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Wnt/ β -catenin signaling and renin-angiotensin system in chronic kidney disease

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Abstract

Purpose of review—Intrarenal activation of the renin–angiotensin system (RAS) plays an essential role in the pathogenesis of hypertension and chronic kidney diseases (CKD). However, how RAS genes are regulated *in vivo* was poorly understood until recently. This review focuses on recent findings of the transcriptional regulation of RAS components as well as their implication in developing novel strategies to treat the patients with CKD.

Recent findings—Bioinformatics analyses have uncovered the presence of putative binding sites for T cell factor (TCF)/ β -catenin in the promoter region of all RAS genes. Both *in vitro* and *in vivo* studies confirm that Wnt/ β -catenin is the master upstream regulator that controls the expression of all RAS components tested such as angiotensinogen, renin, angiotensin converting enzyme (ACE) and angiotensin II type I receptor (AT1) in the kidney. Targeted inhibition of Wnt/ β -catenin, by either small molecule ICG-001 or endogenous Wnt antagonist Klotho, represses RAS activation and ameliorates proteinuria and kidney injury. Blockade of Wnt/ β -catenin signaling also normalizes blood pressure in mouse model of CKD.

Summary—These recent studies identify Wnt/ β -catenin as the master regulator that controls multiple RAS genes, and suggest that targeting this upstream signaling could be an effective strategy for the treatment of patients with hypertension and CKD.

Keywords

Renin-angiotensin system; Wnt/ β -catenin; chronic kidney disease; hypertension; renal fibrosis

INTRODUCTION

The renin–angiotensin system (RAS) is a hormone system that regulates blood pressure and fluid balance [1–3]. It is well recognized that intrarenal RAS activation is associated with the development and progression of chronic kidney disease (CKD) by blood pressure-dependent and –independent mechanisms [4–8]. RAS consists of several components

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Conflict of interest

There are no conflicts of interest.

including angiotensinogen (AGT), renin, angiotensin-converting enzyme (ACE) and the angiotensin II (Ang II) type 1 and type 2 receptors (AT1 and AT2). Ang II is the principal effector peptide of RAS and exerts its actions upon binding to AT1 and AT2 receptors [9–11].

Given the importance of RAS activation in the pathogenesis of CKD, the mainstay of current clinical therapy for CKD is using anti-RAS remedies, such as ACE inhibitors (ACEI) or AT1 receptor blockers (ARB). These treatments are proven to be beneficial for patients with CKD, protect kidney against proteinuria and renal function decline, and retard the progression to end-stage renal disease (ESRD) [12–14]. However, considerable numbers of patients are still not being adequately treated and often progress toward ESRD. One of the underlying reasons could be a compensatory renin expression following anti-RAS therapy via a homeostatic mechanism [15,16]. Renin can mediate profibrotic response by an angiotensin-independent mechanism via binding on its own membrane receptor, the prorenin/renin receptor (PRR) [17]. Therefore, a novel strategy to simultaneously target multiple RAS genes is necessary and essential for the effective treatment of patients with CKD.

Wnt/ β -catenin signaling is an evolutionarily conserved signaling cascade that plays a crucial role in regulating organ development, injury repair and tissue homeostasis [18,19]. In the kidney, multiple Wnts are upregulated and β -catenin is activated after injury, and this activation of Wnt/ β -catenin signaling is closely associated with RAS activation, suggesting a potential connection between these two events [5,20,21]. In this review, we discuss the role of intrarenal RAS activation in the pathogenesis of CKD, highlight new findings on RAS regulation by Wnt/ β -catenin, and propose a novel strategy to treat patients with CKD by targeting Wnt/ β -catenin.

INTRARENAL RAS AND THE PATHOGENESIS OF CKD

RAS activation is a cascade of events, in which two major enzymes, namely renin and ACE, convert AGT to biologically active peptide Ang II (Figure 1). Juxtaglomerular (JG) cells in the kidney express and activate prorenin and secrete renin into the circulation. Renin hydrolyzes AGT to Ang I, which later is converted by ACE to Ang II. Ang II binds to the receptor AT1 and AT2 to mediate biological functions. Through binding to AT1, Ang II induces vasoconstriction, endothelial dysfunction, atherosclerosis, inflammation, fibrosis and apoptosis [9,10]. On the other hand, renin also directly participates in the development of renal fibrosis through binding to its membrane receptor PRR, thereby triggering intracellular signal transduction and inducing transforming growth factor β 1 (TGF- β 1) and matrix gene expression [22,23].

Kidneys have the complete RAS components to form Ang II *in situ* [5,24]. Local synthesized Ang II and renin in the kidney tissues could be much higher than that in the circulation after kidney injury. Ang II specifically binds to AT1 receptor, and induces a series of deleterious effects including elevated intraglomerular pressure, water and solute retention, cell proliferation and hypertrophy, inflammatory responses in glomerular and tubulointerstitial compartments, insulin resistance, and renal fibrosis [25–27]. Furthermore,

renin could independently induce mesangial hypertrophy, glomerulosclerosis and renal fibrosis, since its receptor, PRR, predominantly localizes in mesangium of glomeruli and collecting duct. Renin could also trigger multiple intracellular signal pathways and promotes matrix gene expression via an Ang II-independent mechanism [22,23]. Therefore, it is widely recognized that intrarenal RAS activation plays a pivotal role in the pathogenesis of CKD.

ANTI-RAS THERAPY FOR CKD

Over the last 20 years, anti-RAS remedies are widely used clinically as a standard therapy in hypertension, cardiovascular disorder and kidney diseases. The therapeutic effects of these drugs for CKD often attribute to their anti-hypertension functions. However, it becomes increasingly clear that RAS blockers also have anti-inflammation and anti-fibrosis properties [28–30].

Two classes of drugs that target ACE and AT1 are used in numerous cohort trials and clinical therapy. ACEI inhibits the catalytic activity of ACE and blocks angiotensin I conversion to Ang II, the major pathogenic culprit of RAS. Clinical studies showed substantial renal benefits in patients without diabetes who have advanced renal insufficiency after ACEI treatment [12]. Of interest, renal protection triggered by ACEI, benazepril, was not dependent on blood pressure control. Although acute treatment with ACEI reduces circulating Ang II, long-term ACEI therapy in subset of patients raises Ang II or aldosterone concentrations back to the baseline [31].

AT1 receptor blocker antagonizes the binding of Ang II to its type I receptor, but does not directly affect the synthesis of Ang II. Two randomized, double-blind study, IDNT and the RENAAL trials, investigated ARB therapy in diabetes patients and found the benefits of reducing proteinuria, lowering serum creatinine and slowing the development of ESRD and decreasing mortality [32,33]. To get better therapeutic effect, combination of ACEI and ARB were utilized in several trials. Unfortunately, combined therapy shows no more effects than monotherapy regarding cardiovascular outcome, and even is associated with a worsen composite kidney outcome of developing of acute kidney injury and hyperkalemia, based on the ONTARGET, VALIANT and VA NEPHRON-D trials, as well as a network meta-analysis in cardiovascular and kidney diseases [34–36].

ACEI or ARB also stimulates renin secretion by interrupting Ang II feedback inhibition [15]. In a mouse model, ARB significantly increases plasma renin concentration to more than 20 times basal level in wild-type mice [37]. The compensatory rise of plasma renin activity potentially leads to activation of pathogenic mitogen-activated protein kinase (MAPK) pathway [38] via binding to its receptor PRR [22,23]. This binding of (pro)renin/PRR not only induces the catalytic efficiency of renin in Ang I production and activates inert prorenin to be an active form, but also triggers fibrogenesis by a receptor-mediated, Ang II-independent mechanism [39].

Direct inhibition of renin, the rate-limiting step in RAS activation, has been tested in clinical setting [40,41]. Aliskiren, an orally active renin inhibitor, has been shown to be effective on hypertension, diabetes and CKD [42,43]. However, alikiren appears not to achieve

significant benefits in renal combined cardiovascular diseases compared to ARB alone, but significantly increases the risk of adverse effects, based on the ONTARGET and ALLAY trials [34,44].

REGULATION OF RAS EXPRESSION

RAS genes are regulated by several factors, including hyperglycemia, high-salt, albumin overload, reduced availability of nitric oxide and increased uremic toxin [4,8,45,46]. Recent studies show that loss of Klotho, an antiaging protein, also leads to RAS activation in diseased kidneys [47]. The underlying mechanism responsible for RAS gene regulation still remained to be investigated. A great deal of studies indicates a critical role of oxidative stress signaling in this process.

In rat kidney proximal tubular cells, cellular reactive oxidative species (ROS) generation and p38 MAPK phosphorylation play a role in high glucose-induced AGT gene expression [48]. Albumin overload activates intrarenal RAS in tubular cells through interacting with endocytic receptor megalin/cubulin and a protein kinase C (PKC) and NADPH oxidase-dependent pathway [45]. Advance oxidation protein products (AOPPs) upregulated many components of RAS and increased activity of ACE in proximal tubular cells via the class B scavenger receptor of CD36 and activation of PKC α , NADPH oxidase, and NF- κ B/AP-1 signaling [8]. Indoxyl sulfate, a circulating uremic toxin, stimulates the upregulation of PRR through production of ROS and activation of Stat3 and NF- κ B [46]. Recent studies demonstrate that PRR activation is mediated by cyclooxygenase-2 (COX-2) pathway in kidney cells [49].

The major downstream outcome of RAS activation is TGF- β 1 induction. In addition, Ang II also *trans*-activates epidermal growth factor receptor (EGFR) [50]. In renal fibroblasts, studies show that Ang II induces cell proliferation and matrix expansion through a TGF- β 1-dependent pathway [51]. In mesangial cells, renin increases matrix protein production by upregulation of TGF- β 1 [17], which is independent of its enzymatic action to enhance Ang II generation. In podocytes, Ang II induces cell apoptosis by a mechanism involving TGF- β 1 [11]. Interestingly, recent studies from our laboratory demonstrate that TGF- β 1 also induce RAS gene expressions. *In vitro*, TGF- β 1 induces renin, AGT and AT1 upregulation in proximal tubular cells and fibroblasts (unpublished data). This induction of RAS gene expression by TGF- β 1 is abolished by addition of SIS3, a cell-permeable compound that selectively inhibits Smad3 phosphorylation, suggests a role of TGF- β 1/Smad3 signaling in RAS activation (unpublished data).

WNT/ β -CATENIN AS THE MASTER REGULATOR OF RAS

Although blockade of RAS activation using either ACEI or ARB is an effective therapy to retard progression of CKD, treatment with RAS blockers often causes compensatory upregulation of other RAS components, such as renin, leading to declined effectiveness in the long term [15]. Furthermore, ACEI and ARB only inhibits the activity of these RAS components, but does not affect their expression in the first place. In this context, finding a strategy that represses the expression of RAS components, rather than inhibits their activity, could be an important and potentially more effective therapy for patients with CKD.

We recently found that multiple genes of RAS are direct downstream targets of Wnt/ β -catenin [5], a developmental signaling that plays an essential role in organogenesis, tissue homeostasis, and tumor formation [19,52,53]. In adult kidney, Wnt/ β -catenin signaling is silenced. However, reactivation of Wnt/ β -catenin occurs in wide variety of kidney diseases, including obstructive nephropathy, adriamycin nephropathy, ischemia/reperfusion injury (IRI) and remnant kidney [54–57]. Upon activation, Wnts bind to serpentine receptors, the Frizzled (Fzd) family of proteins, and co-receptors, members of the LDL receptor-related protein (LRP5/6). This ligand/receptor engagement promotes a series of downstream intracellular signaling events involving Dishevelled (Dvl), axin, adenomatosis polyposis coli (APC) and glycogen synthase kinase-3 β (GSK-3 β), ultimately resulting in dephosphorylating of β -catenin [58]. This renders stabilization and nuclear translocation of β -catenin, where it binds to and activates transcription factors of the T-cell factor (TCF) and lymphoid enhancer-binding factor (LEF) families to stimulate the transcription of target genes [59]. The consensus TCF/LEF binding sequence is (A/T)(A/T)CAA(A/T)G. Of particular interest, bioinformatics analyses have uncovered the presence of putative TCF/LEF-binding sites in the promoter regions of all RAS genes including AGT, renin, ACE, AT1, and AT2 (Figure 1). In proximal tubular cells, constitutively activated β -catenin promotes the binding of LEF-1 to these sites, and overexpression of β -catenin or various Wnt ligands induces expression of multiple RAS genes (Figure 1) [5]. These studies identify Wnt/ β -catenin as a master upstream regulator that controls the expression of multiple RAS genes in a synchronized fashion.

In human, Wnt expression and β -catenin nuclear translocation is increased in diseased kidneys, illustrating the activation of canonical Wnt/ β -catenin signaling [55,60,61]. However, whether there are functional genetic polymorphisms in Wnt/ β -catenin/RAS pathway, particularly in the promoter regions of the RAS genes harboring the TCF/LEF-binding sites, deserves further investigation.

THERAPEUTIC IMPLICATION IN COMBATING CKD

Since Wnt/ β -catenin directly controls the expression of multiple RAS genes, blockade of this signaling, in theory, could simultaneously repress RAS activation and may have superior therapeutic efficacy than anti-RAS remedies [5]. Indeed, in numerous animal models of CKD induced by unilateral ureteral obstruction (UUO), adriamycin or IRI, blockade of Wnt/ β -catenin by a small molecule ICG-001, which blocks β -catenin-mediated gene transcription, inhibits fibrosis-related gene expression, reduces collagen deposition and renal fibrosis and improves kidney function [5,54,57,62]. Of interest, transient administration of ICG-001 or late treatment could reduce renal fibrosis and inflammation as well, and restores podocyte functions and abolishes albuminuria [5]. Our recent studies further demonstrate that blockade of Wnt/ β -catenin by ICG-001 displays superior efficacy than ACEI or ARB in mouse models of IRI and adriamycin nephropathy (unpublished data).

The therapeutic efficacy of Wnt/ β -catenin blocker appears to be associated with its inhibition of multiple RAS genes in the kidney [5]. In adriamycin-induced nephropathy, ICG-001 effectively abolishes intrarenal upregulation of major RAS components such as AGT, renin, ACE and AT1 [5]. Therefore, the mode of action of ICG-001 is quite different

from anti-RAS remedies. While ACEI or ARB targets one component of the RAS a time, ICG-001 simultaneously inhibits the expression of all RAS genes. Preliminary studies suggest that ICG-001 is also able to lower blood pressure in rat model of remnant kidney induced by 5/6 nephrectomy (unpublished data). Whether the effect of ICG-001 on blood pressure modulation contributes to its efficacy on CKD, however, remains to be determined.

Klotho, a novel antiaging protein that is highly expressed in the tubular epithelium of normal adult kidneys [63], is found to physically bind to and functionally sequester Wnt ligands, and therefore acts as an endogenous Wnt antagonist that inhibits Wnt/ β -catenin activity [56]. In remnant kidney model and UUO, *in vivo* expression of exogenous Klotho through hydrodynamic-based gene delivery abolishes the induction of multiple RAS proteins including AGT, renin, ACE and AT1 receptor, and normalizes blood pressure [47]. Klotho also inhibits β -catenin activation and ameliorate renal fibrotic lesions [56]. These results suggest a novel and mechanistic linkage between Wnt/ β -catenin and RAS activation in aging and the pathogenesis of CKD [56].

CONCLUSION

Intrarenal activation of RAS plays a critical role in the pathogenesis and progression of CKD. RAS activation is often associated with loss of Klotho and upregulation of Wnt/ β -catenin in the kidney. Recent findings indicate that Wnt/ β -catenin is the master upstream regulator that controls the expression of multiple RAS genes. Therefore, targeting Wnt/ β -catenin would simultaneously repress all RAS genes, thereby normalizing blood pressure, inhibiting inflammation and ameliorating renal fibrosis. Studies on animal models demonstrate that inhibition of Wnt/ β -catenin by ICG-001 displays superior therapeutic efficacy than ACEI or ARB. Although more studies are needed, blockade of Wnt/ β -catenin may hold promise as an effective and novel strategy for the treatment of patients with CKD in the clinical setting.

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KEY POINTS

- Activation of Wnt/ β -catenin signaling is a common pathologic finding in a wide variety of CKD, which is accompanied by intrarenal upregulation of RAS components.
- Wnt/ β -catenin, as a master upstream regulator, controls the expression of multiple RAS genes in the kidney.
- Blockade of Wnt/ β -catenin signaling by small molecule ICG-001 inhibits RAS activation and attenuates renal fibrosis after chronic injury.
- Klotho is an endogenous Wnt antagonist, and loss of Klotho is associated with de-repression of Wnt/ β -catenin signaling, which leads to RAS activation, hypertension and kidney fibrosis.
- As Wnt/ β -catenin regulates many RAS components, targeted inhibition of this signaling could simultaneously inhibit multiple RAS genes, thereby displaying superior therapeutic efficacy.

Figure 1. Wnt/β-catenin signaling regulates the renin-angiotensin system in CKD. (A) Bioinformatics analyses reveal the presence of the consensus TCF binding sites in the regulatory regions of RAS genes. TCF binding sites are highlighted by red color. (B) Expression of constitutively activated β-catenin induces the mRNA expression of multiple RAS genes in tubular epithelial cells. (C) Diagram showing different RAS components and strategies for therapeutic intervention. Panels A and B are adapted from Zhou *et al.* J Am Soc Nephrol 26: 107–120, 2015. Abbreviations: AGT, angiotensinogen; Ang, angiotensin; ACE, angiotensin-converting enzyme; AT1, angiotensin II type 1 receptor; AT2, angiotensin II type 2 receptor; DRI, direct renin inhibitor; ACEI, angiotensin-converting enzyme inhibitor; ARB, AT1 receptor blocker.