EGFR signaling in renal fibrosis

Shougang Zhuang[1,2] and Na Liu[1]

[1]Department of Nephrology, Shanghai East Hospital, Tongji University School of Medicine, Shanghai, China and [2]Department of Medicine, Rhode Island Hospital and Alpert Medical School of Brown University, Providence, Rhode Island, USA

Signaling through the epidermal growth factor receptor (EGFR) is involved in regulation of multiple biological processes, including proliferation, metabolism, differentiation, and survival. Owing to its aberrant expression in a variety of malignant tumors, EGFR has been recognized as a target in anticancer therapy. Increasingly, evidence from animal studies indicates that EGFR signaling is also implicated in the development and progression of renal fibrosis. The therapeutic value of EGFR inhibition has not yet been evaluated in human kidney disease. In this article, we summarize recent research into the role of EGFR signaling in renal fibrogenesis, discuss the mechanism by which EGFR regulates this process, and consider the potential of EGFR as an antifibrotic target.

Kidney International Supplements (2014) **4,** 70–74; doi:10.1038/kisup.2014.13

KEYWORDS: EGFR; fibrosis; kidney; renal epithelial cells; transforming growth factor-β1

Correspondence: *Shougang Zhuang, Department of Medicine, Rhode Island Hospital and Alpert Medical School of Brown University, Middle House 301, 593 Eddy Street, Providence, Rhode Island 02903, USA.*
E-mail: szhuang@lifespan.org

The epidermal growth factor receptor (EGFR/HER1) belongs to a receptor tyrosine kinases protein family that includes HER2/*neu*, HER3, and HER4.[1] At least six EGFR ligands, including transforming growth factor-α (TGF-α), epidermal growth factor (EGF), heparin-binding EGF-like growth factor (HB-EGF), amphiregulin, betacellulin, and epiregualtin, have been identified in kidney cells.[2–5] Depending on the activating ligand, EGFR family members form homodimers or heterodimers with different biological effects.[6] Activation of EGFR can lead to diverse cellular consequences associated with fibrogenesis, including cell proliferation, migration, differentiation, survival, and transformation.[7–8]

EGFR can be activated by several mechanisms under physiological or pathophysiological conditions. Like other tyrosine kinase receptors, EGFR is directly activated by ligand binding, inducing activation of the intrinsic kinase domain and subsequent phosphorylation of specific tyrosine residues. These phosphorylated residues initiate activation of intracellular pathways, including the extracellular signal-regulated kinases1/2 (ERK1/2), the Janus kinase/signal transducers and activators of transcription (STAT), and the phosphatidylinositol-3 kinase/AKT.[1,7,9] On the other hand, EGFR can be transctivated by stimuli that do not directly interact with the EGFR ectodomain, including G protein–coupled receptor ligands (i.e., endothelin, lysophosphatidic acid, thrombin),[10] cytokines (i.e., interleukin-8),[11] and oxidants (i.e., H_2O_2).[12] EGFR transactivation is involved in metalloprotease-mediated shedding of EGF-like ligands.[9] In addition to ligand-dependent mechanisms, EGFR can be directly activated by Src phosporylation on Tyr 845. This mode of EGFR activation has been reported in association with sustained activation of EGFR in renal epithelial cells exposed to angiotensin II (Ang II).[13]

Over the past two decades, numerous studies have been conducted to elucidate the role of EGFR in renal diseases. Initial studies revealed that EGFR activation is critically involved in mediating renal regeneration and functional recovery after acute kidney injury.[14–15] Recent studies also showed that EGFR activation contributes to the pathogenesis of renal interstitial fibrosis, glomerular diseases, including diabetic nephropathy, hypertensive nephropathy, glomerulonephritis, and tubular diseases such as polycystic kidney disease.[16–20] The role of EGFR signaling in acute kidney injury and glomerular disease has been recently reviewed in other articles.[8,21] Here, we will summarize the role of EGFR signaling in renal interstitial fibrosis, and mechanisms

involved and the therapy potential of EGFR inhibition in renal fibrosis.

EXPRESSION OF EGFR AND EGFR LIGANDS IN A DISEASED KIDNEY

The location of EGFR and its ligands in the kidney has been described in the normal kidney. Constitutive EGFR expression was detected in glomeruli, tubules, and interstitium of normal human kidneys[22] and is normally expressed in several cell types in the kidney that include renal epithelial cells, podocytes, renal interstitial fibroblasts, and mesangial cells.[23] EGFR ligands, such as EGF, TGF-α, and HB-EGF are also expressed in renal tubules.[24–26] Other EGFR ligands such as amphiregulin, betacellulin, and epiregualtin are detected in the kidney at low levels, but their nephron location remains unclear.[3]

Upregulated renal expression and *de novo* expression of EGFR and its ligands has been observed in nearly all rodent-kidney injury models.[8,21] Those models include unilateral ureteral obstruction (UUO)-induced renal fibrosis,[27] Ang II-induced renal damage,[28] nephrotoxic nephritis,[23] diabetic nephropathy,[29] ischemia/reperfusion injury.[15] Cellular location of increased expression EGFR and its ligands vary with the type of kidney disease. In experimental renal fibrosis, there is an upregulated expression of EGFR by interstitial cells and renal epithelial cells.[27] In the mode of nephrotoxic serum-induced nephritis, increased EGFR was detected in podocytes and HB-EGF was in parietal epithelial cells and podocytes.[23] Tubular expression of EGFR and multiple EGFR ligands such as EGF, TGF-α, amphiregulin, and HB-EGF are also upregulated in the epithelium of a murine autosomal-recessive polycystic kidney disease model.[4] Moreover, a high level of TGF-α in renal tubules was reported in Ang II-induced renal fibrosis.[28]

Increased EGFR expression/activation under pathological conditions is either transient or persistent.[8,21] Although transient EGFR activation is seen in acute and mild injured kidneys,[8,21] its sustained expression is most observed in the kidney subjected to severe and chronic kidney damage.[13,27] In a mouse model of unilateral urethral obstruction-induced renal fibrosis, EGFR expression and phosphorylation increased gradually after urethral ligation and was persistent for at least 21 days.[27] However, EGFR activation after injury is not due solely to its increased expression, as EGFR phosphorylation is still increased even if EGFR levels are normalized.[27]

The mechanism responsible for sustained expression/activation of renal EGFR after chronic injury remains incompletely understood. We have recently demonstrated that administration of MS-275, a highly selective inhibitor of class I histone deacetylases (HDACs), reduced expression and phosphorylation of EGFR in the damaged kidney after UUO injury *in vivo* and cultured renal epithelial cells *in vitro*.[30] Gilbert *et al.*[29] also indicated that application of vorinostat, a pan-HDAC inhibitor, also resulted in reduced EGFR protein and mRNA levels, and attenuated cellular proliferation in cultured renal tubular cells. Although the molecular mechanism by which the class I HDAC inhibitor suppresses EGFR phosphorylation (activation) remains unclear, vorinostat was reported to attenuate the EGFR transcript and induce its ubiquitination and targeted it predominantly to lysosome degradation in tumor cell lines[31] In line with this observation, inhibition of a class II HDAC member, HDAC6, also accelerates segregation of EGFR from early endosomes to the late endosomal and lysosomal compartments for degradation.[32,33] These studies therefore suggest that both transcriptional and post-translational mechanisms are involved in the modulation of EGFR.

EGFR SIGNALING IN RENAL FIBROSIS

Renal fibrosis is the final common pathway in end-stage renal disease, characterized by aberrant activation and growth of the renal fibroblasts and overproduction of extracellular matrix proteins.[34] Increasing evidence indicates that activation of EGFR signaling is critically involved in the development and progression of renal fibrosis. Lautrette *et al.*[28] demonstrated that the chronic infusion of Ang II causes renal lesions such as glomerulosclerosis, tubular atrophy and/or dilation with microcyst formation, and interstitial fibrosis accompanied by severe proteinuria. These effects of Ang II were reduced in mice overexpressing a dominant-negative form of EGFR. Similar to this observation, mice overexpressing a dominant-negative EGFR construct exhibited significantly less tubulointerstitial injury in the kidney compared with wild-type littermates after subtotal renal ablation. In our study using the model of UUO-induced renal fibrosis, we found that pharmacological inhibition or genetic reduction of EGFR activity markedly reduced the expression of α-smooth muscle actin, a hallmark of activated fibroblasts, and deposition of fibronectin and collagen I, two extracelular matrix proteins, in the kidney.[16] Finally, Flamant *et al.*[35] indicated that treatment with the EGFR inhibitor gefitinib prevented the development of renal vascular and glomerular fibrosis and the decline in renal function in rats with NO deficiency–induced hypertension.

EGFR ligands have also been reported to mediate renal fibrosis and deterioration in renal function. In the model of Ang II-induced renal fibrosis, increased expression of TGF-α and its sheddase, metalloprotease-17, was observed in mice. Specific deletion of TGF-α or inhibition of TGF-α cleavage with a specific metalloprotease-17 inhibitor substantially reduced Ang II-induced renal damage,[28] suggesting that TGF-α may be a key factor between Ang II signaling and EGFR transactivation during Ang II-induced nephropathy. TGF-α expression was also increased in mice after nephron reduction in the lesion-prone FVB/N strain of mice, and EGFR inhibition reduced fibrotic lesion,[36] indicating that TGF-α acts in the genetic predisposition to chronic kidney disease (CKD) progression. In addition, sustained expression of HB-EGF in myofibroblasts during remodeling of the peri-infarct region of the remnant kidney model has also been reported,[37] suggesting a potential role for this EGFR ligand in the progression of CKD.

Signaling pathways downstream of EGFR have been studied in animal models of renal fibrosis. As stated above,