Published in final edited form as:

J Urol. 2011 June ; 185(6): 2320–2328. doi:10.1016/j.juro.2011.02.044.

Cell death serves as a single etiological cause of a wide spectrum of congenital urinary tract defects

Qiusha Guo¹, Piyush Tripathi¹, Edward Poyo^{1,2}, Yinqiu Wang¹, Paul F. Austin³, Carlton M. Bates⁴, and Feng Chen^{1,*}

¹Renal Division, Department of Internal Medicine, Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO, USA

²Department of Biomedical Engineering, School of Engineering, Washington University in St. Louis, MO, USA

³Division of Pediatric Urology, Department of Department of Surgery, Washington University School of Medicine, St. Louis, MO, USA

⁴Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, PA

Abstract

Purpose—We genetically disrupted the Wolffian duct (WD) in mice to study the affected organogenesis processes and to test the hypothesis that cell loss can be the developmental basis for a wide spectrum of congenital anomalies in the kidney and urinary tract.

Materials and Methods—We use *Hoxb7-Cre* transgenic lines (*HC1* and *HC2*) to induce diphtheria toxin (DT) production from a *ROSA^{DTA}* allele, disrupting the wolffian duct and derivatives.

Results—The first set of mutants (*HC1;ROSA^{DTA/+}*) exhibited agenesis of the kidney, ureter, and reproductive tracts. The second set of mutants (*HC2;ROSA^{DTA/+}*) exhibited diverse defects, including renal agenesis/hypoplasia, hydronephrosis, hydroureter, ureter-vas deferens fistulas in males and ureter-oviduct/uterus fistulas in females. The phenotypic differences correspond to the degree of apoptosis-induced caudal truncation of the wolffian duct, which is less severe and more variable in *HC2;ROSA^{DTA/+}* mice. Whenever the wolffian duct failed to reach the urogenital sinus, the ureter failed to separate from the wolffian duct, suggesting that ureteral migration along the common nephric duct to the cloaca and the subsequent common nephric duct degeneration constitute the only pathway for separating the ureter and WD derivatives.

Conclusions—The diverse and severe defects observed emphasize the central role of the wolffian duct in providing progenitors and signals for urogenital development. These results also indicate that the quantitative difference in cell death-induced caudal truncation of the wolffian duct can lead to a wide range of qualitatively distinct defects, and that cell death can serve as a single etiological cause of a wide spectrum of congenital kidney and urinary tract defects.

Keywords

Congenital anomalies diphtheria toxin; mesoderm; urogenital abnormalities; wolffian ducts

*Corresponding Author: Feng Chen, Department of Internal Medicine/Renal Division, Campus Box 8126, Washington University School of Medicine, St. Louis, MO 63110, USA. Phone: (314) 362-3162; Fax: (314) 362-8237; fchen@dom.wustl.edu.

Introduction

Congenital anomalies of the kidney and urinary tract represent a significant cause of morbidity and mortality in the pediatric population.¹ These anomalies occur in many forms, including renal agenesis, renal hypoplasia, horseshoe kidney, hydronephrosis, hydroureter, vesicoureteral reflux (VUR), and others. Interestingly, multiple defects can be found within an affected family or even within an affected individual.¹ Despite recent advancements, the genetic or environmental determinants in most cases are still difficult to ascertain.¹ Excessive alcohol consumption during pregnancy can cause birth defects (fetal alcohol syndrome) that include congenital urinary tract defects.² At least in the nervous system most abnormalities are attributed to cell death due to ethanol toxicity.^{3, 4} How cell death may lead to urinary tract defects has not been directly studied in fetal alcohol syndrome or other conditions involving congenital urinary tract defects.

The Wolffian duct, also called mesonephric or nephric duct, emerges from the intermediate mesoderm to connect the mesonephros to the cloaca. During mid gestation, the ureteric bud (UB) branches out from the WD (at about 28 days in human and E10.5 in mice) and invades the metanephric mesenchyme within the intermediate mesoderm. The UB branches inside the MM to form the collecting system and induce nephron formation.^{5–7} The UB stalk develops into the ureter. The WD and (MD) are present in both genders until sexual differentiation, when MD develops into the female reproductive tract consisting of the oviduct, uterus and upper third of the vagina, and the WD differentiates into the male genital tract, including the vas deferens, epididymis, and rete testis.

Cell loss is common in diseased tissues as a consequence of either necrosis or apoptosis.⁸ Cell loss of sufficient magnitude impairs organ function, resulting in clinically overt diseases.⁸ Surgical, chemical, and genetic cell ablation have been used to study the roles of specific cell populations and to generate disease models.⁹ Among these techniques genetically controlled cell ablation offers the highest precision and consistency. The A chain of diphtheria toxin (DTA) causes apoptosis through its inhibition of the eukaryotic elongation factor 2.^{10, 11} Since rodents have no DTA receptors, transgene controlled production of DTA induces apoptosis in target cells without affecting their neighbors.¹¹ In this study, we use mice with WD-specific expression of Cre to induce DTA production and apoptosis in WD cells. In addition to revealing a central role of the WD in urogenital development, our results also showed that a single factor—cell death—can lead to a wide spectrum of congenital anomalies of the kidney and urinary tract.

Materials and Methods

Mouse strains and sample collection

All animal studies have been approved by the Institutional Animal Care and Use Committee at Washington University and conducted according to NIH guidelines. The *Hoxb7-Cre-EGFP* (*HC1*), *Hoxb7-Cre* (*HC2*), and the *ROSA^{DTA}* allele were described previously.^{11–14} Mice carrying *HC1* (or *HC2*) were crossed with mice carrying *ROSA^{DTA}* to produce embryos/mice (designated as mutants) producing DTA in the WD and derivatives. Their littermates with no DTA production were controls. The *ROSA^{LacZ}* transgene¹⁵ was used to fate map Cre-expressing cells. *ROSA^{DTA}* encodes an attenuated DTA, DTA176, which prevents unintended cell death due to potential minor leakage of the promoters used.¹¹

Histological Analysis

For histological analyses embryos were fixed with 4% paraformaldehyde and embedded in paraffin. Sections of 5 μ m were collected and stained following standard protocol. For immunohistochemistry, sections were stained with an anti-BrdU antibody (Developmental

Studies Hybridoma Bank, 1: 200 ratio) and an anti-Pax2 antibody (Covance, Princeton, New Jersey, 1:200). Appropriate AlexaFluor488 or 555-conjugated secondary antibodies (Molecular Probe, Eugene, Oregon, 1:1,000 ratio) were used to detect the corresponding primary antibodies. Whole-mount immunostaining was carried out with the anti-Pax2 antibody and an anti-E-Cadherin antibody (BD Transduction Laboratories™, 1:200 ratio) as described.¹⁶

β-galactosidase assay

β-galactosidase assay was performed as previously described.¹⁷ Briefly, embryos or tissues were collected from timed pregnant females and fixed in 0.2% glutaraldehyde for 15–30 minutes. After permeabilization (10% TritonX™ 100 in phosphate buffer) embryos and tissues were stained in X-gal solution (50 mM potassium ferricyanide, 50 mM potassium ferrocyanide, 1 mg/ml X-gal) and post-fixed with 4% paraformaldehyde.

Proliferation and Apoptosis

BrdU was injected intraperitoneally into pregnant mice (100 µg/g body weight) 30 minutes before harvest and was detected by a mouse monoclonal anti-BrdU antibody (Developmental Studies Hybridoma Bank, 1: 200 ratio). TUNEL analysis was performed on paraffin-embedded sections using the ApopTag® plus peroxidase *in situ* apoptosis detection kit. Proliferation index is presented as the number of BrdU-positive cells per 100 cells counted, and apoptosis index as number of TUNEL-positive cells per 100 cells counted.

Statistical analysis

TUNEL and BrdU immunohistochemistry results were statistically analyzed using student's *t*-test. A *p* value of 0.05 or less was considered significant. At least 3 sets of samples were analyzed for each data point.

Results

Cell death in WD induced by Diphtheria Toxin

To study the impact of cell loss during nephrogenesis, we generated mice carrying a *Hoxb7-Cre* transgene (*HCI*) and a *ROSA^{DTA}* allele with a *loxP*-flanked transcriptional stop cassette placed between the ubiquitous *ROSA* promoter and the DTA coding sequence. This stop cassette prevents DTA transcription and subsequent cell death. When Cre is present, recombination between the *loxP* sites removes the stop cassette, leading to DTA transcription, translational arrest, and subsequent apoptosis (Fig. 1A). The *HCI* transgene produces Cre specifically in the WD, as demonstrated by β-galactosidase assay on samples from mice carrying *HCI* and *ROSA^{LacZ}* (Fig. 1B–E).¹⁵ Thus, mice carrying both *HCI* and *ROSA^{DTA}* (mutants) would have DTA expression and cell death in the WD and its derivatives, including the ureteric epithelium. However, their littermates carrying 1 or none of these alleles (controls) would not have any transgene-induced cell loss.

Cell death in WD results in congenital urogenital defects

Newborn mice carrying both *HCI* and *ROSA^{DTA}* had kidney and ureter agenesis (Fig. 2A–D). The vas deferens and the seminal vesicles were also missing in the male mutants (Fig. 2B). Since the male reproductive tract derives from the WD, the severe developmental disruption of these structures is not surprising. However, the testis was not affected. While the ovary develops normally in female mutants, the uterus is missing (Fig. 2D), indicating the complete dependency of the development of the uterus (a müllerian duct derivative not targeted by DTA) on the WD.

Since almost no 2 transgenes behave exactly the same way, to further examine if potential minor differences in the level of WD disruption would lead to different outcomes, we generated a second system in which a different *Hoxb7-Cre* transgene (*HC2*)¹² was used to induce DTA production and cell death in the WD. Interestingly, *HC2;ROSA^{DTA/+}* mutants exhibitd wide range of defects (Fig. 2E–L). Besides agenesis, *HC2;ROSA^{DTA/+}* mutants also have renal hypoplasia, hydronephrosis and hydroureter. Most of these defects are characteristic for congenital anomalies of the kidney and urinary tract in humans.

Fistulae between ureter and reproductive tracts

In *HC2;ROSA^{DTA/+}* mutants the kidney was connected to the testis instead of the bladder, in 50% of the males and to the ovary in 39% of the females (Fig. 3). These aberrant connections were apparently the results of fistulae formed between the ureter and the vas deferens/epididymis (Fig. 3B, J and N) or uterus/oviduct (Fig. 3D, L and P). Fistulas start to form in mid gestation, as revealed by whole-mount immunostaining for Pax2 in the urogenital ridges of the developing embryos at E12.5 (Fig. 3E–H). DTA induced cells death in the WD affected its extension toward the cloaca, leading to caudal truncation of the WD. In most *HC2;ROSA^{DTA/+}* mice, the caudal truncation of the WD prevent its contact with the urogenital sinus and the formation of the CND (Fig. 3).

In some mutants with disrupted CND formation, the ureteral bud could still reach the MM to initiate nephrogenesis. In such male mutants, the UB-derived ureter was never separated from the WD. As the WD differentiated into the male reproductive tract below the testis, the UB and the male reproductive tract were connected via a fistula. This connection disrupts the urinary path to the bladder and inevitably results in severe urinary tract obstruction and lethality when presented bilaterally. In females with disrupted CND formation fistulae between the ureter and the female reproductive tract occurred. These malformations differ from those in the males because the female reproductive tract derives from the MD but not the WD. MD emerges at E12.5 near the anterior end of the urogenital ridge in mice. It extends caudally alongside the WD and reaches the cloaca area at about E13.5.

When CND is missing due to the caudal truncation of the WD, instead of elongating towards the urogenital sinus (Fig. 3E), the MD turns to follow the ureters (Fig. 3 F–H), and eventually formed the fistulae between the ureters and oviducts/uterus at later stages in mutant females (Fig. 3L and P). Such fistulas in both genders disrupt the urinary path to the bladder, leading to severe urinary tract obstruction. However, urinary tract obstruction also occurred in *HC2;ROSA^{DTA/+}* mice without fistulae (Fig. 2 and data not shown). Such obstructions are not directly caused by WD caudal truncation. Rather, they appear to result from the apoptosis induced structural and functional alterations in the ureter that eventually affect pyeloureteral peristalsis.

Variations in cell death within the WD correspond to congenital urogenital defects

To confirm that the variation in mutant phenotypes is associated with the differences in levels of cell death, we have examined proliferation and apoptosis on sections from control and mutant mice. The proliferation rate in the WD was significantly reduced in mutants (Fig. 4). More importantly, significantly more WD cells were undergoing apoptosis in the *HC1;ROSA^{DTA/+}* mutants. Excessive cell death was also detected in the MM of these mutants. The *HC2;ROSA^{DTA/+}* mutants also demonstrated significantly reduced proliferation and significantly increased apoptosis in the WD (Fig. 5). Reduced proliferation likely reflects the fact that DTA forced cells that would otherwise proliferate into the apoptosis pathway. Compared to the *HC1;ROSA^{DTA/+}* mutants, alterations in the *HC2;ROSA^{DTA/+}* mutants were less dramatic and more variable. The higher and more uniform level of apoptosis found in the *HC1;ROSA^{DTA/+}* mice is consistent with the more

severe and consistent defects (bilateral kidney and ureter agenesis) in these mutants. The lower and more variable levels of apoptosis in the *HC2;ROSA^{DTA/+}* mutants appear to be the underlying reason for the milder and more variable defects found in the *HC2;ROSA^{DTA/+}* mutants. Similar differences were detected as early as E9.5 (data not shown). Variations in cell death of the WD at earlier stages lead to various degree of caudal truncation of the WD, which, in turn, causes a wide range of defects.

Discussion

In this report, we use genetically controlled tissue-specific diphtheria toxin expression to disrupt the WD during embryogenesis to assess directly the impact of cell death on urogenital development. We found that various levels of cell death in the WD result in congenital urogenital defects ranging from agenesis to milder malformation of multiple urogenital organs. These results provide support for the hypothesis that a single factor, cell death, can be the cause of a wide spectrum of congenital anomalies of the kidney and urinary tract.

The severe agenesis and malformation of multiple urogenital organs in the mutants emphasize an essential role of the WD in urogenital development. Phenotypic differences observed in the 2 lines may result from subtle variations, beyond current experimental detection limits, in the timing and completeness of Cre expression. Regardless of the origins, such variations led to differences in the level apoptosis in targeted cell populations (Fig. 4–5) and subsequently in phenotypic outcomes.

In mutants from both lines variations in apoptosis in the WD would lead to the cumulative effect of shortening of the WD at its caudal end to different degrees (Fig. 3F). The WDs in most *HC1;ROSA^{DTA/+}* mutants fail to extend to the UB budding site, resulting in renal agenesis. Caudal truncation of the WD in *HC2;ROSA^{DTA/+}* mutants is less severe but more variable. When the WD extends beyond the UB budding site but fails to reach the cloaca, nephrogenesis can occur in the absence of ureter-bladder connection, resulting in ureteral fistulas. If the WD manages to reach the cloaca, nephrogenesis and ureter-bladder connection can occur, although frequently accompanied by renal hypoplasia, hydroureter, and hydronephrosis (Fig. 1 and data not shown). These later defects may result from the ongoing DTA-induced apoptosis in the ureteral bud derivative and the subsequent negative effects on MM differentiation. Thus, quantitative differences in apoptosis and caudal truncation can translate into qualitatively distinct outcomes (renal agenesis, urogenital fistulas, or other urinary tract defects) in affected individuals.

In humans, fistulas of the genitourinary tract can involve multiple structures, such as kidney, ureter, bladder, urethra and the reproductive ducts. These fistulae can be congenital or secondary to infection, inflammatory disease, neoplasms, trauma or surgery.^{18–21} Congenital ureteral fistulae connected to the male or the female reproductive tracts are also called ureteral ectopia and their etiology is unclear in most patients.^{22–25} In many male *HC2;ROSA^{DTA/+}* mutants, the CND was missing while the ureteral bud reached the MM to initiate nephrogenesis. In such mutants, the UB failed to separate from the WD, resulting in aberrant connection of their derivatives, the ureter and the male reproductive tract. Thus, the transient CND is critical for the separation of the UB and the WD during ureter maturation. The developmental sequence involving the vertical migration of the UB along the CND, lateral migration of the ureteral bud away from the WD and the degeneration of the CND²⁶ appears to be the only pathway for the separation of the ureter and WD derivatives.

After emerging at E12.5 near the anterior end of the urogenital ridge, the MD normally follows the WD toward the cloaca area.^{27–30} The directional change in MD elongation to

follow the ureter in the event of WD caudal truncation (Fig. 3) suggests that the ureter retains the guidance ability of the WD for MD development. While previous studies suggest that the WD does not contribute cells to the MD,²⁷ the presence of the ureter-oviduct/uterus fistulas raises the possibility that direct cell-cell exchange between the WD and the MD becomes possible under pathological conditions. Apoptosis in some of the WD cells may compromise the integrity of the WD, providing the tightly associated MD entry points and establishing the fistulas between WD and MD derivatives.

Conclusions

Urogenital defects associated with WD cell death emphasize the central role of the WD in providing progenitors and signals for urogenital development. Although the WD signals for regulating MD are still unclear, the ureter appears to be able to provide such signals for MD guidance. The observed ureter-oviduct/uterus fistulae suggest that cell death allows otherwise impossible direct epithelial connection between WD and MD. Results from this study also indicate that quantitative differences in cell death-induced caudal truncation of the WD can lead to a wide range of qualitatively distinct defects and that cell death can serve as a single etiological cause of a wide spectrum of congenital kidney and urinary tract defects.

Acknowledgments

Drs. M. R. Capecchi, A. P. McMahon, and P. Soriano for the *ROSA^{DTA}*, *Hoxb7-Cre*, and the *ROSA^{LacZ}* mice, respectively.

References

1. Miyazaki Y, Ichikawa I. Ontogeny of congenital anomalies of the kidney and urinary tract, CAKUT. *Pediatr Int*. 2003; 45:598. [PubMed: 14521544]
2. Hofer R, Burd L. Review of published studies of kidney, liver, and gastrointestinal birth defects in fetal alcohol spectrum disorders. *Birth Defects Res A Clin Mol Teratol*. 2009; 85:179. [PubMed: 19180632]
3. Smith SM. Alcohol-induced cell death in the embryo. *Alcohol Health Res World*. 1997; 21:287. [PubMed: 15706739]
4. Sulik KK, Cook CS, Webster WS. Teratogens and craniofacial malformations: relationships to cell death. *Development*. 1988; 103(Suppl):213. [PubMed: 3074910]
5. Schedl A. Renal abnormalities and their developmental origin. *Nature Reviews genetics*. 2007; 8:791.
6. Dressler GR. The cellular basis of kidney development. *Annu Rev Cell Dev Biol*. 2006; 22:509. [PubMed: 16822174]
7. Staack A, Donjacour AA, Brody J, et al. Mouse urogenital development: a practical approach. *Differentiation*. 2003; 71:402. [PubMed: 12969333]
8. Hetts SW. To die or not to die: an overview of apoptosis and its role in disease. *Jama*. 1998; 279:300. [PubMed: 9450715]
9. O'Kane CJ, Moffat KG. Selective cell ablation and genetic surgery. *Curr Opin Genet Dev*. 1992; 2:602. [PubMed: 1525514]
10. Lee P, Morley G, Huang Q, et al. Conditional lineage ablation to model human diseases. *Proc Natl Acad Sci U S A*. 1998; 95:11371. [PubMed: 9736743]
11. Wu S, Wu Y, Capecchi MR. Motoneurons and oligodendrocytes are sequentially generated from neural stem cells but do not appear to share common lineage-restricted progenitors in vivo. *Development*. 2006; 133:581. [PubMed: 16407399]
12. Yu J, Carroll TJ, McMahon AP. Sonic hedgehog regulates proliferation and differentiation of mesenchymal cells in the mouse metanephric kidney. *Development*. 2002; 129:5301. [PubMed: 12399320]

13. Zhao H, Kegg H, Grady S, et al. Role of fibroblast growth factor receptors 1 and 2 in the ureteric bud. *Dev Biol.* 2004; 276:403. [PubMed: 15581874]
14. Shakya R, Jho EH, Kotka P, et al. The role of GDNF in patterning the excretory system. *Dev Biol.* 2005; 283:70. [PubMed: 15890330]
15. Soriano P. Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nature Genetics.* 1999; 21:70. [PubMed: 9916792]
16. Jia Q, McDill BW, Li SZ, et al. Smad signaling in the neural crest regulates cardiac outflow tract remodeling through cell autonomous and non-cell autonomous effects. *Dev Biol.* 2007; 311:172. [PubMed: 17916348]
17. Wang Y, Tripathi P, Guo Q, et al. Cre/lox recombination in the lower urinary tract. *Genesis.* 2009; 47:409. [PubMed: 19415630]
18. Dwyer PL, Rosamilia A. Congenital urogenital anomalies that are associated with the persistence of Gartner's duct: a review. *Am J Obstet Gynecol.* 2006; 195:354. [PubMed: 16890546]
19. Mori M, Matsubara K, Abe E, et al. Prenatal diagnosis of persistent cloaca associated with VATER (vertebral defects, anal atresia, tracheo-esophageal fistula, and renal dysplasia). *Tohoku J Exp Med.* 2007; 213:291. [PubMed: 18075232]
20. Shibata T, Nonomura K, Kakizaki H, et al. A case of unique communication between blind-ending ectopic ureter and ipsilateral hemi-hematocolpometra in uterus didelphys. *J Urol.* 1995; 153:1208. [PubMed: 7869501]
21. Yu NC, Raman SS, Patel M, et al. Fistulas of the genitourinary tract: a radiologic review. *Radiographics.* 2004; 24:1331. [PubMed: 15371612]
22. Billmeyer BR, Nygaard IE, Kreder KJ. Ureterouterine and vesicoureterovaginal fistulas as a complication of cesarean section. *J Urol.* 2001; 165:1212. [PubMed: 11257679]
23. Braslis KG, Stephens DA. Uretero-fallopian fistula: an unusual complication of open ureterolithotomy. *J Urol.* 1993; 150:1900. [PubMed: 8230531]
24. Steckel J, Badillo F, Waldbaum RS. Uretero-fallopian tube fistula secondary to laparoscopic fulguration of pelvic endometriosis. *J Urol.* 1993; 149:1128. [PubMed: 8483229]
25. Lazarevski MB, Badiev B. Obstetric ureterouterine fistula: a case report. *Am J Obstet Gynecol.* 1996; 174:1657. [PubMed: 9065152]
26. Batourina E, Tsai S, Lambert S, et al. Apoptosis induced by vitamin A signaling is crucial for connecting the ureters to the bladder. *Nat Genet.* 2005; 37:1082. [PubMed: 16186816]
27. Orvis GD, Behringer RR. Cellular mechanisms of Mullerian duct formation in the mouse. *Dev Biol.* 2007; 306:493. [PubMed: 17467685]
28. Carroll TJ, Park JS, Hayashi S, et al. Wnt9b plays a central role in the regulation of mesenchymal to epithelial transitions underlying organogenesis of the mammalian urogenital system. *Dev Cell.* 2005; 9:283. [PubMed: 16054034]
29. Jacob M, Konrad K, Jacob HJ. Early development of the mullerian duct in avian embryos with reference to the human. An ultrastructural and immunohistochemical study. *Cells Tissues Organs.* 1999; 164:63. [PubMed: 10352885]
30. Kobayashi A, Kwan KM, Carroll TJ, et al. Distinct and sequential tissue-specific activities of the LIM-class homeobox gene *Lim1* for tubular morphogenesis during kidney development. *Development.* 2005; 132:2809. [PubMed: 15930111]

Fig 1. Genetically controlled ablation of WD by Cre-mediated production of DTA

Cre expression induces the production of DTA and the apoptosis of targeted cells(A).

Expression of *Hoxb7-Cre-EGFP* transgene as revealed by *ROSA^{LacZ}* reporter(B–E). The β -galactosidase activity in *Hoxb7-Cre-EGFP; ROSA^{LacZ}* E10.5 embryo (B), E11.5 (C), E12.5 (D) and E13.5 urogenital ridges. The *Hoxb7-Cre-EGFP* transgene driven Cre expression is specifically detected in WD(arrows) and UB(arrowheads), but not in the MM.

Fig 2. Targeted disruption of the WD causes broad range of urogenital defects by two separate *Hoxb7-Cre* transgenes

Instead of forming the normal urogenital systems in the control newborns (A and C), agenesis of the kidneys, ureters and reproductive ducts was found in both males (B) and females (D) *HC1;ROSA^{DTA/+}* mutants. Meanwhile, comparing to the control newborns (E and I), use of the second *Hoxb7-Cre* transgene (*HC2*) caused less severe but more variable defects in the urogenital system, including hydroureter (F and J), hypoplasia (G and H), hydronephrosis (J), renal agenesis (H, K and L) and unusual fistulas (arrowheads in G and K) formed between ureter and vas deferens (G) as well as the ureter and oviduct/uterus (K). K: kidney; U: ureter; T: testis; B: bladder; O: ovary. VD: Vas Deferens.

Fig 3. Cell exchange between WD and the MD can occur during the development of urogenital system, at least under pathologic conditions

Dissection of urogenital system of control (A and C) and *HC2;ROSA^{DTA/+}* mutant (B and D) E18.5 embryos. Fistulas formed between ureter and vas deferens in male (B) and oviducts/uterus in female (D) mutants. Pax2 staining of urogenital ridge at E12.5 demonstrates that instead of elongating towards the urogenital sinus in the control (E), MD turned to follow direction of ureter (boxed areas in F), and eventually formed fistulas between the ureters and oviducts/uterus in females or ureters and vas deferens in males at later stage in the *HC2;ROSA^{DTA/+}* mutants. Higher magnification pictures of the boxed areas in F are shown in E-H. Arrowheads indicate the migrating cells of the tip of developing MD duct are invading and merging toward the ureter. I-P: E-Cadherin staining of urogenital ridge at E14.5 and E18.5 reveals abnormal connections between ureters and reproductive ducts in *HC2;ROSA^{DTA/+}* E14.5 and E18.5 mutants. B: bladder; FRD: female reproductive duct; G: gonad; K: kidney; MRD: male reproductive duct. O: ovary. U: ureter.

Fig. 4. *In situ* detection of apoptosis and proliferation in WD of *HCl;ROSA^{DTA/+}* mutant embryos

Control and mutant embryos at E10.5 (A–F), E11.5 (G–L) and E12.5 (M–R) were analyzed for apoptosis and proliferation using the TUNEL assay and BrdU incorporation, respectively. Transverse sections were stained with H&E for histological analysis (A, D, G, J, M and P). Histological sections in *HCl;ROSA^{DTA/+}* E10.5 (D) and E11.5 (J) mutants demonstrate presence of WD and MM, while only MM exists in E12.5 (P) mutants. *HCl;ROSA^{DTA/+}* mutant embryos have much less BrdU-positive cells (green cells in E, K and Q) in WD compared to control (B, H and N). *Pax2* expression (red cells in B, E, H, K, N and Q) marks the locations of MM and WD. Proliferation index was calculated as the percentage of nuclei that incorporated BrdU. Extensive apoptosis was detected at E10.5 (F), E11.5 (L) and E12.5 (R) for *HCl;ROSA^{DTA/+}* mutant embryos in the WD and MM area compared with their control littermates (C, I and O). Statistic analysis of proliferation and cell death in *HCl;ROSA^{DTA/+}* mutant embryos (red columns) and their control littermates (blue columns) are shown in S and T. K: kidney; U: ureter.

Fig. 5. *In situ* detection of apoptosis and proliferation in WD of *HC2;ROSA^{DTA/+}* mutant embryos

Control and mutant embryos at E10.5 (A–F), E11.5 (G–L) and E12.5 (M–R) were analyzed for apoptosis and proliferation. Transverse sections (A, D, G, J, M and P) were stained with H&E for histological analysis. The histological sections in *HC2;ROSA^{DTA/+}* E10.5 (D), E11.5 (J) and E12.5 (P) mutants show the presence of WD and MM. *HC2;ROSA^{DTA/+}* mutant embryos have fewer BrdU-positive cells (green cells in E, K and Q) in WD compared to controls (B, H and N), as well as more apoptosis (green in F, L and R) compared to control littermates (C, I and O). *Pax2* expression (red cells in B, E, H, K, N and Q) marks the locations of MM and WD. Statistic analysis of proliferation and cell death in *HC2;ROSA^{DTA/+}* mutant embryos (red columns) and their control littermates (blue) is illustrated in S and T.