Epidermal growth factors in the kidney and relationship to hypertension

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MEMBERS OF THE EPIDERMAL GROWTH factor (EGF) family are small mitogenic proteins involved in a number of mechanisms such as normal cell growth and differentiation. Their effects are mediated via autocrine, paracrine, or endocrine mechanisms. ErbB receptors have been recognized as targets in anticancer therapy and are now used in the treatment of breast and colon malignancies (93). The name and gene symbol ErbB (Table 1) was derived from a viral oncogene to which these receptors are homologous: erythroblastic leukemia viral oncogene. Multiple epithelial cancers, including kidney carcinoma, are associated with the EGFR-EGF axis (61, 73, 111, 134). In addition to cancer biology, EGFR receptor (EGFR) activation is critical in acute kidney injury (AKI) and chronic kidney diseases (CKD; Refs. 71, 108, 130). Here we discuss recent results supporting functional interactions between EGF-family proteins and blood pressure control in the kidney, as well as provide details of some mechanisms underpinning such interactions and the clinical consequences of these modulations. Particular emphasis is placed onto the mechanisms of regulation of sodium transport in the kidney by the EGF family. Hence we aim to provide critical insight into the physiological control of kidney function by EGF and its related growth factors.

ErbB Receptors

The EGFR family, also known as ErbB receptors (91), consists of four transmembrane receptors that belong to the receptor tyrosine kinase superfamily and includes EGFR (ErbB1/HER-1), ErbB2 (neu/HER-2), ErbB3 (HER-3), and ErbB4 (HER-4). EGFR as well as other receptors in this family is a transmembrane receptor that consists of an extracellular domain with conserved two cysteine-rich domains necessary for ligand binding; a single membrane-spanning domain, which has a passive role in signaling and functions as an “anchor” of receptor in plasma membrane (53), and a cytoplasmic protein tyrosine kinase domain, where six tyrosine autophosphorylation sites are located (Fig. 1). After ligand binding, ErbB receptors dimerize, which is a required critical step for intrinsic receptor tyrosine kinases to be activated and thereby specific tyrosine-containing residues to become autophosphorylated (12). Phosphorylated ErbB receptors can recruit different adapter proteins with Src homology domain-2 (SH2) or phosphotyrosine binding domains (PTB; Ref. 8). Signals from dimerized, activated ErbB receptors lead to activation of multiple intracellular signal transduction pathways. Depending on the pairing of ErbB family receptors (homodimers or heterodimers; Fig. 1), there can be downstream stimulation of different combinations of signaling pathways (43). To some extent, EGFR is expressed in every nephron segment. Although EGFR is the predominant ErbB receptor found in the normal adult mammalian kidney tubule, ErbB2, ErbB3, and ErbB4 are also expressed in the kidney but mainly localize to the distal and connecting tubules and collecting ducts (87, 130, 131, 133). Figure 2 demonstrates representative immunohistochemical staining for ErbB2 receptor in the kidney cortex of Sprague-Dawley rat. A number of various mechanisms control ErbB receptors trafficking and degradation (64, 99) as well as polarized distribution (18, 127) in the renal epithelial cells.
Regulation of ErbB receptors function is controlled by their ligands, members of the EGF-related peptide growth factor family (39). All EGF-family members, like many other growth factors, are derived from membrane-bound precursor proteins (97, 128). There are at least 12 ligands identified so far that can bind ErbB receptors and induce dimerization of distinct functional heterodimers (27). Heparin binding-EGF (HB-EGF), betacellulin, and epiregulin can bind to either EGFR or ErbB4. Neuregulins-1 and -3 and -4 are specific only for ErbB4 (71). Currently, no ligands have been identified for ErbB2 homodimers. However, ErbB2 is the preferred heterodimerization partner of other ErbB family members and ErbB2-containing heterodimers have the strongest signaling output (36, 114). Interestingly, the sequence of the ErbB3 catalytic domain suggests that this receptor does not have receptor tyrosine kinase activity (17). Thus ErbB3 may function as a platform for heterodimerization and subsequent transphosphorylation by other members of the ErbB family. Hence ErbB2 and ErbB3 can be activated through heterodimerization (6, 17).

Most of the ErbB-family ligands are expressed in the kidney (see Table 2 for some examples), and their expression could be dramatically changed during renal development or in pathological conditions (49, 71, 130). EGF and its related growth factors are present in the lumen at levels much higher than in plasma. However, ErbB receptors are expressed at the basolateral surfaces of tubules in normal adult kidneys (see Fig. 2). This may serve as a protective mechanism for the distal nephron, which can be disturbed under some pathophysiological conditions. One of the examples of apicobasal polarity abnormalities is mislocalization of ErbB receptors that is observed in a variety of genotypic and phenotypic animal models as well as in humans with polycystic kidney disease (PKD; Ref. 127). Another potential mechanism that emerges under some pathological conditions is a disturbance of epithelial cellular integrity. Following tubular injury, tight junctions can be disrupted and ErbB receptor ligands can traverse to the basolateral surface and consequently activate corresponding receptors.

**Activation of ErbB Receptors and Consequent Physiological Functions**

As ErbB ligands exist as inactive transmembrane precursors, they need proteolytic cleavage of their ectodomain to be released as mature soluble ligands. All EGF-family members are synthesized as membrane-anchored precursors that can be processed by specific metalloproteinases to release the soluble bioactive factors from the cell surface. This cleavage is performed by a disintegrin and metalloproteinase (ADAM) family members (9, 72, 80, 90) and matrix metalloproteinases (MMPs; Refs. 55, 104) and tightly regulated by different factors. ADAM-dependent EGF ligand shedding can be induced by activation of G protein-coupled receptors (GPCRs), such as ANG II (ATR) or endothelin-1 (ETR) receptors (Fig. 3). A crucial role for ANG II-dependent EGFR transactivation in chronic kidney disease was demonstrated in mice overexpressing the dominant negative form of EGFR (57). MMPs, a large family of proteolytic enzymes that have the capability to degrade extracellular matrix proteins, are also implicated in regulating nephrin formation and the pathogenesis of kidney diseases (13, 107, 121). For instance, it was shown recently that chronic administration of MMP inhibitors...
delays the progression of, and may even reverse, hypertension and diabetic nephropathy (125).

Activation of ErbB receptors by binding to their ligands promotes several biological responses. The physiological role of ErbB receptors is well established, especially in cancer. What is more, the role of ErbB-family members in renal development, physiology, and pathophysiology is also well recognized (130). Under physiological conditions, ErbB activation may mediate either beneficial or detrimental effects to the kidney (130). ErbB signaling is critically involved in cell signaling, cell growth, proliferation, and renal electrolyte homeostasis. Once activated by site-specific phosphorylation, ErbB receptors serve as molecular integrators through either direct phosphorylation of target molecules or by serving as scaffolds for adaptor proteins. The great diversity of ligands and receptor dimer pairs allows activation of numerous signaling pathways that coordinately regulate complex processes including developmental growth control and adult homeostasis.

Effects of EGF-Family Members in the Kidney

The involvement of EGFR signaling in AKI and CKD development has been recently thoroughly reviewed (108). Although the precise mechanisms underlying effects of EGF signaling are not completely clear, numerous studies evidence abnormal EGF-EGFR axis functionality during progression and development of these diseases. Changes in EGFR expression and EGFR phosphorylation were detected in a variety of experimental models of AKI (15, 41, 44, 108) and CKD (16, 42, 56, 63, 109). Pivotal role of HB-EGF expression and EGFR signaling is also demonstrated in rapidly progressive glomerulonephritis (10, 31); abnormalities of apico-basal polarity and expression of EGF-EGFR axis were also described in the PKD epithelia (68, 126, 133).

What is more, EGF-family growth factors are involved in regulation of various epithelial ion channels in the kidney. For instance, EGF stimulates store-operated Ca\textsuperscript{2+} channels in human mesangial cells through an intracellular signaling mechanism involving tyrosine kinase and PKC (66). Later it was shown that EGF activates store-operated Ca\textsuperscript{2+} channels by a PLC-dependent, but inositol 1,4,5-trisphosphate receptor-independent, pathway (60). Polycystin-2, critical in PKD, can be also activated in response to EGF in the kidney epithelial cells (67). The physiological relevance of EGF-induced activation of TRPP2 was supported by animal studies in which homozygous deletion of the Egr gene resulted in cystic dilatation of collecting ducts (112, 113). A role for ErbB receptor activation in the regulation of Mg\textsuperscript{2+} channels in the kidney has also been demonstrated. Genetic analysis has revealed that the melastatin transient receptor potential 6 (TRPM6) channel is mutated in patients with primary hypomagnesemia and secondary hypocalcemia (92, 122). Furthermore, the direct effects of EGF on TRPM6 channels were reported (46, 47, 110) and it was demonstrated that the EGFR inhibitor erlotinib is capable of affecting TRPM6 regulation and thereby altering Mg\textsuperscript{2+} handling in vivo (22).

EGF-Dependent Regulation of Epithelial Na\textsuperscript{+} Channel-Mediated Sodium Transport in the Kidney

The epithelial Na\textsuperscript{+} channel (ENaC) activity is the rate-limiting step for Na\textsuperscript{+} reabsorption across many epithelia, including the distal nephron (7, 101, 102). Dysfunction and aberrant regulation of this channel lead to a spectrum of

<table>
<thead>
<tr>
<th>Protein</th>
<th>Expression Reported</th>
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<tbody>
<tr>
<td>TGF-\alpha</td>
<td>TAL, DCT, CNT, CDs, papilla</td>
</tr>
<tr>
<td>HB-EGF</td>
<td>Podocytes, mesangial cells, parietal epithelial cells, DCT, PCT, CDs</td>
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<tr>
<td>Amphiregulin</td>
<td>PCT, CDs, podocytes, mesangial cells</td>
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<tr>
<td>Epiregulin</td>
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TGF-\alpha, transforming growth factor-\alpha; HB-EGF, heparin binding-epidermal growth factor; PCT, proximal convoluted tubule; TAL, thick ascending limb of Henle’s loop; DCT, distal convoluted tubule; CNT, connecting tubule; CDs, collecting ducts.
EGF FAMILY ACTIVATION IN THE KIDNEY AND HYPERTENSION

EGF Family Ligands and ENaC Activity

EGF (0.1, 0.5, 1, 10, and 100 ng/ml) and TGF-α also resulted in dose-dependent decreases in ENaC-mediated transepithelial current (59). Liu et al. (62) also reported that EGF-family ligands have dose-dependent biphasic effects on amphibian A6 cells. Both TGF-α and EGF chronically decreased sodium transport in a dose-dependent manner. The IC_{50} was 4.7 ng/ml for TGF-α-induced inhibition and 110 ng/ml for EGF-induced inhibition (62). A number of studies analyzed concentrations of EGF-family ligands in plasma and urine of rodents and human. For instance, it was shown that concentrations of EGF in serum of 60-day-old male mice ranged between 0.264 and 0.503 ng/ml (84); significant age and gender variability of EGF level was also noted by the authors. Considering the fact that ErbB receptors localize at the basolateral side and concentration in the lumen is at least 10-fold higher, these concentrations could represent the same range as levels required for ENaC modulation.

At least acute effects of EGF correlate with reactive oxygen species (ROS) production since pretreatment with the nonselective NADPH oxidase activity inhibitor apocynin blunted both generation of ROS and increase in ENaC-mediated current in response to EGF (48). Most likely, an increase in hydrogen peroxide (H_{2}O_{2}) levels mediates this effects since it was shown that H_{2}O_{2} is critical for ENaC activity (23, 65, 69, 103). Furthermore, EGF had no effect in Rac1 knockdowned principal cells (48). It appears that small GTPase Rac1, which has been shown to be implicated in the development of many renal and cardiovascular diseases (54, 76, 95, 96), is a critical protein in transmission of the signal from EGF to ENaC. Rac1 might mediate its effects on ENaC either through the MAPK pathway (62, 100), WAVE proteins (52), or ROS production (48), since Rac1 is also one of the key subunits of the NADPH oxidase complex.

Role of EGF-EGFR Axis in the Development of Hypertension

A link between EGF-EGFR signaling pathway and renal vasculature and nephron functions was identified in several studies. Previous studies revealed that renal cortical expression of EGFR was increased in both prehypertensive and hypertensive salt-sensitive (SS) rats (129). Immunohistochemical analysis demonstrated that EGFR expression was increased in SS rats compared with Sprague-Dawley and Dahl/Rapp salt-resistant rats. Following addition of EGF, autophosphorylation of the EGFR was further enhanced in the primary cells derived from SS rats and this effect was especially strengthened in the renal vasculature (129). Previous EGF binding studies also revealed that SS rats (105) and the spontaneously hypertensive Lyon rats exhibited increased levels of EGFR in freshly prepared kidney and aortic tissue membranes compared with normotensive or hypertensive strains (106). Microarray-based global gene expression analysis in deoxycorticosterone acetate (DOCA)-salt-induced hypertensive rats further demonstrated that chronic treatment with an EGFR inhibitor AG1478 leads to inhibition of the majority of genes associated with development of renal dysfunction and hypertension in this model (5).

Thus expression of EGFR is associated with hypertensive pathology and can potentially play a role in kidney damage associated with high blood pressure. However, it is hard to define whether changes in gene/protein expression are a consequence of hypertension or the primary basis of the diseases. Previous excellent review by Cowley and Roman (19) discussed potential lines of evidence, which could help to evaluate...
ENaC activity is one of the major factors causing salt-sensitive hypertension. Thus, as we recently demonstrated, ENaC activity is significantly enhanced in SS rats fed a high-salt diet compared with salt-resistant SS.13BN consomic strain and SS rats fed a low-salt diet (82). Importantly, the servo-controlled approach, which allowed us to determine the contribution of renal perfusion pressure (RPP) in the development of hypertension and renal injury in SS rats, provides evidence that ENaC is essential for the development of salt-sensitive hypertension. In the servo-controlled studies, the system maintains the RPP to the left kidney of the instrumented SS rat at control level, whereas RPP to the right kidney increases in response to a high-salt diet. Therefore, both left and right kidneys are exposed to an identical systemic neurohormonal and metabolic environment, but controlled levels of RPP within the left kidney protected it from the high pressure. At least, β-ENaC expression (as analyzed by immunohistochemistry) was increased in the uncontrolled kidneys compared with controlled left kidneys (82).

Fig. 5. Scheme illustrating the hypothesized mechanisms responsible for involvement of EGF and ENaC in development of salt-sensitive hypertension.
on both diets (82), which is consistent with upregulation of EGFR expression (105, 129). To directly evaluate the role of EGFR in the development of hypertension and its effect on ENaC activity, EGFR was intravenously infused and blood pressure was continuously monitored. In addition, ENaC activity was assessed at the end of experiments, when EGFR was continuously infused during several days. Infusion of EGFR decreased ENaC activity, prevented the development of hypertension and attenuated renal glomerular and tubular damage (82). As shown on Fig. 4, in vivo EGFR infusion prevents development of salt-induced hypertension (Fig. 4A) in this strain and decreases ENaC activity in collecting ducts, which might mediate EGF effects on blood pressure (Fig. 4, B and C).

We believe that these findings advance our understanding of salt-sensitive hypertension and provide some insight into its molecular basis (Fig. 5). Importantly, recent studies in mice demonstrated that the EGFR monoclonal antibody Cetuximab given intraperitoneally for 8–10 days raised the blood pressure of mice on normal-salt diet by 25 mmHg and that of those on high-salt diets by 34 mmHg (85). The authors proposed that downregulation of ENaC by arachidonic acid metabolites (83, 124) mediates these effects (85). Thus it appears that EGF-EGF signaling is involved in blood pressure regulation not only in salt-sensitive but also in salt-resistant animals.

Conclusion

ErbB family members are implicated in the development of cancer and cardiac and renal diseases such as AKI, CKD, PKD, and hypertension. Therefore, the therapeutic potential of targeting the ErbB receptors and EGFR-family signaling pathways needs further detailed investigations. Undoubtedly, many questions remain regarding regulation of sodium reabsorption and hypertension by ErbB ligands. For instance, receptor specificity plays the key role in these mechanisms. Furthermore, it is not clear if different ligands targeting the same homo- or heteromer work as a complex and whether they have different affinities and different efficacies depending on the target conformation. Also, transactivation of ErbB receptors and the crucial roles of ADAM and MMP proteases are understudied. In addition, we would like to emphasize that special caution should be paid during the treatment of cancer patients with antibodies vs. ErbBs of inhibitors of EGF-ErbB signaling since inhibition of EGF-ErbBs axis could result in development of hypertension as a side effect in patients with predisposition to salt-sensitive hypertension.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

Author contributions: A.S. conception and design of research; A.S. drafted manuscript; A.S., O.P., D.V.I., and T.S.P. edited and revised manuscript; A.S., O.P., D.V.I., and T.S.P. approved final version of manuscript; O.P. and D.V.I. prepared figures.

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EGF IN THE KIDNEY AND HYPERTENSION


