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EDUCATIONAL REVIEW

Remnant nephron physiology and the progression of chronic kidney disease

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Abstract In chronic kidney disease, ongoing failure of individual nephrons leads to the progressive loss of renal function. This process results in part from a cellular and molecular response to injury that represents an attempt to maintain homeostasis but instead initiates a program that damages the nephron. As nephrons are lost, compensation by the remaining nephrons exacerbates glomerular pathophysiology. The delivery of excessive amounts of biologically active molecules to the distal nephron and tubulointerstitium generates inflammation and cellular dedifferentiation. Energy requirements of hyperfunctioning nephrons exceed the metabolic substrate available to the renal tubule, and inadequacy of the local vascular supply promotes hypoxia/ischemia and consequent acidosis and reactive oxygen species generation. In this way, mechanisms activated to maintain biological balance ultimately lead to demise of the nephron.

Keywords Hypertrophy · Fibrosis · Reactive oxygen species · Chronic kidney disease · Nephron

Introduction

Chronic kidney disease (CKD) progresses through three phases: (1) an initial injury stimulates (2) repair mechanisms that may be misdirected, impairing nephron function. As nephrons are lost, (3) the remaining nephrons respond, compensating for decreased function with further changes in activity that may be deleterious for nephron survival. While these three phases may overlap temporally, their mechanisms are distinct. The initial injury may or may not be identifiable at the time the patient presents with CKD [1]. Importantly, the *sine qua non* of CKD progression is the third phase, involving the ongoing, sequential loss of nephrons; this phase is the topic of the present review. Clinicians have long noted that CKD progresses in a fairly linear fashion. If one graphs the reciprocal of creatinine as a function of time, after several sequential determinations the slope of the resulting line often can be used to predict when the patient will require dialysis or transplantation (Fig. 1 shows a hypothetical graph; it should be noted that in practice patients may not follow this pattern either because of accelerated loss of function as end-stage nears [2] or variability in patterns of kidney function decline [3]). This observation suggests that a pathway exists by which nephron function is inexorably lost. The events involved in this pathway generate a vicious cycle of nephron failure, physiological adaptation via hypertrophy and hyperfunction, and the negative effects of that adaptation, causing further nephron failure. The present review will focus on mechanisms within the kidney that maintain this cycle of events. The systemic factors that contribute to progression in CKD have been reviewed recently elsewhere [4].

The structural complexity of the kidney supports multiple functions that are balanced by forces both within and outside of the kidney. The symptoms of chronic uremia are a reflection of this complexity, and of how events far from the kidney are disrupted and, in turn, further affect kidney function. The central tenet in understanding this process is that kidney function is tightly regulated in a manner that maintains homeostasis. At the level of the whole organism, this regulation seeks to maintain optimal body composition by compensating for injury. Thus, as kidney tissue is lost, hormonal mechanisms that maintain this homeostasis "instruct" the remaining tissue to carry an increased workload. At the level of the individual nephron, the close anatomical

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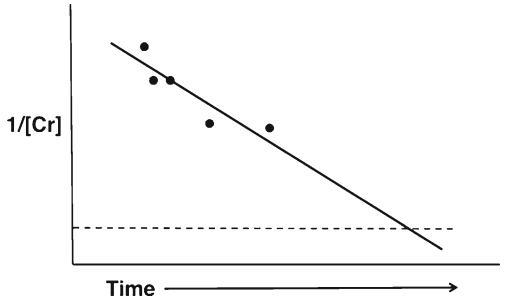

Fig. 1 Loss of renal function is linear as kidney disease progresses. The *solid line* shows a linear relationship between the reciprocal of the serum creatinine concentration (*1/[Cr]*) and time. The *dashed line* shows an arbitrary hypothetical point, where renal function is about 10 % of normal, when dialysis is required. After several creatinine determinations (*solid circles*) are made over an interval of time, a prediction regarding when dialysis will be needed can be made, based on where the slope of the solid line intersects with the dashed line

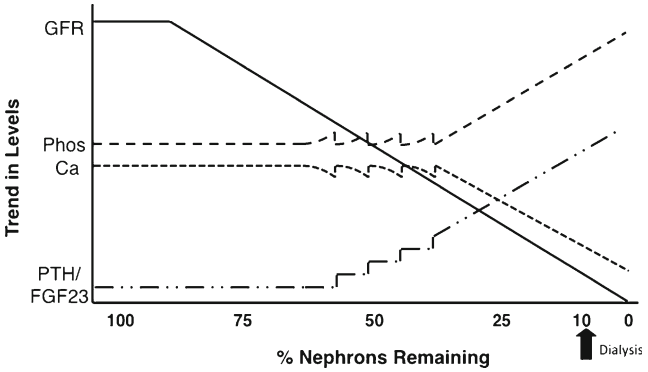

Fig. 2 Idealized depiction of hormonal compensation for nephron loss and disruption of homeostasis. The *Y-axis* shows arbitrary points representing levels of glomerular filtration rate (*GFR*), phosphorus (*Phos*), calcium (*Ca*), and parathyroid hormone (*PTH*) or fibroblast growth factor-23 (*FGF23*). After an initial interval, remnant nephron hypertrophy is unable to compensate for nephron loss, and renal function (GFR) begins to decline. At a later point, increased Phos and decreased Ca repetitively stimulate increased PTH, normalizing Ca and Phos levels. However, at some point the capacity of the remaining nephrons to excrete Phos also fails, with attendant increased Phos, decreased Ca, and rising PTH levels. In a similar manner, for other homeostatic mechanisms, at some point the remaining nephron mass can no longer compensate, but hormonal responses continue, leading to extension effects. These additional effects have a negative impact on body systems, including those of the kidney

relationship of glomerular and tubular vasculature in the same nephron supports glomerulotubular balance, the mechanism by which filtration and reabsorption in each nephron are closely matched.

Nephron loss in the pathogenesis of uremia

An illustration of how this balancing act leads to the uremic state was provided by Bricker and colleagues in several elegant reviews [5, 6] that characterize the unwanted effects of exaggerated attempts at homeostatic regulation. In Fig. 2, the solid line shows the hypothetical relationship between the percentage of nephrons remaining in the kidney and measured total kidney glomerular filtration rate (GFR). During initial nephron loss, hypertrophy of the remaining nephrons maintains GFR. However, at some point, the limit of this adjustment is reached, and as more nephrons are lost GFR decreases. Without significant intervention the subsequent decrease in kidney function is linear with respect to time.

Each homeostatic function of the kidney is governed at the level of the nephron. Thus, if total body sodium and volume increases, the response requires increased sodium excretion by each individual nephron. If a person ingests a large amount of sodium (say, by eating a pizza), hormonal regulation is activated to increase each nephron's salt excretion. Similarly, if the number of nephrons is reduced, the total body sodium will remain constant or rise; in either case, the per-nephron sodium load will be increased. Hormonal regulation stimulates the remaining nephrons to "work harder" in order to excrete sufficient sodium, in the same manner that would be required after ingestion of a sodium load by a healthy subject. Given the large margin for salt handling, the ability to compensate can remain until about 90 % of the nephrons are lost. For another molecule, phosphate, this ability to compensate is not as well maintained. As shown in Fig. 2, when nephrons are lost the plasma phosphate level rises and that of calcium falls. A series of events mediate an increase in parathyroid hormone (PTH) and fibroblast growth factor-23 (FGF23) secretion, which stimulates phosphate excretion to normalize plasma phosphate and calcium. A new steady state is reached, with higher levels of PTH and FGF23. With continued nephron loss, hormone levels increase in a step-wise fashion until the maximal ability of the tubule to respond by increasing phosphate excretion is reached. In the absence of therapeutic intervention, phosphate level increases, calcium level decreases, and PTH and FGF23 levels rise steadily in a vain attempt to compensate.

This simplified version of what occurs with calcium and phosphorous metabolism illustrates what Bricker termed the "amplification" or "magnification" hypothesis [5, 6]. Several important points can be drawn from this illustration. First, the nephron, as the basic unit of regulation of the kidney, can adapt to changing conditions to maintain homeostasis. Second, the cost of this adaptation is a rise in the levels of regulatory hormones. Third, with the loss of each nephron, compensation by the remaining, intact nephrons requires further hormonal response to drive greater nephron function. In the case of PTH and FGF23, excessive levels, in addition to affecting phosphate excretion, increase bone resorption, cause pruritus, and affect other organ systems. These extension effects contribute greatly to the symptoms, morbidity, and mortality of uremia. Thus, understanding

how and why nephrons are lost is key to understanding the pathogenesis of the uremic state.

Structural events in nephron loss

Nephron loss may result from an initial injury that is either glomerular or tubular in nature. Glomerular disease may initiate because of inflammation, thrombogenesis, primary podocyte dysfunction, or vascular injury; or it may involve a combination of these factors, such as in systemic lupus erythematosus. Several mechanisms for how this injury may decrease nephron function have been postulated. Kriz and colleagues have proposed that denuding of the glomerular capillary after podocyte loss leads to the formation of synechiae, adhesions of the denuded endothelium to Bowman's capsule. The resulting adhesion permits extrusion of plasma proteins directly into the periglomerular space, leading to reactive processes that may include inflammation and fibrogenesis [7, 8].

Alternatively, injury may initiate in the tubule or tubulointerstitium. Toxic, inflammatory, or ischemic injury damages tubular cells and structures to the extent that they are not able to regenerate. A number of studies have demonstrated the presence of atubular glomeruli [9]. In some cases, these may represent congenital abnormalities, a finding of anatomically normal but non-functioning structures, but they also may result from tubular injury. In the latter case, such glomeruli are likely transitional forms toward total nephron loss [10].

Nephron hypertrophy is a progression factor

In order to compensate for nephron loss, the remnant, intact nephrons must increase their activity either functionally or by increasing their mass. After unilateral nephrectomy, compensation by hyperfiltration of remnant glomeruli occurs within hours. Beginning by the end of the first day, DNA synthesis and protein expression begin to increase. It is not clear whether the primary event is nephron hypertrophy or hyperfiltration (reviewed in [11]). If it is the latter, mechanical stretching with hyperfiltration is sufficient to induce hypertrophic signals [12]. One major mediator of this hypertrophic response is the renin–angiotensin–aldosterone system (RAAS), which can regulate both hemodynamics and reactive cell hypertrophy/hyperplasia [13]. The RAAS stimulates multiple pathways that mediate hypertrophy [11], the production of fibrogenic cytokines [14], and the generation of reactive oxygen species (ROS), all of which will be discussed in this review. Alternatively, toxic or inflammatory injury may start with the tubule, or glomerular disease may cause tubular damage. In the latter case, increased glomerular filtration of plasma proteins has been proposed to lead to tubulointerstitial injury via increased delivery of plasma proteins to the tubule. A number of potential explanations have been proposed for this phenomenon, including the delivery of biologically active molecules causing inflammation and oxidation (Fig. 3).

Importantly, the distinction between a primarily glomerular and primarily tubular lesion breaks down in considering the pathogenesis of chronic nephron loss. Even if the primary lesion is tubular, the remaining nephrons must increase their circulation and filtration in order to maintain the GFR. Glomerular capillary surface area increases to meet this increased demand, but the terminally differentiated podocytes usually cannot proliferate to cover this increased area. They must instead undergo hypertrophy. If the hypertrophic response is insufficient, areas of the glomerular basement membrane (GBM) are denuded of their epithelial cover. This result would promote synechia formation as discussed above. In support of this model, it has long been known that even nephron loss due to progressive tubulointerstitial disease leads to proteinuria [15] as a result of hyperfiltration and the increased flux of plasma proteins across the GBM. Further, secondary glomerulosclerosis may occur consequent to any form of nephron loss. Thus, the end result of either glomerular or tubular injury is a common pathway of nephron hypertrophy that invokes a cycle of cellular dedifferentiation, inflammation, endothelial dysfunction, increased metabolic demand, tissue hypoxia, acidosis, and the generation of ROS. All of these responses to nephron loss may contribute to progression.

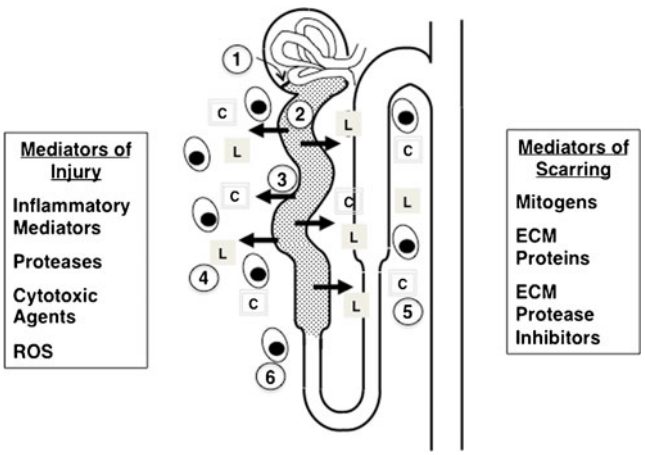

Fig. 3 Structural events in nephron loss. Glomerular hypertrophy and podocyte loss lead to the formation of synechiae (*1*, indicated by *curved arrow*). Increased filtration of protein (*2*, *shaded area*) leads to its extrusion directly into the tubulointerstitium through the synechiae or through its delivery to the renal tubules (*3*), where it may activate cells or be transported into the tissues. Biologically active lipids (*L*, *4*) or cytokines (*C*, *5*) are present as a result of reabsorption, local production by tubular and other resident cells, or generation by inflammatory cells (*6*) that are recruited to the tubulointerstitium. A number of "mediators of injury" (*ROS* reactive oxygen species) contribute to tissue loss, and "mediators of scarring" (*ECM* extracellular matrix) alter the balance between extracellular matrix synthesis and degradation, leading to fibrosis

The cellular response to injury

Dedifferentiation

Cells respond to stress and injury by altering their phenotype. In many cases, they initially have an epithelial phenotype that involves transport and/or secretory/hormonal processes; in others, they serve a structural purpose. When stressed, the cells may become somewhat less differentiated in a process that is called epithelial-to-mesenchymal transition (EMT) (for more detail on EMT, see [16]). This term is actually a misnomer, since the original, relatively quiescent cell may have had more or less epithelial characteristics and may not become a true mesenchymal cell. Nevertheless, the term is widely used.

The dedifferentiated cells tend to show a generalized activation involving proliferation, migration, and cytokine production. These functions, which are considered to represent a response to injury, clearly manifest fewer of the specialized purposes that the cells would carry out in stable health. Often, the dedifferentiation represents an ineffective or counterproductive response that is the cellular basis of *misdirected repair* [1]. The cells may undergo *apoptosis*, as is the case with peritubular capillary rarefaction and some responses to tubular or mesangial injury. Considerable controversy exists regarding the origin of these cells (the tubule, the interstitium, or distant sites) and how they contribute to fibrosis [17, 18]. Depending upon their origin and the degree of differentiation, cells that undergo EMT may recruit other fibrogenic cells and secrete profibrotic factors, or they may produce more ECM proteins or have less ECM protease activity, all changes leading to scar accumulation. Potential roles in these pathways are played by transforming growth factor-beta (TGF-β), angiotensin II, FGF-2 (basic FGF), platelet-derived growth factor, endothelin, and many other cytokines.

Inflammation

Several lines of evidence point to a central role for inflammation in CKD. In focal segmental glomerulosclerosis, progression of CKD correlates better with the degree of tubulointerstitial inflammation than it does with the severity of glomerular sclerosis [19]. The levels of the inflammatory markers tumor necrosis factor-alpha receptor 2 and interleukin-6 and the white blood cell count are elevated in CKD [20], although it is not certain whether these changes represent a contribution to, or are a result of, CKD progression. It has been proposed that pro-inflammatory molecules are delivered to the distal nephron in CKD, reabsorbed by the tubule, and concentrated in a manner that promotes inflammation (Fig. 3). In a way, this is an extension to the tubule of the periglomerular inflammation resulting from misdirected filtration, in the model proposed by Kriz and colleagues. Macrophages, which along with dendritic cells are present even in the normal kidney and which could participate in inflammatory signaling [21], are recruited to the injured tubulointerstitium by chemoattractants/chemokines [22, 23]. It is generally agreed that the complement system plays a part in CKD, but it is less clear whether its role is more important for disease initiation or for progression in a final common pathway. Abbate and colleagues [24] have reported that chronic fibrosis is attenuated in C3-deficient mice with overload proteinuria. Rather than attributing this protection to decreased activity of C3 affecting the distal tubule, the authors cite a protective effect on the glomerulus leading to decreased glomerular passage of plasma proteins in the C3-null mice. In contrast, Boor and colleagues [25] evaluated unilateral ureteral obstruction, a primary tubulointerstitial model, in C5-deficient mice. They attributed amelioration of disease, at least in part, to inhibition of TGF-β1 production by the tubular cells, suggesting a role for C5 in chronic, fibrotic events. Since the first model is initiated with glomerular overload and the second with tubular injury, these results are consistent with an interpretation that the site of protection is the part of the nephron that is injured in each model. Regardless of the target effect involved, inflammation appears to play a role in both the initiation and the maintenance of CKD.

Endothelial dysfunction

Deterioration of the renal microcirculation is a potentially important contributor to CKD progression. The vascular supply to the nephron is critical for its function, not only because of the importance of tissue perfusion in maintaining cell viability, but also for the ways in which the anatomical relationship between the vessel and the nephron promotes the selective excretion and reabsorption of plasma water constituents. In the nephron, blood circulates first through the glomerulus and subsequently through the peritubular capillaries to perfuse the tubulointerstitium. This arrangement provides the opportunity to achieve glomerulotubular balance. To achieve this balance, increased glomerular filtration (decreasing water content) increases oncotic pressure in the efferent arteriole and peritubular capillaries, which promotes proximal tubular fluid reabsorption; decreased glomerular filtration achieves the opposite effect. The negative impact of increased tubular transport is that the extraction of oxygen by this system is extensive, and the renal medulla in particular is a relatively hypoxic environment. Thus, any further loss of perfusion poses a significant risk for the kidney. Glomerular damage may decrease flow through the efferent arteriole, leading to poor perfusion of the tubular portion of the nephron. Tissue damage or fibrosis in the tubulointerstitium may further decrease distal nephron

perfusion. It is not clear whether subsequent endothelial cell apoptosis represents a response to poor perfusion or a direct effect of the disease processes on the vessel, but such apoptosis does occur and leads to peritubular capillary rarefaction, a central event in nephron loss [26].

Increasing evidence supports a role for disordered endothelial function and decreased angiogenesis in CKD [27, 28]. Microvascular disease causes mild tissue ischemia, potentially stimulating an angiogenic response. This could be favorable in some cases, but a number of angiogenic cytokines may also induce pro-inflammatory and pro-fibrotic responses [29], outweighing the potential pro-angiogenic benefits. The balance between angiogenic and anti-angiogenic signals may be an important determinant of outcome. Angiogenic factors such as vascular endothelial cell growth factor (VEGF) are produced acutely during experimental renal disease and may promote inflammation and fibrogenesis, but during the healing phase of the disease they could contribute to tissue regeneration [26]. Endostatin, an anti-angiogenic protein, is found at relatively high levels in CKD patients, and higher levels correlate with increased rates of disease progression [30]. In the experimental Thy1.1 rat nephritis model, early expression of angiogenic factors in the glomerulus and dysregulation of the tubulointerstitial angiogenic response were noted, with increased tubular expression of a protein that downregulates hypoxia-inducible factor 1-alpha (HIF-1α) [31] (see section on "Hypoxia-stimulated signal transduction" below). The role of angiogenic or anti-angiogenic factors in responding to vessel rarefaction remains a subject of considerable investigation. The sequential regulation of such factors is a potentially useful approach to CKD management and resolution [26, 32].

Recent studies regarding endothelial dysfunction have focused on the role of asymmetric dimethylarginine (ADMA), a naturally occurring inhibitor of nitric oxide (NO) synthase. NO is an important vessel relaxant that helps maintain normal blood pressure and tissue perfusion, including that in the glomerulus [33]. Plasma levels of ADMA are predictive of the severity of CKD progression, as well as of the incidence of CKD-associated cardiovascular disease [34]. An endogenous inhibitor of ADMA, dimethylarginine dimethylaminohydrolase, decreases the rate of experimental CKD progression in rat subtotal nephrectomy [35]. Thus, in our proposed model, nephron loss may induce changes in the renal microcirculation, and such changes will undoubtedly contribute to nephron loss.

Biochemical events in progressive nephron loss

Unmet metabolic demands of the tubule

Impaired circulation could exacerbate metabolic problems in the tubule. In hypertrophy, renal mass increases in both the glomerulus, where total capillary length and surface area increase, and the tubule, where increased functional demand stimulates increased mass. These responses can have significant effects on remnant renal function. We have discussed the importance of podocyte hypertrophy in "covering" the increased glomerular capillary surface, in order to prevent synechia formation and glomerulosclerosis. In the tubule, the increased mass and increased workload lead to increased metabolic needs. Although the kidney constitutes less than 1 % of the mass of the human body, renal blood flow receives 25 % of cardiac output at rest, and renal oxygen consumption significantly exceeds its relative mass. It is widely accepted that the high relative blood flow is necessary for the kidney to serve as an effective filter for maintaining homeostasis, but the metabolic requirements of the kidney receive less attention. In order to provide sufficient energy for the selective transport of myriad salts—particularly sodium [36]—organic acids, and other plasma constituents, large quantities of ATP are consumed. These must be generated by aerobic metabolism. Thus, oxygen and glucose are required. Patients with normal kidney function and those with CKD have similar systemic oxygen consumption (each group, about 10,000 μmol O_2/min), but normal renal oxygen consumption is 391 μmol O_2/min/kidney, compared with a value of 177 μMol O_2/min/kidney in CKD. Decreased renal blood flow in CKD is compensated by a higher percentage oxygen extraction. Renal oxygen consumption in CKD, when corrected for creatinine clearance, was higher, supporting the notion that the increased transport demand on the remnant tubule increases its oxygen requirement [37]. These results suggest that the availability of oxygen, glucose, and phosphate may become rate-limiting considerations for nephron function. A switch to anaerobic metabolism (i.e., with incomplete metabolism of glucose and decreased generation of ATP per mole of glucose) will lead to additional pathways of nephron loss, as discussed below.

Acidosis

Acidosis is a common aspect of CKD and has significant metabolic and physiological consequences [38]. Increasingly, reports associate acidosis with disease progression and its treatment with delayed progression [39]. Controlled trials suggest that bicarbonate or citrate supplementation in patients slows the decline of GFR in patients [40–42]. Experimental evidence concurs with these findings. The results suggest that local tissue acidification plays a significant role in CKD. Given the progressively increasing likelihood in CKD of anerobic metabolism and both local and systemic acidosis, renal tissue acidosis can be anticipated to occur or increase. It has been proposed that ammonia binds to the C3 component of complement, leading to the alternative pathway of complement activation. Ammoniagenesis in response to local acidosis would thus

activate the complement pathway and cause tissue damage. In support of this hypothesis, local deposition of complement is decreased in bicarbonate-supplemented experimental rats [43]. As an alternative, or perhaps complementary, mechanism, luminal acidification in an animal model enhances renal tubular superoxide production (see following section on Oxidative metabolism and ROS), and bicarbonate feeding alkalinizes the urine and reduces both tubular superoxide production and proximal tubule damage [44]. Further, the production of additional cytokine mediators of progression is decreased by either decreasing dietary titratable acid or increasing dietary alkali supplementation ([45], and references contained therein).

Oxidative metabolism and ROS

One mechanism by which hypoxia may lead to CKD progression is the generation of ROS. These molecules are potential oxidizing agents that include oxygen itself. We generally consider ROS to be represented by three unstable forms: the hydroxyl radical (OH•; note that it does not carry a negative charge like the hydroxyl ion, and is far more active), the superoxide anion (O_2^-), and hydrogen peroxide (H_2O_2). ROS normally are present in fairly low, physiological amounts and are regulated by a number of mechanisms. For example, superoxide dismutase catalyzes the reaction of superoxide to hydrogen peroxide plus water, and catalase metabolizes hydrogen peroxide to water plus molecular oxygen. ROS are generated during the normal course of biological processes and serve an important signal transduction role. For instance, angiotensin II stimulates the production of the superoxide ion through an action on NAD(P)H oxidases, subsequently activating mitogen-activated protein (MAP) kinases and leading to cellular hypertrophy [46]. An increase in the generation of ROS acts upon oxidation-sensitive sites of various proteins, altering thiol residues to change molecular configuration and protein–protein interactions. These in turn can alter the binding affinities of proteins for their receptors. Alternatively, the activity of enzymes such as phosphatases may be affected, enhancing the longevity—and therefore the activity—of phosphorylation-activated molecules such as MAP kinases.

ROS may contribute to disease pathogenesis in several ways [47, 48]. At low concentrations, ROS may mediate the activity of cytokines and hormones that have deleterious effects on cell function. At higher concentrations, approximately in the range of 50–100 μM, ROS may interact with and activate (or inactivate) proteins that are not the "intended" targets of receptor-initiated signals, stimulating other events that mediate untoward events in the cell. Finally, at even higher doses, ROS may indiscriminately interact with and create unstable metabolites of cellular molecules. As an example, it has been proposed that membrane lipid peroxidation is a mechanism that promotes and propagates cellular damage in disease. However, while in vitro experiments have shown that such reactions may occur, it is not clear whether and how such high levels of ROS are generated in vivo.

Numerous studies have suggested that ROS are generated during models of kidney disease [49] and that antioxidants may slow or prevent progression [50]. The importance of ROS as a primary mechanism of progression remains a subject of intense study. Various potential mechanisms of ROS generation have been proposed. An important one is hypoxia. At first glance, the generation of ROS when the amount of substrate for this action (oxygen) is reduced seems to be a paradox. The explanation lies in the role of mitochondria. Mitochondria are major oxygen sensors in mammalian cells. When oxygen tension is reduced, complex III at the mitochondrial inner membrane generates superoxide by adding an electron to molecular oxygen. This reaction occurs more frequently in hypoxia because complex III enzymes are stabilized. Importantly, the mitochondria are responsive to low levels of oxygen (hypoxia) rather than the absence of oxygen (anoxia) in order to alter cell metabolism under conditions that are threatening, rather than in the presence of total anoxia where shutting down cell function may be too late to preserve the cell. Thus, sufficient substrate (oxygen) is present to support such a response [51].

Other potential contributors to the generation of ROS do not require hypoxic conditions. Among these, non-hypoxic forms of stress are prominent [52] and may be self-propagating [53]. TGF-β expression may be stimulated by a variety of factors and activates NADPH oxidase [54]. The delivery of serum proteins, such as cytokines, or other biologically active molecules, such as ferrous iron, to the tubules, in addition to promoting inflammation, may provoke or propagate the generation of ROS. Macrophages activated by inflammation are a generous source of ROS. Another potential non-hypoxic stimulus is the RAAS. Angiotensin II stimulates ROS production which in turn stimulates MAP kinase activity, promoting cell proliferation. These same events also stimulate the expression of molecules that stabilize HIF-1α (cf. Wolf [46]). Alternatively, systemic angiotensin may stimulate local renal tubular angiotensinogen production, which in turn generates hypertension, production of ROS, proximal tubular cell apoptosis, and tubulointerstitial fibrosis. These events are blocked by catalase, a free-radical scavenger, supporting a central role for ROS [55]. In diabetic mice, catalase also blocks the hypoglycemia-induced expression of a caspase (a critical mediator of apoptosis) [56], preventing apoptosis [57]. Administration of a cell-permeable superoxide dismutase also decreases progression after ischemic renal injury [58]. Uremia itself might play a sustaining or amplifying role in progression. Carbamylated low-density lipoprotein promotes oxidative stress in endothelial cells [59].

Treatments that have attempted to oppose ROS generation include antioxidants such as ascorbic acid (vitamin C) [60] and omega-3 fatty acids [61].

Hypoxia-stimulated signal transduction

Another mechanism of progression involves protein-mediated signaling pathways activated by hypoxia-inducible factors (HIFs). The HIF molecule is a heterodimeric transcription factor. The β subunit, also known as the arylhydrocarbon receptor nuclear translocator, or ARNT [62], is stable, but the α-subunit of the dimer is oxygen-sensitive. In normoxia, the HIF-α chain is rapidly eliminated (half-life of approximately 5 min) by a pathway in which oxygen-dependent prolyl hydroxylase modifies specific HIF-α residues, permitting HIF-α interaction with the von Hippel–Lindau tumor suppressor, pVHL, and subsequent ubiquitin-mediated proteasomal degradation. Under hypoxic conditions this oxygen-requiring hydroxylation is inhibited, thereby stabilizing the expression and activity of HIF α-chains.

The tissue environment in the tubulointerstitium is particularly vulnerable to hypoxia/ischemia because it is a tissue region with little reserve oxygen capacity and already may have a relatively low oxygen tension under normal physiological conditions [63]. As might be expected, many HIF target genes are expressed in the tubulointerstitium in response to hypoxia. Most prominent among these for clinical nephrologists is erythropoietin; the erythropoietin promoter contains a HIF-responsive element (HRE) that activates the gene when tissue oxygenation decreases. Thus, the fibroblasts and perivascular cells of the tubulointerstitium act as an oxygen sensor and offer an ideal site for an erythropoietin-mediated system that responds to mild-to-moderate decreases in tissue oxygenation caused by anemia. The gene promoter for VEGF, a protein that stimulates the formation of new vessels after injury, also contains HREs.

Studies by several groups have indicated that HIFs play a role in CKD. When the gene is knocked out in mice, several models of chronic kidney fibrosis are ameliorated. Initially, these models were all tubulointerstitial in nature [64]. However, more recently, HIF has been implicated in glomerular disease, at least that of a vascular nature [65]. Explanations for these findings are beginning to emerge. HIFs play a direct or indirect role in the development of a profibrotic phenotype by epithelial cells [66] and also are necessary for optimal TGF-β-stimulated collagen gene activation even in the absence of hypoxia [67]. Complicating this assessment, Nangaku and colleagues have demonstrated in a series of papers that HIFs also may play a role in protecting the renal parenchyma in an earlier stage of the initiation of progression ([32], and references therein). A potential explanation is that angiogenic actions of HIF may be protective in acute injury, whereas chronic actions may be deleterious. Thus, hypoxia may be only one means to elicit the participation of HIFs in CKD, and tissue function may be governed by HIF in complex ways.

Pathways of progression

The mechanisms described above contribute at a cellular, physiological, and biochemical level to CKD progression. Importantly, they are interactive and mutually sustaining in a cycle of response to injury, misdirected repair, and deleterious adaptation. In Fig. 4, the area indicated by the number 1 notes initial injury, and areas 2 and 3 note the factors contributing to either injury or misdirected repair. The role of remnant nephron physiology begins with part 4 ("Loss of Nephrons"). Tubuloglomerular feedback regulates glomerular filtration (part 5). With decreasing nephron number, reactive hypertrophy occurs (part 6) to support increasing single nephron GFR. Increased workload for the nephron in the face of decreased perfusion and lessened delivery of metabolic substrate increases anerobic metabolism (part 7) and the generation of acid and ROS.

All of these processes contribute to nephron loss and to exaggeration of the remnant nephron response. The result is a vicious cycle by which this response contributes to the further demise of the nephron and, ultimately, to the loss of renal function.

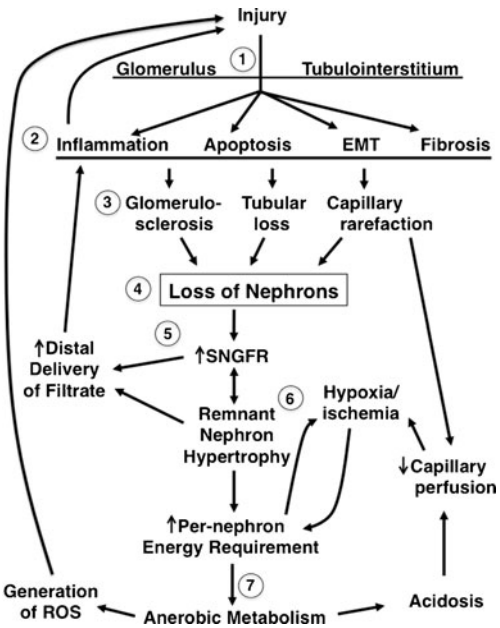

Fig. 4 The vicious cycle of progressive nephron loss. Injury to the nephron affecting either the level of the glomerulus or the tubule (*1*) leads to a set of reactions (*2*) that in turn generate structural consequences (*3*) and nephron failure (*4*). The compensatory response involves increased single nephron GFR (*SNGFR*) and nephron hypertrophy (*5*). Increased metabolic activity in the presence of reduced substrate (*6*) leads to ischemia/hypoxia, acidosis, and the generation of ROS (*7*). These factors then cause further nephron injury and loss. *EMT* Epithelial-to-mesenchymal transition, *ROS* reactive oxygen species

Key summary points

1. The basic unit of kidney loss, as it is with kidney function, is the nephron.
2. As nephrons are lost, endocrine actions and physiological responses drive compensation by the remaining nephrons to maintain total body homeostasis.
3. The consequent, progressive demand on the individual nephron ultimately exceeds the nephron's capacity to respond.
4. Subsequent structural and metabolic failure leads to further nephron damage and loss.

Multiple choice questions (answers are provided following the reference list)

1. In the absence of intervention, the progression of CKD to end-stage usually is:
   a. Inhibited by acidosis
   b. Linear with respect to time
   c. Unaffected by blood pressure
   d. All of the above

2. Oxygen consumption by the whole kidney is:
   a. Increased in CKD
   b. Driven primarily by potassium regulation
   c. Disproportionate to the mass of the kidney
   d. Most important for glomerular filtration

3. Complement activation in CKD is:
   a. A mediator of progression
   b. Exacerbated by acidosis
   c. Related to inflammatory cell infiltration
   d. All of the above

4. Reactive oxygen species (ROS) may affect cell function by:
   a. Reacting with thiol groups on signaling molecules
   b. Binding to structural molecules in the cell
   c. Propagating free radicals through lipid peroxidation
   d. All of the above

5. The renin–angiotensin–aldosterone system may accelerate progressive kidney disease by:
   a. Inhibiting TGF-β production
   b. Preventing apoptosis
   c. Decreasing HIF-1α expression
   d. Promoting cell hypertrophy

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Answers to questions:

1. b
2. c
3. d
4. d
5. d