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The Role of the EGF Family of Ligands and Receptors in Renal Development, Physiology and Pathophysiology

Fenghua Zeng[#], Amar B. Singh[#], and Raymond C. Harris

Department of Medicine, Vanderbilt University, Nashville, Tennessee-37232

Summary

Mammalian kidney expresses all of the members of the ErbB family of receptors and their respective ligands. Studies support a role for ErbB family receptor activation in kidney development and differentiation. Under physiologic conditions, EGFR activation appears to play an important role in the regulation of renal hemodynamics and electrolyte handling by the kidney, while in different pathophysiologic states, EGFR activation may mediate either beneficial or detrimental effects to the kidney. This article provides an overview of the expression profile of the ErbB family of ligands and receptors in the mammalian kidney and summarizes known physiological and pathophysiological roles of EGFR activation in the organ.

Keywords

EGFR; Kidney; Injury; Fibrosis; Cancer

The epidermal growth factor receptor (EGFR) family, also known as ErbB receptors (ErbBs), consists of four transmembrane receptors belonging to the receptor tyrosine kinase (RTK) superfamily and includes EGFR (ErbB1/HER1), ErbB2/Neu/HER2, ErbB3/HER3, and ErbB4/HER4 [1]. All four ErbBs have a common structure, with an extracellular ligand-binding domain, a single membrane-spanning region, a homologic cytoplasmic protein tyrosine kinase domain and a C-terminal tail with multiple phosphorylation sites. Activation of the ErbBs is controlled by their ligands, members of the EGF-related peptide growth factor family [2,3]. These ligands can be divided into three groups. The first group includes EGF, amphiregulin (AR), and transforming growth factor- α (TGF- α), which bind specifically to EGFR; the second group includes betacellulin (BTC), heparin-binding EGF (HB-EGF), and epiregulin (EPR), which exhibit dual specificity in that they bind both EGFR and ErbB4. The third group is composed of the neuregulins (NRGs, also known as heregulins, HRGs) and forms two subgroups based upon their capacity to bind ErbB3 and ErbB4 (NRG-1 and NRG-2) or only ErbB4 (NRG-3 and NRG-4) [4]. Ligand binding to ErbBs induces formation of homo- and heterodimers, leading to activation of the intrinsic kinase domain and subsequent phosphorylation on specific tyrosine residues within the cytoplasmic tail. These phosphorylated residues serve as docking sites for a variety of signaling molecules, whose recruitment leads to the activation of intracellular pathways, including the MAP kinase

Address Correspondence to: Raymond C. Harris, MD., C-3121 Medical Center North, Department of Medicine, Vanderbilt University, Nashville, Tennessee 37232-4794, Office: (615) 343-0030, Fax: (615) 343-7156, E-mail: ray.harris@vanderbilt.edu.

[#]Both authors have contributed equally to this article.

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pathways, JAK/STAT pathways and the phosphatidylinositol-3 kinase pathways, controlling cell proliferation, differentiation, and apoptosis [1,5,6]

All of the EGFR ligands initially present as type I transmembrane proteins, with a “mature” soluble form released to cell matrix or cell culture medium following cleavage by members of the ADAM (A Disintegrin And Metalloproteinase) family of metalloproteinases [7–9]. Each of these soluble peptides of this family of ligands contains at least one characteristic EGF-like motif, defined by six spatially conserved cysteine residues (CX7 CX4–5 CX10–13 CXCX8 C) that generate three peptide loops through the formation of disulfide bonds with the following interactions: C1–C3, C2–C4, C5–C6 [10]. Less homology exists outside the essential cysteine and glycine residues required for the EGF-like confirmation.

Proteolytic release of EGFR ligands represents an important regulatory step for receptor activation [11,12], and blocking the release of ErbB ligands inhibits growth as well as migration in EGFR dependent cell lines [13]. However, many EGFR ligand precursors, including HB-EGF, TGF- α , AR, and BTC, are biologically active even when they are tethered to the plasma membrane, suggesting they may also function as juxtacrine factors [14–18]

High concentrations of EGF are found in urine, and high levels of the EGF precursor, preproEGF, have been detected in mammalian kidneys. EGF production in the adult mouse kidney has been localized to thick ascending limb of Henle (TALH) and early distal convoluted tubule [19]. However, distinct from the basolateral localization of its receptor (EGFR), immunoreactive EGF is also localized at the apical surface of the tubule cells [20–22]. The different expression sites as well as the different cellular location may exclude interaction between EGF released into the urine and its receptor in the normal kidney.

Unlike the studies cited above, in human kidney tissue, EGF mRNA and protein have also been detected in the collecting duct [23,24], and specifically in the intercalated cells of this nephron segment, where EGF is localized to its basolateral surface. Renal artery infusion of EGF in sheep produced natriuresis and diuresis, suggesting a tubular effect [25]. When administered basolaterally to isolated rabbit cortical collecting duct, EGF inhibited sodium transport by inhibiting the epithelial sodium channel (ENaC) [26,27], and also inhibited the hydroosmotic effect of vasopressin [28].

In addition to EGF, other EGFR ligands are also expressed in the mammalian kidney. TGF- α has been implicated in embryonic development, and its expression is low in normal adult kidney [29], with localization to the distal convoluted tubule and the collecting duct [24,30]. HB-EGF has been localized to the distal nephron as well as the apical membrane of proximal tubule epithelial cells and the vasculature [31]. Although other EGFR ligands such as betacellulin, amphiregulin and epiregulin [32,33] are also expressed at low levels in the kidneys, their nephron localization has yet to be described in detail.

Among the four ErbBs, EGFR is the prototypical receptor and is widely expressed in mammalian kidney, including glomerular mesangial cells, proximal tubule and cortical and inner medullary collecting duct, as well as in medullary interstitial cells [34–37]. In the proximal tubule, EGF binding and EGF receptor-associated tyrosine kinase activity are localized to basolateral membrane. Similarly, functional responses in collecting duct are observed only with basolateral administration.

ErbB2 is mainly expressed on human fetal kidney epithelial cells [38,39], with high expression in epithelial structures of both mesenchymal and ureteric bud origin but low or undetectable expression in undifferentiated mesenchyme. There is broad distribution in the adult kidney [39].

ErbB3 is expressed in both fetal and adult kidney, with a somewhat higher expression in the fetal kidney. It has been localized predominantly in the distal tubule and collecting duct, with minimal expression in the proximal tubule and loops of Henle and no detectable expression in glomeruli [40].

ErbB4 is also widely expressed in adult kidney, even though its expression level is relatively lower than in the fetal kidney. In the adult kidney, ErbB4 is highly expressed in the distal and collecting tubules and has moderate expression levels in the proximal tubules and loop of Henle. Expression in the glomerulus is restricted to mesangial cells. [41,42]

ErbBs in renal development

Metanephrogenesis is initiated when the ureteric bud (UB, Wolffian duct), an outgrowth of the mesonephric duct, encounters undifferentiated mesenchyme, designated as metanephric blastema. Subsequently, the UB branches to form the collecting system of the mature kidney, including the intrarenal collecting ducts. The metanephric blastema gives rise to the more proximal nephron from the glomerular capsule to the distal tubule. These processes are regulated by mutually inductive epithelial-mesenchymal interactions [43,44]. The development and branching of the UB is also influenced by interactions between epithelial cells and extracellular matrix [45]. Organ culture studies suggest that a number of growth factors, including several EGF family members, such as EGF, TGF- α , epiregulin and HB-EGF, are expressed during metanephric development and may contribute to nephron development through EGFR activation [46–50].

The impact of EGF, TGF- α and HB-EGF on renal development has been extensively studied. [46,49–51]. In human embryos, immunoreactivity to EGF and TGF- α can be detected in all metanephric structures from the 7th week onward and decreases in differentiating nephrons [52]. These EGFR ligands are capable of inducing tubulogenesis and branching morphogenesis *in vitro* [46,53]. Addition of anti-TGF- α antibodies to the organ culture medium also caused inhibition of ureteric bud branching and tubulogenesis in rat metanephric cultures [49]. HB-EGF is also detected in embryonic kidney and is localized to UB and its derivatives. Both soluble HB-EGF and proHB-EGF induce branching tubulogenesis in UB derived cells, but with different patterns. ProHB-EGF stimulates long tubular structures with few branches, while soluble HB-EGF administration produces abundant short branches. The difference between the branching caused by soluble HB-EGF and that produced by the membrane-bound form may be due in part to juxtacrine receptor activation [51].

All the EGF growth factors listed above exhibit their function through EGFR activation. The importance of EGFR activation in UB tubulogenesis has been studied in several systems [52, 54–56]. *In vitro* cultures of metanephric kidney or specific cell types of the metanephros have indicated a possible role for EGFR activation in metanephric development. Branching morphogenesis of cultured UB cells is largely prevented by inhibition of EGFR kinase activity in conjunction with inhibition of HGF signaling [46]. In EGFR null allele mice, no apparent abnormalities were detected in nephron segments derived from the metanephric mesenchyme (glomeruli, proximal tubules and thick ascending limbs). However, collecting ducts did exhibit a dilated phenotype in which the epithelial lining of these tubules was flattened, losing its normal cuboidal appearance [54]. Collectively, these *in vitro* and *in vivo* studies suggest that EGFR is not required during the early induction of UB formation or the initiation of the metanephric blastema but may play a role in UB development and terminal differentiation.

There still remains uncertainty about the contribution of individual EGFR ligands in renal development. Gene deletion of EGF, TGF- α , and AR produced relatively minor phenotypes [57]. AR has recently been reported to be a downstream gene of WT1 in metanephric development [58], but no renal developmental abnormalities have been reported in AR

knockouts, and triple knockouts of EGF, TGF- α , and AR were viable and healthy, although there were abnormalities in mammary gland development [57]. Similarly, HB-EGF knockouts have no apparent renal developmental phenotype (unpublished data). The fact that multiple growth factors are capable of inducing branching tubulogenesis has also been used to argue for “relative redundancy” and may explain why knockouts of individual growth factors often fail to exhibit obvious abnormalities in kidney development [59–62].

Compared to EGFR, the effects of other ErbBs in renal development have not been extensively studied. One possible reason is that ErbB2 and ErbB4 null mice die in midgestation from neural and cardiac abnormalities prior to the onset of metanephric development [63,64]. In *in vitro* 3D collagen gel culture, HB-EGF induced tubulogenesis only in MDCK II cells expressing the ErbB4 JM-a/CYT-2 isoform, an ErbB4 isoform with the ability to translocate to the nucleus after ADAM-dependent cleavage. [41]

Recently, Sakurai et al. identified HRG- α as one of the growth factors in the conditioned medium from metanephric mesenchyme derived cells that regulates UB tubulogenesis *in vitro* [46,50]. HRG- α is a member of the NRG1 subtype of neuroregulins that signals via ErbB2, ErbB3, and ErbB4 [6] and has been shown to be critical for development of the central and peripheral nervous system as well as the heart [65–67] and mammary gland [68]. Like HB-EGF, HRG is also mainly expressed in UB in the embryonic kidney (our unpublished data) in the same location as ErbB3 and ErbB4 [40–42,47]. In isolated UB culture, HRG promoted growth and maturation of the UB, but did not stimulate branching morphogenesis [50]. A similar effect of HRG was recently reported on MDCK II cells that overexpressed ErbB4 and were grown in a 3D gel [41]. Furthermore, non-branching growth of the UBs induced by HRG treatment is coincident with the loss of expression of GFR-1, a receptor for GDNF (glial cell line-derived neurotrophic growth factor), which suggests that the presence of GFR-1 in cells at the branching tip may be a prerequisite for the UB to undergo branching morphogenesis. Thus, if GFR-1 were ubiquitously expressed along the UB at a relatively high level, the UB would be expected to display an “all-tip” phenotype, resulting in globular growth without any apparent stalk formation. On the other hand, if GFR-1 expression were reduced or non-existent, tip cells would not be able to respond to GDNF and would be expected to display an “all-stalk” phenotype. The loss of this “tip” phenotype appears to be necessary for differentiation of the ureteric bud into collecting duct epithelium and to induce increased transporter and water channel expression. [69]

Functional studies

EGF Receptor Ligands and Renal Physiology

Studies have indicated a functional role for EGFR activation in the regulation of renal hemodynamics. In addition to confirming EGFR expression in glomerular mesangial cells, these studies demonstrated that exposure of these cells to physiologic concentrations of EGF resulted in *in vitro* functional responses characterized by activation of mesangial cell Na⁺/H⁺ exchange and contraction. *In vivo*, EGF administration reduced the glomerular capillary ultrafiltration coefficient, K_f, which, in combination with EGF-induced constriction of both preglomerular and postglomerular arterioles, resulted in significant reductions in the rates of glomerular filtration and renal blood flow [36]. A similar effect upon glomerular hemodynamics was also seen with HB-EGF infusion [70].

As mentioned above, preproEGF is synthesized in the thick ascending limb of Henle and distal convoluted tubule [71]. EGF is detected in urine in concentrations of up to 50 nM [72], of which 50% appears as the mature form (6 kDa) and the rest is present in high molecular weight forms (165, 116, 97, 66, 50, 42, and 30 kDa) [73–75]. In normal tubular epithelial cells, EGFR is expressed at the basolateral surface and thus is not accessible to the EGF present in the lumen.

The presence of EGF in the lumen directly bathing the epithelia (urine) at concentrations several orders higher than that found in plasma suggests that EGF may serve as a cytoprotective agent of the distal nephron, ureter, and bladder because it is produced in the nephron segment just proximal to where urinary acidification is accomplished, and the collecting duct, ureter, and bladder are exposed to a urine pH as low as 4.5 as well as to a hypertonic milieu. Under normal circumstances, EGF produced in the kidney would not interact with EGFRs in the kidney or the distal uroepithelium, due to localization of the receptor to basolateral membranes but would remain within the lumen and be excreted in the urine. The physical segregation of a ligand to the apical solution and its receptor to the basolateral membrane provides a simple system that is then available for activation when epithelial integrity is compromised. Following localized or general tubular injury, cell-cell interactions are disrupted, and EGF could traverse to the basolateral surfaces and activate the EGFRs located there to promote reestablishment of the epithelial barrier. In this regard, EGF significantly increases tight junction function in cultured MDCK II cells [76,77].

One of the most important functions of the kidney is to maintain ion homeostasis. Although a direct role of EGFR activation in the regulation of renal ion transport remains incompletely understood, studies suggest a role of EGF in the modulation of distal nephron sodium transport through inhibition of ENaC [26,78]. In addition, recent studies support a direct role for EGFR activation in the regulation of magnesium (Mg^{2+}) absorption in the kidney. Homeostasis of magnesium levels is tightly regulated and depends on the balance between intestinal absorption and renal excretion. Free extracellular magnesium is filtered at the glomerulus, and 70% is reabsorbed in the thick ascending limb of the loop of Henle. TRPM6 (transient receptor potential cation channel, subfamily M, member 6), is actively involved in Mg^{2+} transport and is localized along the apical membrane of the loop of Henle and the distal convoluted tubule, as well as the brush border of the small intestine [79]. As noted, EGFR is also highly expressed in these regions. A possible correlation between EGFR and Mg^{2+} absorption was first noted when cancer patients treated with cetuximab, a monoclonal antibody against EGFR, occasionally developed hypomagnesemia [80]. Subsequently, certain individuals with primary hypomagnesemia, a rare heterogeneous group of disorders characterized by renal or intestinal Mg^{2+} wasting, have been identified with impaired basolateral sorting of prepro-EGF [81], inadequate renal EGFR activation and thus insufficient activation of the epithelial Mg^{2+} channel TRPM6 [81]. Studies *in vivo* and using isolated renal tubules have also indicated that EGFR activation can regulate proximal tubule phosphate transport [82–84] and gluconeogenesis [35]. In MDCK cells, EGFR activation leads to a specific decrease in the expression of the tight junction integral protein, claudin-2, which results in modulation of paracellular sodium transport [76,85]. Thus, a correlation between EGFR signaling and paracellular ion transport can also be postulated but remains to be confirmed.

Role in kidney injury

Addition of EGFR ligands to renal tubular cells has been shown to promote biological responses *in vitro*, including cell proliferation, migration, matrix production and epithelial-to-mesenchymal transdifferentiation (EMT) [86–88]. Studies have supported a role for EGFR activation as an important mediator of renal repair following injury [89,90]. On the other hand, constitutive/chronic EGFR activation is thought to be potentially important in the evolution of renal diseases that include renal fibrosis, polycystic kidney disease and renal cell cancer [88, 91–94].

A number of studies have investigated the potential role of EGF and/or the EGF family of growth factors in acute renal injury [95–99]. After acute renal injury, both preproEGF mRNA levels and urinary EGF excretion decrease and remain significantly depressed for up to 7 days [100,101], although it has been reported that there is transiently increased processing of

preproEGF to its soluble, active form in response to acute ischemic renal injury [102]. In contrast, HB-EGF expression increases significantly in rat kidney in response to acute tubular injury induced by mercuric chloride, ischemia/reperfusion, aminoglycosides, or folic acid [99,103,104]. TGF- α expression also increases in response to folic acid nephrotoxicity [104]. Exogenous administration of EGF, TGF- α or HB-EGF accelerates recovery from acute ischemic renal injury [85,89]. In addition, waved-2 mice, which contain a point mutation in EGFR that reduces receptor tyrosine kinase activity by >90%, showed much slower renal function recovery after acute renal injury, indicating that functional EGFR activity is an essential component of the kidney's ability to recover from acute injury and that EGFR may regulate genes involved in growth, repair, and cell survival in the kidney [105].

In contrast, a potentially detrimental role for EGFR and its ligands in progressive renal disease was demonstrated in mice with selective kidney proximal tubule overexpression of a dominant negative EGFR (DN-EGFR) construct. In response to subtotal renal ablation, these mice had significantly less tubulointerstitial injury than their wild type littermates [91]. Studies using JunD knockout further supported these findings and indicated that TGF- α -dependent paracrine activation of EGFR is central to this model of progressive nephron injury [106].

Angiotensin II (Ang II) plays an important role in many conditions leading to progressive renal injury [107]. Recent studies in both renal and nonrenal cells have demonstrated that many of the proliferative and mitogenic effects of Ang II are mediated through transactivation of the EGF receptor [107,108]. As described above, several ligands of the EGF receptor family, including HB-EGF and TGF- α , are generated following proteolytic cleavage of the cell surface precursors in response to specific signals that initiate downstream signaling cascades, such as the stimulation of Akt, mitogen-activated protein (MAP) kinase and cell proliferation [107, 108]. Both ligand-dependent EGFR activation and ligand-independent EGFR transactivation through src-mediated pathways appear to be involved in Ang II signaling [109].

Severe renal lesions following chronic Ang II infusion were significantly reduced in transgenic mice expressing a dominant negative EGFR in the proximal tubular cells [107]. Immunohistochemical analysis reveals that Ang II treatment causes marked induction and redistribution of ADAM17 (also known as Tumor necrosis factor- α Converting Enzyme, TACE), which cleaves TGF- α , to the apical membranes of distal renal tubules [107,110]. Inactivation of the gene encoding TGF- α or treatment with the specific TACE inhibitor, WTACE2, prevented lesion development in Ang-II-treated mice [107,110].

Non-physiological EGFR activation has also been implicated in renal cystic diseases (RCD) as a promoter of epithelial hyperplasia in cystic epithelia, renal cyst formation and progressive cyst enlargement in murine models of RCD, as well as human ADPKD (Adult Dominant Polycystic Kidney Disease) and ARPKD (Adult Recessive Polycystic Kidney Disease) [82]. Cystic epithelial cells from patients with ARPKD or ADPKD are unusually susceptible to the proliferative stimuli of EGF. Studies have shown increased expression of a number of EGFR ligands in renal cystic epithelia, including EGF, TGF- α , HB-EGF and amphiregulin [82]. Moreover, the cyst fluid of CKD patients contains mitogenic concentrations of EGFR ligands [111–113]. In addition to these quantitative defects (overexpression), qualitative defects in expression of one or more members of the ErbB receptors may also play an important role in cyst development and/or growth. ErbB receptors have been shown to “mislocalize” to the apical (luminal) surface of cysts, where they are in contact with the ligands present in the cyst fluid [114,115]. In this regard, overexpression of ErbB2 resulted in tubular hyperplasia and the development of renal cysts in transgenic mice [116].

Ligand dependent, non-physiological activation of EGFR has also been associated with renal cell carcinoma (RCC) [117,118]. RCC is the most prevalent form of kidney cancer and is

frequently associated with loss of von Hippel-Lindau (VHL) gene function, resulting in aberrant Hypoxia-Inducible Factor (HIF)-dependent transcriptional activation of genes that contribute to tumor growth and metastasis, including TGF- α [119–121]. Constitutive/chronic EGFR activation may contribute to uncontrolled cell division. In addition, EGFR activation may lead to the inhibition of apoptosis. EGFR is expressed in 50%–90% of RCCs [122,123]. EGFR inhibition sensitizes RCC cells to the cytotoxic effects of various anti-cancer drugs including bortezomib [118]. However, clinical trials using EGFR kinase inhibitors (gefitinib, erlotinib) in RCC have been disappointing to date. Gefitinib (Iressa), a specific small-molecule tyrosine kinase inhibitor (TKI) of EGFR failed to demonstrate any objective responses in a small Phase II study of 16 patients with advanced RCC compared to its 10% to 18.5% response rates in advanced nonsmall cell lung carcinoma (NSCLC) [124]. A similar disappointing outcome came from the Phase II trial using 40 patients and an alternative EGFR TKI, erlotinib [125] as well as Cetuximab, a chimeric mouse/human MoAb that binds EGFR [126].

Conclusion

There is developmentally regulated expression of the ErbB family receptors and respective ligands along the nephron, and *in vitro* and *in vivo* studies have provided insights into the role of ErbB signaling in renal development. Furthermore, studies have indicated important roles for ErbB receptor signaling in regulation of renal physiologic responses, including regulation of renal hemodynamics and tubular function.

ErbB signaling mediates renal cell proliferation, motility and migration, suggesting a role in renal protection and recovery from acute injury. On the other hand, dysregulated ErbB signaling may be involved in the development of progressive fibrotic renal injury, polycystic kidney disease and renal cell cancer. Therefore, depending on localization, type, severity and duration of the inciting stimulus, the same ErbB signaling-mediated cellular mechanisms may result in either a beneficial or a detrimental outcome in the kidney (see Figure).

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1. . EGFR activation and its potential association with renal physiology/pathophysiology

In normal intact epithelial cells, EGFR activation is tightly regulated. Epithelial injury induces cleavage of receptor ligands and results in activation of signaling pathways downstream of EGFR. While acute activation of the receptor is beneficial and may participate in repair due to proliferation and migration, chronic activation is detrimental and may participate in dedifferentiation and dysregulated proliferation (cystic diseases), dysregulated matrix deposition (fibrotic diseases) or carcinogenic transformation (renal cell carcinoma).

Table 1
The expression of ErbBs and their ligands in normal kidney

ErbBs/Ligands	Dev. kidney	Adult kidney			TAL ²	DCT ³	CD ⁴	Cellular localization	reference
		glomeruli	PCT ¹						
ErbB1	+	+	+		-	+	+	basolateral	[46,54,55,127,128]
ErbB2	+	+	+		+	+	+/++	basolateral	[39]
ErbB3	+	-	-		-	+	+	basolateral	[40]
ErbB4	+ UB ⁵	-	+		+	++	++	basolateral	[41,42]
EGF	+	-	-		++	+	+	apical and basolateral	[19,24,52,55,129]
HB-EGF	+ (UB)	-	+		+		+	apical and basolateral	[31,41,47,99,103]
TGF- α	++	-	-		-	+	+	Cytoplasmic and basolateral	[24,49,52,55]

Tissues were scored as a relatively expression level: negative (-); weakly positive (+); moderate to strong positive (++)

- ¹PCT, proximal convoluted tubules;
- ²TAL, thick ascending limbs;
- ³DCT, distal convoluted tubules;
- ⁴CD, collecting ducts;
- ⁵UB, ureteric buds.