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Tubular atrophy in the pathogenesis of chronic kidney disease progression

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Abstract

The longstanding focus in chronic kidney disease (CKD) research has been on the glomerulus, which is sensible because this is where glomerular filtration occurs, and a large proportion of progressive CKD is associated with significant glomerular pathology. However, it has been known for decades that tubular atrophy is also a hallmark of CKD, and is superior to glomerular pathology as a predictor of GFR decline in CKD. Nevertheless there are vastly fewer studies that investigate the causes of tubular atrophy, and fewer still that identify potential therapeutic targets. The purpose of this review is to discuss plausible mechanisms of tubular atrophy, including tubular epithelial cell apoptosis, cell senescence, peritubular capillary rarefaction and downstream tubule ischemia, oxidative stress, atubular glomeruli, epithelial-to-mesenchymal transition, interstitial inflammation, lipotoxicity and Na^+/H^+ exchanger-1 (NHE1) inactivation. Upon obtaining a better understanding of tubular atrophy (and interstitial fibrosis) pathophysiology, it might then be possible to consider tandem glomerular and tubular therapeutic strategies, in a manner similar to cancer chemotherapy regimens, which employ multiple drugs to simultaneously target different mechanistic pathways.

Keywords

apoptosis; CKD; interstitial fibrosis; lipotoxicity; NHE1

Introduction

Over 26 million people in the US are afflicted with chronic kidney disease (CKD), as defined by persistent albuminuria and/or decline in glomerular filtration rate (GFR) [1]. These CKD criteria reflect that glomerular pathology is central to the pathogenesis for most types of CKD in adults and in a significant proportion of children, and that heavy albuminuria and decreased GFR are risks for mortality [2, 3]. In adults, in whom diabetes and hypertension are common, the majority of CKD patients in the US do not undergo a diagnostic renal biopsy and thus, the specific glomerular pathology is often unknown. As a result, some glomerular diseases, such as hypertensive nephrosclerosis, which should be diagnosed according to well-established pathologic criteria [4], are overrepresented in ESRD registries as clinical diagnoses of exclusion [5]. In contrast, in the pediatric population,

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biopsies are more routinely performed for the suspicion of glomerular disease, and the specific etiology is therefore more likely to be known in children. While the distribution of CKD diagnoses differs between children and adults, with a predominance of structural/inherited/tubular disorders, there is still a significant representation of glomerular etiologies in children (Table 1).

Regardless of the primary glomerular disease etiology, tubular atrophy, which is ultimately defined as the disappearance of either individual tubular epithelial cells or entire tubules, often in conjunction with interstitial fibrosis, is an important hallmark of CKD because tubular atrophy has repeatedly been shown to be superior to glomerular pathology as a predictor of CKD progression [6-11]. Prior to the actual disappearance of cells, the initial morphologic features include loss of brush border and apical mitochondria, followed by epithelial simplification to a more cuboidal appearance, which is associated with diminished transport functions [12]. The final degeneration phase is characterized by wrinkling of the tubular basement membrane, influx of inflammatory cells and macrophages, tubular epithelial cell apoptosis and ultimately replacement of the entire area with scar/fibrous matrix proteins [12].

Pathology of tubular atrophy

The first widely recognized report to describe the relationship between tubular atrophy and GFR was a case series from the UK that included 50 patients with biopsy-proven primary glomerulonephritis (GN) [8]. Most patients had the nephrotic syndrome, including 12 with minimal change disease, and 20 with other causes. Fourteen subjects had proliferative GN, including two with fibrocellular crescents. Subjects with associated systemic diseases were excluded. Glomerular pathology was scored for presence of sclerosis on a 0 to 5 scale. Tubular atrophy was defined by the presence of tubular epithelial thinning, pyknotic nuclei or tubular dilation, with or without protein casts. Ten random fields per specimen were observed and scored as the percentage with tubular atrophy features. Tubular atrophy significantly correlated with serum creatinine, creatinine clearance, as well as ability to concentrate and acidify urine [8]. There was also a correlation between glomerular pathology and serum creatinine, creatinine clearance and urine concentration, though the P values were less significant compared to P values associated with tubular atrophy.

This landmark study was followed two years later by a widely cited case series from the US that contained 70 biopsies from patients ranging in age from 8 to 75 years [9]. In contrast to the UK report, this series included patients with underlying systemic diseases, such as diabetes and multiple myeloma. Detailed semi-quantitative histomorphometric analyses were conducted on glomerular and tubulointerstitial compartments [13], which were correlated with inulin and creatinine clearances (as indices of GFR), para-aminohippurate (PAH) clearance (as an index of renal plasma flow), and maximum osmolality and ammonium secretion, as indices of urinary concentration and acidification capacities, respectively. There was a tight correlation between tubular atrophy or interstitial fibrosis with inulin or creatinine clearances, and every patient with an inulin clearance below 60 ml/min/1.73 m^2 showed evidence of some tubular atrophy on biopsy. In contrast, there was a weak relationship between glomerular histology and estimated GFR. Urinary

concentration and acidification capacity also correlated with tubular atrophy, and to a lesser extent with glomerular pathology. PAH clearance correlated with tubular atrophy, particularly if accompanied by vascular disease.

More than 25 years elapsed before a larger, more definitive study was published by Bohle et al [14], which examined the relationship between GFR and quantitative histomorphometric analyses of glomerular and tubulointerstitial pathology. In this series, which contained over 3,000 biopsies with predominately chronic GN, but also included diabetic nephropathy, amyloidosis and primary tubulointerstitial diseases, there was a significant correlation between cortical interstitial volume and serum creatinine. Moreover, glomerular pathology was not associated with elevated creatinine, unless it was accompanied by tubular atrophy and interstitial fibrosis.

Tubular atrophy has also been associated with renal dysfunction in transplanted kidneys. In a study of 146 transplant biopsies performed for clinical indications, with multiple associated diagnoses, Bunnag et al examined a panel of 12 histologic parameters and identified tubular atrophy and interstitial fibrosis as the most predictive markers of eGFR decline [11]. Tubular atrophy has been associated with multiple transplant-specific processes, such as acute cellular rejection, anti-donor antibody-mediated injury, and chronic rejection due to non-immune mechanisms [15]. The prevalence of interstitial fibrosis and tubular trophy is so common in late allograft failure, that the abbreviation IF/TA has now replaced the term chronic allograft nephropathy. However, IF/TA is also an early predictor of renal allograft function. In a protocol biopsy study performed three months post-transplant in 280 patients, 10-year graft survival was 95 % in patients with normal histology, 82 % with IF/TA without transplant vasculopathy, and 41 % with IF/TA plus transplant vasculopathy [16].

One countervailing view is that the importance of tubular atrophy may be exaggerated in the evaluation of CKD pathology and pathophysiology because the tubulointerstitium comprises 90 % of the biopsy area, and features in this compartment are therefore easily observed and measured [17]. In addition, the capacity to examine renal tubular epithelial cells in an in vitro environment has been feasible for decades, which has permitted generation of large amounts of data to support roles for tubule cells in kidney disease pathogenesis. In contrast, the glomerulus encompasses only 5-10 % of biopsy area, which creates a greater probability of sampling error that could be amplified if diseased glomeruli are disproportionately replaced by scar matrix, and remnants of the previously connected nephron remain [12]. Furthermore, many specialized features of glomerular endothelial cells and podocytes still cannot be accurately modeled in cell culture systems, which may preclude generation of reliable in vitro data. These problems have been somewhat, but not wholly circumvented by strategies that employ glomerular-specific conditional knockout mice [18, 19]. Finally, the contribution of medullary tubulointerstitial pathology is largely unknown, since diagnostic biopsies are intended to sample only cortical tissue.

Mechanisms of tubular atrophy

Tubular epithelial cell apoptosis

Over the past 20 years it has repeatedly been shown, in both human biopsies and animal models of progressive kidney diseases, that renal tubular epithelial cells undergo apoptosis [20-29]. Multiple apoptosis pathways have been implicated in tubular atrophy, including recent reports linking proximal tubule endoplasmic reticulum (ER) stress in diabetic nephropathy [29, 30]. Until recently the most compelling argument that apoptosis is the cause of tubular atrophy had been kinetic experiments demonstrating that proximal tubule apoptosis precedes tubular atrophy in mouse models of focal and segmental glomerulosclerosis (FSGS) [22]. A more definitive link between apoptosis and tubular atrophy is provided in a study by Grgic et al, in which mice were genetically engineered to express the simian diphtheria toxin receptor exclusively in kidney epithelial cells, thereby allowing isolated effects of tubule cell apoptosis to be examined following systemic, sublethal diphtheria toxin administration [31]. A single dose resulted in significant proximal tubule apoptosis, most prominently in the S1 and S2 segments, as well as interstitial inflammation with macrophage, T cell and neutrophil infiltrates. These findings were followed by robust tubular epithelial proliferation and recovery, simulating the effects of a single episode of ischemic acute kidney injury (AKI). However, when the diphtheria toxin was given on three occasions over a two week period, which models persistent apoptotic insults from CKD, there was a significant, sustained increase in serum creatinine, and the resulting pathology included interstitial capillary rarefaction, tubular atrophy (characterized by simplified, flattened epithelia, and thick, wrinkled tubular basement membranes) and the appearance of inflammatory infiltrates, fibroblasts and fibrosis within the interstitium [31]. Although the mechanism for this more severe phenotype was not extensively investigated, the authors speculated that the atrophic and regenerating tubules secrete inflammatory and pro-fibrotic cytokines [32, 33], which may perpetuate progressive renal dysfunction (see following discussion). Intriguingly, the multiple dose diphtheria toxin regimen also caused glomerulosclerosis, and there was a statistical correlation between atrophic tubules and scarred glomeruli, elevated creatinine and albuminuria.

Cell senescence

A recent theory for the pathogenesis of a variety of diseases is acceleration of normal aging processes, such as oxidative stress. Senescent cells are defined by acquired absence of the ability to divide, which is associated with telomere shortening and initiation of a DNA damage response. Although not a diagnostic criterion, a lower threshold for apoptosis is generally recognized as a feature of senescent cells. Because multiple stimuli, such as reactive oxygen species (ROS) have subsequently been discovered to cause stress-induced cell senescence, which is characterized by $p16^{Ink4a}$ or p53-induced, p21-dependent cell cycle arrest, these pathways have increasingly been implicated in disease pathogenesis, including CKD [34]. The best example in the kidney literature may be the subset of AKI that proceeds to CKD [35]. Theoretically, in AKI that results in renal function recovery, injured tubular epithelial cells undergo cell death, and are replaced by regenerating cells. On the other hand, tubular epithelial cells that are injured, but survive may adopt a maladaptive, senescent phenotype, which includes secretion of inflammatory cytokines, such as TGF-β,

that then perpetuate progression to CKD. Experimental evidence to support cell senescence as a mechanism of tubular atrophy is scant, but Braun et al showed that tubular atrophy was reduced following ischemic injury in mice with the $p16^{Ink4a}$ gene deleted (*INK4a*$^{-/-}$) compared to wild-type mice, and mice transplanted with kidneys from *INK4a*$^{-/-}$ animals developed decreased tubular atrophy and interstitial fibrosis [36].

Autophagy is a cellular pathway that prevents cell senescence. In response to multiple cell stressors cytosolic proteins, lipids and carbohydrates, as well as damaged organelles are sequestered in double membrane organelles, called autophagosomes, which fuse with lysosomes, and the contents are degraded. In most instances the degradation products are recycled, which promotes cell survival. In rats that underwent unilateral nephrectomy plus induction of diabetes with streptozotocin, proximal tubule cells were noted to contain decreased autophagosomes after three days [37], suggesting that this hypertophic phase, which heralds tubular atrophy, is fueled by decreased autophagy. In several more recent reports, conditional deletion of a key autophagy gene (*Atg5*) has yielded data that suggest a role for autophagy in preventing tubular atrophy. Proximal tubule *Atg5*-null mice developed a modest phenotype at nine months, but significant renal dysfunction with tubular atrophy and renal fibrosis at 24 months [38]. In a separate study, combined *Atg5* deletion from proximal and distal tubules led to a 50 % increase in serum creatinine at one and five months, with cellular evidence of ER and oxidative stress [39]. Finally, *Atg5*$^{-/-}$ mice subjected to intraperitoneal injection of fatty acid-loaded albumin to induce lipotoxicity (see discussion below) developed increased proximal tubule apoptosis and severe histopathology compared to identically treated wild-type mice [40].

Sirtuins are a highly conserved family of NAD^+-dependent deacetylases, which defend against apoptosis, oxidative stress and inflammation by promoting autophagy [41]. Sirtuin-1 (Sirt1) has been extensively studied in the kidney, and is most highly expressed in the inner medulla [42], perhaps as a teliologic counterweight to the hyperosmolar and hypoxic environment. $SIRT1^{+/-}$ mice developed substantial apoptosis and interstitial fibrosis following ureteral obstruction, compared to wild-type mice, and treatment with the Sirt1 activator SRT1720 ameliorated the tubulointerstitial phenotype [42]. Another Sirt1 activator, resveratrol, has also been shown to limit interstitial fibrosis from ureteral obstruction, by deacetylation of Smad3, a downstream activator of TGF-β [43]. In mice that underwent chronic dietary calorie restriction, kidney Sirt1 levels were increased, and this was associated with enhanced proximal tubule autophagy and resistance to apoptosis and oxidative stress [44]. Proximal tubule Sirt1 expression was decreased in streptozotocin-induced type 1 diabetes or the db/db model of type 2 diabetes [45]. Importantly, Sirt1 expression changes preceded the advent of albuminuria, and proximal tubule-specific Sirt1 deletion or overexpression correlated with glomerular histology and albuminuria, implying that proximal tubule Sirt1 regulates glomerular function in diabetic nephropathy. The purported mechanism of crosstalk is through diffusion of nicotinamide mononucleotide (NMN, a NAD precursor) from proximal tubule to glomerulus, which normally preserves podocyte function by suppressing claudin-1. However, an early manifestation of diabetic nephropathy is decreased proximal tubule NMN synthesis, resulting in both impaired NMN secretion to the glomerulus, as well as decreased NAD^+-dependent Sirt1 deacetylation [45].

Peritubular capillary rarefaction and altered blood flow

The vascular microanatomy for each nephron is essentially two sets of capillaries – glomerular and post-glomerular, which are separated by the efferent arteriole (Figure 1). Since the peritubular capillaries represent the sole blood supply to the tubules, glomerular scarring that damages the upstream capillary bed could have downstream consequences. In the previously cited studies by Bohle et al [14] an important additional finding was a significant inverse relationship between post-glomerular capillary area and serum creatinine, implying that obliteration of the peritubular capillary network led to tubular atrophy. These data have subsequently been corroborated in multiple rodent models of CKD [46-48]. Mechanisms of peritubular capillary regression in the context of chronic glomerular injury include reduction of angiogenic and survival factors (vascular endothelial growth factors (VEGFs), fibroblast growth factors (FGFs), angiopoietins, nitric oxide) and/or their receptors [49], secretion of anti-angiogenic factors (endostatin, thrombospondin-1), malfunction of endothelial cells and their progenitors, and impaired endothelial cell-pericyte interactions, leading to detachment of both cell types from basement membrane [50, 51]. Many of these mechanisms result in endothelial apoptosis, which can lead to thrombosis and inflammation.

The benefits of ACE inhibitors and ARBs for CKD therapies are predicated upon reduction of efferent arteriolar tone, resulting in commonly cited mechanisms of normalization of intraglomerular hypertension and amelioration of extracellular matrix deposition. However, it is not as widely recognized that angiotensin blockade increases peritubular capillary blood flow and interstitial pO_2 [52], with a potential manifestation of preventing tubular atrophy.

Tubule ischemia

Peritubular capillary rarefaction logically leads to reduced blood flow, which results in tubule hypoxia and tubular epithelial cell death [22, 53-55]. The S3 segment of the proximal tubule is especially vulnerable, due to the fine balance between low O_2 tension and high metabolic demands, even under ambient conditions. CKD patients are often anemic, which contributes to tissue hypoxia by further reducing the O_2-carrying capacity (blood flow × Hgb concentration × O_2 saturation). Interstitial fibrosis and tubular basement membrane thickening increase the distance for O_2 diffusion, thereby amplifying the effects of diminished blood flow on tubule damage. Disproportionate interstitial fibrosis compared to glomerular pathology may provide an explanation for the previously discussed discordance between glomerular and tubular pathology as predictors of CKD progression.

One consequence of tissue hypoxia is the activation of hypoxia inducible factors (HIFs). The HIF-1 protein is composed of α and β subunits, and HIF-1α undergoes constitutive proteasomal degradation under normoxic conditions. In response to hypoxia, HIF-1αβ heterodimers evade degradation, and act as transcription factors, to stimulate expression of hypoxia-sensitive target genes, such as VEGF and erythropoietin. Multiple animal models of CKD have demonstrated HIF-1 upregulation [56, 57], but it remains unclear whether enhanced HIF-1 activity is salutary or deleterious [50, 58]. For example, kidney-specific deletion of HIF-1α abrogates tubulointerstitial disease from unilateral ureteral obstruction [57], whereas HIF-1α stimulation with cobalt chloride was recently shown to be beneficial

in the rat model of streptozotocin-induced diabetic nephropathy [59]. Explanation for conflicting HIF-1 effects could be related to cell type, context (disease model, kinetics of HIF-1 regulation, timing of interventions), the vast number of HIF-1 target genes (>200), some of which have contradictory functions, as well as opposing effects of individual target molecules. For example, VEGF promotes angiogenesis, which may improve hypoxia, but also triggers inflammatory cascades [50].

Oxidative stress

A mechanism related to hypoxia (and inflammation), which has been implicated in tubular atrophy, is oxidative stress. The most important, and potentially toxic ROS are the superoxide anion ($\cdot O_2^-$), the hydroxyl radical (·OH), and hydrogen peroxide (H_2O_2), which can then react with substrates to form other oxidants, such as peroxynitrite ($ONOO^-$). Oxidative stress can also be accelerated by oxidized target molecules, most notably advanced glycation end products in diabetic nephropathy. During oxidative phosphorylation and electron transport in mitochondria, electrons from reduced forms of nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH2) are transferred to O_2, the vast majority of which is reduced to H_2O. Under normal circumstances, ROS are generated when a very small percentage of electrons are leaked at electron transport chain complexes I and III, and transferred to O_2 to create $\cdot O_2^-$. In pathologic states additional intracellular sources of ROS include (a) redox-activated flavin proteins, and leakage of electrons at the coenzyme Q-complex III interface [60]; (b) oxidases (also known as oxidoreductases), such as acyl CoA oxidase in peroxisomes, NADPH oxidases [61], which localize to phagosomes and plasma membrane compartments in resident cells of glomeruli, tubules and the interstitium [62], and xanthine oxidase; (c) uncoupled nitric oxide synthase, which leads to nitrosylation of intracellular proteins.

The accumulation of ROS results from an imbalance between production and consumption by antioxidant scavengers, such as glutathione, or detoxification by enzymes, such as superoxide dismutase, which reduces $\cdot O_2^-$ to H_2O_2, and catalase, which metabolizes H_2O_2 to $H_2O + O_2$. Under normal conditions low ambient concentrations of ROS have intended, homeostatic functions by oxidizing cysteine residues on proteins, especially kinases and phosphatases, which can alter secondary structure, protein-protein interactions, and ultimately catalysis [63]. At higher intracellular concentrations ROS can modify accidental targets, such as DNA, membrane lipids (through lipid peroxidation) and structural proteins, resulting in apoptosis and necrosis [29, 64]. In some instances, such as ROS derived from NADPH oxidase-4 (NOX4), dual deleterious and beneficial properties have been described with implications for tubular atrophy and interstitial fibrosis [42, 65-67], and the divergent phenotypes may be related to the concentration of ROS generated.

Although a definitive role for oxidative stress in the pathogenesis of tubular atrophy has not been established, the best supportive evidence may be derived from catalase-overexpressing transgenic mice, which showed reduced proximal tubule apoptosis in models of diabetic nephropathy and angiotensin II-mediated hypertensive renal disease [27, 28, 68]. There is also some suggestion that oral antioxidant therapies may be beneficial for CKD progression in humans, though the number of trials is small [69]. On the other hand, a recent trial with

the Nrf2 activator bardoxolone methyl, which has antioxidant and anti-inflammatory properties, demonstrated no effect on progression to ESRD and increased cardiovascular mortality in patients with diabetic CKD [70].

Atubular glomeruli

Another possible mechanism of tubular atrophy in the context of chronic glomerular diseases is related to the disconnection of proximal tubules from the associated glomeruli, which was first observed in ultrastructure experiments from microdissected nephrons [71]. This phenomenon, which is variably referred to as aglomerular tubules, or more commonly, atubular glomeruli, is most often observed with primary tubular disorders. The prototype may be hereditary cystinosis, which results in proximal tubule lysosome accumulation of cystine and apoptosis, followed by development of glomerulotubular stenosis ("swan neck deformity"), and ultimately severing of the glomerulotubular junction [72]. Atubular glomeruli are presumably associated with peritubular capillary disruption, although this has not been investigated in detail, perhaps due to the tedious methods that would be required for such experiments.

In a hypothesis formulated by Kriz to explain tubular atrophy resulting from primary glomerular diseases, "degenerative" diseases are caused by fibrocellular crescents that accumulate along the outer/basolateral surface of the proximal tubule, and can entrap extravasated ultrafiltrate [12]. "Inflammatory" glomerular diseases are characterized by encroachment of crescents upon the glomerulotubular junction. According to both models, atubular glomeruli develop as an intermediate step between the expanding crescent and complete loss of nephron function [12]. The guiding principle is that tubular atrophy directly results from a diseased, adjacent glomerulus, and interstitial inflammation and fibrosis are secondary, rather than causal processes.

Epithelial-to-mesenchymal transition (EMT)

EMT is a process whereby epithelial cells lose apical-basal polarity and cell-cell adhesion, and acquire migratory and invasive properties to become mesenchymal stem cells, which are multipotent stromal cells capable of differentiating into fibroblasts. The EMT concept was described many years ago, and has been unequivocally implicated in embryonic development programs and tumor metastasis [73]. More recently EMT has been hypothesized as a possible mechanism to explain tubular atrophy. The appeal of this hypothesis is that tubule epithelial cells are not actually deleted, but instead convert to a myofibroblast phenotype, and then contribute to scar matrix deposition [74, 75] – one process would therefore explain several aspects of CKD pathogenesis. Because EMT is associated with enhanced ATP generation under hypoxic conditions [76, 77], and pro-fibrotic cytokines generally activate transcription factors that are associated with protection against apoptosis, EMT might therefore convey short-term advantages to a cell submerged in an ischemic state, as described above. Fibrogenic cytokines, most notably TGFβ1, have been detected at increased concentrations in CKD tissue, and multiple studies have demonstrated that exposure of these cytokines to tubular epithelial cells maintained in cell culture results in acquisition of fibroblast features [78]. However, an EMT hypothesis for the pathophysiology of CKD has been difficult to prove in vivo, with most studies revealing

only rare transitioning cells, as demonstrated by simultaneous expression of epithelial and mesenchymal markers [79, 80]. Furthermore, detection of such cells does not permit distinction between EMT versus mesenchymal-to-epithelial transition (MET). There are even fewer examples of cells with concomitant epithelial and mesenchymal features migrating across the tubular basement membrane to the interstitium, where myofibroblasts reside.

The most definitive approach to address the role of EMT in tubular atrophy is with cell lineage tracing techniques, which allow the fate of a cell to be mapped from early development onward. If a heritable marker and expression system is carefully selected (in this case for tubular epithelial cell precursors), then once activated in vivo, the marker will be indefinitely detectable irrespective of subsequent pathologic perturbations. Using such an approach in a unilateral ureteral obstruction (UUO) model, Iwano et al demonstrated that approximately 30 % of injury-induced kidney myofibroblasts were derived from proximal tubule cells [75]. In stark contrast is the report by Humphreys et al, which detected zero proximal tubule-derived myofibroblasts following UUO [81]. This study instead identified the pericyte (when localized to peritubular capillaries) or resident fibroblast (when localized to the interstitium), rather than the tubular epithelial cell, as the source of scar-producing myofibroblasts. So why the discrepancy? The report by Humphreys et al offers some technical advantages that permit firmer conclusions to be drawn, such as (a) knock-in rather than transgenic overexpression of Cre recombinase, (b) expression of *LacZ*, as well as a fluorescently-labeled fate marker, which do not require immunohistochemical localization, and (c) detection of more faithful myofibroblast antigens (α-smooth muscle cell actin, FSP-1 and type I collagen [82] versus FSP-1 and HSP47 [75]). However, this topic remains controversial, and there are abundant data to support and refute EMT as a mechanism of tubular atrophy [83]. On balance, we conclude that EMT cannot be dismissed as a contributor to CKD pathogenesis, particularly for primary glomerular diseases, where it has not been tested as rigorously, but it is unlikely to represent a major mechanism.

Inflammation

Progressive glomerular diseases are generally not accompanied by large interstitial inflammatory infiltrates. Nevertheless, in FSGS interstitial inflammation more tightly correlates with CKD progression than glomerular pathology [84]. Furthermore, there are ample data to support a role for specific inflammatory cells, arising from a pool that includes both resident and recruited circulating inflammatory cells, in the pathogenesis of interstitial fibrosis [17, 85]. Studies implicating inflammatory cells, and associated cytokines and chemokines as direct causes of tubular atrophy are much less abundant. The most commonly invoked inflammatory cells in CKD tubulointerstitial pathophysiology are from the monocyte/macrophage lineage. The purpose of kidney macrophages may be for phagocytosis of damaged cells and matrix after injury, and although this macrophage subpopulation has not been well characterized, some common features include abundant lysosomes and immunomodulation by IL-10 [86, 87]. However, when the inflammation becomes unbridled, other populations of kidney macrophages may be injurious, either through secretion of inflammatory cytokines (e.g., IL-1β, IL-18, TNF-α and C-X-C chemokines), which can stimulate apoptosis and further inflammation [88-91], or an array of

cytokines (e.g., TGF-β, PDGF, IGF-1), which directly stimulate myofibroblast differentiation. As a result it is difficult to determine solely from histologic studies whether inflammatory infiltrates are beneficial or detrimental. The role of specific cytokines and associated signaling pathways has been implicated from microarray studies in human biopsies with tubulointerstitial disease from diabetic nephropathy [92], and has been extensively reviewed recently [93]. There are some data indicating that systematic macrophage depletion is protective for tubular epithelial cells in models of ischemic AKI [94, 95], but similar data for CKD are lacking.

Lipotoxicity

The intracellular accumulation of excess non-esterified fatty acids (NEFA) and metabolites can result in organ dysfunction, and has been well described in liver (leading to hepatic steatosis), pancreatic β-cells (leading to diabetes), and heart (leading to congestive failure) [96]. The association between renal tubular epithelial cell lipid deposition and diabetic nephropathy was described many years ago [97, 98], and lipotoxicity has been implicated in CKD progression [99-102]. At a molecular level, lipotoxicity has been associated primarily with long chain (C12-C22) NEFA accumulation, and cytotoxicity is proportional to acyl chain length and carbon bond saturation [103, 104]. Some, but not all studies indicate that unsaturated NEFA exposure could be cytoprotective [105], perhaps due to preferential partitioning to lipid droplets [106] (see discussion below). This is clinically relevant because plasma concentrations of saturated NEFAs are increased in CKD patients, and are associated with CKD progression, particularly in cohorts enriched for diabetes [107-112].

The mechanisms of NEFA accumulation in tubule cells is an area of active investigation. In cases of glomerular damage that result in albuminuria, a large fraction of the filtered albumin is reabsorbed by the megalin/cubilin/amnionless protein complex expressed on the proximal tubule brush border, and degraded by lysosomes [113]. It has been postulated that in the nephrotic syndrome massive quantities of intracellular albumin exceed lysosomal degradative capacity, which leads to proximal tubule ER stress and secretion of noxious cytokines and chemokines [114]. This protein overload hypothesis is attractive because it directly links glomerular and tubular pathology to inflammatory pathways that perpetuate further renal injury, as discussed in the previous paragraph. However, albumin is heavily bound by NEFA, which permits NEFA to circulate in a soluble complex. Several groups have shown that tubulointerstitial disease can be induced in rats by infusion of NEFA-loaded, but not delipidated albumin [115-117], and in vitro incubation with albumin-bound NEFAs stimulate proximal tubule apoptosis, whereas exposure to delipidated albumin does not [116-121]. The importance of NEFA-bound albumin filtration and internalization is further illustrated in minimal change disease, which does not progress, at least in part, due to absence of tubular atrophy. Despite massive albuminuria, the ultrafiltrate is relatively NEFA-depleted in minimal change disease [122]. In subjects with heavy albuminuria due to diabetic nephropathy or minimal change disease, urine NEFA: albumin ratio was significantly greater in the diabetic nephropathy group, and correlated with tubulointerstitial pathology [123]. These data suggest that proximal tubule uptake of filtered NEFAs, rather than albumin, is the source of tubular toxicity (Figure 2). NEFAs are internalized by vectorial acylation, which involves simultaneous transport (mostly likely by a member of the

fatty acid transporter (FATP) family) and esterification to long chain acyl CoA (LC-CoA) by plasma membrane-localized long chain acyl CoA synthetases. Under normal conditions LC-CoAs are primarily converted to acylcarnitine by carnitine palmitoyl transferase (CPT), which facilitates LC-CoA transport into mitochondria for β-oxidation and ATP generation, as described below.

In addition to NEFA uptake, tubular lipotoxicity may be amplified by increased proximal tubule NEFA synthesis, as shown in animal models of CKD due to type 1 and type 2 diabetes [124, 125]. Sterol regulatory element-binding protein (SREBP)-1c and carbohydrate response element-binding protein (ChREBP) transcription factors, which regulate fatty acid synthesis, have been investigated as likely mechanisms. On the other hand, SREBP-1c, ChREBP and fatty acid synthase mRNA expression have recently been shown to be significantly decreased with declining GFR in a diabetic nephropathy biopsy series [126], thereby casting some doubt regarding lipogenesis as a major mechanism of lipotoxicity in CKD progression.

NEFAs are the preferred substrate for proximal tubule ATP generation under normal circumstances [127-129]. However, in diabetic nephropathy proximal tubule β-oxidation of NEFA is decreased [124, 125], a finding that has been corroborated by human microarray studies, which demonstrate that GFR decline and interstitial fibrosis correlate with decreased NEFA β-oxidation, as well as decreased expression of CPT1a, PPAR-γ and PPAR-α transcripts [92, 126, 129], which encode for proteins that are critical for β-oxidation. One consequence of a block in β-oxidation is the accumulation of lipid precursor molecules, which are potentially pathogenic. For example, LC-CoAs, which are normally shuttled into the mitochondria for β-oxidation by CPT1a in the proximal tubule, are significantly increased in the renal cortex of mouse models of diabetic nephropathy and FSGS [120]. In vitro inhibition of CPT1a, which mimics the lipid metabolism abnormality in CKD, caused proximal tubule epithelial cell apoptosis [120, 129].

Numerous pathways buffer against NEFA accumulation and subsequent lipotoxicity. The first is metabolism of NEFA by β-oxidation to generate ATP (Figure 2). Once energy needs are met, excess NEFA are stored as cytoplasmic organelles comprised of triacylglycerol aggregates, termed lipid droplets [130] (Figure 2). While primarily functioning as an energy reserve by facilitating bidirectional NEFA trafficking throughout the cell, lipid droplets are also an adaptive depot for excess NEFA, and thereby shield against lipotoxicity and apoptosis [131-133]. In adipocytes, the cytoplasm is almost entirely filled with lipid droplets, whereas in epithelial cells lipid droplet capacity is limited, and saturation can therefore lead to lipotoxicity. The most alarming situation may be the combination of ROS generation and intracellular accumulation of NEFA, resulting in lipid droplet storage of peroxidated NEFA, which are cytotoxic [134].

Intracellular accumulation of NEFA and LC-CoA to levels that exceed tubule cell metabolism and storage thresholds can then lead to lipid-induced apoptosis (lipoapoptosis) (Figure 2). Multiple mechanisms of lipoapoptosis have been described in a variety of systems, including enhanced ceramide generation, ER stress, inhibition of several pro-survival kinases (Akt, PI-3 kinase, and AMP-activated protein kinase) [135-137] and ion

transporters (see next paragraph). In diabetic nephropathy, β-oxidation may also be aberrant, resulting in electron transport chain defects and ROS generation [60]. Pharmacologic and genetic studies suggest that stimulation of PPAR-α or PPAR-γ, which enhance NEFA utilization, may improve histopathology and slow CKD progression in humans, as well as animal models of CKD [129, 138-143].

NHE1 inactivation

As mentioned previously, a final common pathway for tubular atrophy is tubular epithelial cell apoptosis, and an invariant feature of apoptosis is cell volume decrease. In response to shrinkage by non-apoptotic stimuli, cells undergo activation of regulatory volume increase (RVI) pathways in an effort to re-expand. It has been suggested that a similar pathway is activated to limit damage from apoptotic stress [144]. Of the well-characterized RVI-associated ion channels and transporters, only the NHE1 Na^+/H^+ exchanger is expressed in proximal tubules [144]. Apoptotic cells also undergo cytosol acidification, which catalyzes pro-apoptotic enzymes [145, 146], indicating that NHE1-regulated Na^+/H^+ exchange might defend against renal tubular epithelial cell apoptosis by intracellular alkalinization, as well as expanding cell volume.

Proximal tubule NHE1 is indeed activated by multiple different apoptotic stressors [147, 148]. NHE1 activation is dependent upon the anchoring of two Arg/Lys-rich cytoplasmic domains to the plasma membrane inner leaflet through binding to negatively charged phosphate groups on PI(4,5)P2 [149-152]. PI-3 kinase phosphorylates PI(4,5)P2, and the PI(3,4,5)P3 product docks the pro-survival kinase Akt, leading to its activation and phosphorylation of downstream targets that inhibit apoptosis. This docking and signal transduction function of NHE1, which is independent of Na^+-H^+ exchange activity, represents one mechanism of proximal tubule defense against apoptotic stress and tubular atrophy [152].

Multiple studies have demonstrated that tubular epithelial cell NHE1 becomes inactivated during apoptosis [147, 148, 153, 154], but the mechanism has been difficult to clarify. Decreased NHE1 mRNA and protein expression were noted in ureteral obstruction models [153, 154], and there is some evidence to indicate that the NHE1 cytosolic tail undergoes caspase-3-dependent degradation [147, 155]. However, the cleavage site has not been mapped, and there are no consensus caspase-3 cleavage sequences within the cytosolic tail. NHE1 inactivation has recently been associated with the previously discussed lipotoxicity model. The link is predicated upon the accumulation of LC-CoAs, which bear structural homology to phosphoinositides, and demonstration that LC-CoAs bind the NHE1 cytosolic tail with greater affinity compared to $PI(4,5)P_2$ [120]. In cultured proximal tubule cells and microinjected oocytes, LC-CoAs compete with $PI(4,5)P_2$ for binding to NHE1, and uncouple the NHE1-$PI(4,5)P_2$ interaction, causing loss of NHE1 activity, which predisposes to apoptosis [120]. In this model, proximal tubule NHE1 therefore acts as a metabolic sensor for lipotoxicity.

Animal models of tubular atrophy

There are a number of rodent models of non-diabetic glomerular diseases that include a progressive phenotype with tubular atrophy (recently reviewed in [156]). In general, these models fall into the categories of transgenic animals, spontaneous/acquired mutations, or induction of a renal phenotype. In some cases, a combination of phenotype induction in a mutant animal has been fruitful for accentuating tubular atrophy [147, 157]. An important consideration is the genetic background of the animal, and in mice, C57BL/6 background tends to be resistant to disease, whereas FVB, 129/sv and Balb/c mice are relatively susceptible [158, 159].

Conclusions

The glomerulus has been the focus of CKD pathology and cell biology investigation for many years. More recently there has been much deserved attention to the podocyte, which appears to be the proximate source of dysfunction in a variety of glomerular diseases. A better understanding of podocyte pathophysiology will undoubtedly lead to treatments for prominent glomerular diseases, such as FSGS, which are badly needed, since state of the art therapeutic regimens have not substantially changed since the advent of ACE inhibitors in the 1980s, and current immunosuppressive agents have substantial toxicities, and may not be effective in a significant number of patients [160]. This review emphasizes the importance of the remainder of the nephron, which has been relatively neglected as a topic for CKD research, despite repeated demonstration over 50 years that tubular atrophy and interstitial fibrosis are better predictors of CKD progression than glomerular pathology.

A reductionist approach to identify CKD therapies, with separate focus on glomerular and tubular compartments, may be sensible for several reasons. First, a panacea (monotherapy) to simultaneously address CKD pathology for the entire nephron, which is comprised from over 20 cell types, is unlikely to be identified. Second, as highlighted in this review, several plausible pathophysiologic mechanisms of tubular atrophy are proposed, and although some may ultimately be proven incorrect, the emergence of a single dominant pathway is unlikely. Third, even if amelioration of tubular atrophy could be achieved, this would expose the glomerulus as the limiting factor, with necessary identification of separate glomerular therapies. As a result, a sanguine approach might be to develop glomerular and tubular therapies in tandem, in a manner similar to cancer chemotherapy regimens, which employ multiple drugs that target different mechanistic pathways. There are no current therapies specifically designed to treat tubulointerstitial pathology, but based upon the importance of tubular atrophy and interstitial fibrosis as drivers of CKD progression, it seems rational to begin the search, which should involve testing in adults and children.

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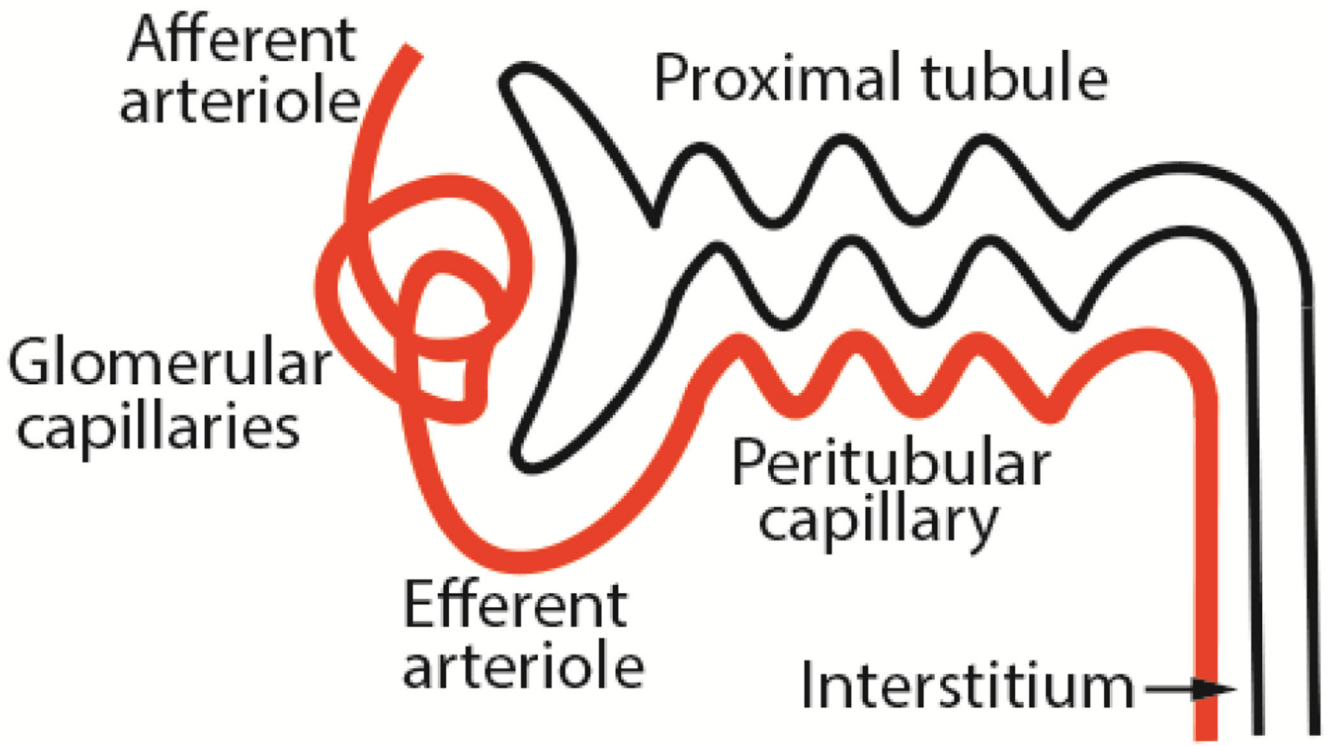

Figure 1.
Schematic diagram of the initial portion of the nephron. Note that the peritubular capillary emanates from the efferent arteriole, and provides blood supply to a parallel tubule.

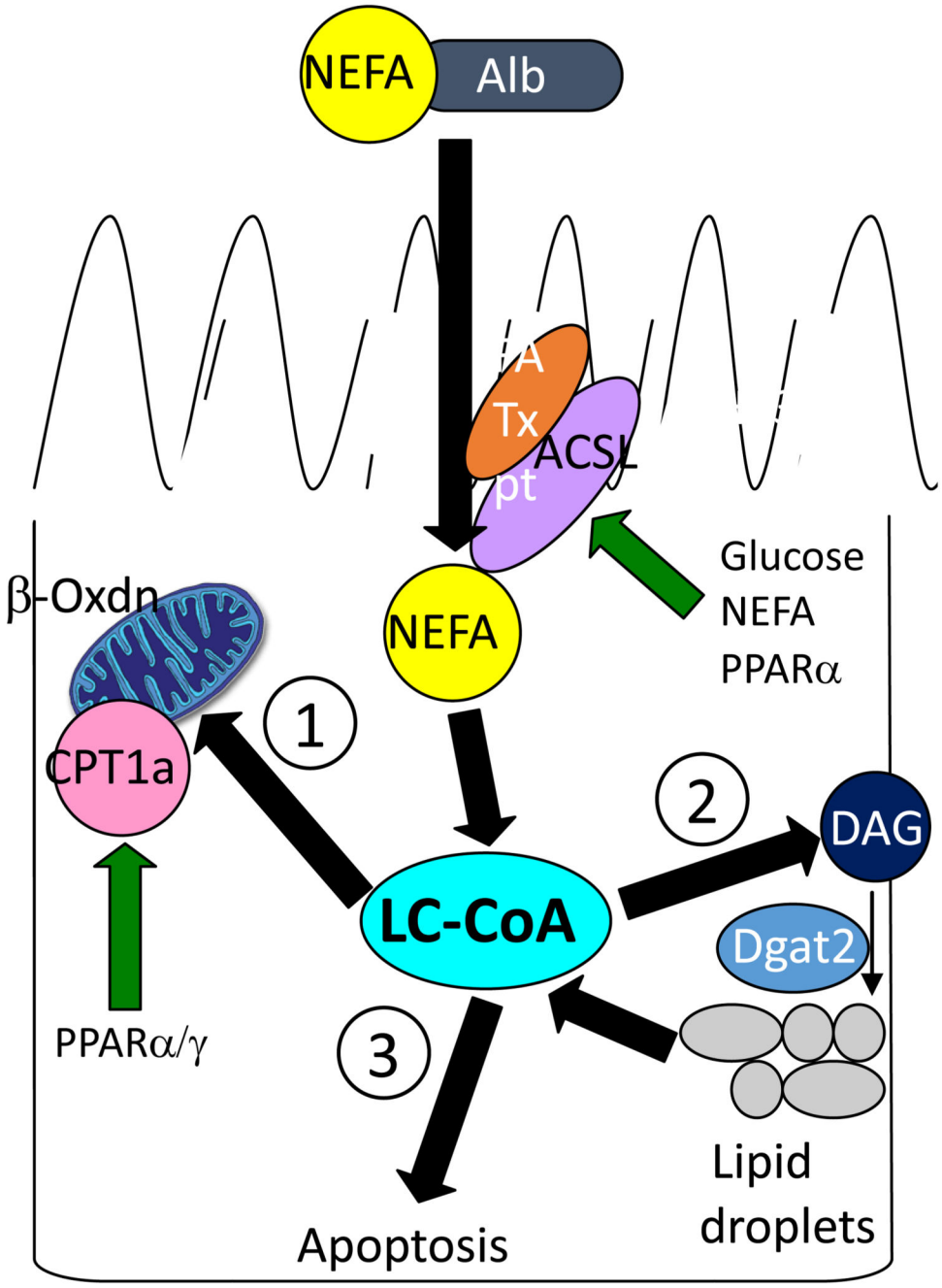

Figure 2.

Model of lipotoxicity-induced apoptosis and tubular atrophy. In progressive albuminuric renal diseases non-esterified fatty acid (NEFA) concentration is increased in proximal tubule cells through luminal reabsorption and/or lipogenesis. NEFA are rapidly converted to long chain fatty acyl CoA (LC-CoA) by long chain acyl CoA synthetases (ACSL). Preferential metabolic pathways include (1) carnitine palmitoyl transferase-1a (CPT1a)-regulated LC-CoA transport into mitochondria for β-oxidation and ATP generation or (2) conversion of diacylglycerol (DAG) to triacylglerol, which is stored in lipid droplets. If these pathways are

blocked or saturated, accumulated LC-CoAs become detrimental and lead to apoptosis (3), through a variety of mechanisms, such as peroxisomal oxidation and reactive oxygen species (ROS) generation, disruption of NHE1-PI(4,5)P2 interaction-mediated cell survival, and ceramide formation.

Table 1

Incidence of ESRD by major causes in pediatric (ages 0-19) and adult (ages 20 and older) populations over the period 2008-2012. Data are derived from 2014 USRDS database and expressed as percentages.

	Pediatric (N=6,204)	**Adult (N=554,689)**
Diabetes	1.0	45.0
Glomerulonephritis	34.3	8.4
Interstitial nephritis/pyelonephritis	5.0	N/A
Hypertensive/large vessel disease	4.5	28.7
Hereditary/congenital/cystic diseases	38.3	2.3

N/A; not available.