ORIGINAL ARTICLE

Factors influencing the development of Multicystic Dysplastic Kidney (MCDK) following urinary tract obstruction in the fetal lamb

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Abstract
Background Creating obstructive uropathy (OU) during glomerulogenesis in the fetal lamb results in multicystic dysplastic kidney (MCDK) at term. We explored this using immunohistochemical techniques.
Method OU was created in fetal lambs at 60-day gestation, ligating the urethra and urachus. The kidneys of MCDK lambs, 60-day gestation fetal lambs, full-term lamb (145 days), term sham-operated lambs, and adult ewes were evaluated by HE staining, and immunohistochemistry with paired box genes 2 (PAX2) and CD10.
Results Multiple cysts were found in the MCDK model. CD10 was expressed in proximal tubular epithelial cells, glomerular epithelial cells, and medullary stromal cells in the kidneys of 60-day gestation fetal lambs and full-term lambs and adult ewes. PAX2 expression was found in ureteric buds, C- and S-shaped bodies, epithelial cells of collecting ducts, and Bowman's capsule of fetal kidneys at 60-day gestation, but only in the collecting ducts of full-term fetal lambs and adult ewes. Both CD10 and PAX2 were expressed in the cystic epithelial cells of the MCDK model.
Discussion PAX2 expression in cystic epithelial cells suggests that cyst formation is associated with disturbed down-regulation of PAX2 in the nephrogenic zone epithelial cells during the renal development in the OU model.

Keywords Multicystic dysplastic kidney · MCDK · Kidney · PAX2 · CD10

Introduction

Multicystic dysplastic kidney (MCDK) is one of the most common renal anomalies leading to chronic renal failure in children. The diagnosis is based on the characteristic histology of the abnormal and incomplete differentiation of metanephric mesenchymal tissue and ureteric buds, with fibromuscular tissue surrounding the cysts. MCDK can regress spontaneously, but when MCDK occurs bilaterally,

it leads to the development of Potter's syndrome. MCDK is often detected on routine fetal ultrasound examination, occurring between 1 in 3640 and 1 in 4300 live births [1], which is a high rate among the wide range of congenital anomalies of the kidney and urinary tract (CAKUT). Although the causes of dysplastic kidneys in humans are unknown, it is believed that the urinary tract obstruction is intimately involved, based on animal models utilizing ligation of the urinary tract during embryonic development, which results in dysplastic kidneys. Dysplastic kidneys in humans are also often associated with obstructive uropathy. Our previous studies have also demonstrated MCDK development following obstruction of the urinary tract of fetal lambs [2].

In previous fetal lamb studies, these cysts were thought to be caused by urine accumulation in the developing nephron due to urinary tract obstruction, resulting in physical dilatation of the tubules and collecting ducts [3]. Electron microscopy studies in our fetal lamb OU model suggested that the cysts were derived from dilated proximal tubules [4]. CD10 has been found to be a useful antibody for identifying

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proximal tubules using immunohistochemistry [5], and we have demonstrated CD10 in the epithelial cells of the MCDK cysts [6].

PAX2 is a transcription factor required for renal development. Mutations in the PAX2 gene cause renal aplasia, dysplastic kidneys, and MCDK [7]. It is the factor most likely to be involved in the development of MCDK.

In this study, CD10 and PAX2 were explored in the obstructive uropathy (OU) model in fetal lambs and compared with normal term lambs, sham-operated term lambs, and adult sheep's kidneys.

Materials and methods

Approval was obtained from the Animal Ethics Committee of the Wellington School of Medicine and Health Sciences, the University of Otago Wellington, New Zealand (approval numbers WAEC 2–11, 3–18). The following groups of animals were used: term OU (MCKD) lambs ($n=6$), normal 60-days gestation lambs ($n=5$), sham model term lambs ($n=4$), normal 145-days gestation (term) lambs ($n=4$), and the left kidneys from normal adult ewes ($n=2$).

Timed gestation (60 day gestation ± 1 day) ewes were transported from the farm to the laboratory 24–48 h before the operation. Our preoperative and anesthetic management has been reported previously [2, 8]. The MCDK models were created as follows. The uterus was exposed through a left flank incision in the ewe, with the caudal end of the lamb being delivered through a transverse hysterotomy. The fetal urethra and urachus were ligated as previously described [2, 8]. The lambs were returned to the uterus, and the uterine and abdominal incisions were closed. The sham models were exposed through a transverse hysterotomy at 60 days gestation to the same extent as required to create an OU model, for the mean time taken to create our OU model, and returned to the uterus without urinary tract obstruction. Normal 60-day gestation fetal lambs and the sham-operated term lambs were simply euthanized as previously described [2, 8] and processed as outlined below.

The ewes were anesthetized again at 145-day gestation (term), and the lambs were delivered via cesarean section. The lambs were then sacrificed by injecting pentobarbital into their umbilical vein, as we have previously described [2, 8]. The lambs' and ewes' kidneys were then removed and fixed in 10% formalin. The kidneys were divided longitudinally, and samples were taken from the cut surface of the kidney, processed for light microscopy in paraffin blocks, and sliced at 3 μm for light microscopy. Sections were deparaffinized in xylene and rehydrated in a graded series of alcohol, followed by H_2O_2. The sections were then either stained with hematoxylin and eosin (H&E) or subjected to the immunohistochemical analysis. For immunohistochemistry,

we used anti-PAX2 rabbit monoclonal antibody, ab79389 (Abcam), 1:500 dilution, for 60 min, and CD10 monoclonal antibody, 56C6 (Nichirei bioscience), undiluted solution, for 60 min [6, 12].

Results

The number of samples

Six lambs were used for the MCDK model, with the urachus and urethra being ligated at 60 days gestation. There were 4 normal term (145 days) lambs, 5 normal lambs sacrificed at 60-day gestation, 4 sham-operated term lambs and 2 adult ewes that were used as controls. The kidneys of fetal lambs at day 60 of gestation were evaluated at the maximum cleavage plane of both kidneys because of their small size, and only the left kidneys were evaluated histologically in the term lambs and adults.

Morphological changes of the kidney in fetal lambs: immunohistochemistry

The nephrogenic zone (NZ), containing ureteric buds, metanephric mesenchymal cells, and C- and S-shaped bodies were observed in the sub-capsular regions of the kidneys of lambs at 60 days gestation (Fig. 1a). We also identified mature glomeruli and tubules deep in the cortex and near the medulla. The NZ had disappeared in the kidneys of term fetuses (Fig. 1b, c). The histology of the kidneys in the Sham model lambs was no different from normal controls (Fig. 1d).

CD10 was strongly expressed in the tubular epithelial cells at 60 days gestation, and its expression was also observed in the glomerular epithelial cells, Bowman's capsule epithelial cells, and medullary stromal cells (Fig. 2a). CD10 expression was also observed in the tubular epithelial cells, glomerular epithelial cells, Bowman's capsule epithelial cells, and medullary stromal cells both in normal term lambs' kidneys, sham model lambs' kidneys and the kidneys of the adult sheep (Fig. 2b, c, d) (Table 1).

PAX2 expression was observed in the ureteric buds, C- and S-shaped bodies, collecting duct epithelial cells, and some Bowman's capsule epithelial cells at 60-day gestation in the fetal kidneys (Fig. 3a). In the normal term lambs' kidneys, the sham model lambs' kidneys, and the kidneys of adult sheep, PAX2 expression was observed only in the collecting ducts (Fig. 3b, c, d) (Table 2).

In the MCDK models, there were many cysts in which fibromuscular tissue surrounded the primitive ducts (Fig. 4a, b). In the epithelial cells of the cysts, CD10 expression was

Fig.1 Nephrogenic zone (H&E staining, scale bar: 500 μm). **a** Fetus on day 60 of gestation. **b** Normal term fetus (145 days gestation). **c** The Sham model lambs' kidneys (145-day gestation) also presented renal tissue that was no different from normal controls. **d** Adult sheep. On day 60 of gestation, the NZ is present in the renal cortex and contains ureteric buds, metanephric mesenchymal cells, C-shaped bodies, and S-shaped bodies. The thinning of NZ occurs over the course of the pregnancy, and it disappears well before term. In the adult sheep kidney, tubular development is advanced, as the tubular epithelium is large, and the distance between glomeruli is increased
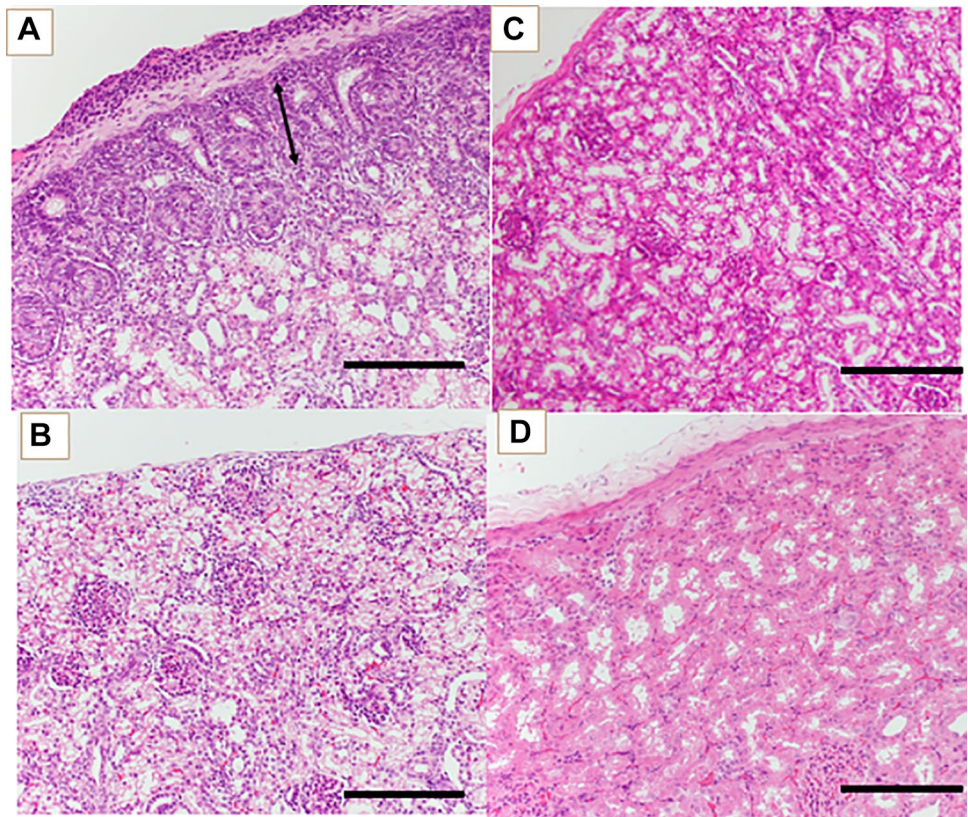

Fig.2 CD10 immunostaining (scale bar: 100 μm). **a** In the fetal kidney on day 60 of gestation, strong expression of CD10 was observed in the tubular epithelial cells, and its expression was also observed in the glomerular epithelial cells, Bowman's capsule epithelial cells, and medullary stromal cells. **b** Similarly, CD10 expression was observed in the tubular epithelial cells, glomerular epithelial cells, Bowman's capsule epithelial cells, and medullary stromal cells in term lamb kidneys on day 145 of gestation. **c** Similarly, CD10 expression was observed in the tubular epithelial cells, glomerular epithelial cells, Bowman's capsule epithelial cells, and medullary stromal cells in sham model lambs' kidneys. **d** CD10 expression was also observed in the tubular epithelial cells, glomerular epithelial cells, Bowman's capsule epithelial cells, and medullary stromal cells in adult sheep
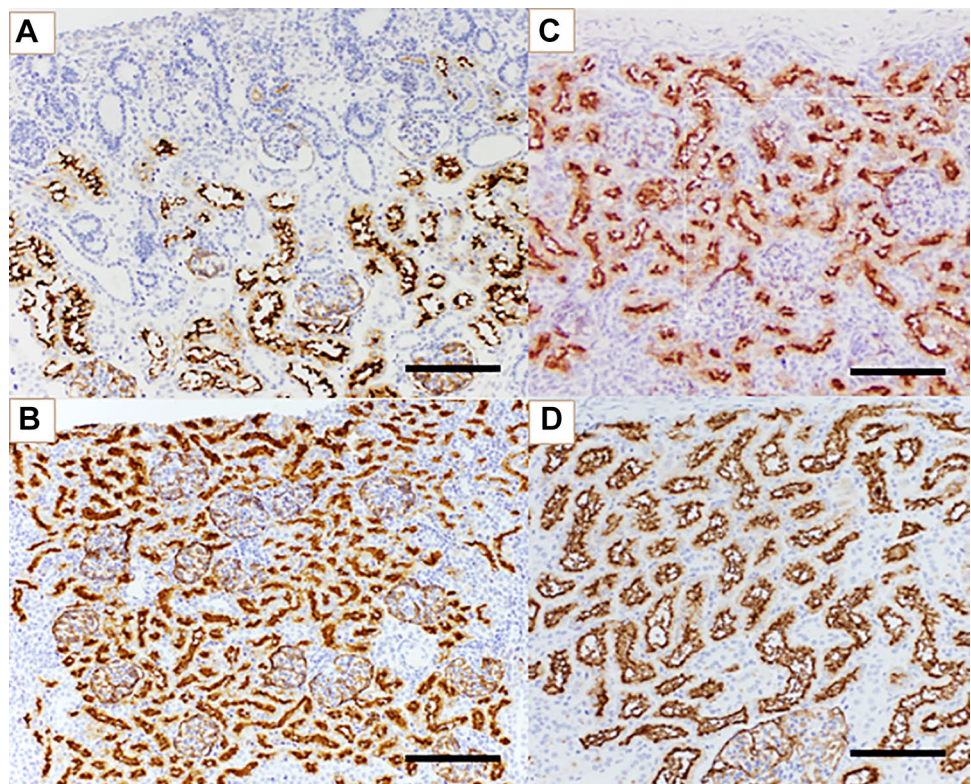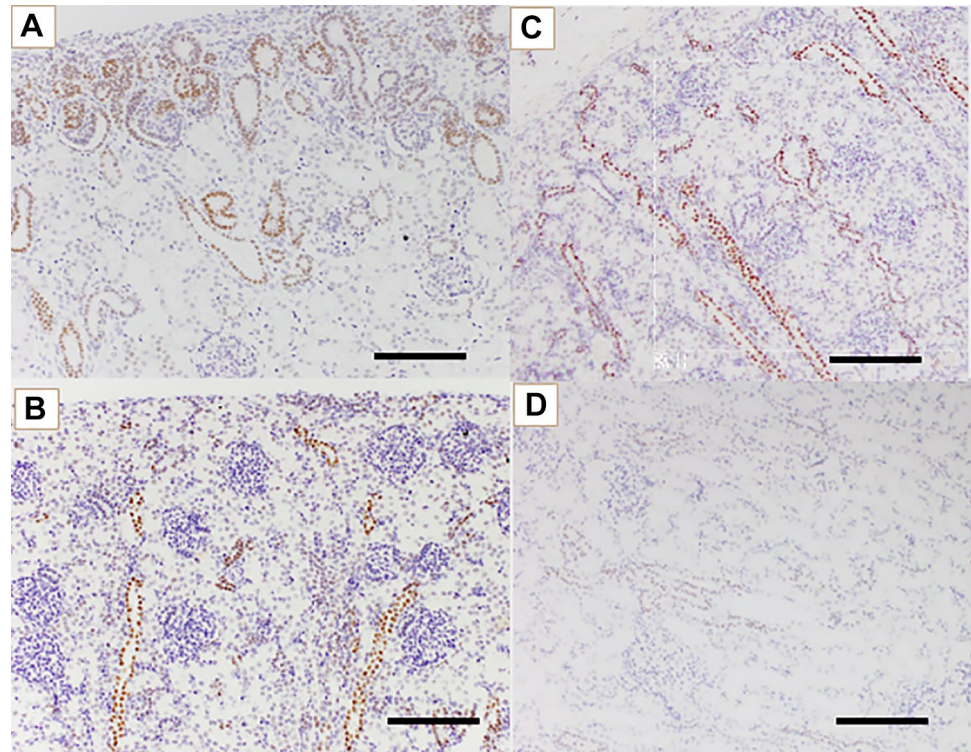
Table 1 Sheep models and CD10-positive cells

	Term MCDK	60-Day gestation	Term gestation	Sham model	Adult (control)
Epithelial cells of the cyst wall	+ +	−	−	−	−
Tubular epithelial cells	+ +	+ +	+ +	+ +	+ +
Glomerular epithelial cells	+	+	+	+	+
Bowman's capsule epithelial cells	+	+	+	+	+
Medullary stromal cells	+	+	+	+	+

Fig.3 PAX2 immunostaining (scale bar: 100 μm). **a** In the fetal kidney on day 60 of gestation, PAX2 expression was observed in the ureteric buds, C-shaped bodies, collecting duct epithelial cells, and some Bowman's capsule epithelial cells. **b** In the term lamb kidney on day 145 of gestation, PAX2 expression was observed only in the collecting duct. **c** PAX2 expression was observed only in the collecting duct in sham model lambs' kidneys, also. **d** PAX2 expression was also observed only in the collecting duct in adult sheep

Table 2 Sheep models and PAX2-positive cells

	Term MCDK	60-Day gestation	Term gestation	Sham model	Adult (control)
Epithelial cells of the cyst wall	+	−	−	−	−
Ureteric bud	NP	+ +	NP	NP	NP
C- and S-shaped body	NP	+ +	NP	NP	NP
Collecting duct epithelial cells	+ +	+ +	+ +	+ +	+ +
Bowman's capsule epithelial cells	+	+ +	+	+	+

NP not present

observed (Fig. 4c, d). PAX2 was also expressed in these cells (Fig. 4e, f).

Discussion

Mammalian kidney development begins with the interactions between the Wolffian (mesonephric) duct and the
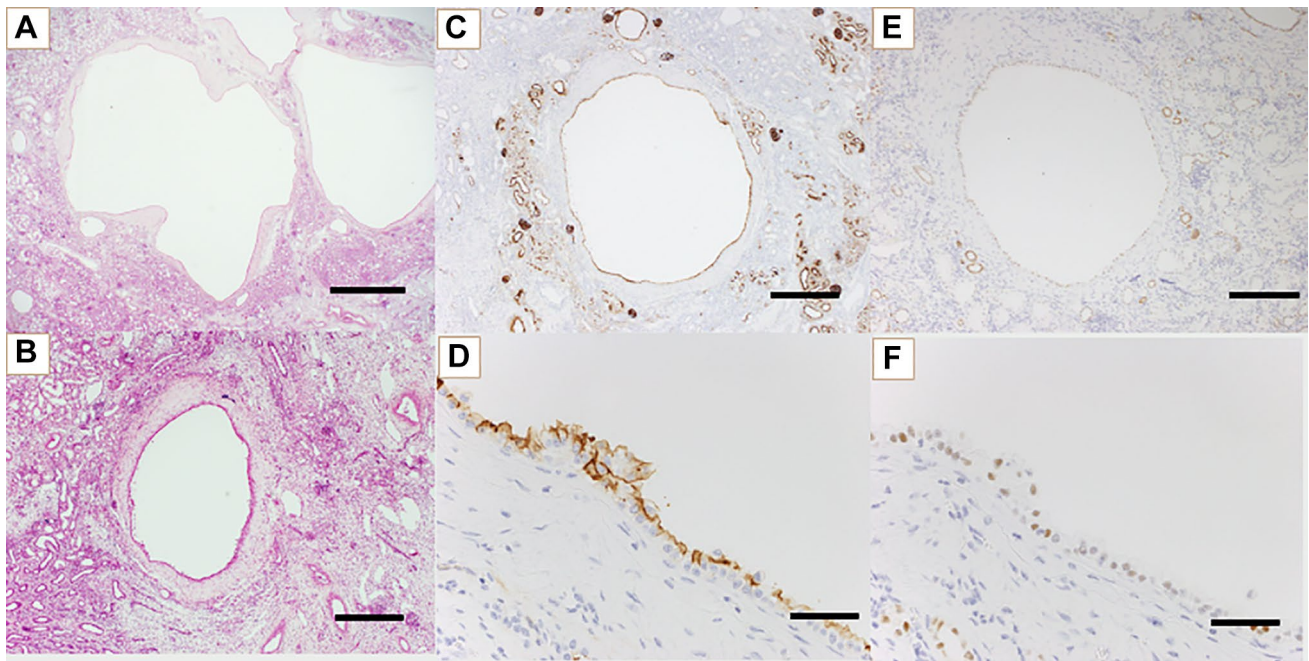
Fig.4 MCDK models. Urinary tract obstruction on day 60 of gestation resulted in many cysts surrounded by fibromuscular tissue surrounding the primitive ducts at term **a** (H&E staining, scale bar: 1000 μm). **b** (H&E staining, scale bar: 500 μm). **c** CD10 expression was observed in the cystic epithelial cells. (CD10 immunostaining, scale bar: 500 μm). **d** (CD10 immunostaining, scale bar: 50 μm). **e** PAX2 expression was also observed in the cystic epithelial cells (PAX2 immunostaining, scale bar: 500 μm). **f** (PAX2 immunostaining, scale bar: 50 μm)

metanephric mesenchyme [9, 10]. The branching ureteric bud invades the metanephric mesenchyme, inducing the mesenchymal–epithelial transition (MET). Following the invasion of the ureteric bud, the metanephric mesenchyme aggregates to form the renal vesicles, which then develop into the C- and S-shaped bodies. These form the glomeruli and proximal tubules, the loop of Henle, and distal tubules that fuse with the branching ureteric bud. This differentiates into the collecting ducts which join the renal pelvis to form the kidney [9].

During these developmental processes, it is clear that various transcription factors are repeatedly expressed and then diminish [10, 11]. One of these transcription factors, PAX2, has been reported to play roles in the development of the central nervous system, spine, eyes, and kidneys. PAX2 is essential for renal development. Mouse embryos with no expression of PAX2 have renal aplasia, which is lethal. In heterozygous mutations, there is renal hypoplasia [7]. PAX2 is also protective against apoptosis of ureteric bud cells [12]. Additionally, mutations in PAX2 gene are most commonly reported to be responsible for congenital anomalies of the kidney and urinary tract (CAKUT), including renal agenesis, dysplastic kidney, and MCDK. PAX2 mutations are found in approximately 6–10% of cases of renal hypoplasia and dysplasia [13]. It is also said to cause the autosomal

dominant inherited renal coloboma syndrome, which is a very rare disease [14].

CD10 has been shown to be a useful immunohistochemical antibody in the identification of proximal tubules. In recent years, cell isolation has also been studied and used as an indicator of proximal tubules [5].

Here, we performed immunohistochemical analysis to assess the expression of CD10, and PAX2 in term kidneys in our fetal lamb obstructive uropathy model, and further evaluated them in serial sections. Normal kidneys at 60-day gestation, term lambs, sham-operated term lambs, and adult ewes were similarly assessed as controls. CD10 was found to be expressed in the proximal tubule of embryos on day 60 of gestation, term lambs, sham-operated term lambs, and adult ewes. In normal lambs, PAX2 did not express in the renal tubules at any stage of development, but was expressed in the collecting ducts in normal 60-day gestation and term lambs, and adult sheep, as well as in the ureteric buds, C- and S-shaped bodies, collecting duct epithelial cells, and some Bowman's capsule epithelial cells at 60 days gestation in the fetal kidneys. Both CD10 and PAX2 were expressed in the cyst epithelial cells of the MCDK model. This strongly suggests that there is a discrepancy between our previous hypothesis that the cysts in the MCDK model originated from tubules and collecting ducts by physical expansion, and the present findings [3]. CD10 has been demonstrated

to be expressed in glomerular epithelial cells and tubular epithelial cells [6, 15], and PAX2 has been demonstrated in the ureteric bud and collecting duct lineage and in the medullary expression of the C- and S-shaped bodies that form the glomerulus and the proximal tubule early in the renal development [10, 16]. This suggests that the MCDK model likely expresses PAX2 as a growth signal in the C- and S-shaped bodies, which should be down-regulated during embryonic development, and that PAX2 is persistently expressed in the proximal tubule without down-regulation when urinary tract obstruction is produced at about 60-day gestation in the fetal lamb. In two earlier studies, excessive PAX2 expression caused cystic diseases, but reduced cystogenesis was observed in a murine model of MCDK upon reducing the dosage of PAX2 gene [17, 18], which supports the results of this study.

However, these present findings are speculative, and it is not yet clear how the urinary tract obstruction affects PAX2, and therefore, genetic studies are also warranted. We used a large animal model, the sheep model, because the relatively long gestation allows time for the renal changes to develop. However, genetic studies using knockout models are also needed. An ovine knockout model has not yet been developed.

Conclusion

Immunohistochemical analysis of PAX2 was helpful in investigating the developmental processes leading to the development of MCDK. The occurrence of cystic epithelial cells in MCDK may be due to persistent expression of PAX2 as a growth signal in proximal tubular epithelial cells that fails to undergo down-regulation in the face of urinary tract obstruction.

Declarations

Conflicts of interests None.

References

1. Hains DS, Bates CM, Ingraham S, Schwaderer AL (2009) Management and etiology of the unilateral multicystic dysplastic kidney: a review. Pediatr Nephrol 24(2):233–241

2. Kitagawa H, Pringle KC, Koike J, Zuccollo J, Nakada K (2001) Different phenotypes of dysplastic kidney in obstructive uropathy in fetal lambs. J Pediatr Surg 36(11):1698–1703

3. Kitagawa H, Pringle KC, Koike J, Zuccollo J, Seki Y, Fujiwaki S et al (2003) Optimal timing of prenatal treatment of obstructive uropathy in the fetal lamb. J Pediatr Surg 38(12):1785–1789

4. Kitagawa H, Pringle KC, Zuccolo J, Stone P, Nakada K, Kawaguchi F et al (1999) The pathogenesis of dysplastic kidney in a urinary tract obstruction in the female fetal lamb. J Pediatr Surg 34(11):1678–1683

5. Van der Hauwaert C, Savary G, Gnemmi V, Glowacki F, Pottier N, Bouillez A et al (2013) Isolation and characterization of a primary proximal tubular epithelial cell model from human kidney by CD10/CD13 double labeling. PLoS ONE 8(6):e66750

6. Aoba T, Koike J, Nagae H, Kitagawa H, Pringle KC (2008) Expression of CD10 in Developmental Kidney in Fetal Lambs. J Dev Kidney 16(1):36–38

7. Narlis M, Grote D, Gaitan Y, Boualia SK, Bouchard M (2007) PAX2 and PAX8 regulate branching morphogenesis and nephron differentiation in the developing kidney. J Am Soc Nephrol 18(4):1121–1129

8. Tanaka K, Manabe S, Ooyama K, Seki Y, Nagae H, Takagi M et al (2014) Can a pressure-limited V-A shunt for obstructive uropathy really protect the kidney? J Pediatr Surg 49(12):1831–1834

9. Kawaguchi K, Obayashi J, Kawaguchi T, Koike J, Seki Y, Tanaka K et al (2018) The role of the ureteric bud in the development of the ovine fetal kidney. J Pediatr Surg 53(12):2502–2506

10. Nishiya Y, Kawaguchi K, Kudo K, Kawaguchi T, Obayashi J, Tanaka K et al (2021) The Expression of Transcription Factors in Fetal Lamb Kidney. J Dev Biol 9(2):22

11. Morizane R, Miyoshi T, Bonventre JV (2017) Concise Review: Kidney Generation with Human Pluripotent Stem Cells. Stem Cells 35(11):2209–2217

12. Torban E, Eccles MR, Favor J, Goodyer PR (2000) PAX2 suppresses apoptosis in renal collecting duct cells. Am J Pathol 157(3):833–842

13. Renkema KY, Winyard PJ, Skovorodkin IN, Levtchenko E, Hindryckx A, Jeanpierre C et al (2011) Novel perspectives for investigating congenital anomalies of the kidney and urinary tract (CAKUT). Nephrol Dial Transplant 26(12):3843–3851

14. Sanyanusin P, McNoe LA, Sullivan MJ, Weaver RG, Eccles MR (1995) Mutation of PAX2 in two siblings with renal-coloboma syndrome. Hum Mol Genet 4(11):2183–2184

15. Faa G, Gerosa C, Fanni D, Nemolato S, Marinelli V, Locci A et al (2012) CD10 in the developing human kidney: immunoreactivity and possible role in renal embryogenesis. J Matern Fetal Neonatal Med 25(7):904–911

16. O'Brien LL, McMahon AP (2014) Induction and patterning of the metanephric nephron. Semin Cell Dev Biol 36:31–38

17. Dressler GR, Wilkinson JE, Rothenpieler UW, Patterson LT, Williams-Simons L, Westphal H (1993) Deregulation of Pax-2 expression in transgenic mice generates severe kidney abnormalities. Nature 362(6415):65–67

18. Ostrom L, Tang MJ, Gruss P, Dressler GR (2000) Reduced Pax2 gene dosage increases apoptosis and slows the progression of renal cystic disease. Dev Biol 219(2):250–258

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