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Progression and fibrosis

Molecular pathways of chronic kidney disease progression☆

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ABSTRACT

Chronic kidney disease is characterized by the progressive loss of functional nephrons. This loss means that the remaining nephrons are put under stress and are forced to adapt in order to maintain kidney function. Over the time, the strains imposed by these adaptations result in a vicious circle in which the loss of damaged nephrons results in the damage of the so far healthy nephrons. Hence, the rate of chronic kidney disease progression depends on the ability of the remaining nephrons to cope with stress. This article reviews the molecular pathways involved in the compensation and deterioration process after nephron reduction. In particular, we examine the role of mammalian target of rapamycin complex (mTORC)/serine-threonine protein kinase AKT, epidermal growth factor receptor (EGFR) and unfolded protein response pathways, as well as the pleiotropic function of Lipocalin 2. We also discuss the dual role played by some of these pathways in acute and chronic kidney disease. Finally, the relevance of these experimental finding to human chronic kidney disease is discussed.

1. Introduction

Chronic kidney disease is characterized by a progressive decline in renal function to end stage renal disease that can occur irrespective of the cause of the renal damage (diabetes, hypertension, ischemia or immune diseases), once a critical number of nephrons has been lost. Chronic kidney disease represents a worldwide concern: over 7 million people in the European Community are affected by chronic renal failure and 300,000 are undergoing renal replacement therapy, either by dialysis or transplantation. Grossly, 10% of the adult population is estimated to suffer from chronic kidney disease [1]. These patients display an increased risk factor for cardiovascular diseases and death. Moreover, the quality of life of patients with chronic kidney disease remains poor, due in large part to a complex set of deleterious sequelae. Faced with the persistently poor outcome of end stage renal disease, current clinical research efforts focus on preventive strategies to slow down the rate of progression of chronic kidney disease. Elucidating the molecular pathways underlying the progression of chronic kidney disease is the first step for the development of new therapeutic and pharmacological targets and a key challenge for medical planning.

2. Mechanisms of chronic kidney disease progression: importance of nephron loss

The mechanisms of chronic kidney disease progression remain poorly understood. Attempts to dissect the molecular basis of chronic kidney disease have been facilitated by the development of several experimental models of renal deterioration. Among these, the remnant kidney model is a mainstay, since nephron reduction characterizes the evolution of most human chronic kidney disease. Consequently, this model recapitulates many features of human chronic kidney disease, including hypertension, proteinuria, glomerular and tubulo-interstitial lesions. Over the last 50 years, this model has led to the discovery of critical pathways and, more importantly, to the design of therapeutic strategies to slow down the progression of chronic kidney disease, such as the widely clinically used renin-angiotensin inhibitors [2].

Experimental models of nephron reduction have shown that the reduction in the number of functional nephrons triggers molecular

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and cellular events that promote compensatory growth of the remaining ones to maintain kidney function [3]. However, over time, the strain imposed by these adaptations results in mechanical and metabolic stresses that lead to a vicious circle in which the loss of the residual nephrons results in the damage of healthy nephrons (overwork hypothesis) [3,4]. Hence, the rate of chronic kidney disease progression crucially depends on the ability of remaining nephrons to cope with stresses.

3. Role of haemodynamic adaptation

Since the pioneer works from Brenner et al., it has been known that nephron reduction leads first to an adaptive response through the increase of the blood flow and the intracapillary glomerular pressure which end in higher single nephron glomerular filtration rate [3]. The strain imposed by these adaptations results in mechanical constraints of glomerular cells that ultimately lead to glomerular damage, albuminuria and further nephron loss [5]. By their position and function, podocytes are particularly exposed to such mechanical stresses that try to counteract through a complex regulation of their actin cytoskeleton. Based on these observations, we hypothesized that, during nephron reduction, the fate of podocytes might depend on their ability to engage an adaptive genetic program to cope with the constraints imposed by the increase in single nephron glomerular filtration rate. Among the possible programs, we focused our attention on AKT kinases because:

- these kinases deliver anti-apoptotic signals and mediate cytoskeleton rearrangement [6–8];
- they appear to be positively regulated by the core proteins of the slit diaphragm [9].

4. mTORC/AKT pathway: experimental proofs

AKT proteins are conserved cytosolic serine-threonine kinases that regulate many cellular processes including survival, proliferation, migration and cytoskeleton organization [6]. In mammals, three distinct genes encode AKT homologs: *Akt1*, *Akt2* and *Akt3*, respectively. AKT activation requires, first, its recruitment to plasma membrane, which is initiated by phosphatidyl-inositol-3-kinase (PI3K), then, the phosphorylation on Thr^{308} and Ser^{473} by 3-phosphoinositide-dependent protein kinase-1 (PDK1) and mammalian target of rapamycin (mTOR) complex 2 (mTORC2), respectively [8]. Once activated, AKT proteins phosphorylate several substrates to regulate multiple cellular functions. Indirect evidences suggest a role of AKT in podocyte physiology. In vitro, AKT pathway inhibition reduces podocyte viability [10]. It has been also shown that, in cultured podocytes, AKT can be activated by nephrin, a protein that contributes to the slit diaphragm stability [9]. Notably, nephrin is sought to transduce extracellular signals, such as mechanical constraints, to the intracellular compartment of the podocyte [10]. By applying different experimental models of nephron reduction to genetically modified mice, we showed that AKT2, but not AKT1, activation by mTORC2 plays an essential role in podocyte cytoskeleton maintenance, survival and adaptation to environmental constraints [11]. In fact, *Akt2* gene deletion leads to severe albuminuria and worsened glomerular lesions after nephron reduction. Mechanistically, we observed that AKT2 triggers a compensatory program involving mouse double minute 2 (MDM2), glycogen synthase kinase 3 (GSK3) and ras-related C3 botulinum toxin substrate 1 (RAC1). Consistent with these observations, previous studies have highlighted the important function played by RAC1-GTP in the maintenance of podocytes cytoskeleton organization and foot processes in mice and humans [12,13]. It is worth noting that AKT2 mediates insulin signalling and that the specific deletion of the insulin receptor in podocytes mirrored the glomerular lesions observed during diabetic nephropathy [14]. Hence, by integrating many messages ranging from mechanical to metabolic stresses, AKT2 acts as a central node of podocyte signaling.

5. mTORC/AKT pathway: from mice to humans

A major problem encountered in patients treated with sirolimus, a mTORC inhibitor and one of the most widely used immunosuppressive treatments, is its adverse effect on the kidney, particularly in patients with compromised renal function. In fact, several studies have reported that kidney transplant recipients might develop proteinuria upon sirolimus treatment [15]. Our translation studies have provided a rational basis to this observation. In fact, we have demonstrated that, by preventing the activation of AKT2 in kidney, sirolimus leads to marked proteinuria and podocyte loss in transplanted recipients with severe nephron reduction [11]. Interestingly, as in mice, mTORC2 rather mTORC1, seems to be involved in AKT activation and in the deleterious effect of sirolimus. Sequential biopsies allowed us to demonstrate that AKT activation may anticipate the toxicity of sirolimus. Hence, in order to prevent to the adverse effects of mTORC inhibitors, we suggest evaluating the state of AKT2 activation (phosphorylation) in all kidneys of patients suffering from chronic kidney disease with compromised renal function.

6. Role of residual proteinuria

Convergent evidences from clinical and experimental studies indicate that albuminuria and proteinuria are not simply markers of chronic kidney disease progression, but active players in the evolution of the disease [16]. Mechanistically, it has been proposed that the proteins that escape into the glomerular filtrate have a toxic effect on tubular cells and that, once damaged, tubular cells lead to the development of interstitial fibrosis and inflammation [17]. The increased production of endothelin-1, monocyte chemoattractant protein 1 (MCP-1), RANTES (regulated upon activation normal T cell expressed and secreted), or complement components have been involved in this toxic effect [17]. More recently, a few studies have shown that the unfolded protein response is also activated in tubular cells exposed to albumin [18–20], but the pathophysiological role of such activation remains unknown.

7. Endoplasmic reticulum stress/Lipocalin 2 pathway: experimental proofs

The endoplasmic reticulum is a signalling platform that responds to various cellular stresses by inducing a coordinated response, the unfolded protein response [21]. During the unfolded protein response, inositol-requiring enzyme 1α (IRE1α) promotes the phosphorylation of c-JUN and the specific splicing of the unfolded protein response transcription factor X-box binding protein 1 (XBP1). Besides, protein kinase R (PKR)–like kinase (PERK) phosphorylates eukaryotic translation-initiation factor 2α (eIF2α): this reduces general translation but promotes translation of activating transcription factor 4 (ATF4), which activates the CCAAT/enhancer-binding protein homologous protein (CHOP). If this adaptive response cannot overcome endoplasmic reticulum stress, it triggers apoptotic cell death. Using experimental models of proteinuric nephropathies, we have very recently uncovered a novel endoplasmic reticulum stress pathway critically involved in chronic kidney disease progression [22]. In fact, we observed that