A Look at Transactivation of the EGF Receptor by Angiotensin II

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A growing number of clinical and experimental studies show that the renin–angiotensin system (RAS) is involved in the progression of CKD.[1] In fact, pharmacological inhibitors of the RAS such as angiotensin-converting enzyme inhibitors and angiotensin II (Ang II) type I receptor (AT1R) blockers are the most reliable and effective tools known to attenuate progression of parenchymal changes in CKD.[1] However, despite the clinical evidence, understanding how RAS inhibition exerts its renoprotective function at the molecular level remains unclear.

Theoretically, Ang II binds to AT1R, a G protein–coupled receptor, predominantly expressed by renal cells.[2] Activation of AT1R mediates the majority of Ang II actions through activation of phospholipase C, generation of inositol triphosphate and diacylglycerol, and an increase in intracellular Ca^{2+}, which in turn stimulates protein kinase C (PKC). In addition, activation of AT1R leads to tyrosine phosphorylation and stimulates mitogen-activated protein (MAP) kinases and growth responses. However, because AT1R lacks intrinsic tyrosine kinase activity, it is not clear how AT1R stimulates extracellular signal kinases 1 and 2 (Erk1 and 2). Several experimental findings suggest that activation of AT1R promotes transactivation of the EGF receptor (EGFR).[2–5] This transactivation is likely mediated by metalloproteinase-dependent release of EGFR ligands such as EGF, TGF-α, and heparin-binding EGF (HB-EGF) from their cell membrane–bound precursors and intermediary signaling molecules including intracellular Ca^{2+}, PKC, and cytosolic tyrosine kinases such as Src kinases.[4,5] The contribution of each of these molecular pathways to Ang II–mediated transactivation of EGFR in the kidney with CKD is not known.

In the normal adult kidney, high concentrations of EGF are found in urine, and high levels of the EGF precursors are detected in the apical surface of thick ascending limb of Henle and early distal convoluted tubule.[5–7] In addition, TGF-α is localized to the distal convoluted tubule and the collecting duct, whereas HB-EGF is localized to the proximal and distal tubules.[5,8] EGFR is the prototypical receptor among four members of the receptor tyrosine kinase superfamily and widely expressed in the glomerular mesangium, proximal tubule, collecting duct, and medullary interstitial cells.[5] Interestingly, distinct from the apical localization of its ligands, EGFR is localized to the basolateral surface of tubular cells, especially in the proximal tubule. Therefore, different expression sites, as well as different cellular locations, complicate interpretations of interactions between EGFR and its ligands in the kidney under pathologic and experimental conditions.

The addition of EGFR ligands to the medium of cultured tubular cells results in activation of EGFR, leading to cell proliferation/hypertrophy, migration, matrix production, and epithelial–mesenchymal transition (EMT).[5] As these results suggest, transitory activation of EGFR-regulated genes may be involved in recovery from acute kidney injury.[9] In contrast, prolonged activation of EGFR is associated with progressive parenchymal changes of notable pathology in CKD.[7,10] The latter is demonstrated in diabetic animals treated with an EGFR tyrosine kinase inhibitor,[11] as well as by a histone deacetylase inhibitor,[12] in which blockade and attenuated expression of EGFR significantly suppresses diabetes-associated kidney enlargement. Terzi *et al.*[6] also demonstrated this using mice with targeted expression of a dominant negative EGFR (DN-EGFR) transgene in the proximal tubules. After subtotal (~75%) nephrectomy and an ischemia–reperfusion injury, less tubulointerstitial changes develop in these mice than in wild-type controls. Through subsequent independent experiments using JunD gene deficient mice[13] and a genome-scan analysis,[14] Terzi and colleagues proposed that activation of EGFR by paracrine TGF-α plays a pivotal role in development of tubulointerstitial changes after subtotal nephrectomy, at least in FVB/N mice, which are highly susceptible to renoablation.

Ang II–dependent transactivation of EGFR has also been shown to play a role in renal lesions after Ang II infusion. Lautrette *et al.*[7] reported that Ang II induced pro–TGF-α and its sheddase, ADAM17, in the apical membranes of distal tubule, activated EGFR and downstream MAP kinases, and generated tubulointerstitial changes in the kidneys of wild-type mice after long-term Ang II infusion. On the other hand, all experimental procedures such as targeted expression of DN-EGFR in the proximal tubule, genomic deletion of the TGF-α gene, and systemic treatment with an ADAM17 inhibitor significantly attenuated development of Ang II–induced renal lesions by inhibition of EGFR phosphorylation. Although this study indicated a potentially detrimental role of cross-talk between Ang II and EGFR in the progression of parenchymal changes in CKD, the paradoxical occurrence in

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the kidney of a paracrine link between EGFR in the proximal tubule and Ang II–induced TGF-α in the distal tubule remains to be explained.

In this issue of *JASN*, Chen *et al.*[15] identified another pathway of Ang II–mediated transactivation of EGFR in the kidney after Ang II infusion. Previously, they had demonstrated in cultured tubular cells stably transfected with an AT1R expression vector that Ang II transactivated EGFR through HB-EGF shedding and independently activated the TGF-β signaling pathway, resulting in tubular cell hypertrophy.[8] Ang II–mediated transactivation of EGFR (^{pY1173}EGFR) by HB-EGF shedding seemed plausible because all of the components involved in this process were colocalized to one cell. In the present study, however, ^{pY1173}EGFR activity was short term and not sufficient to promote progressive renal fibrosis.[15] Instead, AT1R activation led to another, sustained transactivation of EGFR (^{pY845}EGFR) by a reactive oxygen species (ROS)-dependent phosphorylation of Src within proximal tubular cells, which in turn stimulated TGF-β1 expression via activation of the Erk pathway. Targeted deletion of *EGFR* gene in the proximal tubules and systemic inhibition of EGFR with the tyrosine kinase inhibitor erotinib significantly decreased TGF-β–dependent renal fibrosis after Ang II infusion or diabetes induction. Additionally, Src phosphorylation has been reported to regulate dedifferentiation of proximal tubular cells such as increases in vimentin expression and migration through the EGFR/Akt pathway.[16] This may be partially involved in the antifibrotic effects of EGFR inhibition in the kidney because dedifferentiation of tubular cells is considered as an intermediate-state of EMT,[17] which is significantly associated with subsequent renal fibrosis.[18] Of interest, induced TGF-β1 is thought to promote ROS production,[19] possibly yielding a vicious cycle of (Ang II-)ROS-Src-EGFR-Erk-TGF-β1 expression in the proximal tubules in the fibrogenic kidney.

Either genetic or pharmacologic inhibition of EGFR in this study suppressed another Ang II–mediated transactivation of EGFR by TGF-α shedding, especially in the proximal tubular cells. In contrast, genomic deletion of the *TGF-α* gene, which also significantly attenuates Ang II–induced renal lesions as described above,[7] could not suppress those actions by HB-EGF shedding and ROS-dependent phosphorylation of Src in the kidney after Ang II infusion. Therefore, the relative contribution of each of these pathways to transactivate EGFR that led to the progressive parenchymal changes in the kidney with CKD still leaves some uncertainty.

It also remains unclear whether global inhibition of TGF-β activity, which is currently being tested in clinical trials, will prove to be effective and safe, whereas RAS inhibition is clinically, but not satisfactorily, renoprotective.[1] Recently, inhibition of platelet-derived growth factor receptors by treatment with a tyrosine kinase inhibitor significantly attenuated renal injury in an experimental animal model.[20] Because the findings by Chen *et al.*[15] strongly suggest that the feed-forward mechanism of the EGFR/Erk pathway launched by AT1R activation is responsible for promoting the progression of TGF-β–dependent renal fibrosis, EGFR may be a potential therapeutic target for CKD as an alternative to Ang II and TGF-β.

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DISCLOSURES

None.

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See related article, "EGFR Signaling Promotes TGFβ-Dependent Renal Fibrosis," on pages 215–224.

Managing Microvascular Complications of Diabetes with MicroRNAs

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DNA and proteins have long been viewed as the movers and shakers in genomic studies, with RNA sometimes seen as no more than mere messengers shuttling information between the two. This view, however, dramatically changed when the key roles of microRNAs (miRNAs) in gene expression were gradually disclosed over the last decade.[1,2]

miRNAs are short ~22 nucleotide noncoding RNAs, which constitute a relatively new class of small RNAs that act as post-transcriptional regulators of gene expression. Most miRNAs in animals share a common biogenesis pathway in which miRNAs are transcribed by RNA polymerase II as precursor molecules. These precursors, also known as pri-miRNAs, fold into hairpin structures and are further processed by the endonuclease, Drosha, into pre-miRNAs. The pre-miRNAs are exported from the nucleus to the cytoplasm, where they are cleaved by the endonuclease, Dicer, to yield mature miRNAs. The mature miRNA is then loaded into the RNA induced silencing complex, comprised of the Argonaute family of proteins, where it is able to recognize specific mRNA targets. In general, miRNAs negatively regulate their target mRNAs through Watson-Crick base pairing of nucleotides 2–8 of the miRNA (the seed sequence) with complementary sequences within the target mRNA's open reading frame and 3′ untranslated region.

Dysregulation of a single miRNA can influence an entire signaling network. This is because individual miRNAs have multiple targets and thus can exert robust control over complex biological pathways by targeting multiple interrelated proteins. Indeed, it is becoming increasingly apparent that the aberrant expression of a single miRNA may be causally related to a variety of disease states such as cancer, cardiac diseases, and more recently, kidney diseases. In the kidney, miRNAs play important roles in a variety of pathologic conditions. For example, the consequences of inhibition of miRNAs in the glomerulus was recently examined using conditional deletion of Dicer, the RNAs essential for miRNA biosynthesis, in podocytes by several groups.[3–5] Overall, it was reported that podocyte-specific deletion of Dicer leads to increased proteinuria, podocyte effacement, reduced slit diaphragm protein expression, and ultimately renal failure. As well, a podocyte-specific knockout of Drosha leads to collapsing glomerulopathy,[6] further establishing the importance of regulated miRNAs in podocytes.

With regard to diabetic nephropathy (DN), Natarajan and colleagues[7] were the first to report a role for a specific miRNA. Their group reported that miR-192 was upregulated in mesangial cells *in vitro* and in glomeruli from streptozotocin (STZ)-induced and *db/db* mouse models of DN. miR-192 has been shown by others to also modulate Smads and fibrogenesis in DN.[8,9] Natarajan and colleagues also convincingly demonstrated that miR-192 targets the E-box repressor Smad-1 interacting protein. More recently, the same group has reported that miR-216a was upregulated by TGF-β in experimental models of DN.[10] Our group has identified miR-93 as a signature miRNA in the diabetic milieu.[11] Expression of miR-93 is increased in experimental models of diabetes both *in vitro* and *in vivo*. We also identified vascular endothelial growth factor-A (VEGF-A) as a putative target of miR-93 in the kidney. Using transgenic mice containing VEGF-LacZ bicistronic transcripts, inhibition of glomerular miR-93 by peptide-conjugated morpholino oligomers elicits increased expression of VEGF.

In this issue of *JASN*, Wang *et al.*[12] provide new insights into the role of the miR-29 family in DN. They report that members of the miR-29 family are downregulated in response to TGF-β stimulation in cultured proximal tubular epithelial cells, podocytes, and mesangial cells. This is accompanied by a concomitant increase in the expression of the validated miR-29 targets, collagens I, III, and IV. The authors report that ectopic overexpression of miR-29 results in increased expression of E-cadherin. Interestingly, a correlative assessment was made between miR-29 expression and treatment of the uninephrectomized STZ-diabetic rats with losartan and fasudil, a Rho kinase inhibitor. The renoprotective effects of fasudil correlate with an increase in

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