4 are expressed as normal by adjacent mesenchymal cells. The population of ureteric cells expressing Bmp4 originates in the tail bud, and Bmp4 expression around the UB stalk is needed for upregulation of MSC cytoskeletal proteins several days later. The periurothelial location of Bmp4-expressing cells is similar to the pattern of Tshz3 expression, and Bmp4 increases mRNA levels encoding Tshz3 in organ cultures of embryonic renal tracts. In wild-type embryonic renal tracts, Bmp4 induces the differentiation of ureteric SMCs in association with phosphorylation of Smad1, 5, and 8 yet these phosphorylated proteins are present in the wall of the proximal ureter in Tshz3 null mutant mice.

Putting all of the data together, one can envisage a signaling pathway starting with Shh in the urothelium and ending with ureteric SMC differentiation, with Tshz3 downstream of Bmp4 but upstream of myocardin and SMC contractile protein synthesis. As depicted in Figure 2, the simplest model includes an autocrine loop in which mesenchymal cells generate Bmp4, which then enhances SMC differentiation by upregulating Tshz3, which itself stimulates myocardin expression. It is possible that Bmp4 induction of Tshz3 is mediated by
pSmad and Smad1, 5, and 8, although other pathways, such as those involving mitogen-activated protein kinase/extracellular signal-regulated kinase, may be implicated.

Bmp4 not only enhances ureteric SMC differentiation but is also essential for urothelial differentiation. In the Tshz3 null mutant model, the expression of Bmp4 transcripts is preserved in the proximal ureter, as is urothelial differentiation, as assessed by Upk expression. This firstly demonstrates that urothelial differentiation can be uncoupled from muscularization. Second, these observations are consistent with an alternative explanation of how Bmp4 enhances SMC differentiation. Here, under the influence of Bmp4, the nascent urothelium would send a yet-to-be-defined paracrine signal, which then induces SMC differentiation in a Tshz3-dependent manner. Finally, with respect to Bmp4, it is important to note this protein has other actions on renal tract development, including correct positioning of the UB and enhancement of ureter elongation as well as glomerulogenesis. Weber et al. reported BMP4 missense changes in individuals with malformations including renal agenesis, dysplasia, and hypoplasia.

**TSHZ3 AND THE DIVERSITY OF MUSCLE IN THE RENAL TRACT**

Tshz3 is expressed in differentiating SMCs in the distal and proximal parts of the ureter, and Shh signaling is required for normal SMC differentiation along the whole ureter. Despite these observations, in Tshz3 mutant mice, myogenesis occurs almost normally in the distal ureter despite the absence of SMCs in the proximal ureter. This suggests that other molecular pathways exist that make Tshz3 functionally less important in the distal ureter. In fact, the arrangement of muscle bundles in the ureter varies along its length, consistent with the notion that SM-forming pathways show regional differences. In the renal pelvis, muscle fibers run obliquely, and as the pelvis merges with the ureter itself, “bundles of different orientation lie side by side, so that the ureter consists of braided bundles of muscle fibers arranged in interlacing spirals.”

In the distal ureter there exists an inner longitudinal SM layer surrounded by circular muscle, and longitudinal SM bundles line the urothelial tube as it traverses the intrinsic (detrusor) bladder muscle wall. Similar considerations apply to the urinary bladder where, although Shh signaling is implicated in detrusor myogenesis and Tshz3 is expressed in the embryonic bladder, bladder muscle formation appears grossly normal in Tshz3 null mice.

**Other Genes are Involved in Ureter Muscularization**

Angiotensin II signaling through the angiotensin II receptor type 2 is required for apoptotic modeling of the ureter, although there is no deregulation of programmed cell death in Tshz3 null proximal ureters. The T-box18 (Tbx18) transcription factor is expressed in ureteral mesenchyme, and in Tbx18 null mutant mice some ureteric mesenchymal cells fail to aggregate around the ureter and instead mislocalize to the kidney periphery; furthermore, proliferation of the epithelia and mesenchyme is reduced. The expression of Shh, Ptc1, and Bmp4 transcripts are downregulated in Tbx18 mutant ureters, so Tbx18 acts upstream of Shh and Bmp4. Bmp4 expression is also dependent on Gata2 and deletion of Id2, a Bmp4 target gene, causes congenital hydronephrosis. In Discs large homologue one (Dlgh1) null mice, fetal ureters are short with misaligned SM bundles. Dlgh1 encodes a PDZ domain protein expressed in nascent urothelium and SMCs, but it is unclear how it controls ureteric development. Of note, whereas Dlgh1 mutant ureters lack stromal cells between the urothelium and the SMC layer, stromal cells expressing retinaldehyde dehydrogenase 2 are present in Tshz3 null mutant ureters (Figure 2).

Later in ureter development, other molecules play roles in muscle function and these include angiotensin II signaling through angiotensin II receptor type 1 and calcineurin subunit 1b, a component of the calcium-dependent serine-threonine phosphatase. When these molecules are perturbed, hydronephrosis tends to occur postnatally, as opposed to Tshz3 mutants in which it presents several days before birth. However, the hydronephrosis in angiotensinogen-deficient mice is rescued by restoration of angiotensin II in the brain, suggesting that central mechanisms rather than an intrinsic ureteric SM defect are causative. Finally, although an early report suggests that genetic deletion of Adams-1, encoding for a protease associated with the extracellular matrix, led to excess collagen fibers between ureteric epithelia and SMCs, a more recent report failed to find the gene expressed in the ureter, instead attributing hydronephrosis to a failure of renal medulla maturation.

**TEASHIRT-3 AND CONGENITAL DISEASES AFFECTING RENAL TRACT SM**

Hydronephrosis is a common finding in human fetal anomaly scans. It is usually transient, perhaps reflecting the normal high flow of fetal hypotonic urine and/or a modest delay in SM maturation. Rarely, persistent hydronephrosis is a sign of bladder outflow obstruction, usually caused by posterior urethral valves. Experimental fetal bladder outflow obstruction leads to striking detrusor and ureteric muscle overgrowth. In other organ systems, myocardin is implicated in myocyte hypertrophy, leading to the possibility that this molecule, and therefore Tshz3, plays roles in similar responses in the anatomically obstructed developing urinary tract.

More commonly, congenital hydronephrosis is a manifestation of either VUR or pelvureteric junction obstruction (PUJO; also called ureteropelvic junction obstruction). Indeed, congenital PUJO occurs in 0.3% to 0.4% of births. We speculate that active propulsion of tubular fluid from the renal pelvis to the bladder becomes most critical toward the end of human gestation, when the fetal head usually rests in the
mother’s (skeletal) pelvis. Thus, fetal urine must often travel against gravity and, should peristalsis fail, “functional obstruction” will follow, manifested by hydrenephrosis. Hence, one hypothesis is that hydrenephrosis in the context of PUJO is the result of a failed peristaltic mechanism. Indeed, studies of pelviureteric junctions resected at operation for congenital PUJO report histology with various muscular anomalies, including deranged arrangement of muscle bundles,\(^8\) SM hypertrophy and fibrosis,\(^9,10\) and a lesser number of muscle bundles than normal.\(^11\)

The sequences and chromosomal loci of the three human \(TSHZ\) genes are known (\(TSHZ1\)q22.3; \(TSHZ2\)q213.2; \(TSHZ3\)q13.11). Of relevance to \(TSHZ3\) being a candidate malformation gene are reports of two individuals with chromosomal rearrangements disrupting the 19q locus where \(TSHZ3\) resides: one individual had hydrenephrosis and the other had a multicystic dysplastic kidney.\(^12,13\) Intriguingly, families exist with dominant inheritance of PUJO,\(^14\) and we suggest they should be screened for \(TSHZ\) family mutations.

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DISCLOSURES

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