Brief Reviews

Epidermal Growth Factor Receptor Signaling in the Kidney
Key Roles in Physiology and Disease

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Signaling through epidermal growth factor (EGF) receptors (ErbB receptors; EGFRs) is important for fundamental cellular functions, such as proliferation, migration, growth, and differentiation. In human biology, ErbB signaling is involved in normal growth and development, as well as in the initiation and progression of disease. Based on the aberrant expression in a variety of malignant tumors, ErbB family members have been recognized as targets in anticancer therapy and are now used in the treatment of breast and colon malignancies.

Other than tumor biology, ErbB signaling is critically involved in renal electrolyte homeostasis. Moreover, ErbB family members are implicated in the development of end organ damage, as occurs in hypertension and atherosclerosis. Therefore, the therapeutic potential of targeting ErbB receptors and ErbB signaling pathways may go beyond the field of oncology. In this review, we report on the physiological and disease-related aspects of renal ErbB signaling, with attention to potential benefits and downsides of systemic ErbB inhibition in the healthy and diseased kidney.

ErbB Receptors: Upstream and Downstream Signaling

The ErbB receptor family belongs to subclass I of the receptor tyrosine kinase superfamily, incorporating epidermal growth factor (EGF) receptor (EGFR; HER1; ErbB1), HER2/neu (ErbB2), HER3 (ErbB3), and HER4 (ErbB4). All of the ErbB receptors have a common extracellular ligand-binding site, a single membrane-spanning region, and a cytoplasmic protein tyrosine kinase domain. Upon ligand binding, ErbB receptors undergo conformational changes that induce the formation of receptor homo- or heterodimers. As a consequence, the intrinsic tyrosine kinase domain is activated, phosphorylating specific tyrosine residues within the cytoplasmic tail of the receptor. These autophosphorylated residues serve as docking sites for signaling molecules, of which the recruitment activates intracellular signaling pathways. Individual ErbB receptors are able to discriminate between binding ligands, and phosphorylation of different tyrosine residues occurs upon binding of different ligands to the same ErbB receptor. Moreover, receptors are ligand selective, and differing receptor dimers can be formed. As such, a variety of downstream signal transduction pathways can be selectively activated.

Thus far, 11 ligands for ErbB receptors have been identified. EGF, transforming growth factor (TGF) α, heparin-binding EGF-like growth factor (HB-EGF), amphiregulin (AR), betacellulin, epigen, and epiregulin preferentially bind to the EGFR, whereas neuregulin (NRG) family ligands (NRG-1, NRG-2, NRG-3, and NRG-4) bind to ErbB3 and ErbB4 but not to the EGFR (Figure 1). ErbB2 is unique in the family, because it has no known ligand but forms a heterodimer with other ErbB receptors. As a stabilized complex, it can then induce downstream signaling. ErbB3 lacks intrinsic kinase activity but can be phosphorylated by other ErbB receptors on heterodimerization. It is important to note that all of the ErbB ligands exist as inactive transmembrane precursors, requiring proteolytic cleavage of their ectodomain to be released as mature soluble ligands. This cleavage is performed by ADAM (a disintegrin and metalloprotease) family members. ADAM9, 10, 12, 15, 17, and 19 have been demonstrated to cleave ErbB ligands, thereby regulating ErbB ligand availability. ADAM-dependent EGFR ligand shedding can be induced by factors that bind G protein-coupled receptors (GPCRs), such as angiotensin II binding the angiotensin II type 1 receptor (AT1R). Therefore, GPCR-induced, ADAM-assisted EGFR activation has been termed “transactivation.” Depending on the tissue, different ADAMs may be involved, because ADAM17 was implicated in angiotensin II–induced EGFR transactivation in the kidney and ADAM12 in the heart. At present, it is not fully elucidated how GPCRs activate ADAMs. It has been demonstrated that Gq and second messengers, such as Ca2+ and reactive oxygen species, are required for angiotensin II–induced, ADAM17-dependent HB-EGF shedding in EGFR transactivation. In addition, GPCR binding was shown to alter ADAM17 activity by modulating its phosphorylation status through extracellular signal–regulated kinase via Thr735 phosphorylation.

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Depending on the ErbB ligand and the combination of ErbB receptor homodimer and heterodimer, distinct downstream pathways can be activated, including the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase signaling pathways (Figure 2; reviewed in Reference 1). These cytoplasmic pathways translate signals to the nucleus, changing the activity status of transcription factors, thereby determining gene transcription and, thus, cellular behavior. In ErbB signaling, important downstream transcription factors are c-jun, c-fos, c-myc, nuclear factor κB, and signal transducer and activator of transcription.1 Through these signaling cascades, ErbB receptors control cellular processes that can lead to both beneficial and detrimental outcomes.

**Physiology: Renal Organogenesis**

ErbB family members are crucially involved in nephrogenesis. EGFR knockout mice suffer from impaired epithelial development in several organs, including the kidney, and die at midgestation or shortly after birth.11 In human nephrogenesis, the EGFR was detected in collecting ducts in both early and late gestational stages,12,13 whereas ErbB2 was expressed in proximal tubules and collecting ducts.13 ErbB4 was only reported in the ureteric bud and developing tubules of embryonic rat kidneys.14 EGF and TGF-α were prominently expressed during both early and late nephrogenesis, with strong expression in developing tubules and glomeruli.12,13 Interestingly, AR was expressed in developing glomeruli only at 18 weeks of gestation.16 Figure 3 provides an overview of ErbB family member expression during human nephrogenesis.

In vitro models advocate a role for ErbB signaling in nephrogenesis. Recombinant EGF and TGF-α stimulate growth of cultured embryonic kidney cells,17 whereas epiregulin and AR enhance ureteric bud branching morphogenesis.18 In vitro inhibition of EGFR tyrosine kinase activity or TGF-α reduced cell tubulogenesis and ureteric bud development.19,20 Interestingly, AT1R binding by angiotensin II...
induced ureteric bud branching in vitro, a processes that depended on EGFR phosphorylation.\textsuperscript{21} Unfortunately, none of the in vitro studies provided insight into the exact functional mechanism by which ErbB signaling influences nephrogenesis.

**Physiology: Electrolyte Homeostasis**

Ligand-dependent EGFR signaling has diverse roles in human renal physiology. EGFR modulates glomerular hemodynamics and renal metabolism,\textsuperscript{22} whereas TGF-\(\alpha\) has been shown to be crucial for survival of renal medullary cells during osmotic stress.\textsuperscript{23} HB-EGF determined tubular transepithelial resistance by adjusting the configuration of tight junction proteins in tubules,\textsuperscript{24} a general mechanism to manage the settings for paracellular ion conductance. Moreover, EGFR could specifically control sodium reabsorption across distal nephron epithelia by adjusting epithelial sodium channel activity.\textsuperscript{25} This finding was in line with an expected function for EGF in renal tubular physiology, as based on the strong EGF expression in renal proximal tubular cells (RPTC), the thick ascending limb of Henle’s loop, and distal convoluted tubules (DCT) of normal kidneys.\textsuperscript{26} Moreover, EGF had been detected as a released soluble form in the urine, which suggested that EGF secretion by tubular epithelial cells serves paracrine signaling. The robust EGFR expression along the basolateral membrane of distal tubular cells and in collecting ducts further supported this speculation.\textsuperscript{27} Only recently, has it been discovered that EGF-EGFR signaling is critically involved in renal magnesium homeostasis.

Magnesium (Mg\textsuperscript{2+}) levels are tightly regulated by the kidney. Approximately 80% of plasma Mg\textsuperscript{2+} is filtered by the glomeruli, and \(\approx95\%\) of prouinary Mg\textsuperscript{2+} is reabsorbed passively. Although the DCT reabsorbs only \(\approx10\%\) of filtered Mg\textsuperscript{2+}, it does so in an active manner. Because virtually no reabsorption takes place beyond the DCT, this active Mg\textsuperscript{2+} reabsorption is critical in fine tuning the amount of Mg\textsuperscript{2+} that is excreted into the urine.\textsuperscript{28} Cellular Mg\textsuperscript{2+} entry occurs through an Mg\textsuperscript{2+}-permeable channel termed transient receptor potential melastatin (TRPM) 6, a close homolog of TRPM7, which was already known to be involved in cellular Mg\textsuperscript{2+} homeostasis.\textsuperscript{29,30} Interestingly, TRPM7 was reported to be part of aldosterone-mediated development of renal inflammation and fibrosis through Mg\textsuperscript{2+}-sensitive pathways, indicating pathophysiological relevance.\textsuperscript{31} In the kidney, TRPM6 is predominantly localized along the luminal membrane of DCT cells, which is in favor of the postulated function as being the gatekeeper in renal Mg\textsuperscript{2+} homeostasis.\textsuperscript{30} In addition, patients with mutations in the gene encoding TRPM6 developed hypomagnesemia.\textsuperscript{29} The critical link between EGF and Mg\textsuperscript{2+} handling was only recently uncovered after analysis of a Dutch family with isolated recessive renal hypomagnesemia, a disorder that leads to hypomagnesemia because of renal Mg\textsuperscript{2+} wasting.\textsuperscript{27} Homozygosity-based mapping strategy revealed a mutation in the pro-EGF gene, where the highly conserved proline in the cytoplasmic \(1067^\text{PNP}{1070}\) motif was substituted by a leucine. The mutation disturbed basolateral sorting of pro-EGF, leading to a diminished basolateral release of EGF, seriously hampering EGFR-dependent activation of the basolaterally localized EGFR. As a consequence, TRPM6 was insufficently activated, with a decreased cellular Mg\textsuperscript{2+} influx as a final outcome.\textsuperscript{27} (Figure 4). It was shown recently that EGF-mediated stimulation of TRPM6 occurs via signaling through Src kinases and Rac1, thereby redistributing endomembrane TRPM6 to the plasma membrane.\textsuperscript{32} The functional existence of this reabsorption mechanism was supported by a cohort study of 98 patients with colorectal cancer who were treated with anti-EGFR monoclonal antibodies, because most patients developed hypomagnesemia resulting from renal Mg\textsuperscript{2+} wasting.\textsuperscript{33} Based on the abundance of EGFR and its ligands in tubular epithelial cells, it is conceivable that EGFR signaling is involved in the homeostasis of other electrolytes as well. In this light, ADAMs also come into sight, because EGFR signaling depends on activity of these sheddases in cleaving inactive EGFR ligand precursors from the cell membrane. Corroboratively, ADAM19\textsuperscript{34} and ADAM17 (unpublished observations) have been shown to be highly expressed in distal tubular epithelial cells of healthy human kidneys.
Mechanism of EGF-dependent tubular Mg\(^{2+}\) reabsorption. EGF regulates Mg\(^{2+}\) reabsorption through activation of the basolateral EGFR, leading to TRPM6 activation and apical Mg\(^{2+}\) influx. Subsequently, Mg\(^{2+}\) is extruded via a putative Na\(^{+}\)/Mg\(^{2+}\) exchanger on the basolateral side, which finalizes the process of Mg\(^{2+}\) reabsorption.

Renal Disease

Tissue Distribution of ErbB Family Members
Constitutive EGFR expression was detected in glomeruli, tubules, and interstitium of most normal human kidneys,\(^{35}\) and EGFR upregulation was noted in various forms of glomerulonephritis and in allograft nephropathy.\(^{36,37}\) In the latter, marked EGFR expression was found in glomerular fibrotic lesions, and tubular EGFR correlated with the extent of interstitial fibrosis. HB-EGF was strongly expressed in the glomerular mesangium of patients with glomerulonephritis, but absent in normal kidneys. Moreover, mesangial HB-EGF correlated with the extent of mesangial proliferation, and cultured mesangial cells that were exposed to recombinant HB-EGF proliferated and synthesized collagen types I and III.\(^{38}\) TGF-\(\alpha\) and the EGFR were strongly expressed in primitive tubules in human kidney dysplasia\(^{39}\) and in cyst epithelial cells of patients with autosomal dominant polycystic kidney disease.\(^{40}\)

Contrary to the expression pattern of HB-EGF, TGF-\(\alpha\), and the EGFR, EGF was strongly expressed in the RPTCs and DCTs of normal kidneys, whereas it was absent in RPTCs in reflux nephropathy. Furthermore, decreased tubulointerstitial EGF expression correlated with severity of apoptosis.\(^{41}\) In line with these findings, an increase in EGF mRNA was strongly associated with a decrease in tubulointerstitial apoptosis in chronic renal disease, whereas EGF decrease was associated with a decrease in renal function.\(^{42}\) To date, the human renal tissue expression of AR, beta-catenin, epigen, and epiuregulin has not been investigated. It must be remarked that, although the local availability of EGFR ligands modulates EGFR signaling, the expression of these ligands as assessed by immunohistochemistry could reflect both cellular production and cellular uptake.

The dissociating tissue expression profile of the EGFR and its ligands in normal versus diseased human kidneys, together with the observed correlations with parameters of fibrosis, suggests that EGFR signaling is implicated in the pathophysiology of renal fibrosis. Interestingly, EGFR ligands are inversely regulated, suggesting different functions for ligands that bind the same receptor.

Experimental Interventions in ErbB Signaling
Genetic and pharmacological ErbB targeting have provided functional insight into the versatile role of ErbB signaling in the kidney. Transgenic mice that carry a kidney tube-specific dominant-negative EGFR isoform function normally under basal conditions yet display reduced tubular dilatation after subtotal nephrectomy and reduced tubular atrophy, interstitial fibrosis, and mononuclear cell infiltration after induction of renal ischemia.\(^{43}\) In rats with NO deficiency–induced hypertension, treatment with an EGFR tyrosine kinase inhibitor prevented the development of renal vascular and glomerular fibrosis and the decline in renal function.\(^{44}\) This protective response was associated with normalized downstream MAPK activity and reduced collagen I expression. Diabetic rats that received an EGFR tyrosine kinase inhibitor showed a reduction in tubular epithelial cell proliferation, glomerular enlargement, and kidney weight.\(^{45}\) Waved-2 mice, expressing a point mutation in the EGFR that reduces receptor tyrosine kinase activity by \(\sim 90\%\), have normally developed kidneys. However, when the Waved-2 mutation was introduced into the murine orpk mutation model of autosomal recessive polycystic kidney disease, a substantial decrease in cyst formation and improvement of renal function were observed.\(^{46}\) Also, treatment with an EGFR tyrosine kinase inhibitor could reduce cyst formation and prevent renal function decline. This effect was more profound when EGFR signaling inhibition was combined with ADAM17 inhibition, expectedly because of decreased EGFR ligand availability.\(^{47}\) Interestingly, ErbB2 inhibition also resulted in reduced renal cyst development, and cultured autosomal dominant polycystic kidney disease cells showed reduced migration after EGFR or ErbB2 inhibition.\(^{48}\)

Altogether, constitutive ErbB signaling is needed for normal development, and inhibition of ErbB activity is beneficial in experimental renal fibrotic and cystogenic disorders. Nevertheless, a certain basal level of ErbB activity seems needed to recover from harmful insults, because Waved-2 mice with HgCl\(_2\)-induced acute nephrotoxicity had more severe tubular injury with a concomitant decrease in recovery of renal function when compared with their wild-type littermates.\(^{49}\) Therefore, ErbB signaling may initially serve processes of tissue repair but may lead to excess tissue fibrosis and functional deterioration under overcompensating reparative actions. It still needs to be determined whether these experimental observations also apply for human ErbB signaling.

Transactivation of the EGFR in Renal Disease and Hypertension
Angiotensin II has a well-recognized role in the development of renal fibrotic lesions. Historically, most of its effects have been attributed to hemodynamic regulation, and, indeed, renal
fibrotic lesions can be identified in most hypertensive patients. Only recently was it discovered that angiotensin II can also induce EGFR transactivation via AT1R-induced ADAM-dependent shedding of membrane-bound EGFR ligands. EGFR transactivation occurs in various kidney cell types and in vascular smooth muscle cells. In the latter, EGFR transactivation induced cell hypertrophy and migration, which play a role in the development of vascular lesions, as can be seen in atherosclerosis and hypertension. In rat glomerular afferent arterioles, EGFR signaling contributed to intracellular calcium influx, which is part of the contractile response to angiotensin II. Moreover, GPCR-induced EGFR transactivation promoted vasoconstriction both in vitro and in vivo, and angiotensin II–induced hypertension in rats was attenuated by treatment with EGFR antisense nucleotides. Furthermore, leptin-induced hypertension and monocrotaline-induced pulmonary hypertension were reduced by pharmacological EGFR tyrosine kinase inhibitors, whereas endothelin-induced hypertension was attenuated in Waved-2 mice. In rats transgenic for human renin and angiotensinogen (dTGR hypertensive rats), blood pressure and associated renal and cardiac tissue damage could be reduced by pharmacological inhibition of MAPK p38, an important signal molecule downstream the EGFR.

In the kidney, wild-type mice developed severe fibrotic lesions after infusion of angiotensin II, on both glomerular and interstitial level, whereas mice expressing a kidney tubule-specific dominant-negative isoform of the EGFR were protected from these lesions. In addition, it was shown that TGF-α knockout mice, as well as mice that were treated with a pharmacological ADAM17 inhibitor, were protected from angiotensin II–induced renal lesions.

Although TGF-α appeared critically involved in angiotensin II–induced renal lesions in vivo, in vitro experiments using glomerular mesangial cells demonstrated a key role for HB-EGF in the induction of fibronectin synthesis, which is a prominent player in the development of glomerulosclerosis. Moreover, renal tubular epithelial cell hypertrophy was shown to depend on HB-EGF–induced transactivation of the EGFR, not on TGF-α. Intriguingly, angiotensin II–induced EGFR transactivation can activate TGF-β signaling pathways, thereby linking 2 of the major effectors of fibrosis.

**ErbB Signaling in Mechanisms of Renal Repair**

Although interventional studies have provided convincing evidence for a detrimental role of ErbB signaling in renal disease, ErbB activity is also implicated in the underlying processes of renal repair. In RPTCs, EGF, HB-EGF, and epiregulin can activate the EGFR and induce regenerative proliferation and migration. EGFR activation via AR or HB-EGF was a prerequisite for induction of full RPTC motility, and HB-EGF, AR, and epiregulin induced proliferation of cultured mesangial cells. In vivo, HB-EGF enhances renal tubular cell regeneration and repair after renal ischemia. Moreover, EGFR activation by HB-EGF protected kidney cells from apoptosis in a cell-cell or cell-matrix deprived environment, indicating that HB-EGF has cytoprotective effects. In rat unilateral ureteral obstruction, EGF expression was decreased when compared with normal kidneys. Administration of EGF during acute tubular injury and after relief of unilateral ureteral obstruction attenuated tubular damage and accelerated tubular regeneration. In addition, subcutaneous injection of EGF or TGF-α increased the recovery of renal epithelial cells. Although folic acid–induced acute renal injury caused an increase in expression of the EGFR, HB-EGF, and TGF-α, EGF was completely depressed. The consequences of EGF signaling could be species dependent, because EGF administration during hydronephrosis potentiated renal cell death in mice but cell survival in rats.

**ErbB Signaling: Beneficial or Detrimental to the Kidney?**

Signaling through ErbB receptors serves bidirectional outcomes. ErbB signaling was proposed as an unfavorable mechanism in the development of renal disease when upregulated ErbB family member expression was identified in fibrotic conditions. Interventional animal models supported this assumption, because ErbB inhibition was beneficial for renal fibrotic disorders. On the other hand, ErbB signaling is engaged in mechanisms of repair, suggesting a role in renal protection and recovery from injury. Depending on localization, type, severity, and extent of the environmental stimulus, it is likely that the same ErbB signaling–mediated cellular mechanisms can cause beneficial and detrimental outcomes. For example, RPTCs need proliferation and migration to exert repair; however, the long-term effects of the same processes could also result in fibrosis and loss of functional tissue. As such, ErbB signaling could carry out repair after injury, whereas overcompensation may lead to unnecessary tissue damage. Similar functional profiles have been demonstrated for other crucial mediators of tissue repair and fibrosis, such as macrophage activity and TGF-β signaling.

**Targeting ErbB Activity: Benefits and Downsides for the Kidney**

ErbB inhibition is of therapeutic value in experimental renal fibrotic disorders, positioning ErbB signaling as a promising target of intervention in human fibrotic kidney disease. At present, no Food and Drug Administration–approved indications exist yet for the kidney. In the treatment of kidney-unrelated tumors, ErbB inhibition provoked minor renal disturbances in electrolyte balance. Almost all of the patients with colorectal carcinoma who were treated with EGFR inhibitors developed hypomagnesemia because of reduced activity of EGFR-dependent renal Mg2+ channels. In another population, EGFR inhibitors induced collapsing glomerulonephritis, focal segmental glomerulosclerosis, and acute tubular necrosis, albeit in a minority of patients. During pregnancy, the occurrence of anhydramnion has been reported under anti-ErbB2 (HER2) treatment, which may relate to the role of ErbB2 in fetal kidney development.

These data further confirm that physiological ErbB signaling is needed for renal electrolyte homeostasis and maintenance of kidney integrity, illustrating that systemic ErbB targeting is likely to come at a price. In this respect, the discovery that different intracellular tyrosine kinases are phosphorylated on binding of different ligands to the same...
ErbB receptor is crucial. As such, rather than inhibiting all of the ErbB signaling pathways, selectively targeting individual signaling cascades comes into view. A clinical example used in renal transplantation is the immunosuppressive agent rapamycin, which inhibits the signaling molecule mammalian target of rapamycin downstream of ErbB receptors. Alternatively, beneficial ErbB signaling pathways could be selectively activated, thereby appreciating the versatile role of ErbB signaling. Finally, based on the widespread functions of ErbB signaling in human biology, the development of strategies to traffic ErbB inhibitors to specific sites is of great importance. Based on the ongoing efforts to unravel the precise mechanism of ErbB signaling, mainly driven by the cancer research community, it is expected that the best days of ErbB targeting are yet to come.

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Disclosures
None.

References


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