The Renal Collecting Duct Rises to the Defence

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Keywords

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Abstract

When injury occurs, it implies that attack has overcome defence. Tubulointerstitial injury plays important roles in acute kidney injury (AKI) and chronic kidney disease (CKD) and is the common pathway leading to end-stage renal disease, but how the renal tubulointerstitium defends against attack is poorly understood. Emerging evidence suggests that collecting ducts (CDs), which modify urine from nephrons and drain into ureter, could be key defenders protecting tubulointerstitium from injury; furthermore, the canonical renal vitamin A signalling physiologically confined to CDs could be a key regulator of this protective machinery. This hypothesis can be tested by in vitro, in vivo and clinical studies, particularly by repressing or boosting key molecular regulators in CDs, to observe the resulting phenotypes in models of AKI and CKD. Further investigation of this hypothesis could lead to new strategies for diagnosis, prevention and treatment of AKI and CKD.

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Background

Kidneys and renal medicine are often regarded as the “victims of their own success.” Kidneys have remarkable functional reserves, and renal medicine is among the most successful in replacement therapies when kidneys do fail [1]. Patients with end-stage renal disease (ESRD) on dialysis or kidney transplantation can maintain life, but these therapies are costly, donor organs are in short supply and mortality rates are high – young patients (20–49 years old) on these therapies are 16–25 times more likely to die in 1 year than the age-matched general population [2]. Rates of acute kidney injury (AKI) and chronic kidney disease (CKD) are increasing worldwide, and the intercalated AKI-CKD syndrome continues to cause more ESRD and mortality [3, 4]. The nephrology community has been promoting the “0by25” initiative for AKI (zero preventable deaths by 2025)” [5], but few effective strategies exist to prevent AKI, CKD and the progression to ESRD.

Why are AKI and CKD such intractable problems? To answer this question, advice from past presidents of the American Society of Nephrology is worth noting: we must “emphasise innovation” [6] and “build new paths to kidney health” [7].

“Modern medicine can learn from traditional practices”, concluded GP-TCM, European Union’s first ever Coordination Action on modernisation of traditional Chinese medicine (TCM) under its Seventh Framework Programme [8]. As a nephrologist and the principal investigator of the consortium, I think that innovation in modern nephrology could be catalysed by the wisdom from *Yellow Emperor’s Inner Canon*, a TCM classic inscribed in the Memory of the World Register, United Nations Educational, Scientific and Cultural Organization [9], which says: “When there is sufficient protective *qi* inside, pathogenic factors have no way to hurt the body; when pathogenic factors cause damage, the internal *qi* must be deficient”. Herein, *qi* means the internal mechanisms of the body that defend against attack.

However, in nephrology, most of the past work on AKI and CKD focused on how attacks damage, with relatively less consideration of the defending mechanisms in the kidney. In particular, although tubulointerstitial injury plays important roles in AKI [4] and progressive tubulointerstitial damage is the common pathway to ESRD [10], the protective mechanisms by which the renal tubulointerstitium defends against a variety of injurious stimuli is poorly understood.

Hypothesis

Inspired by recent findings of my laboratory [11–14], I hypothesise that collecting ducts (CDs) are key to the defence of the tubulointerstitium and are regulated by the canonical vitamin A signalling mediated by retinoic acid (RA) and RA receptors (RARs).

Evidence Supporting a Key Defence Role for CDs

Studies on AKI and CKD have traditionally focused on glomeruli, proximal tubules and the vasculature, leaving distal tubules and CDs largely neglected. In particular, CDs are the only tubules that span much of the kidney and are ideally placed to protect the whole tubulointerstitial compartment.

Importantly, in addition to principal cells that regulate water homeostasis and intercalated cells that regulate acid-base balance, CDs are equipped with specialised pro-repair and pro-regenerative mesenchymal stem cells [15] and defence molecules, for example, antimicrobial β -defensins [16] and anti-fibrotic microRNAs [17]. Expression of many genes crucial for renal development, for example, *Pax2*, *Wnt4* and *Wnt7b*, is also confined to CDs

in the adult kidney [18]. Could these genes adapt to protective roles? Furthermore, CDs are equipped with RA/RAR physiological signalling [11, 12]. A defence role for RA/RARs is highlighted by the susceptibility to pyelonephritis and urolithiasis in rats fed a vitamin A-deficient diet [19]. Since RA is effective in treating many models of AKI and CKD [20–22], endogenous RA/RARs in CDs may have been evolutionarily selected for protection against injury. To pinpoint roles for RA/RARs in CD cells, my laboratory has catalogued RA/RAR-dependent mRNAs in CD cells at a pan-genomic level, which support a critical role for the RA/RAR signalling in maintaining gene expression implicated in defence against infection (*Ppbbp*, *Lcn2*, *Upk3b*), inflammation (*Ppbbp*, *Cpm*, *Muc20*) and fibrosis (*Bmp7*) [11]. Thus, RA/RAR signalling in CDs, including its target genes, may serve as an important regulator for defence against tubulointerstitial damage.

Emerging evidence suggests that renal RA/RAR signalling plays important roles in AKI and CKD. In resolving AKI, CDs are relatively spared and renal RA/RAR activity is increased [21]. Conversely, in progressive CKD induced by unilateral ureteral obstruction, renal RAR expression decreases [22], and in mice with diabetic nephropathy there is a kidney-specific impairment of RA/RAR signalling [23]. Work in my laboratory has shown that endogenous RA/RAR activity is conserved in both mouse and human CD cells, including cortical and medullary CD cells and CD-derived mesenchymal stem cells (Xu [13], unpublished data). Furthermore, albumin specifically and dose-dependently represses RA/RAR activity in CD cells, suggesting that albuminuria, the leading risk factor for CKD progression, may contribute to CKD progression by repressing RA/RAR activity in CDs (Xu [13], unpublished data). In addition, angiotensin II, aldosterone, endotoxin and high glucose, which all contribute to CKD, dose-dependently repress RA/RAR signalling in CD cells, while gentamicin and aristolochic acid, which are known to induce AKI, increase signalling in cultured CD cells (Xu [13], unpublished data). Thus, RA/RAR signalling in CD cells appears to be a convergence point of regulation by AKI and CKD risk factors and may be a novel target for AKI and CKD prevention and treatment.

Central Questions and Key Experiments Proposed to Test the Hypothesis

CDs are structurally and functionally important for the kidney. They serve to drain urine from nephrons to ureters and plays fundamental roles in maintaining fluid

Fig. 1. A hypothesised protective role for the canonical vitamin A signalling in CDs and proposed points for experimental intervention in order to test the hypothesis [11, 12, 20]. Vitamin A (Rol) is metabolised to RA via 2 oxidation steps. While the first step is reversibly catalysed by retinol dehydrogenases (Rdh) and dehydrogenase/reductase (SDR family) member 3 (Dhrs3), the second step converting retinaldehyde (Ral) into RA is catalysed by aldehyde dehydrogenase family 1 subfamily a1–3 (Aldh1 a1, a2 and a3) and is irreversible. RA binds RARs in heterodimers of retinoid X receptors (RXR) and RAR to regulate gene expression. In RAR/RXR heterodimers, RXRs are silent. Thus, RA-activated RAR/RXR signalling is also simplified as RA/RAR signalling. By tetracycline-inducible, CD-specific promoter-driven overexpression of a dominant negative mutant RAR (to antagonise RARs) and an

RA-metabolising enzyme, for example, Cyp26a1, b1 or c1 (to eliminate RA), the RA/RAR signalling in CDs can be repressed on demand – this approach will be suitable to examine roles for the RA/RAR signalling in CDs when it is relatively high; by conditional, CD-specific deletion of Dhrs3 or over-expression of Rdh or Aldh (to increase RA synthesis), the RA/RAR activity in CDs can be boosted – this approach will be suitable to examine roles for the RA/RAR signalling in CDs when it is repressed. Proposed points of intervention are highlighted by red fonts. CD, collecting duct; Rdh, retinol dehydrogenases; Dhhrs3, dehydrogenase/reductase 3; Rol, retinol (vitamin A); Ral, retinaldehyde; RA, retinoic acid; RAR, RA receptor; RXR, retinoid X receptor; AKI, acute kidney injury; CKD, chronic kidney disease.

and electrolyte homeostasis. Thus, any experiments proposed to test the hypothesised new protective roles for CDs must first ensure that the classical structural and functional roles for CDs are preserved. To determine the protective roles for CDs, it is important to identify potential protective effectors in CDs and test their functions and such experiments must be designed carefully at the molecular, subcellular and cellular levels. To begin with, at the molecular level, I propose to test the roles for *Pax2*, *Wnt4*, *Wnt7b* and the RA/RAR signalling in CDs as protective effectors in AKI and CKD models [11–14, 18], while other candidate genes and signals are to be identified. I would like to propose the following key questions: How do CD cells maintain resilience? How do CDs protect other tubulointerstitial cells? What are the roles for CD-specific gene expression, RA/RAR signalling and CD-derived microvesicles and mesenchymal stem cells in renal tubulointerstitial defence? How do these mechanisms interact? How is CD defence regulated in AKI and CKD? Is failure of CD defence a cause of more severe AKI, chronicity and CKD progression? Do CDs secrete defence molecules into the urine and express defence biomarkers in biopsied renal tissue? Can these biomarkers predict prognosis and guide treatment? Finally, can novel therapies be devised to prevent AKI, AKI transition to CKD and CKD progression to ESRD by modulating CD defence?

To test whether and how CD cells, including principal cells, intercalated cells and CD-derived mesenchymal stem cells, protect other renal cells, co-culture of these CD-derived cells and other cells in the presence of various types of stress and nephrotoxins could be a useful in vitro model. Conditional, CD-specific deletion, silencing or overexpression of *Pax2*, *Wnt4*, *Wnt7b* and other genes of interest in animal models will establish in vivo proof of concept of the roles of these genes in the CDs in AKI and CKD. To determine whether RA/RAR signalling in CDs plays an important role in AKI and CKD, spatiotemporal changes of renal RA/RAR activities in different AKI and CKD models could be visualised and quantified in RA/RAR activity reporter mice [12, 24]; and as illustrated in Figure 1, AKI and CKD models could be induced in transgenic mice in which RA/RAR signalling in CDs is conditionally repressed or boosted.

To examine the specificity of any proposed protective role for CDs, it is important to compare roles for specific genes and signals in CDs versus other tubules. For example, repressing microRNA biogenesis in CDs but not in proximal tubules leads to renal fibrosis, supporting a specific role for CDs in supplying anti-fibrotic microRNAs [17, 25]. In contrast, deleting the TGF- β type II receptor in either CDs or proximal tubules leads to similar pro-fibrotic phenotypes, suggesting that the anti-fibrotic ef-

fect of the TGF- β type II receptor in CDs is not unique [26, 27].

Many genes (e.g., *Pax2*, *Wnt4* and *Wnt7b*) and signals (e.g., the RA/RAR signalling) in CDs that are potential protectors are indispensable in renal development. Thus, silencing genes or repressing RA/RAR signalling non-conditionally may lead to malformation of the foetal kidney and embryonic lethality thus preventing further studies on their roles in adult AKI and CKD. To test my hypothesis, reliable conditional gene knockout or transgenes to facilitate conditional gene expression changes specifically in adult renal CDs will be needed. However, CD-specific gene modification remains a challenge. Promoters of *Hoxb7*, *Aqp2* and *Atp6v1b1* are most commonly used to selectively regulate gene expression in CDs, but the promoter activity of *Hoxb7* is not strictly confined to CDs [28]; *Aqp2* is only expressed in CD principal cells and CD-derived mesenchymal stem cells [15], while *Atp6v1b1* is only expressed in CD intercalated cells and some hybrid cells [29]. Thus, it remains a challenge to find pan-CD cell-specific promoters. Based on single-cell transcriptomic analysis of different mouse CD cells [29] to target all major CD cells during adulthood, the best approaches may still be inducible deletion or overexpression of target genes mediated by *Hoxb7* promoter or both *Aqp2* and *Atp6v1b1* promoters.

Clinically and in animal models, whether CD-derived urinary microvesicles in AKI and CKD could report activation or failure of the defence capacity and thus predict disease prognosis is worthy of investigation. CDs and other tubules micro-dissected from biopsies of patients or animal models of AKI and CKD could be subjected to in-depth analysis to guide discovery of novel mediators of CD defence. Finally, retinoids and non-retinoid therapies mobilising protective mechanisms in CDs could be explored for the prevention and treatment of AKI and CKD.

Conclusions

A new role for CDs in protecting the renal tubulointerstitium is proposed, supplementing their role in controlling urinary fluid and electrolyte composition. Further investigation of this hypothesis could lead to a paradigm shift in understanding kidney health and may lead to new strategies for diagnosis, prevention and treatment of AKI and CKD. Although this paper focuses on the kidney, CDs are also important in protecting the heart [30]. Given that non-immune defence

mechanisms are generally overlooked in modern medicine, it is hoped that this Special Article will inspire not only nephrologists but also other physicians and medical scientists alike, to perceive health and disease in light of the balance and imbalance between attack and defence.

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Author Contributions

Dr. Qihe Xu conceptualised and wrote this paper.

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