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BASIC SCIENCE REVIEW

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Molecular and cellular pathophysiology of obstructive nephropathy

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Abstract Congenital obstructive nephropathy remains one of the most-important causes of renal insufficiency in children. This review focuses on the unique interactions that result from urinary tract obstruction during the period of renal development in the neonatal rodent. Following unilateral ureteral obstruction (UUO), growth of the obstructed kidney is impaired and compensatory growth by the intact opposite kidney is related directly to the duration of obstruction. Development of the renal vasculature is delayed by UUO, and the activity of the intrarenal renin-angiotensin system is enhanced throughout the period of obstruction. Glomerular maturation is also delayed by UUO, and nephrogenesis is permanently impaired. The effects of UUO on the developing tubule are also profound, with a suppression of proliferation, stimulation of apoptosis, and the maintenance of an immature phenotype by tubular epithelial cells. Expression of tubular epidermal growth factor is suppressed and transforming growth factor-β1 and clusterin are increased. Maturation of interstitial fibroblasts is delayed, with progression of tubular atrophy and interstitial fibrosis resulting in part from continued activation of the renin-angiotensin system and oxygen radicals. Future efforts to prevent the consequences of congenital urinary tract obstruction must account for the dual effects of obstruction: interference with normal renal development and progression of irreversible tubulointerstitial injury.

Key words Development · Obstructive nephropathy · Growth · Angiotensin

Introduction

Congenital obstructive nephropathy is the principal cause of renal failure in infants and children [1]. Whereas in the adult kidney chronic urinary tract obstruction leads to tubular atrophy and interstitial fibrosis, urinary tract obstruction in the maturing kidney also permanently impairs renal development [2, 3]. Normal renal development in the human involves the formation of the metanephric kidney, with onset of nephrogenesis during the first trimester, and completion of nephrogenesis by the 34th week of gestation. Congenital obstruction of the urinary tract in the human most commonly develops at the ureteropelvic junction: this may be the result of either intrinsic or extrinsic impediment to urine flow. Although far less common, posterior urethral valves constitute the most-important cause of bladder outlet obstruction. The mechanisms underlying the embryological development of obstruction remain to be elucidated, and are not the subject of this review. In some cases of congenital obstructive nephropathy, renal maldevelopment (such as renal dysplasia) may be modulated by factors other than the primary obstruction to urine flow.

A variety of animal models have been created to investigate the pathophysiology of congenital obstructive nephropathy. The timing of renal development in the fetal sheep is similar to that in the human (nephrogenesis is completed before birth), and the size of the animal allows fetal manipulation. The guinea pig also completes nephrogenesis before birth, but its small size makes fetal manipulation difficult, necessitating neonatal studies. The opossum has been investigated, because fetal development in marsupials is largely extrauterine, thereby allowing more-convenient access for surgical intervention. In the rat and mouse, most nephrons are formed postnatally, such that experimental urinary tract obstruction in the neonatal period is analogous to that arising in the human in midtrimester. While no one model is ideal, the present review will focus on studies performed in neonatal rodents, which have allowed elucidation of a number of the renal molecular and cellular consequences of urinary tract obstruction in early development. The experimental surgical obstruction of a single ureter (UUO) most closely parallels human ureteropelvic junction obstruction, which is characterized by hydrone-

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phrotic rather than dysplastic alterations in the renal parenchyma.

Renal growth and counterbalance

It has been over 50 years since Hinman [4] described compensatory growth of the intact opposite kidney following UUO in the rat. Chronic UUO in the fetal sheep impairs growth of the obstructed kidney and stimulates compensatory growth of the opposite kidney [5]. A similar response is noted in the guinea pig, in which the impairment of growth of the obstructed kidney and compensatory growth of the opposite kidney are inversely proportional to the age of the animal at the time of obstruction [6]. It would therefore appear that while the developing kidney is more susceptible to the effects of ipsilateral UUO, adaptive growth by the opposite kidney is also enhanced. We have shown recently in the neonatal rat that UUO impairs growth of the obstructed kidney and stimulates growth of the opposite kidney in direct proportion to the duration of obstruction [7]. Chronic UUO in the neonatal rat reduces DNA content of the obstructed kidney, and increases that of the intact opposite kidney [2]. Fetal compensatory renal growth has been demonstrated also in humans [8], and adaptive growth of the opposite kidney has been proposed as a sensitive index of the severity of obstruction in infants with ureteropelvic junction obstruction [9].

There are fundamental differences between normal and compensatory renal growth. Silber and Malvin [10] demonstrated that compensatory growth is reversible, whereas normal growth is not. However, we showed that contralateral nephrectomy in the neonatal guinea pig with partial UUO preserves renal growth (but less than a normal kidney remaining after contralateral nephrectomy) [11]. It is likely that growth of the single partially obstructed kidney in this case represents the net effects of slowed growth due to ipsilateral UUO and enhanced growth due to contralateral nephrectomy.

Recent studies indicate that whereas angiotensin acts as a growth factor in normal renal development [12], it is not necessary for compensatory renal growth in the neonatal mouse [13]. Compensatory renal growth in the neonate is primarily hyperplastic, whereas that in the adult is hypertrophic [14]. It is likely that insulin-like growth factor-1 plays a role in neonatal (but not adult) compensatory renal growth [15].

Vascular development

Fetal development of the renal vasculature is characterized by a diffuse distribution of renin along the length of the afferent arterioles and the interlobular arteries, with gradual localization to the juxtaglomerular region only in the early neonatal period [16]. Renal nerve activity is increased in early development [17], and is responsible at least in part for increased renal renin activity [18]. Interference with the activity of the renin-angiotensin system (RAS) during this critical period results in abnormal development of the renal vasculature [19, 20]. The RAS is normally highly active during early development, which contributes to a high renal vascular resistance in the fetus and neonate [21].

Chronic UUO in the neonatal rat results in a rapid and sustained increase in renal renin gene expression [2], and persistence of the fetal pattern of renin distribution [22]. In addition to increased immunolocalization of renin along the microvasculature, chronic UUO in the neonatal rat also increases the number of renin-secreting cells [23]. This is modulated by renal nerve activity [24]. Relief of obstruction reduces the extent of renin distribution along afferent arterioles of the neonatal rat (R.L. Chevalier, unpublished observations) and normalizes the renin content of the postobstructed neonatal guinea pig kidney [25]. Activation of the intrarenal RAS is therefore dynamically modulated by tubular fluid flow, intratubular pressure, or mechanical distention of the tubule.

Chronic UUO in the neonatal rat or guinea pig results in a marked increase in renal vascular resistance of the obstructed kidney and vasodilatation of the intact opposite kidney [11, 26]. Chronic UUO in the guinea pig increases angiotensin-dependent renal vasoconstriction in the obstructed kidney independent of renal nerves [27]. Vasodilatation of the opposite kidney may be mediated by renal nerves or contralateral renal renin suppression [2, 26]. Chronic inhibition of angiotensin converting enzyme in the neonatal guinea pig with chronic partial UUO prevents the reduction in blood flow to the obstructed kidney, indicating that angiotensin plays a major role in vasoconstriction [28]. However, renal blood flow is not normalized 10 days after relief of temporary UUO in the neonatal guinea pig, and enalapril has no salutary effect, suggesting that additional vasoconstrictors are involved in the postobstructed kidney [25]. However, angiotensin converting enzyme inhibition reduces vascular resistance of the intact opposite kidney, which may be the result of an increased sensitivity of angiotensin II receptors in response to relief of obstruction [25]. Thromboxanes have been shown to act as additional modulators of renal vascular resistance following ipsilateral [29, 30] or in rats with congenital spontaneous hydronephrosis [31]. Interestingly, vasodilator prostaglandins appear to contribute to vasodilatation of the intact opposite kidney [32].

The role of nitric oxide, a potent vasodilator, in modulation of renal vascular tone depends on whether ureteral obstruction is unilateral or bilateral. Following release of bilateral ureteral obstruction, renal nitric oxide synthase activity is decreased [33], whereas following UUO, nitric oxide synthase activity is increased, thereby counteracting the vasoconstrictor responses described above [34]. It appears that one of the salutary effects of enalapril on the obstructed kidney is a consequence of increased nitric oxide generation [35]. Following 24 h of UUO, glomerular soluble guanylate cyclase activity is increased (through angiotensin II stimulation), while

phosphodiesterase activity is reduced [36]. By increasing the production of cyclic GMP, a potent vasodilator, these responses would also counter vasoconstrictors generated following UUO. Although renal blood flow is normalized 6 weeks after relief of temporary UUO in the neonatal guinea pig, renal growth remains impaired [37]. This indicates that persistent ischemia cannot account for the impaired renal growth resulting from UUO: alterations in renal growth factor expression are more likely to play a significant role.

Glomerular development

Following induction of glomeruli, which are formed from mesenchyme in the clefts of S-shaped bodies, glomerular capillaries increase in number, while podocytes become progressively flattened [38]. This is associated with increasing surface area for glomerular filtration and a significant increase in glomerular filtration rate (GFR) [39].

Chronic UUO interferes with both nephrogenesis and with terminal maturation of glomeruli. Human fetuses with severe obstructive nephropathy have reduced numbers of glomeruli [40]. Moreover, in both the fetal rabbit and fetal sheep, chronic UUO decreases the number of nephrons [41, 42]. In the neonatal rat (in which 90% of nephrons are formed postnatally), 5 days of UUO reduces the number of nephrons by 40% [43]. Most importantly, although relief of obstruction does not lead to "catch-up" nephrogenesis, GFR of the postobstructed kidney is normal, indicating significant hyperfiltration by remaining nephrons [43]. In the neonatal guinea pig (in which nephrogenesis is complete before birth), severe partial UUO reduces the number of perfused glomeruli by 16% and decreases GFR by 80% at 3 weeks [11]. After 8 weeks' partial UUO, the number of perfused glomeruli is reduced by 34%, and GFR is reduced by 95% [37]. Relief of 10 days of obstruction does not increase the number of perfused glomeruli at 8 weeks of age, but GFR is normalized [37]. These findings highlight the irreversible nature of nephron loss resulting from chronic UUO in the developing kidney, regardless of whether obstruction is present during or after the completion of nephrogenesis. Marked hyperfiltration by remaining nephrons takes place in the postobstructed kidney despite the presence of a normal contralateral kidney. The primary concern is that over time hyperfiltration may lead to progressive glomerular sclerosis, which is more marked in immature than in adult rats subjected to unilateral nephrectomy 5–11 months previously [44, 45].

Five days of UUO in the neonatal rat delays terminal maturation of glomeruli [43], while immature glomeruli are not present beyond the completion of normal nephrogenesis in the rat (14 days), even in obstructed kidneys. As shown in Fig. 1a, administration of epidermal growth factor (EGF), 0.1 mg/kg per day, to neonatal rats with UUO prevented the delay in glomerular maturation. However, EGF did not augment the number of glomeruli in the obstructed kidney (Fig. 1b). Expression of this growth factor is suppressed by UUO, and delayed glomerular maturation may be a secondary consequence.

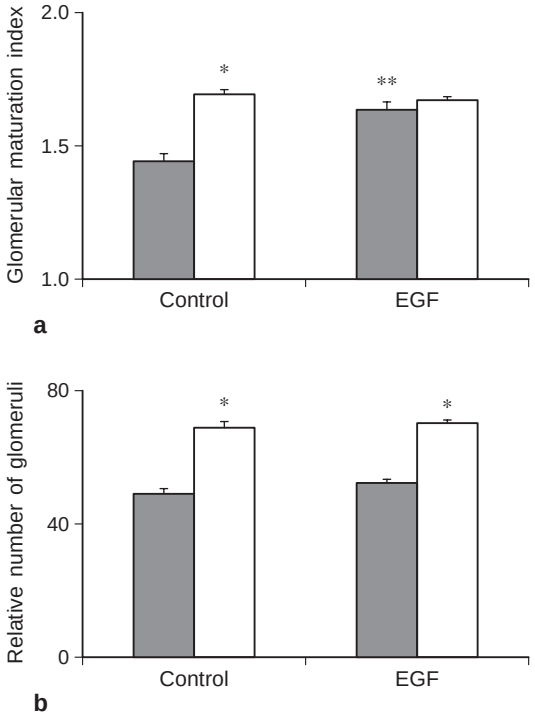

Fig. 1 a, b Effects of unilateral ureteral obstruction (UUO) and exogenous epidermal growth factor (*EGF*) on glomerular maturation and number of glomeruli in neonatal rats. Sprague-Dawley rats underwent UUO within the first 48 h of life and received either EGF (0.1 mg/kg per day, n=5) or saline (control, n=7) for the following 7 days as described previously [94]. Weighted glomerular maturation score and the relative number of glomeruli per transverse kidney section were determined in each group as described previously [41, 95]. Data are presented as mean±SEM, with obstructed kidneys in *black* and intact opposite kidneys in *white*. *P<0.05 vs. UUO kidney; **P<0.05 vs. control. **a** Glomerular maturation was significantly delayed by UUO in the control group, but treatment with EGF prevented this delay. **b** The number of glomeruli was significantly reduced by UUO and EGF had no effect on nephrogenesis

Tubular development

Organ growth is the result of a balance between cellular proliferation and programmed cell death (apoptosis). The bulk of renal growth is due to proliferation and hypertrophy of tubular epithelial cells. Tubular maturation is characterized by the progressive disappearance of markers normally expressed only in early development. These include clusterin, a large glycoprotein [46], and KS, a kidney-specific gene with unknown function [47]. In addition, tubular maturation involves the polarized expression of a number of transporters, such as Na-K-ATPase [48], and receptors, such as EGF receptors [49].

In early renal development, mesenchymal cells expressing vimentin undergo phenotypic transformation to become polarized tubular epithelial cells [50]. Converse-

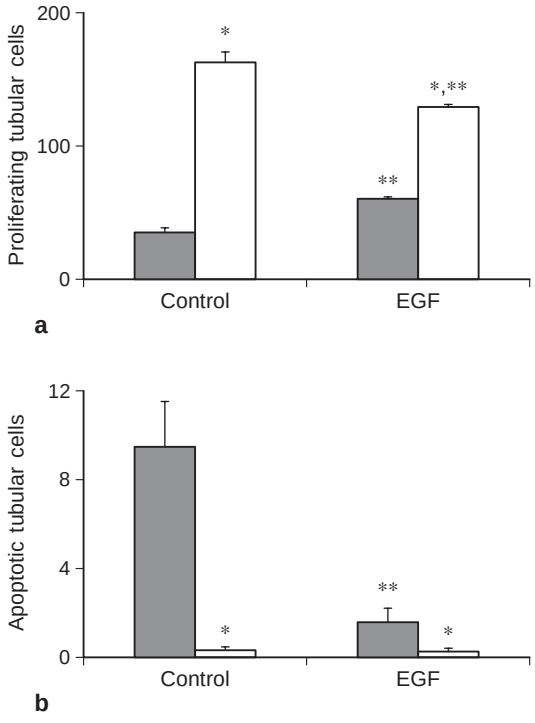

Fig. 2 a, b Effects of UUO and exogenous EGF on renal tubular cell proliferation and apoptosis in neonatal rats. Sprague-Dawley rats underwent UUO within the first 48 h of life and received either EGF (0.1 mg/kg per day, *n*=6) or saline (control, *n*=6) for the following 7 days. Data (previously published [94]) are presented as mean±SEM, with obstructed kidneys in *black* and intact opposite kidneys in *white*. **P*<0.05 vs. UUO kidney; ***P*<0.05 vs. control. **a** Relative tubular cell proliferation is represented by nuclei staining for proliferating cell nuclear antigen. Cellular proliferation was markedly suppressed in the obstructed kidney, but increased 74% with EGF treatment. In contrast, cellular proliferation in the intact opposite kidney was reduced 20% by EGF treatment. **b** Relative tubular cell apoptosis is represented by nuclei identified by the TUNEL technique. While tubular apoptosis was increased over 30-fold in the obstructed compared with the intact opposite kidney, EGF treatment reduced apoptosis in the obstructed kidney by over 80%

ly, as a consequence of chronic UUO, tubular epithelial cells in the neonatal rat undergo epithelial-mesenchymal transformation and express vimentin [51]. In addition to acquisition of this mesenchymal characteristic, tubular epithelial cells in the obstructed kidney express the EGF receptor on the luminal as well as the basolateral surface, consistent with loss of normal polarity [52]. Additional evidence of UUO inducing renal tubular cell immaturity is the persistence of KS in tubules of neonatal rats subjected to chronic UUO [47].

Chronic urinary tract obstruction stimulates apoptosis of renal tubular epithelial cells in animal models and in humans [3, 53]. The loss of polarity and acquisition of mesenchymal characteristics predispose cells to break free of the basement membrane. This process has been termed *anoikis* or "homelessness" [54]. The change in cell shape is a potent stimulus to initiation of apoptosis. An additional factor likely contributing to apoptosis of tubular cells is a reduced expression in scattered dilated tubules of bcl-2, an oncoprotein that normally inhibits apoptosis [55, 56]. There is circumstantial evidence that apoptosis of renal tubular cells progresses to tubular atrophy in obstructive nephropathy [57]. It is therefore not surprising that factors countering apoptosis are also activated by UUO. One potential candidate is clusterin, which binds epithelial cells together and to the basement membrane after they have become detached [58, 59]. Chronic UUO in the neonatal rat results in a progressive increase in renal clusterin expression, which is localized to scattered collecting ducts [2, 3].

Whereas UUO in the adult rat induces an increase in cellular proliferation and DNA content, UUO in the neonate markedly suppresses tubular cell proliferation and DNA content [3]. It is likely that this difference is due to greater interstitial macrophage proliferation in the adult than the neonate, as well as to a greater increase in tubular cell apoptosis in the neonate [3]. One of the reasons for a reduction in renal tubular cell proliferation may be a suppression of EGF expression by distal tubular cells. This growth factor is normally not expressed in the rat kidney until several days of age, after which expression increases linearly [2]. Chronic UUO inhibits this normal increase in EGF expression [2], and relief of 5 days of UUO in the neonatal rat does not normalize tubular EGF expression, proliferation, or apoptosis 28 days later [43]. However, administration of EGF to neonatal rats with persistent UUO stimulates tubular cell proliferation while inhibiting apoptosis [51] (Fig. 2). Of interest, EGF administration also reduces vimentin and clusterin expression by renal tubular cells in the obstructed neonatal rat kidney [51].

Although the RAS is already activated by UUO, administration of exogenous angiotensin II stimulates renal cellular proliferation while further stimulating apoptosis in the neonatal hydronephrotic kidney [60]. In contrast, administration of angiotensin II has no effect on renal tubular cell proliferation or apoptosis in the adult rat with UUO [60]. These age-related differences may be due in part to changing distribution of angiotensin receptors. Following birth, there is a progressive increase in the renal expression of AT1 receptors, which mediate vasoconstriction and renal cellular proliferation [61, 62]. In contrast, following birth there is a rapid decrease in renal expression of AT2 receptors, which inhibit cell growth and induce apoptosis [61–64]. Although AT1 and AT2 receptors are downregulated 24 h following UUO in the neonatal rat, AT1 receptor expression and binding progressively increase, despite increased renal production of angiotensin II [65]. In contrast, expression of AT2 receptor decreased regardless of the presence of UUO [65].

Interstitial development

Maturation of renal interstitial fibroblasts depends on their phenotypic transformation from myofibroblasts to fibroblasts [66, 67]. As a result of this process, renal interstitial fibroblasts in the rat no longer express either vimentin or

α-smooth muscle actin after approximately 14 days of age [66]. However, as a consequence of chronic UUO from birth, α-smooth muscle actin continues to be produced by fibroblasts until 28 days [2]. Interstitial fibroblasts may undergo phenotypic transformation in the adult as well: obstruction of single nephrons in the adult rat induces the expression of α-smooth muscle actin by fibroblasts surrounding the dilated tubular loops [68]. Moreover, dilated tubules in kidneys from infants with severe congenital obstructive nephropathy are also surrounded by myofibroblasts [69]. These data indicate a close relationship, or "crosstalk", between the renal tubular epithelial cell and the adjacent interstitial extracellular matrix.

Progressive renal injury resulting from urinary tract obstruction

Over time, chronic UUO leads to tubular atrophy and interstitial fibrosis. Even short-term (3–5 days) complete UUO in the neonatal rat leads to persistent tubular atrophy and interstitial fibrosis, and the degree of injury is directly related to the duration of obstruction [7]. Phenotypic transformation of fibroblasts to myofibroblasts has been shown to play a role in progressive deposition of extracellular matrix [67]. Chronic UUO in the neonatal rat increases interstitial deposition of collagen types I, III, and V, and leads to progressive interstitial fibrosis [70]. This deposition of extracellular matrix is a consequence of both increased synthesis and reduced degradation [71].

Transforming growth factor-β1 (TGF-β1), a cytokine that enhances the deposition of extracellular matrix and also inhibits its degradation [72], is produced by renal tubular epithelial cells and interstitial fibroblasts [73, 74]. This action may be mediated at least in part through stimulation of α-smooth muscle actin production by interstitial fibroblasts [72, 75]. Renal expression of TGF-β1 is markedly increased by ureteral obstruction in the fetal sheep [76], as well as the neonatal or adult rat [2, 73]. Renal tubular TGF-β1 receptor expression is also increased by UUO, which may serve to amplify the effect [77]. In addition to its fibrogenic actions, TGF-β1 stimulates apoptosis of epithelial cells, which may also contribute to progressive tubular atrophy [78]. Relief of obstruction attenuates, but does not normalize, tubular expression of TGF-β1, clusterin, and vimentin– all of which are evidence of persistent tubular injury [43]. We have demonstrated recently that administration of EGF to neonatal rats with chronic UUO reduces tubular TGF-β1 expression as well as the extent of renal interstitial fibrosis [51]. This underscores the delicate interrelationship between growth factors in determining the progression of tubulointerstitial injury as well as in modulating renal development.

Activation of the RAS contributes significantly to fibrotic injury in a variety of organs, including the heart and kidney [79]. Angiotensin stimulates the expression of TGF-β1 and α-smooth muscle actin [80, 81], while AT1 receptor inhibition in the neonatal rat with UUO reduces the renal expression of TGF-β1 [82]. Although inhibition of the RAS has been shown to attenuate renal injury in the adult rat with UUO [83–85], we found no improvement in the growth of the obstructed neonatal rat kidney, and growth of the intact opposite kidney was actually impaired, due to the dependence of the developing kidney on an intact RAS [82]. Using neonatal mice with varying numbers of functional copies of the angiotensinogen gene, we have recently demonstrated that renal interstitial fibrosis resulting from chronic UUO is attenuated by decreasing angiotensinogen expression [13]. However, renal interstitial fibrosis was reduced by only 50%, even in rats with zero copies of angiotensinogen, indicating that other factors also contribute significantly to the fibrogenic process [13].

Additional mechanisms for progression of renal fibrosis in the hydronephrotic kidney include the release of compounds by infiltrating macrophages. An example of this is the increased renal expression of tissue inhibitor of metalloproteinase following 3 days of UUO in the rabbit [86]. Enhanced production of the inhibitor may account for the impaired extracellular matrix degradation in the obstructed kidney [87]. Macrophages may be recruited to the interstitium by the release of adhesion molecules, such as intercellular adhesion molecule-1, from proximal tubular epithelial cells [81].

An increase in reactive oxygen species in the obstructed kidney is also likely to play a significant role in the progression of tubulointerstitial injury. Following the release of 24 h of either unilateral or bilateral ureteral obstruction, renal malondialdehyde levels (a measure of free radical production) are increased [88, 89]. However, antioxidant enzyme activity is decreased in the hydronephrotic kidney, an effect that can be mimicked by incubation of isolated tubules with TGF-β1 [90]. Most interesting was the finding that stretching proximal tubular cells in vitro suppressed catalase expression, suggesting that mechanical forces imposed on tubular epithelial cells in the obstructed kidney could contribute to renal oxidant injury [90]. Since obstructive nephropathy can result in significant sodium wasting and volume contraction, we examined the effect of sodium depletion on renal antioxidant enzyme activity in rats subjected to chronic UUO [91]. We found that while sodium depletion increased several renal antioxidant enzymes in intact rats, UUO not only reduced baseline antioxidant activities, but also prevented the normal response to sodium depletion [91]. However, other compensatory mechanisms may come into play in the obstructed kidney. Clusterin has been shown to protect against oxidative stress in isolated renal tubular cells [92], and renal clusterin expression is markedly increased by UUO [3].

Summary

Figure 3 is a schematic outline of the proposed major cellular effects of UUO on the developing kidney. With

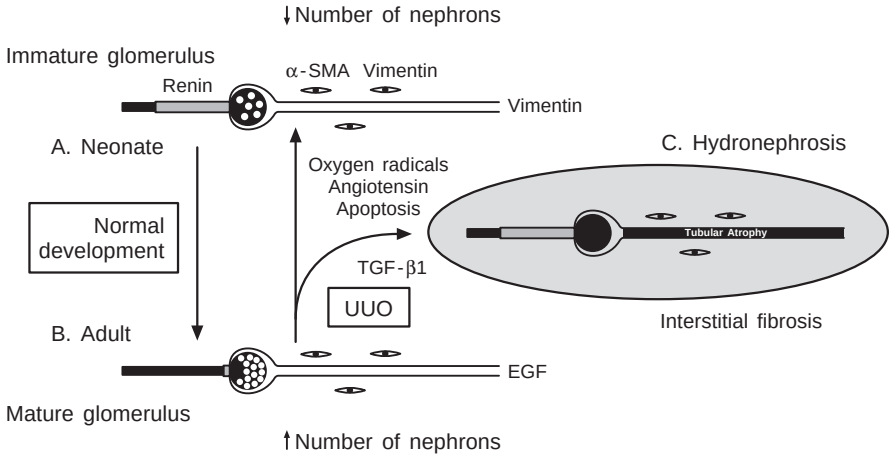

Fig. 3 Proposed scheme of effects of UUO on the developing kidney. *α-SMA*, α-Smooth muscle actin; *TGF-β1*, transforming growth factor-β1

normal renal development (*arrow* from *A* to *B*), nephrogenesis proceeds with increasing numbers of nephrons. The renal vasculature matures as evidenced by a disappearance of renin from the length of the afferent arteriole and its localization to the juxtaglomerular region. Glomerular maturation is characterized by proliferation of glomerular capillary loops and flattening of the podocytes. Tubular maturation is reflected by a disappearance of vimentin and the increasing expression of EGF. Interstitial fibroblasts lose their expression of α-smooth muscle actin and vimentin. As a consquence of UUO, the progression of normal nephron maturation is delayed or arrested (*arrow* from *B* to *A*). In addition, UUO induces increased production of oxygen radicals, angiotensin and TGF-β1, and stimulates tubular apoptosis, leading to tubular atrophy and interstitial fibrosis in the hydronephrotic kidney (*curved arrow* to *C*). The molecular mechanisms responsible for these effects remain to be defined. It is likely that the initiating stimulus is reduced tubular fluid flow (with accumulation of certain signalling molecules in the tubular lumen), increased hydrostatic pressure, or stretch of the tubule. The renal tubular epithelial cell is almost certainly the central "processor" of these stimuli, feeding signals to the vasculature, glomerulus, and surrounding interstitium [93]. Future efforts to attenuate or prevent the consequences of urinary tract obstruction on the developing kidney must take into account the dual effects of obstruction: interference with normal developmental programs and progression of irreversible injury.

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