ErbB receptors and their growth factor ligands in pediatric intestinal inflammation

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Abstract

The ErbB tyrosine kinases (epidermal growth factor receptor (EGFR), ErbB2/HER2, ErbB3, and ErbB4) are cell surface growth factor receptors widely expressed in many developing mammalian tissues, including in the intestinal tract. Signaling elicited by these receptors promotes epithelial cell growth and survival, and ErbB ligands have been proposed as therapeutic agents for intestinal diseases of pediatric populations, including inflammatory bowel diseases (IBD), necrotizing enterocolitis (NEC), and inflammation associated with total parenteral nutrition (TPN). Furthermore, emerging evidence points to reduced ErbB ligand expression and thus reduced ErbB activity in IBD, NEC, and TPN models. This review will discuss the current understanding of the role of ErbB receptors in the pathogenesis and potential treatment of pediatric intestinal inflammation, with focus on the altered signaling in disease and the molecular mechanisms by which exogenous ligands are protective.

Inflammatory disorders of the intestine are a major source of morbidity and mortality in the pediatric population. A significant proportion of inflammatory bowel disease (IBD) diagnoses are in children and adolescents, with an apparent increase in incidence over the last two decades [1,2]. Necrotizing enterocolitis (NEC) affects up to 10% of premature infants [3] and has up to 30% mortality [4]. Sepsis and infection are major complications of parenteral nutrition [5], which in rodent models are associated with tumor necrosis factor (TNF)-driven intestinal inflammation [6]. Together, these conditions represent a major burden on public health in the developed world.

The most widely-used therapeutic approaches for these disorders generally focus on anti-inflammatory agents (e.g., corticosteriods or anti-TNF biologicals for IBD) and supportive care. However, currently available agents are not effective in all patients, and relapses are common. Furthermore, significant long-term safety concerns with a number of drugs (possible growth retardation with corticosteriods [7], risk of lymphoma with biologicals [8],

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etc.) limit options with a significant number of patients. Thus, new approaches to treating pediatric intestinal inflammation are desperately needed.

The mixed effectiveness of exclusively anti-inflammatory agents in IBD and NEC may, in part, be because a major element of disease pathophysiology—damage to the intestinal epithelium—is not a direct target of these approaches. For example, mucosal healing is a desirable endpoint in IBD, and in some studies it predicts long-term remission [9,10], but it is generally expected to occur through endogenous mechanisms secondary to the inhibition of inflammation. Thus, strategies aimed specifically at protecting or repairing the epithelium are attractive options to augment anti-inflammatory treatment in these diseases. A major class of molecules which has been studied in this regard are the ErbB family of receptor tyrosine kinases (RTKs) and their cognate ligands, which promote intestinal epithelial cell growth [11,12], survival [13,14], and restitution/wound healing [15,16]. They may therefore be good models for therapeutic agents for intestinal diseases of pediatric populations. Intriguingly, accumulating evidence makes it clear that defects in ErbB signaling occur, and may be causal, in multiple intestinal inflammatory conditions [17–20]. Thus, replacement or reactivation of the ligands and receptors might be effective at directly promoting mucosal healing.

The ErbB family includes the prototypic member epidermal growth factor (EGF) receptor (R)/ErbB1 as well as ErbB2, ErbB3, and ErbB4 (Figure 1A). ErbBs transduce signals from outside the cell by recognizing, with varying affinities and specificities, growth factors from the EGF and heregulin/neuregulin (NRG) families. Most ErbB ligands are detectable in the intestine [6,17]. After ligand binding, ErbBs signal as dimers (both homo- and hetero-dimers) through increased kinase activity and autophosphorylation on c-terminal cytoplasmic tyrosines; these phosphotyrosine residues then provide docking sites for downstream substrates and adapter proteins. The four ErbBs all share significant homology, but have distinct individual properties. EGFR and ErbB4 each have exclusive (EGF and NRG4 respectively, for example) as well as shared (betacellulin and HB-EGF, for example) ligands, while ErbB3 binds a subset of the NRG family. ErbB2 has no known ligand [21], and ErbB3 has greatly attenuated kinase activity relative to ErbB 1, 2, and 4 [22], and thus these family members are thought to signal primarily in hetero-dimers. These distinct properties, as well as a variable set of downstream molecules that can attach to c-terminal phosphotyrosine docking sites [23] provide intricate networks of ligand/receptor/target combinations that are just beginning to be understood.

ErbB activation in cultured intestinal cells promotes cellular outcomes that would be expected to be protective during inflammation (Figure 1B). For example, in colon epithelial cells EGFR stimulates proliferation [12,24], reduction in cytokine-induced apoptosis [13], and migration/wound healing [15,16], both *in vitro* and *in vivo*. Selective responses can be invoked by particular ligand/receptor combinations; NRG4, which exclusively activates ErbB4 [25], signals for mouse colonocyte survival but not proliferation or migration [17]. This suggests that, with better understanding of the relative effects of different ligands and receptors, a significant degree of selectivity in response can be obtained.

Inflammatory bowel disease

EGFR's capacity to promote healing of gastrointestinal ulcers has been appreciated since the 1980s [26]. Since then, a number of studies using rodent models have demonstrated a protective role in intestinal inflammation. Mice with defective EGFR activity [27,28] or lacking the EGFR ligand TGF-α [29] are more susceptible to experimental colitis, while mice over-expressing TGF-α [30] or rats treated with exogenous EGF [31] are resistant. EGFR is furthermore responsible for the effects of some other treatments; for example, proteins derived from the probiotic organism *Lactobacillus rhamnosus GG* ameliorate dextran sulfate sodium colitis in mice by an EGFR- and Akt-dependent mechanism [32]. In that model, the mechanism involved stimulation of metalloproteinase-mediated ligand release [33], which is a common theme for EGFR transactivation by other pathways such as G protein coupled receptors [34] or toll-like receptor/MyD88 signaling [35].

Multiple cellular effects of EGFR signaling contribute to its protective effects in colitis. In addition to promoting epithelial cell survival, proliferation, and restitution, EGF rescues colitis-associated perturbations of transepithelial ion transport [36]. Furthermore, ErbB signaling is not only a target of inflammation but also plays an immunomodulatory role; for example, amphiregulin signals through EGFR to support regulatory T cell function [37].

ErbB2-4 also play protective but distinct roles in murine colitis models. Epithelial-specific knockout of ErbB2 or ErbB3 does not show profound effect on the onset of injury in DSS colitis, but long-term recovery is dramatically impaired in these mice [38]. Additionally, ErbB4 activation, by i.p administration of its selective NRG4 ligand, blocks cytokine-induced colonocyte apoptosis and reduces injury in acute DSS colitis [17]. This response is dependent on PI3K/Akt signaling similar to results seen with EGF. However, unlike EGF, NRG4 has no effect on cell proliferation or migration, apparently acting strictly through an anti-apoptotic pathway. Together these results indicate that ErbB family members play distinct but complementary roles in protecting or repairing the intestinal mucosa.

Evidence from several studies shows that ErbB signaling is altered in IBD, though the results have not always been in agreement. Multiple studies of clinical specimens have reported reduced levels of EGFR or EGFR ligands in ulcerative colitis (UC) or Crohn's disease (CD) patients [19,39]. For example, a recent study of over fifty patients each in healthy control, CD, and UC groups showed reduced serum EGF in the IBD patients [20]. We have also noted a reduction in the ErbB4-specific NRG4 ligand in active UC and CD compared to uninflamed controls [17]. On the other hand, some groups have described increased expression of specific ligands such as amphiregulin in inflamed tissue [40] or increased EGFR [41] and EGFR ligand [42] in rodent colitis models. These apparently contradictory results may be in part a result of assaying expression at different phases of disease (e.g., acute vs. chronic vs. recovery), but may also reflect the natural complexity and interdependency of the different ErbBs and their ligands. For example, we find increased ErbB4 receptor expression in colitis [43] but downregulation of its ligand NRG4, which in IL-10$^{-/-}$ mice actually results in a relative decrease in ErbB4 activation [17]. A thorough understanding of coordinate changes in multiple ligands and receptors, as has recently been attempted in the context of TPN [6], will likely be informative and necessary.

Importantly, EGF was effective in a 2003 double-blind clinical trial with ulcerative colitis [44]. Twelve patients per group were given either EGF or vehicle daily (by enema) for two weeks in combination with oral mesalamine. By two weeks, 10/12 of the EGF patients compared to 1/12 controls were in remission. Remission was maintained for >3 months. As proof of concept, this trial demonstrates the potential effectiveness of ErbB ligands for treating IBD.

Nectotizing Enterocolitis

EGF is present in human milk [45], which is protective against NEC, and premature infants with NEC have reduced intestinal and serum EGF [46]. Two EGFR ligands, EGF and HB-EGF, have been extensively tested in rodent models of NEC. Most studies have used the "formula feeding/hypoxia" (FFH) rat model, in which prematurely delivered rat pups are fed by formula and subjected to several rounds of hypoxia and cold stress, with or without variations including LPS exposure along with formula [47,48]. This protocol induces a reproducible and highly penetrant NEC-like pathology.

Luminal EGF treatment is protective in FFH NEC [49], reducing disease onset and severity and blocking ileal epithelial apoptosis and histological damage [50]. It also improves many of the model's physiological symptoms and molecular signatures. NEC-associated increase in bile acids is ameliorated by EGF [51], as are the loss of barrier integrity and dissolution of tight junctions within the epithelium [52], and the overexpression of IL-18 [53]. Furthermore, a recent study showed that in both human and mouse NEC, EGFR levels are significantly reduced, but amniotic fluid is protective against mouse NEC by dampening TLR4 signaling in an EGFR-dependent manner [18]. Thus there is ample evidence that loss of EGF-driven signaling is associated with NEC development, and replacing this signal may be therapeutic. In fact, a limited 2007 trial with recombinant EGF in severe NEC showed improvement in several clinical parameters [54].

Similar to EGF, HB-EGF administration reduces experimental NEC incidence, severity, and barrier defects [55] in rat pups. This is accompanied by reductions in epithelial apoptosis [56] and increased proliferation and migration [11]. Microcirculation in the intestine, normally compromised in the FFH model, is preserved [57], as are repair-inducing signaling pathways such as MAPK and PI3K [58]. Similar to the rat data, FFH NEC experiments with transgenic mice either over-expressing [59] or deficient [60] in HB-EGF confirmed a protective role for this growth factor.

There has been some disagreement in the literature over the relative effectiveness of EGF vs. HB-EGF [47,48], which may in part be due to differences in the experimental conditions used, for example the presence or absence of LPS added in the FFH model. This variability with apparently small changes suggests the need to expand the study of ErbB ligands in NEC to additional models such as the recently described dithizone/*Klebsiella* mouse protocol [61], in order to test which compounds work well over a range of experimental systems and thus maximize translatability.

Parenteral Nutrition

Intestinal atrophy and barrier dysfunction as a result of TPN appear particularly amenable to improvement with EGF. Treatment rescues the crypt proliferative index [62], reduces mucosal atrophy and bacterial translocation [63], and partly reverses loss of Gln uptake [64] in rats given TPN. The trophic effects of EGF can apparently be enhanced by combined administration of other growth factors such as GLP-2 [65]. As a leaky barrier and associated sepsis are major complications of TPN in pediatric patients [66], these results definitely suggest a role for ErbB signaling in improving outcomes of patients who cannot tolerate enteral nutrition. For example, TPN adds additional complexity to the problem of managing short bowel syndrome after massive resection, and EGF has been shown to be effective in supporting intestinal adaptation in rodent models of SBS with TPN [67,68].

A recent string of papers from the Teitelbaum lab using a mouse model of TPN have demonstrated not only a protective role for EGF in the model, but also a causative role for loss of EGFR activity in the onset of pathology. In this model, animals fed only parenteral nutrition develop intestinal inflammation characterized by decreased proliferation and increased apoptosis in the epithelium, and at the molecular level by reduced signaling through the PI3K/Akt cascade [69]. Forced activation of Akt improved disease, as did supplementation with glutamine [70]. Since both Akt phosphorylation [71] and glutamine transport [64] are targets of EGFR signaling, these results suggest a defect in that pathway, which is what was found. In TPN mice, there is a TNF-driven, TNFR1-dependent loss of EGFR signaling [6]. This was accompanied by decreased expression of most ErbB ligands including EGF, TGF-α, HB-EGF, NRG1, and NRG4. Exogenous EGF improved pathology and survival, interestingly in a TNFR2-dependent manner.

The observation that ligands for ErbB3 and ErbB4 (e.g., NRG1, NRG4) are also altered in TPN suggests that signaling through these receptors is likely dysregulated in disease as well. Loss of NRG4 and an accompanying reduction in ErbB4 signaling would be consistent with our own observations in colitis [17] and NEC models (Castle, McElroy, and Frey, unpublished observations). However, the specific role that altered ErbB3 or ErbB4 signaling might play in TPN is still an open question.

Potential issues with targeting ErbBs in inflammation

Despite numerous in vitro and animal studies over the last two and a half decades and a few small but promising clinical trials, only limited progress has been made towards translating the mucosal protective effects of ErbB RTK signaling into therapeutic use. In large part this is due to reluctance to chronically promote the activity of receptors which, when mutated or over-expressed, may be oncogenic. This is certainly a valid concern, especially given the predisposition of patients with inflammation of the intestine to colorectal cancer development [72,73]. However, several lines of evidence argue against CRC promotion being a major issue for GF therapy: the fundamental difference between mutation of a pathway and activation of the endogenous wild type signaling, possible immuno-modulatory effects of GF treatment, and the idea that the anti-tumorigenic effects of wiping out inflammation may be much greater than any tumor promoting effects.

EGFR, ErbB2, and ErbB3 are indeed over-expressed or expressed in the form of constitutively active mutants in many cases of intestinal neoplasia [74–76]. The role of ErbB4 in CRC is as yet less clear, but its expression has been reported in tumors with high levels in a subset [77]. Thus, the ErbB family members are at least candidate proto-oncogenes. However, there are likely differences between expression at supra-physiologic levels or expression of constitutively active mutants versus activation of wild-type receptor with ligand. Mutant or hyper-expressed receptors exhibit defects in location or duration of signaling [78], and ErbB mutations which inhibit ligand-stimulated down-regulation resulting in inappropriately sustained signaling have been described [79]. In contrast, application of exogenous ligand to wild-type receptor triggers a more acute, physiological response, which in the case of wild type receptors is terminated over time [78]. Furthermore, it was recently shown that loss of endogenous EGFR signaling can in fact promote colon carcinogenesis in mice. When crossed with the EGFR hypomorphic Waved-5 allele, IL-10$^{-/-}$ animals displayed, increased inflammation, colonic crypt hyperproliferation, increased DNA damage in enterocytes, and dramatically accelerated onset of colitis-associated tumors [28]. Similar results were observed in the AOM-DSS model. Thus, while excessive, "always-on" EGFR activity may contribute to tumorigenesis, insufficient signaling through this pathway contributes to chronic inflammation and, in the end, is also tumorigenic. As ErbB signaling levels appear to be reduced in IBD patients [17,19,39], application of ErbB ligands may actually be anticarcinogenic in this setting. Further study into this complex issue is warranted.

In addition to concerns regarding unintended effects of growth factor signaling, protein growth factors may present difficulties with bioavailability, stability, and convenient administration. In the single clinical trial of EGF for ulcerative colitis published to date, it was given in an enema preparation daily for 2 weeks [44], which is not a convenient means for wide-scale treatment. Alternative approaches using coated beads designed to release growth factor only in the lower intestinal tract are possible alternatives. Additionally, promoting ErbB signaling through indirect mechanisms may be a useable approach. For example, EGFR transactivation by *Lactobacillus rhamnosus GG*-derived soluble proteins is effective in mouse models of colitis [32], as is *Lactobacillus*-fermented milk [80]. Evidence suggests that the protective effects of these probiotic-derived proteins is through stimulating local release of EGFR ligand.

Conclusions

Since the observation, decades ago, that urogastrone/EGF is a trophic factor for the intestinal epithelium, growth factor treatment of intestinal inflammatory disorders has been a theoretical possibility. Recent advances defining how EGF-like growth factors and their ErbB receptors affect—and are affected by—inflammatory signaling suggest that this signaling axis still has great potential to treat the epithelial damage in settings such as IBD, NEC, or TPN, perhaps in combination with anti-inflammatory agents to form a two-pronged attack on disease. Ongoing work towards understanding signaling specificity and selective activation mechanisms will likely open the way towards clinical use of ErbB signaling.

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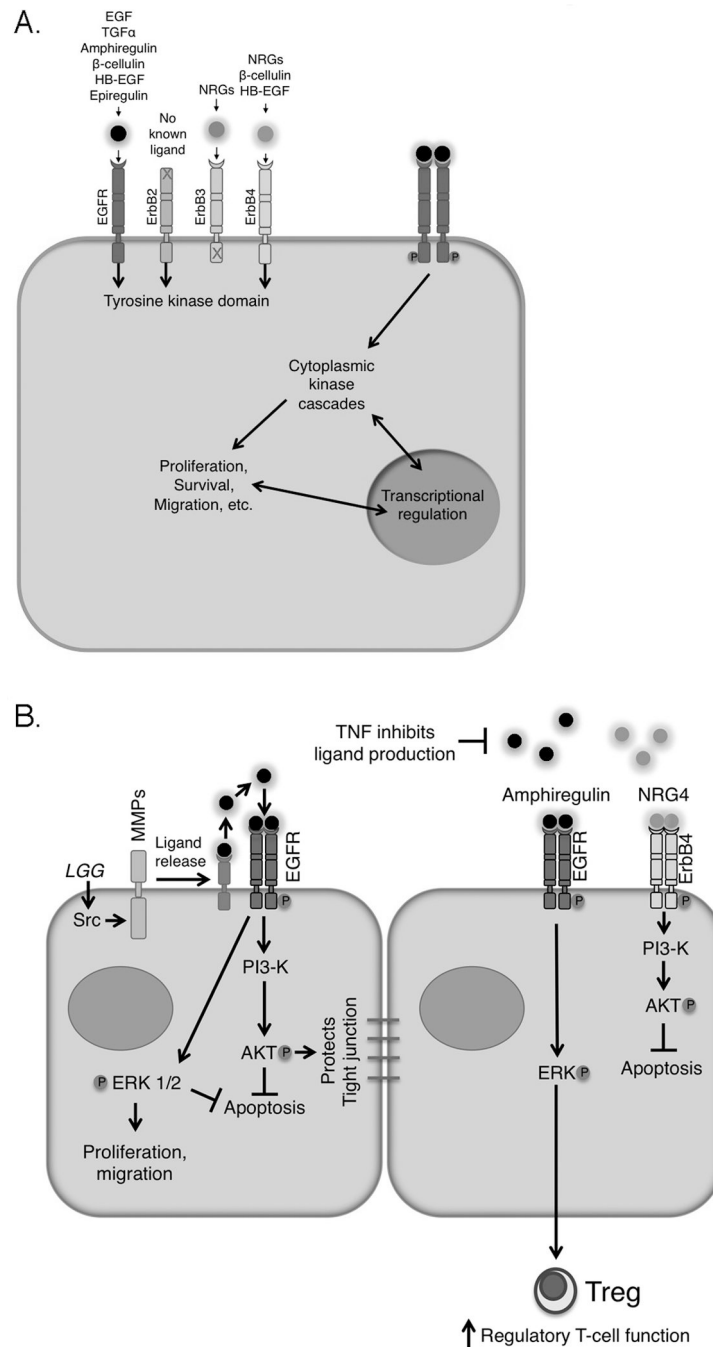
Figure 1.
ErbB receptors and their ligands play key roles in intestinal epithelial integrity. (A) The
ErbB family consists of four receptors which bind a suite of ligands with varying specificity.
Ligand binding promotes homo- and hetero-dimer formation, autophosphorylation, and
downstream signaling involving both cytoplasmic kinases and transcriptional changes. (B)
Multiple protective mechanisms are triggered by ErbB activation, including ERK MAPK
and PI3K/Akt signaling. These cascades restrict apoptosis, promote proliferation and
migration, maintain barrier integrity, and contribute to immune system regulation.

Metalloproteinase-dependent ErbB ligand cleavage and release can be triggered by extracellular signals such as probiotic/commensal bacteria (*Lactobacillus rhamnosus GG* shown as example). However, inflammatory cytokines can inhibit expression of multiple ErbB ligands, potentially limiting responses in the absence of exogenous ligand.