Angiotensin II as a Morphogenic Cytokine Stimulating Renal Fibrogenesis

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ABSTRACT
Inhibitors of the renin-angiotensin-aldosterone system attenuate glomerulosclerosis and interstitial fibrosis. Although the mechanisms underlying their antifibrotic effects are complex, angiotensin II (Ang II) emerges as a major profibrogenic cytokine. Ang II modulates renal cell growth, extracellular matrix synthesis, and degradation by multiple fibrotic pathways. One of the main targets of Ang II in renal fibrosis is TGFβ. Many, but not all, of the stimulatory effects of Ang II on fibrogenesis depend on the induction of TGFβ and its downstream mediators of matrix accumulation, inflammation, and apoptosis. However because of the difficulty in targeting TGFβ, connective tissue growth factor (CTGF), a downstream mediator of TGFβ, has become a more promising antifibrotic target. Ang II can directly induce expression of renal CTGF and mediate epithelial-mesenchymal transition. Other profibrotic factors stimulated by Ang II include endothelin-1, plasminogen activator inhibitor-1, matrix metalloproteinase (MMP)-2, and a tissue inhibitor of metalloproteinase-2. Finally, connections among Ang II, hypoxia, and the induction of hypoxia-inducible factor-1α contribute to fibrogenesis. A better understanding of the multiple morphogenic effects of Ang II may be necessary to develop better strategies to halt the progression of renal disease.


Chronic kidney disease (CKD) is increasing worldwide. Although the cause of its progression is multifactorial,1–3 activation of the renin-angiotensin-aldosterone system (RAAS) is a key effector and RAAS inhibitors are among the best-studied renoprotective agents.4–7 Although the RAAS is complex, with new peptides such as angiotensin 1-7 and angiotensin IV exhibiting profibrotic effects by binding to specific receptors as well as observations that aldosterone and even renin itself can stimulate fibrogenesis in the kidney,8–12 and angiotensin II (Ang II) blockade can stimulate renin release,13 the review presented here focuses exclusively on Ang II as the major component of the RAAS effect on morphogenesis. There are several excellent reviews covering the other components of the RAAS and their profibrotic actions in the kidney.8–10,14 The classic view of Ang II as a vasoactive agent involved in local and systemic hemodynamic regulation needs extension to encompass its properties as a morphogenic cytokine with an active role in renal pathology.16,17

Ang II was first described 2 decades ago as stimulating the hypertrophy of tubular cells and the associated increase in collagen secretion.18,19 Fibrosis is the final common pathway leading to renal diseases of diverse etiology, including inflammation, hemodynamics, and metabolic injury.20–22 Ang II modulates renal cell growth and extracellular matrix (ECM) synthesis or degradation.17,23–25 For example, overexpression of renin and angiotensinogen in rat glomeruli leads to expanded ECM without inducing systemic hypertension.26,27 The stimulatory effects of Ang II on increased collagen expression depend on various mechanisms. In addition to angiotensin-converting enzyme (ACE), which is the most well known enzyme capable of Ang II formation, other non-ACE Ang II-generating pathways are currently under study.28 ACE inhibitors also diminish cellular infiltrates and inflammatory markers in many models of renal injury.29

ANG II AND TGFβ

The interactions between Ang II and the TGFβ axis are multiple and complex (Figure 1).29–31 TGFβ is a fibrogenic and anti-inflammatory cytokine and plays a critical role in the pathophysiology of renal injury.32,33 Ang II stimulates transcription and synthesis of TGFβ, particularly TGFβ1, in cultured murine proximal tubular cells and also upregulates specific receptors for TGFβ, further enhancing its profibrogenic action.34–36 Ang II directly stimulates complex interactions in the

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TGF axis (Figure 2). Ang II directly stimulates transcription of the gene encoding TGFβ by activating specific transcriptional motifs (activator protein-1 binding sites in the TGFβ1 promoter). Furthermore, Ang II engages p38 mitogen-activated protein kinase (MAPK) and c-jun N-terminal kinase signaling for thrombospondin-1, which in turn leads to an increased release of active TGFβ from its latent complex. Finally, Ang II stimulates the expression of chemokines such as monocyte chemoattractant protein-1 (MCP1). MCP1 action itself induces TGFβ. The stimulatory effects of Ang II on collagen expression and cellular hypertrophy also depend on TGFβ expression. Ang II stimulates proliferation of cultured renal fibroblasts and increases mRNA encoding of TGFβ, fibronectin, and collagen type I. TGFβ subsequently stimulates matrix accumulation and inflammation.

ANG II AND EPITHELIAL-MESenchymAL TRANSITION

Innovative experiments demonstrate that more than one third of local fibroblasts in renal interstitial fibrosis originate from tubular epithelial cells through a process called epithelial-mesenchymal transition (EMT). The molecular mechanisms of EMT have been reviewed in detail. EMT is important in early stages of interstitial fibrosis, producing tubular atrophy and the disappearance of epithelial cells. One important mediator of EMT is TGFβ, and Ang II contributes to EMT through induction of this profibrotic factor.

In vitro treatment of HK2 tubular cells with Ang II for 3 days also causes the transition to myofibroblast-like cells. Blockade of the MAPK cascade, using the specific inhibitors SB203580 for p38, PD98059 for extracellular signal-regulated kinase (ERK) 1/2, and SP600125 for c-jun N-terminal kinase, diminishes Ang II-induced EMT. The blockade of the RhoA/Rho-associated kinase (ROCK) pathway, by transfection of a RhoA dominant-negative vector or by ROCK inhibition with Y-27632 or fasudil, also inhibits EMT caused by Ang II. MAPKs and ROCK inhibitors block connective tissue growth factor β (CTGF) overexpression induced by Ang II. As a side, the macula densa is also critical for RAAS physiology, and there is evidence in a rat model that treatment with candesartan increases volume and number of macula densa cells compared with tubular cells. Therefore, the transdifferen-
tiation from tubular cells to macula densa cells can be controlled by angiotensin type 2 receptors (AT2Rs).68,69 EMT is also antagonized by hepatocyte growth factor.60,61 and Ang II inhibits its expression.62 In a more complex paradigm, Ang II within minutes can also rapidly stimulate the downstream mediators of the TGFβ signaling pathway, Smad2/3, independently of the paracrine action of TGFβ.53,64 The pathophysiological interposition of this pathway is unclear. Because renal fibrosis is a relatively slow and persistent process, progression likely depends primarily on persistent TGFβ-dependent Smad activation.65

The therapeutic effect of inhibiting the RAAS system on renal fibrosis is mediated by the inhibition of TGFβ production, in addition to lowering BP.3,65 There was an expectation that selective inhibition of TGFβ would have an enhanced antifibrotic effect.65 Several elegant animal studies clearly demonstrate the benefit of anti-TGFβ antibodies after the development of nephropathy, thereby showing that an anti-TGFβ approach might be useful clinically in patients with progressive nephropathy.66–68 However, data on clinical anti-TGFβ therapy in several fibrosis-associated diseases, including diabetic nephropathy, are disappointing because of a lack of efficacy or because of side effects or other properties.67

**ANG II AND CTGF**

CTGF has also emerged as a new antifibrotic target because it is more than a downstream mediator of TGFβ.68–78 CTGF, also known as CCN2,72 belongs to the CCN family of early response genes and acts as a multifunctional mediator with biologic activities including regulation of cellular apoptosis and proliferation, angiogenesis, migration, adhesion, and fibrosis.70,72,74,77 It is not expressed normally in the healthy kidneys, but its expression is induced in human renal diseases, including glomerulonephritis, glomerulosclerosis, and diabetic nephropathy, and its levels of expression correlate with the severity and progression of renal fibrosis.74 In vivo data reveal that Ang II-infused rats overexpress CTGF in glomeruli, tubules, and renal arteries as well as during tubular injury with and elevated fibronectin deposition.69,74 Treatment with an angiotensin type 1 receptor (AT1R) antagonist diminishes CTGF and fibronectin and ameliorates tubular damage.69 In rats with immune complex nephritis, renal overexpression of CTGF is diminished by the ACE inhibitor, quinapril, and correlates with a diminution in fibrosis.68 In cultured mesangial and tubular epithelial cells, Ang II also increases mRNA encoding CTGF and collagen type I in tubular epithelial cells,74 Ang II mediates the induction of CTGF and functionally associates with cellular hypertrophy, representing a possible contribution to renal damage on the way to progressive fibrosis.69–72,74–78

**ANG II, CTGF, AND SIGNALING PATHWAYS**

Initially, CTGF was mainly understood to act downstream of TGFβ to regulate ECM synthesis.4,8 The Smad proteins are essential components of this intracellular signaling pathway, acting as transcription factors for TGFβ-mediated responses, including fibrosis.71 In a rat model of remnant kidney disease, Ang II induces tubular mRNA encoding CTGF and collagen I, and the subsequent proteins are upregulated by phosphorylated Smad2/3 but downregulated by Smad7; that is, overexpression of Smad7 abolishes Ang II-induced phosphorylation and upregulation of CTGF and collagen I.79 Additionally, Ang II also induces a rapid activation of Smad2/3, CTGF, and collagen type I in tubular epithelial cells lacking the TGFβ gene, which is blocked by the addition of an AT1R antagonism, losartan, or inhibitors to ERK (PD98059) and p38 (SB203580) but not by inhibitors to AT2R (PD123319) or c-jun N-terminal kinase (SP600125). These findings demonstrate the existence of a TGFβ-independent, AT1R-mediated ERK/p38 MAPK crosstalk pathway for Ang II-mediated CTGF and collagen type I expression. Interestingly, in knockdown experiments, the ability of Smad3 but not Smad2, to inhibit Ang II-induced CTGF and collagen type I expression further reveals an essential role for Smad3 in Ang II-mediated renal fibrosis.79 Smad7 is also identified in vivo and in vitro as a negative regulator of TGFβ signaling, including the induction of CTGF.80,81 Transfection or overexpression of Smad7 results in diminished upregulation of CTGF, fibronectin, and collagen type I and blocks TGFβ-induced ECM production and renal fibrosis caused by Ang II.79–81 These data indicate that Smad signaling is a common mechanism of Ang II-mediated fibrosis in renal diseases and that the blockade of Smad activation could be another important antifibrotic target.80

Finckenberg et al. also identified a calcineurin inhibitor-dependent pathway for Ang II-mediated induction of CTGF in transgenic rats harboring human renin and angiotensinogen genes in the heart and kidney.82 Serum and glucocorticoid-inducible kinase (SGK1) are highly expressed in fibrotic tissues including the kidney. In human kidney fibroblasts Ang II enhances expression of SGK1 and CTGF.83 In SGK1 wild-type mouse fibroblasts, Ang II stimulates transcript and protein abundance of CTGF that is significantly blunted in SGK1-null fibroblasts.83 Thus, SGK1 presumably contributes to the profibrotic effect of Ang II.83

A serine-threonine kinase, Rho-kinase, also signals a pathway toward inflammatory and proliferative changes. In several animal models treatment with Rho-kinase inhibitors diminishes glomerular and tubulointerstitial inflammation and fibrosis, including a decrease of TGFβ and ECM proteins.58,59,84–89 Ang II through AT1Rs is involved in the intracellular signaling of the small guanosine triphosphatase Rho and its downstream effector Rho-kinase.84 In rats after Ang II infusion, treatment with the Rho-kinase inhibitor Y-27632 decreases proinflammatory and
profibrotic mediators including CTGF.84,90

ANG II AND CTGF INHIBITION

Pentoxifylline is a potent inhibitor of CTGF. Lin et al. observed that pentoxifylline84 inhibits CTGF expression by interfering with Smad3/4-dependent CTGF transcription through protein kinase A, blocking the profibrogenic effects of CTGF on renal cells.84,90 In cultured mesangial cells, IL-1β inhibits Ang II-mediated production of collagen type IV through CTGF downregulation and increases collagen type IV degradation through the regulation of MMP-9.91 Furthermore, the addition of eicosapentaenoic acid and docosahexaenoic acid suppresses Ang II and arachidonic acid-induced profibrogenic genes, including CTGF and TGFβ, in human mesangial cells, further implicating the regulatory involvement of the Ang II-TGFβ axis.92,93 Interestingly, a recently identified oxidized LDL receptor in LOX1-null mice that is upregulated by Ang II further upregulates Ang II activity; Ang II infusion decreases mRNA encoding CTGF, AT1R expression, and phosphorylation of p38 and p44/42 MAPKs.94 Therefore, CTGF has emerged as a promising antifibrotic target beyond its importance as a downstream mediator of TGFβ.95

ROLE OF AT1R AND AT2R

Ang II mainly acts through its binding to two specific receptors, AT1R and AT2R.92 AT1R is responsible for most of the pathophysiological effects of Ang II by promoting proliferation, inflammation, and fibrosis (Figure 1).79,94 The role of the AT2R is not completely defined. AT2R is involved in inhibition of cell growth and inflammatory cell recruitment in the kidney.79,96–99 A complete discussion of the various Ang II receptors is beyond the scope of this review and can be found elsewhere.95,97,100 Ang II, through AT1Rs, upregulates many proinflammatory genes such as vascular cell adhesion molecule-1, intercellular adhesion molecule-1, IL-6, and MCP1 through the activation of several intracellular signaling systems, including the nuclear factor-kB (NF-kB), MAPK cascade, Rho proteins, and redox pathways.79,85,102–104 Ang II induces plasminogen activator inhibitor (PAI)-1 and tissue inhibitor of metalloproteinase (TIMP)-196 through AT1. There are emerging data that AT2Rs also transduce proinflammatory effects and are involved in promotion of fibrosis and hypertrophy.9,105,106

In murine models of renal fibrosis, AT2R-null mice show increased renal fibrosis and fibrocyte infiltration with a concomitant upregulation of renal transcripts encoding collagen type I. Fibrocyte numbers in bone marrow also increase in AT2R-null mice. In contrast, pharmacologic inhibition of AT1R with valsartan decreases the degree of renal fibrosis and the number of fibrocytes in kidney and bone marrow. In isolated human fibrocytes, inhibition of AT2R signaling by Ang II regulates ECM accumulation mediated by the endogenous production of profibrotic growth factors.95

Although the Ang II-TGFβ-axis through AT1R and AT2R is pathophysiologically coherent for understanding development of renal fibrosis, therapeutic strategies blocking TGFβ actions have not afforded the expected beneficial effects. Therefore, CTGF has emerged as a promising antifibrotic target beyond its importance as a downstream mediator of TGFβ.95 Beyond this, experimental and clinical studies investigating the effect of Ang II receptor blockers and studies in AT1R-null mice indicate a further involvement of the AT2R in the progression of CKD.98

Whereas Ang II stimulates increased expression of collagen type I, inhibition of AT1R decreases collagen synthesis and the loss of AT2R signaling paradoxically increases renal injury and mortality in CKD. This was confirmed in a model of 5/6-nephrectomized comparing the effects of the AT1R and AT2R blockade.103 Treatment with an AT2R antagonist enhanced glomerulosclerosis, cortical collagen content, and increased phospho-Erk2 and PAI-1 in comparison to treatment with an AT1R blocker.102,103 Beneficial effects achieved with AT1 blockade seem to contribute not only to AT1R blockade but also to an increase of Ang II effects transduced through the AT2R.98 In SJL mice with antiglomerular basement membrane nephritis, treatment with an AT1 blocker completely abolishes the antifibrotic effects of AT1R activation represented by ECM deposition and expression of TGFβ and PAI-1.107 Counteractivation of AT2Rs in this model by increased Ang II under AT1R blockade likely confers an antifibrotic protection.103 By contrast, in a model of spontaneously hypertensive stroke-prone rats, treatment with an AT2R agonist preserves renal structure by preventing inflammatory cell infiltration, collagen accumulation, and the neoeexpression of vimentin; it also prevents increased plasma renin activity and accumulation of urinary acute-phase proteins.100

Consequently, the role of AT2Rs seems more complex. In cultured podocytes, Ang II-mediated induction of the receptor for advanced glycation end products (RAGE) by a NF-kB binding site at −1519 on the RAGE promoter is dependent on the AT2R.108 Further experimental data confirm that AT2Rs are involved in inflammatory cell recruitment in the kidney. AT2R, but not AT1R, antagonists diminish the number of inflammatory cells in different animal models, such as systemic infusion of Ang II and unilateral ureteral obstruction.109–113 This correlates closely with the activation of the NF-kB pathway by Ang II, which is activated by the AT1R or AT2R or both.105,106,108,109,112–114 NF-kB inhibition attenuates renal interstitial inflammation and hypertension.113 This finding suggests the possibility that blockade of Ang II generation by an ACE inhibitor or by combined blockade of angiotensin receptors,116 as well as by the inhibition of the NF-kB pathway, is necessary to fully stop the inflammatory process. Above all, not only angiotensin receptor blockade but also reduced Ang II formation is of major relevance in the development of renal fibrosis. In addition, vita-
Ang II can directly or indirectly induce other factors that in turn mediate fibrosis, particularly MCP-1, endothelin, and osteopontin. In several models of kidney fibrosis with increases in local Ang II, endothelin simultaneously increases endothelin-1 (ET-1). In a model of severe Ang II-dependent hypertension in Ren-2 transgenic rats, the role of Ang II, ET-1, and L-type calcium channels in the development of glomerular, vascular, and tubulointerstitial fibrosis is observed after treatment with irbesartan, bosentan, and a selective endothelin receptor antagonist. Ang II and L-type calcium channels modulate fibrosis selectively in the tubulointerstitial and perivascular compartments, respectively. Treatment with the ET-1 receptor antagonist prevents fibrosis in all three compartments, pointing to a major role of ET-1 in the development of Ang II-induced renal fibrosis. The importance of ET-1 was confirmed by Gagliardini et al. in uninephrectomized rats with streptozotocin-induced diabetes using combined therapy with the ACE inhibitor, lisinopril, and the ET-1 receptor antagonist, avosentan, although significant side effects have been seen with endothelin antagonists when used to clinically treat diabetic nephropathy.

Ang II can also exert a strong stimulatory effect on the transcription of PAI-1, a member of the serpin superfamily of serine protease inhibitors crucially involved in pathologic ECM deposition. Considerable evidence suggests that despite its main function in regulating intravessel fibrinolysis, an up-regulation of PAI-1 can be an important mediator of tissue fibrosis. Continuous infusion of Ang II in rats induced fibrosis concomitant with a significant increase in levels of expression in glomerular PAI-1 and cyclooxygenase-2 (COX-2). This effect is associated with increased binding of the ubiquitous RNA-binding protein human-antigen R to the mRNAs of PAI-1 and COX-2 in the cytoplasmic fractions of renal homogenates from Ang II-treated rats. Ang II promotes an increase in nuclear-cytoplasmic shuttling of human-antigen R that is blocked by the protein kinase C inhibitor, rottlerin, and the AT1R antagonist, valsartan, but is unaffected by AT2R antagonists. Because COX-2-derived prostaglandins participate in the pathogenesis of inflammatory processes in addition to maintaining renal and vascular homeostasis, the Ang II-induced stabilization of PAI-1 and COX-2 may critically contribute to the Ang II-triggered fibrosis and inflammation.

Furthermore, Ang-II treatment induces in vivo and in vitro alterations in the matrix MMP-2 and TIMP-2 balance that favor TIMP-2 overactivity and thereby lead to a disturbed balance between ECM synthesis and degradation resulting in fibrosis. Moreover, Ang-II-mediated changes in the production of MMP-2 and TIMP-2 occur through AT1Rs and a TGFβ1-dependent mechanism, and not through hemodynamic effects.

**ANG II AND OTHER PROFIBROTIC FACTORS**

**Figure 3.** Schematic overview of EGFR transactivation by Ang II. Ang II binds extracellularly to the G-protein-coupled AT1Rs that lack an intracellular kinase domain. This activation leads, through unclear mechanisms, to a translocalization of the metalloproteinase TACE from the perinuclear, cytoplasmic space to the cell surface. Ang II also may stimulate expression of TACE. The cell-surface localization of TACE has a longer half-life and can now act on the membrane-associated pro-TGFα, releasing soluble TGFα. In turn, TGFα binds to the EGFR, leading to autophosphorylation of the C-terminal end, recruiting cytoplasmic mediators that bind to phosphotyrosine residues of the receptor through SH2 or PTB domains. The result is activation of various kinase pathways including mammalian target of rapamycin/S6 kinase, phosphatidylinositol-3-kinase-dependent kinase/Akt, and ERK 1/2. Interestingly, it has been previously shown that Ang-II-mediated injury associates with activation of these kinases. Modified from reference 125.
tive receptor and did not influence receptor downregulation.\textsuperscript{119} Norman et al. concluded that Ang II potentiates EGF-induced mitogenesis at one or more postreceptor steps.\textsuperscript{126} In 1990, we described in a murine proximal tubular cell line that pretreatment with Ang II further enhanced EGF-induced cell division by approximately 40\%.\textsuperscript{19} Although there are similarities between EGF and Ang II in the induction of immediate early genes, EGF stimulates mitogenesis whereas Ang II induces hypertrophy in these proximal tubular cells.\textsuperscript{127} These early findings provide evidence of a potential interaction between Ang II and EGF-mediated signaling, and Chen and colleagues recently found evidence for a role of heparin-binding EGF (HB-EGF) in Ang II-induced tubular hypertrophy.\textsuperscript{128}

Axel Ulrich’s group discovered a pathway in 1996 for how G-protein coupled receptors such as the AT1R could phosphorylate and activate the EGFR.\textsuperscript{129} Ang II was initially not studied, but the investigators found that ET-1, lysophosphatic acid, and thrombin all activate G-coupled receptors lacking the kinase domain by phosphorylating tyrosine residues on the EGFR and thereby activating other downstream kinases.\textsuperscript{130} These findings were rapidly extended to Ang II.\textsuperscript{129} For example, in cardiac fibroblasts, Ang II stimulates tyrosine phosphorylation of the EGFR, a process called “transactivation.”\textsuperscript{131} Further studies reveal that stimulation of G-protein coupled receptors leads to EGFR phosphorylation through activation of MMPs.\textsuperscript{132} After ligand binding to the G-protein coupled receptors, MMPs are activated, inducing cleavage of pro-HB-EGF, which liberates a soluble HB-EGF that activates the EGFR.\textsuperscript{132,133}

Launette and colleagues studied transgenic mice overexpressing a dominant-negative form of the EGFR.\textsuperscript{124} Those mice that could not activate the EGFR or downstream kinases were protected from glomerulosclerosis, mononuclear cell infiltration, tubular fibrosis, and atrophy induced by 2 months of Ang II-infusion. Ang II-infused EGRF mutant mice exhibited significantly less proteinuria compared with Ang II-treated wild-type mice, but tail-cuff-measured arterial BP was identical in both groups. These results indicate that the damaging effects of Ang II-infusion are mediated by EGFR transactivation rather than by systemic hypertension. Launette et al. next studied potential mechanisms by which Ang II transactivates the EGFR during renal injury.\textsuperscript{124} Incubation of a rat liver cell line with Ang II induces secretion of TGF\(_{\alpha}\), a ligand of the EGFR. TGF\(_{\alpha}\) is released from a larger integral membrane precursor called proTGF\(_{\alpha}\).

An important enzyme in cleaving TGF\(_{\alpha}\) from its transmembrane precursor is TNF\(_{\alpha}\) converting enzyme (TACE), first identified in the processing of membrane-bound precursors of TNF\(_{\alpha}\).\textsuperscript{134} Launette and co-workers then tested whether a pharmacologic EGFR antagonist and a specific TACE inhibitor prevented Ang II-mediated kinase activation of renal injury \textit{in vivo}.\textsuperscript{124} First, Ang II-infusion stimulated a marked increase in tubular expression (ascending limb of Henle’s loop and distal tubule) of TGF\(_{\alpha}\) without a concomitant increase in mRNA, suggesting that more membrane-bound proTGF\(_{\alpha}\) is converted to active TGF\(_{\alpha}\). In parallel, immunostaining for TACE, but not mRNA, increased in Ang II-infused mice at the same tubular location as TGF\(_{\alpha}\).

It appears that Ang II-infusion leads to a TACE translocalization from the perinuclear compartment to the cell surface. Treatment of Ang II-infused animals with a TACE inhibitor blunts Ang II-induced TGF\(_{\alpha}\) accumulation, EGFR phosphorylation, and renal damage without influencing BP. Similar effects are also observed in TGF\(_{\alpha}\)-null mice in which Ang II fails to transactivate the EGFR. Because Ang II-infusion is a nonphysiological model of kidney injury, the authors finally studied a renal ablation model in mice (75\% reduction of renal mass).\textsuperscript{125} TGF\(_{\alpha}\) and TACE levels, but not EGF protein, increases 2 months after ablation at the point in time when the renal injury appears.\textsuperscript{124} Treatment with an AT1R antagonist prevents these changes, indicating that renal ablation leads to intrarenal RAAS activation.\textsuperscript{25}

In summary, these elegant experiments suggest the following signal transduction pathway shown in Figure 3: Ang II binds to G-protein coupled AT1R, leading to a redistribution of TACE from the cytoplasm to the cell surface. How this works is unclear. Cell surface-associated TACE has an increased half-life and cleaves adjacent membrane-associated proTGF\(_{\alpha}\) to release active TGF\(_{\alpha}\). In turn, TGF\(_{\alpha}\) binds to the EGFR in an autocrine or paracrine manner and activates the receptor-associated kinase cascade, leading to phosphorylation of phosphatidylinositol 3-kinase/Akt, ERK 1/2, and mammalian target of rapamycin/S6 kinase.\textsuperscript{135} This rather complicated signal transduction pathway goes from the outside (Ang II) to the inside (TACE), again to the outside (TGF\(_{\alpha}\)), and finally returns to the inside of the cell with EGFR phosphorylation.\textsuperscript{125} Nature is complicated.

**ANG II, HYPOXIA, AND HYPOXIA-INDUCIBLE FACTOR-1\(\alpha\)**

Hypoxia-inducible factor (HIF)-1\(\alpha\) is responsible for inducing various genes supporting cell survival mechanisms, such as glucose metabolism, vasomotor regulation, angiogenic growth, and anemia.\textsuperscript{135,136} Ang II directly stimulates the expression of HIF-1\(\alpha\) by post-transcriptional mechanisms, which were demonstrated in several kidney cell lines including pathways mediated by AT2Rs and reactive oxygen species-dependent activation of the phosphatidylinositol 3-kinase—protein kinase B/Akt pathway.\textsuperscript{53,137} Active oxygen species act as second messengers in kidney damage caused by Ang II and mediate the induction of HIF-1\(\alpha\) by cytokines. Infusion of Ang II into mice causes renal HIF-1\(\alpha\) activation and oxidative stress, which is diminished by addition of antioxidants in a cell culture model of tubulointerstitial cells.\textsuperscript{88} In a heterozygous mouse model for Morg1, which is a major regulator of HIF-1\(\alpha\) expression,\textsuperscript{138} increased expression of HIF-1\(\alpha\) and HIF-1\(\beta\) leads to par-
tial protection from renal ischemia reperfusion injury. This is associated with enhanced expression of mRNA encoding erythropoietin and reduction of tubulo-interstitial cytokines. In unilaterally nephrectomized rats with acute glomerulonephritis, preinduction of HIF-1α results in attenuated tubular and glomerular damage mediated by Ang II. Another model using subtotal nephrectomized rats with subsequent induction of HIF-1α attenuates proteinuria and structural renal damage. Therefore, HIF-1α seems to be cytoprotective in acute injury models of the kidney, yet HIF-1α also promotes fibrosis in an experimental model of chronic renal injury after unilateral ureteral obstruction, which seems to be the result of HIF-1α-mediated stimulation of EMT. In animal experiments simulating the aging kidney, hypoxic damage correlates with increased activation of HIF-regulated genes such as vascular EGF or glucose transporter. HIF-1α is also involved in the development of renal fibrosis utilizing activation mechanisms such as Ang II itself or through Ang II-mediated hemodynamic changes.

Hypoxia per se mediates induction of HIF-1α, whereas RAAS activation enhances tubulointerstitial hypoxia, pointing to a vicious cycle. Up to now it is not clear how far HIF-1α acts as an agonist or antagonist of renal fibrosis. There is definite evidence, mainly in models of acute kidney injury, for its renoprotective capabilities, yet it also is a mediator in models of chronic renal damage or the aging kidney in which stable activation of HIF-1α enhances kidney fibrosis.

Possible new antifibrotic therapies might target the CTGF or other signaling pathways such as the Smad signaling system or the Rho/Rho kinase pathway. Protective effects of treatment with AT1R antagonists and ACE inhibitors seem to be the result of inhibition of renal CTGF in various experimental models of kidney damage. In patients with IgA nephropathy, high urine levels of CTGF and TGFβ correlate with the degree of tubulointerstitial damage. Similar data are found in patients with cardiac myofibrosis in which serum CTGF levels are a marker of cardiac dysfunction. In experiments with mice with type 1 or type 2 diabetes, a blockade of CTGF with antisense oligonucleotides leads to reduced expression of genes involved in matrix expansion, such as fibronectin, type I collagen, and PAI-1, contributing to significant reversal of mesangial expansion in diabetic nephropathy. More in vivo data are needed to evaluate the expected beneficial effects of CTGF blockade, with a special focus on the renal inflammatory response. In addition, a better understanding of the potential antiproteinuric, antifibrotic, and anti-inflammatory effects of AT2R antagonism might enable development of an innovative strategy for the treatment of CKD. Finally, inhibition of Ang II-mediated EGFR transactivation by TACE inhibitors may be a promising novel therapeutic strategy.

DISCUSSIONS

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