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Responses of Proximal Tubular Cells to Injury in Congenital Renal Disease: Fight or Flight

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Abstract

Most chronic kidney disease in children results from congenital or inherited disorders, which can be studied in mouse models. Following 2 weeks of unilateral ureteral obstruction (UUO) in the adult mouse, nephron loss is due to proximal tubular mitochondrial injury and cell death. In neonatal mice, proximal tubular cell death is delayed beyond 2 weeks of complete UUO, and release of partial UUO allows remodeling of remaining nephrons. Progressive cyst expansion develops in polycystic kidney disease (PKD), a common inherited renal disorder. The PCY mutant mouse (which develops late-onset PKD) develops thinning of the glomerulotubular junction in parallel with growth of cysts in adulthood. Renal insufficiency in nephropathic cystinosis, a rare inherited renal disorder, results from progressive tubular cystine accumulation. In the *Ctns* knock out mouse (a model of cystinosis), proximal tubular cells become flattened, with loss of mitochondria and thickening of tubular basement membrane. In each model, persistent obstructive or metabolic stress leads ultimately to the formation of atubular glomeruli. The initial “fight” response (proximal tubular survival) switches to a “flight” response (proximal tubular cell death) with ongoing oxidative injury and mitochondrial damage. Therapies should be directed at reducing proximal tubular mitochondrial oxidative injury to enhance repair and regeneration.

Keywords

atubular glomeruli; cystinosis; obstructive nephropathy; mitochondria; polycystic kidney disease

Introduction

The majority of kidney disease in children is the result of congenital urinary tract anomalies or inherited renal disorders, with highly variable rates of progression into adulthood. The generally accepted paradigm for the progression of adult kidney disease is focused on the pathophysiology of renal fibrosis: nephron loss through the accumulation of interstitial extracellular matrix and glomerular sclerosis [1]. Currently, unilateral ureteral obstruction (UUO) serves as the most widely-used animal model of chronic kidney disease, with renal interstitial fibrosis serving as the primary end-point for studies of gene deletion, overexpression, or pharmacologic inhibitors [1]. In contrast to the distal nephron, the proximal tubule has a greater concentration of mitochondria and reduced endogenous antioxidant defenses [2; 3]. We have recently demonstrated that chronic UUO in the adult mouse induces oxidative stress in the proximal tubule of the obstructed kidney, leading to tubular collapse and cell death by apoptosis, autophagy, and necrosis [4; 5]. This results in rapid loss of renal parenchyma within 7 to 14 days, and the formation of atubular glomeruli

due to destruction of the glomerulotubular junction and remodeling of Bowman's capsule by sealing of the urinary pole [4]. Notably, the increase in interstitial matrix components does not exceed 15% of total volume fraction of the obstructed kidney, whereas there is a 65% reduction in proximal tubular mass [5]. Inhibition of transforming growth factor- β 1 receptor in the adult mouse attenuates proximal tubular loss and formation of atubular glomeruli in the obstructed kidney [6]. Many renal disorders (glomerular and tubulointerstitial) involve proximal tubular injury and the formation of atubular glomeruli. These include congenital nephrotic syndrome, diabetes, IgA and membranous nephropathies, pyelonephritis, obstructive nephropathy, and renal artery stenosis [7]. These studies suggest that preservation of the proximal tubule (and in particular the glomerulotubular junction) may prove to be a more promising objective in slowing the progression of kidney disease than is the attenuation or reversal of fibrosis.

Congenital Obstructive Nephropathy

Congenital obstructive nephropathy is the leading identifiable cause of chronic kidney disease in children. Since the onset of pediatric kidney disease generally occurs before completion of nephrogenesis or renal maturation, the dynamics of renal development must be considered in relation to the underlying disorder. Clinical studies suggest that renal interstitial fibrosis is a later consequence of ureteropelvic junction obstruction, generally developing in children over one year of age [8]. Of significance, proximal tubules are smaller in kidneys from those children with ureteropelvic junction obstruction who develop deterioration in renal function [8]. Complete UUO in the neonatal mouse delays nephron maturation (Fig. 1A, B), and proximal tubular mitochondrial loss and cell death are delayed during the first two weeks of life [9]. However, in the following two weeks of persistent UUO, proximal tubules sustain continued oxidative stress, mitochondrial injury, tubular atrophy (Fig. 1D, E, G, H), and undergo progressive cell death. It is likely that a shift from anaerobic glycolysis to oxidative metabolism in the developing proximal tubule contributes to the increasing susceptibility of this nephron segment to oxidative injury during maturation [10; 11]. Following chronic *partial* UUO in the neonatal mouse, a model that more closely reflects clinical obstructive nephropathy, the formation of atubular glomeruli is further delayed (3-6 weeks) [12]. These studies indicate that therapeutic interventions to slow or prevent nephron loss in congenital obstructive nephropathy should be initiated prior to maturation of the kidney.

Certain caveats exist that require a distinction between infant and adult disease. Inhibition of endogenous angiotensin, the primary available intervention to slow progression of renal disease, can actually aggravate obstructive injury in the neonate [13]. Moreover, transforming growth factor- β 1 (TGF- β 1) receptor inhibition in neonatal murine UUO also exacerbates obstructive renal injury, in contrast to a salutary effect in the adult [6]. Thus, although dysregulation of angiotensin or TGF- β 1 contributes to progressive kidney disease in the adult, these pathways are necessary for normal renal maturation and ought not be disrupted at this stage of development.

Polycystic Kidney Disease

Polycystic kidney disease (PKD) is a relatively common inherited renal disorder, with both autosomal recessive (early onset) and autosomal dominant (late onset) mutations. Atubular glomeruli develop in a rat model of PKD [14], and expansion of cysts is thought to cause compression and tubular obstruction of adjacent nephrons in PKD [15; 16]. To test this hypothesis, proximal tubular injury was assessed in mutant mice with late onset (PCY) PKD. The PCY mice develop cysts after 5 weeks of age, and glomerulotubular junction integrity decreases linearly with increasing cyst area (Fig. 2A, B), with only 25% normal and 25% atubular glomeruli present by 30 weeks of age (onset of renal failure) [17]. This is

associated with a marked reduction in fractional proximal tubular mass (Fig. 2C, D). These findings are consistent with a role for progressive tubular obstruction causing proximal tubular injury and formation of atubular glomeruli in adult PCY mice.

Nephropathic Cystinosis

Cystinosis is a rare congenital metabolic disorder resulting from a mutation in cystinosisin, a lysosomal cystine carrier, which results in progressive nephron injury and renal failure by the second decade. Development between 6 and 12 months of age of the so-called “swan neck,” consisting of an attenuated glomerulotubular junction is the characteristic renal lesion of cystinosis [18]. A recent report of cystinotic children undergoing renal transplantation revealed that most glomeruli in every patient were atubular, likely the result of chronic proximal tubular injury due to cystine accumulation [19].

The development of an animal model of nephropathic cystinosis was recently shown to be dependent on genetic background. *Ctns*^{-/-} mice on a C57BL/6 genetic background develop glucosuria, phosphaturia, and decreased glomerular filtration rate [20]. Morphometric analysis of kidneys from *Ctns*^{-/-} C57BL/6 mice reveals remodeling of cells of the glomerulotubular junction, with oxidant injury, pronounced flattening, loss of mitochondria, and thickening of tubular basement membrane (Fig. 1C, F, I) [21]. This is paralleled after 6 months of age by a progressive decrease in proximal tubular mass and mitochondrial superoxide production. The formation of atubular glomeruli is a terminal event, developing after 12 months of age (following the loss of 50% of the fractional proximal tubular mass).

A New Paradigm

The most widely applied paradigm for the progression of kidney disease is based on the central role of myofibroblast activation which leads to interstitial fibrosis and nephron loss; thus therapies are targeted at inhibiting pro-fibrotic or stimulating anti-fibrotic molecular pathways [22]. However, recent morphometric analysis of the obstructed adult mouse kidney has revealed rapid massive injury to the proximal tubule, with the loss of 65% of proximal tubular mass following 2 weeks of UUO, but with only a 15% increase in interstitial matrix volume fraction [4; 5]. Congenital renal disorders are superimposed on the normal process of nephrogenesis and renal maturation, which involves a more rapid growth of the proximal tubule than any other nephron component [23; 24]. As originally proposed by Walter Cannon at the level of the organism, and applied at the cellular response by Goligorsky [25], epithelial cells can respond to stress by “fight” or “flight”. Thus, the maturing proximal tubule, with its greater proliferative response, immature mitochondria, and greater glycolytic metabolism than the adult, maintains its integrity despite the ischemic and oxidative stress produced by UUO [26]. Compared to the adult mouse kidney subjected to UUO, integrity of the proximal tubule of the neonatal kidney is preserved during the first 2 weeks of obstruction (Fig. 3A), but cell death and formation of atubular glomeruli similar to that in adults follows with maturation through the 3rd and 4th weeks (Fig. 3B). Release of partial UUO in the neonatal mouse prevents progressive proximal tubular injury and leads to remodeling of the kidney [12]. The “fight” response is sustained with remodeling of the injured nephron if the stress (UUO) is removed (Fig. 3D). In contrast, maturation in the face of continued obstructive stress switches proximal tubular cells to a “flight” response, characterized by cell death and tubular atrophy (Fig. 3E).

The PCY mouse develops progressive proximal tubular loss in adulthood, in parallel with enlargement of cysts, which compresses adjacent nephrons and results in obstructive injury and formation of atubular glomeruli. In contrast, as with obstructive renal injury, the proximal tubular response in the *Ctns*^{-/-} mouse results from metabolic stress, cellular adaptation is markedly different. Proximal tubular cells become extremely flattened, with

loss of mitochondria and thickening of the tubular basement membrane. While this maintains integrity of the proximal tubule well into adulthood (“fight”) (Fig. 3C), loss of reabsorptive function results in Fanconi syndrome with continued accumulation of cystine in downstream proximal tubular cells eventually leading to tubular collapse (“flight”). In this model, then, both the formation of atubular glomeruli and interstitial fibrosis are terminal events (Fig. 3E).

These three murine models of congenital renal disease promote the proximal tubule as a target through which the slowing or prevention of progression of renal failure may be accomplished. Optimal intervention may be achievable before maturation is complete, while the proximal tubule retains growth potential and its mitochondria are more resistant to oxidative injury. We are currently utilizing the models of neonatal UUO and *Ctns*^{−/−} mice to study the effects of antioxidants targeted to mitochondria on preservation of proximal tubular integrity. Attachment of the lipophilic triphenylphosphonium cation to a coenzyme Q derivative can achieve mitochondrial concentrations over 100-fold plasma levels [27], and may lead to new approaches to the management of congenital renal disorders.

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Figure 1. Glomerulotubular junction and proximal tubules in mice: response to unilateral ureteral obstruction (UUO) and cystinosis

A-C: periodic-acid Schiff (PAS) staining; D-F: semithin plastic sections; G-I: immunostaining for 4-hydroxynonenal (4-HNE), an indicator of oxidative stress. A, D, G: normal kidney from 14-day-old mice; B, E, H: kidney from 14-day-old mice following complete ureteral obstruction; C, F, I: kidneys from cystinotic mice

A. PAS staining is specific for carbohydrates, including those of the proximal tubule brush borders and the tubular basement membranes (TBM), which in normal kidney is present as a thin red layer on the basal surfaces of epithelial cells.

B. Ureteral obstruction brings about degeneration of the proximal tubules, which is accompanied by thickening of their TBM (arrows).

C. Cystinosis results in thinning of proximal tubule cells, with thickening of their TBM (arrows). (shown in a three-month-old animal)

D. In a mouse nephron, tall epithelial cells normally compose a portion of Bowman's capsule at the urinary pole and extend to form the proximal tubule. Such cells are characterized by abundant, rod-shaped mitochondria (shown at higher magnification in the inset).

E. With ureteral obstruction, thin, mitochondrion-poor cells occupy Bowman's capsule at the urinary pole, and the remaining proximal tubule cells begin to lose their polarity, their mitochondria becoming more spheroidal (inset).

F. Cystinotic nephropathy results in the formation of the "swan-neck" deformity, comprising an initial proximal-tubule segment with severely flattened epithelial cells underlain by a thickened TBM (arrow). (shown in a four-month-old animal).

G. 4-HNE staining in normal kidney is restricted to the vasculature, with no reactivity present in the proximal tubules (PT).

H. After two weeks of obstruction, proximal tubules display strong immunostaining; this kidney was perfused with nitroblue tetrazolium, resulting in deposition within the proximal tubules (PT) of blue diformazan crystals, generated by superoxide activity within the epithelial cells.

I. In this 16-day-old cystinotic mouse, epithelial cells have not begun to thin, but 4-HNE expression is present in the glomerulotubular junction (at asterisks).

Scale bars in A, B, C, G, H, and I each equal 50 μm ; scale bars in D, E, and F represent 25 μm .

Figure 2.

A and C, wild-type (CD-1) mouse; B and D, PCY polycystic kidney mouse. Compared to wild-type (A, PAS stain), the proximal tubule of PCY mouse is atrophic with wrinkled basement membrane (B). *Lotus tetragonolobus* lectin stains the dense population of proximal tubules in wild-type kidney (C), but staining is sparse in renal parenchyma remaining between multiple large cysts in PCY kidney (D). Scale bars represent 100 μm.

Figure 3.

Scheme showing nephron response to obstructive injury (unilateral ureteral obstruction [UUO] or polycystic kidney disease [PKD]) or metabolic injury (cystinosis). Schematic nephrons are depicted from fetal/neonatal life (A) through completion of nephrogenesis (B and C) and late maturation (D and E). Adaptive “fight” (green) or “flight” (red) responses to injury are based on the paradigm of Goligorsky [25]. Persistent tubular obstruction or metabolic stress results in ultimate nephron loss (E, “flight”), whereas release of obstruction leads to remodeling of the proximal tubule (D, “fight”).