



# Source and Impact of the EGF Family of Ligands on Intestinal Stem Cells

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Epidermal Growth Factor (EGF) has long been known for its role in promoting proliferation of intestinal epithelial cells. EGF is produced by epithelial niche cells at the base of crypts *in vivo* and is routinely added to the culture medium to support the growth of intestinal organoids *ex vivo*. The recent identification of diverse stromal cell populations that reside underneath intestinal crypts has enabled the characterization of key growth factor cues supplied by these cells. The nature of these signals and how they are delivered to drive intestinal epithelial development, daily homeostasis and tissue regeneration following injury are being investigated. It is clear that aside from EGF, other ligands of the family, including Neuregulin 1 (NRG1), have distinct roles in supporting the function of intestinal stem cells through the ErbB pathway.

**Keywords:** Epidermal Growth Factor, neuregulin 1, intestinal stem cells, signaling, niche, ErbB, tissue regeneration, organoids

## INTRODUCTION

A balance between cell proliferation and differentiation is exquisitely controlled in the intestinal epithelium throughout life (Beumer and Clevers, 2021). This is coordinated by a system of cues from surrounding niche cells that includes Paneth cells in the epithelium (Sato et al., 2011), diverse populations of stromal cells (Hageman et al., 2020; McCarthy et al., 2020a; Sphyris et al., 2021), enteric neural cells (Van Landeghem et al., 2011; Talbot et al., 2020) and macrophages (De Schepper et al., 2018; Sehgal et al., 2018). These signals act on the epithelium to modulate stem cell function and cell fate acquisition in progenitor cells (Tetteh et al., 2016). This complex array of cellular inputs has the ability to support the enormous expansion of the intestinal tract during development (Chin et al., 2017) and the strong proliferative response occurring during tissue repair following damage to ensure integrity of the epithelium (Hageman et al., 2020). Maintenance of the barrier and adequate tissue function is vital to prevent systemic infection from enteric pathogens and adequate digestion and nutrient absorption. Stem cells, which reside in the base of intestinal crypts, either self-renew or generate transit-amplifying progenitor cells that ultimately differentiate and generate the diversity of secretory and absorptive differentiated cell types required for a functional epithelium (Beumer and Clevers, 2021). Interplay of key signals from the WNT, Notch, Epidermal Growth Factor (EGF), and Bone Morphogenetic Protein (BMP) signaling pathways regulate the survival, self-renewal and differentiation of these cells to ensure a balance of cell types (Holik et al., 2013; Horvay and Abud, 2013; Tian et al., 2015; McCarthy et al., 2020b; Beumer and Clevers, 2021). The exact mechanisms

of how signals are produced, what cell types secrete and receive signals and how tissues respond to promote the process of regeneration are being investigated. Some of the cell types that secrete molecules that either augment or inhibit WNT and BMP signaling have been described. EGF is present in Paneth cells and has primarily been shown to influence proliferation (Abud et al., 2005; Basak et al., 2017), but it is becoming increasingly clear that other ligands from this family can also influence the diversity of cells within the epithelium (Jardé et al., 2020; Holloway et al., 2021). Whether these ligands have distinct or functionally redundant activities and how signals influence the epithelium in different contexts is still being investigated. In this review, we discuss current evidence on the cellular source and role of ligands from the EGF family and how they interact with receptors in the epithelium to influence cellular proliferation, stem cell identity and lineage differentiation.

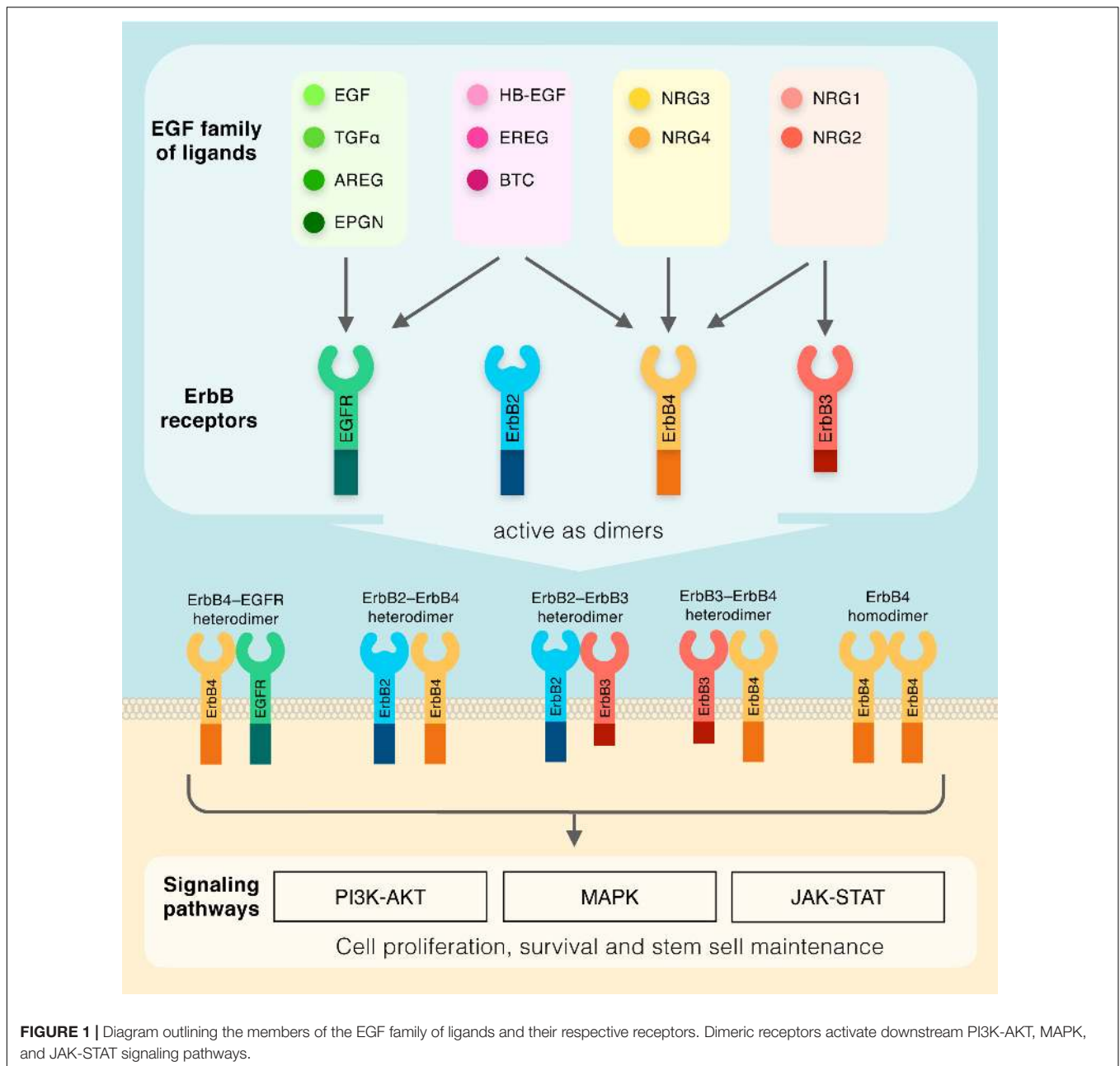
## THE EGF FAMILY OF RECEPTORS AND LIGANDS

The EGF family of ligands includes eleven structurally related proteins, namely EGF, transforming growth factor  $\alpha$  (TGF- $\alpha$ ), amphiregulin (AREG), epigen (EPGN), heparin-binding EGF-like growth factor (HB-EGF), epiregulin (EREG), betacellulin (BTC), and the neuregulins (NRG1-4) (Figure 1). These molecules have in common similar EGF-like motifs, and, due to their membrane-anchored nature, can act in a juxtacrine manner between two neighboring cells, or, in an autocrine/paracrine mode via proteolytic cleavage of the external EGF-like domain, which results in its release in the extracellular compartment (Singh and Harris, 2005; Rayego-Mateos et al., 2018). The EGF-like protein drives cellular signal transduction through the ErbB subclass of the Receptor Tyrosine Kinase superfamily, which consists of four members EGFR (also known as ErbB1), ErbB2, ErbB3, and ErbB4 (Downward et al., 1984; Schechter et al., 1984; Semba et al., 1985; Kraus et al., 1989; Plowman et al., 1993). The EGF family of ligands can be classified into four sub-groups based on distinct receptor binding specificities: (1) the ligands which recognize ErbB1 only (EGF, TGF $\alpha$ , AREG, and EPGN); (2) the ligands binding to both ErbB1 and ErbB4 (HB-EGF, EREG, and BTC); (3) the ligands which are specific for both ErbB3 and ErbB4 (NRG1 and NRG2); and (4) the ligands activating ErbB4 only (NRG3 and NRG4) (Figure 1). It should be noted that no ligands have been identified for ErbB2 to date. Nonetheless, all ErbB receptors contain an extracellular ligand binding site, a single membrane spanning region and a cytoplasmic tyrosine-kinase-containing domain (Lemmon et al., 2014). Upon ligand-induced conformational change, ErbB receptors form homodimers or heterodimers (Figure 1), which activates the intrinsic kinase domain, resulting in the phosphorylation of specific tyrosine residues within the cytoplasmic tails (Lemmon et al., 2014). Phosphorylated residues serve as docking sites for a range of molecules and regulatory proteins involved in various cascades of intracellular signaling, including MAPK, PI3K-AKT, and JAK-STAT (Singh and Harris, 2005; Iwakura and Nawa, 2013). These complex signaling routes regulate many key cellular functions,

including cell proliferation, cell death and stem cell maintenance, which are essential for numerous body systems.

## LOCATION AND FUNCTION OF SIGNALS AND RECEPTORS DURING MAMMALIAN HOMEOSTASIS

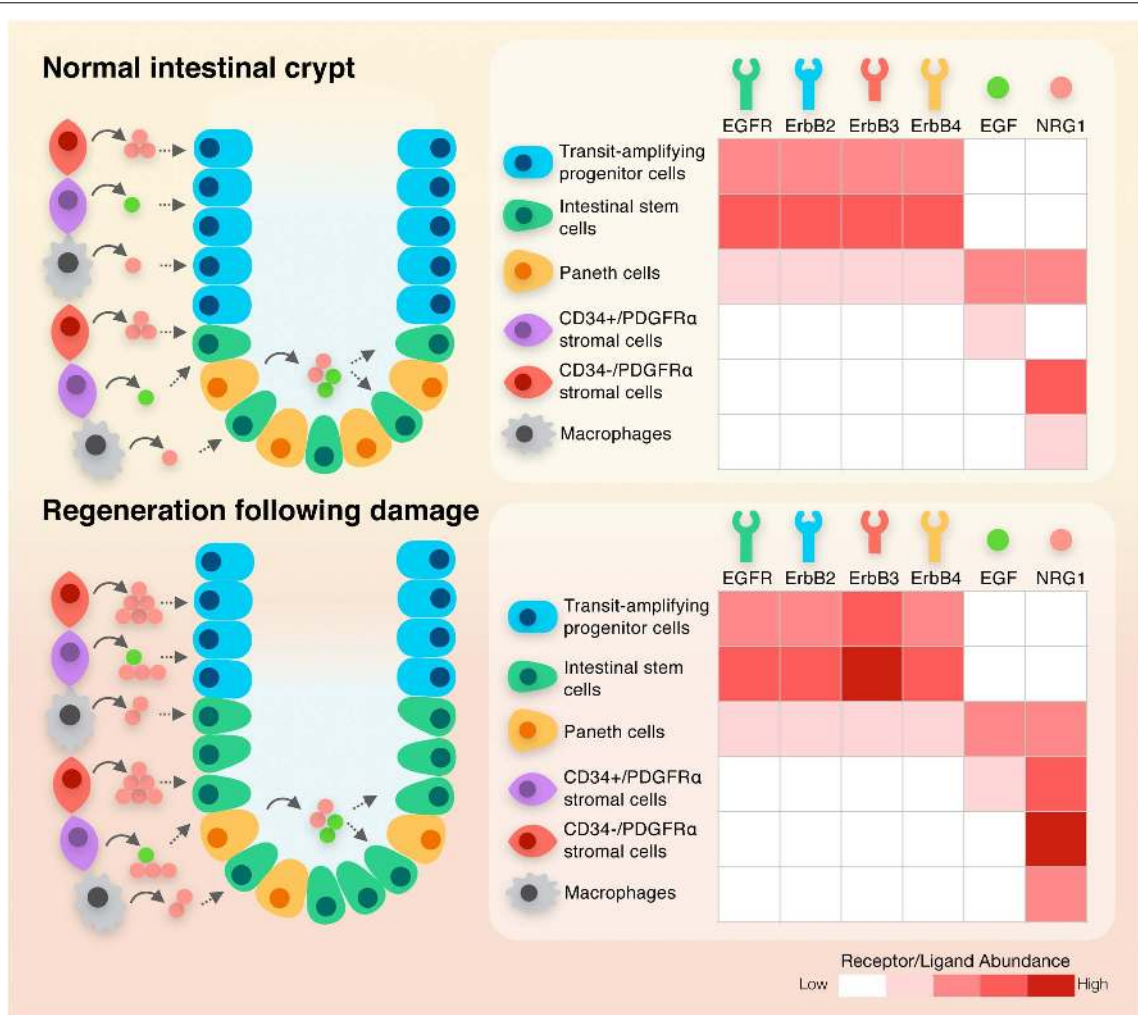
In mice and humans, Paneth cells are localized within the epithelium adjacent to intestinal stem cells in the base of crypts. These specialized cells secrete WNT3, EGF and NRG1 during intestinal homeostasis (Sato et al., 2011; Jardé et al., 2020; Figure 2). Although initially identified as a key constituent of the niche, it is clear that when Paneth cells are depleted *in vivo*, other cellular sources can compensate for this loss, resulting in the maintenance of an intact epithelium (Durand et al., 2012; Farin et al., 2012). The stromal compartment of the adult intestinal tract contains sub-populations of cells including FOXL1+ telocytes that secrete WNT2B and the WNT signaling potentiator RSPO3 (Aoki et al., 2016; Shoshkes-Carmel et al., 2018) and PDGFR $\alpha$ + cells (Greicius et al., 2018; McCarthy et al., 2020b) that also secrete WNT2B and RSPO3. Mesenchymal cells marked by GLI1 also secrete WNT in the colon (Degirmenci et al., 2018). Single cell sequencing has revealed distinct sub-populations that express different levels of PDGFR $\alpha$ , with those expressing higher levels being localized to the villi, and CD34+ PDGFR $\alpha$ <sup>low</sup> cells residing at the base of crypts, where they express *Grem1* and antagonize BMP signaling. These cells described as trophocytes are capable of fully supporting the growth of organoids *ex vivo* (McCarthy et al., 2020b). A similar sub-population of fibroblasts associated with crypts was also recently identified in the colon (Brugger et al., 2020). MAP3K2 expressing cells also reside at the base of crypts where they secrete RSPO1 (Wu et al., 2021). Macrophages identified by expression of CD11b and CSF1R are also closely associated with crypts and when depleted, loss of intestinal stem cells are observed (De Schepper et al., 2018; Sehgal et al., 2018). A unifying global expression analysis of the EGF family of ligands in both epithelial and mesenchymal intestinal niche sub-populations during intestinal homeostasis is currently missing. Re-analysis of recently published single cell RNA sequencing datasets would help understanding the complex ligand dynamics in the intestinal tract (Kinchen et al., 2018; Kim et al., 2020; Buslinger et al., 2021; Yu et al., 2021). Studies, which focused on individual ligands, have shown extremely low levels of EGF can be detected in mesenchymal cell populations, while NRG1 is expressed at relatively high levels in stromal cells, including PDGFR $\alpha$ + cells, and is observed in macrophages (Jardé et al., 2020). Both EGF and NRG1-expressing cells are also found in the developing human intestinal tract, with EGF localized in the epithelial villus domain and NRG1 detected in PDGFR $\alpha$ + cells within the subepithelial mesenchyme underlying crypts (Holloway et al., 2021; Yu et al., 2021). Other members of the NRG family of ligands display exclusive low expression in the mesenchyme (NRG2) or in the epithelium (NRG4) while NRG3 is absent in both compartments in adult intestinal tissues. AREG shares a similar expression pattern with NRG1, with an enrichment in



subepithelial myofibroblasts and in rare F4/80+ macrophage-enriched cells but with limited expression in the epithelium (Inatomi et al., 2006; Shao and Sheng, 2010; Yang et al., 2017). EREG is also localized in an uncharacterized subpopulation of mesenchymal cells and is weakly expressed in the epithelium (Xian et al., 1999; Kallincos et al., 2000; Xia et al., 2003; Lee et al., 2004). In contrast, BTC, HB-EGF, and TGF $\alpha$  are enriched in the intestinal epithelium. The expression pattern of EPGN in both mesenchymal and epithelial compartments is currently unknown. Interestingly, EGFR/ERBB receptors are present within the epithelial cells of the epithelium (Figure 2). EGFR is enriched in stem and progenitor cells while ERBB2 and ERBB3 are detected throughout the crypt – villus axis

(Suzuki et al., 2010; Jardé et al., 2020). Taken together, these data suggest a model where mesenchymal-secreted molecules, which includes NRG1, NRG2, AREG, and EREG, act on epithelial cells *via* a paracrine mechanism while epithelial-produced ligands such as EGF, BTC, HB-EGF, and TGF $\alpha$  regulate cellular function in an autocrine manner. It is important to note that the processes and signaling pathways regulating ligand production during intestinal homeostasis in these particular sub-types is not clearly defined. In addition, a comparative analysis of ligand-mediated downstream signaling pathways in normal intestinal cells is not available.

Numerous mouse knockout studies have investigated the functional roles of individual ligands (summarized in Table 1).



**FIGURE 2 |** Schematic diagram depicting the expression of ligands and receptors within the different cellular compartments of the intestinal crypt during normal homeostasis and following regeneration.

There is likely to be some functional redundancy given triple knockout of three of the ligands EGF, AREG, and TGF $\alpha$  has little effect on the intestinal epithelium with animals being viable and fertile (Luetteke et al., 1999). Similarly, single knockout of the other EGF ligands does not affect embryonic development, with the exception of *Nrg1*, which results in aberrant heart and neural development leading to embryonic lethality (Meyer and Birchmeier, 1995; Erickson et al., 1997). Although knockout of EGF in mice produces little effect on the epithelium, the loss of NRG1 under homeostatic conditions produces a significant reduction in proliferative stem and progenitor cells (Jardé et al., 2020; Table 1). Loss of each individual ERBB receptor is lethal and produces severe defects with significant intestinal abnormalities observed (Gassmann et al., 1995; Lee et al., 1995; Miettinen et al., 1995; Threadgill et al., 1995; Erickson et al., 1997; Riethmacher et al., 1997). Genetic background has a considerable effect on the penetrance of phenotypes (see Table 1) with knockout of EGFR exhibiting variable phenotypes from peri-implantation lethality to viable animals with multi-organ

defects (Sibilia and Wagner, 1995; Threadgill et al., 1995). Individual intestinal-specific knockout of ERBB receptors has also been reported to have minimal phenotypic effects during tissue homeostasis, suggesting some functional redundancy (Lee et al., 2009; Zhang et al., 2012; Almohazey et al., 2017; Srivatsa et al., 2017). However, double and/or triple knockout combinations that are intestinal-specific will be required to confirm this.

## ROLE OF THE EGF FAMILY OF LIGANDS AND RECEPTORS DURING REGENERATION FOLLOWING INJURY

The intestinal epithelium is a selective permeable barrier that permits uptake of nutrients from the luminal contents while forming a barrier against the toxic by-products of digestion and pathogenic bacteria (Beumer and Clevers, 2021). As the

**TABLE 1 |** Knockout mouse models of EGF family of ligands and receptors and their associated phenotypes.

Ligand	Phenotype	Study
<i>Egf</i>	<i>Egf</i> null mice are viable and fertile and display no overt phenotype, including in the gastrointestinal tract.	Luetteke et al., 1999
<i>Areg</i>	<i>Areg</i> KO mice are viable and fertile and display no overt phenotype, including in the gastrointestinal tract.	Luetteke et al., 1999
<i>Areg</i>	Loss of <i>Areg</i> significantly decreases the number of regenerating crypts following radiation-induced injury.	Shao and Sheng, 2010
<i>Tgfa</i>	<i>Tgfa</i> mutant mice are viable and fertile, but exhibit hair and eye defects.	Luetteke et al., 1993
<i>Egf/Areg/Tgfa</i>	Triple KO mice survive to maturity and display hair and eyes abnormalities consistent with single <i>TGFα</i> KO.	Luetteke et al., 1999
<i>Epgn</i>	Homozygous mutant mice are viable and fertile, and display no abnormal phenotype.	Dahlhoff et al., 2013
<i>HB-Egf</i>	KO mice are viable and fertile. Normal gastrointestinal tract architecture, but heart valve malformation.	Jackson et al., 2003
<i>HB-Egf</i>	Mutant mice are viable, but exhibit heart malformation.	Iwamoto et al., 2003
<i>Ereg</i>	Epiregulin null mice are morphologically normal and display no overt abnormal phenotype, including in the gut. However, KO mice display increased susceptibility to DSS-induced intestinal damage.	Lee et al., 2004
<i>Ereg</i>	KO mice are viable, but display chronic dermatitis.	Shirasawa et al., 2004
<i>Btc</i>	Mutant mice are viable, fertile and display normal growth. No overt phenotype.	Jackson et al., 2003
<i>Nrg1</i>	Mutant animals die during development and display heart and nervous system aberrant phenotypes.	Meyer and Birchmeier, 1995
<i>Nrg1</i>	Embryonic lethality of mice deficient in Neuregulin Igl domain. Abnormal heart and cranial nerve development.	Kramer et al., 1996
<i>Nrg1</i>	<i>Nrg1</i> null embryos die at E10.5 due to abnormal heart development.	Erickson et al., 1997
<i>Nrg1</i>	Inducible loss of <i>Nrg1</i> in adults affects intestinal cell proliferation and stem cell maintenance during tissue homeostasis and regeneration.	Jardé et al., 2020
<i>Nrg2</i>	<i>Nrg2</i> KO pups are viable but significantly smaller than their littermates. Analysis of major organs revealed no obvious changes.	Britto et al., 2004
<i>Nrg2</i>	Mutant mice are viable, but exhibit behavioral disorders.	Yan et al., 2018
<i>Nrg3</i>	KO mice are viable and fertile, but exhibit behavioral disorders.	Hayes et al., 2016
<i>Nrg4</i>	<i>Nrg4</i> mutant mice are viable, but display metabolic disorders.	Wang et al., 2014
Receptor	Phenotype	Study
<i>Egfr</i>	<i>Egfr</i> KO on a CF-1 background results in peri-implantation death. On a 129/Sv background, homozygous mutants die at mid-gestation due to placental defects. CD-1 mutants live for up to 3 weeks and show abnormalities in numerous tissues, including the gastrointestinal tract.	Threadgill et al., 1995
<i>Egfr</i>	Embryonically lethal, but some mutant mice survive for up to 8 days after birth and display abnormal development, including in the gut.	Miettinen et al., 1995
<i>Egfr</i>	<i>Egfr</i> mutant fetuses on a 129/Sv background are retarded in growth and die at mid-gestation due to placental defects. Some mice on a 129/Sv - C57BL/6 background survive until birth and to postnatal day 20 on a 129/Sv - C57BL/6 - MF1 background. Newborn mutant mice display skin and lung phenotypes, but normal gastrointestinal tract.	Sibilia and Wagner, 1995
<i>Egfr</i> (i)	Mice harboring intestinal specific loss of <i>Egfr</i> are viable and display no obvious gut abnormalities.	Srivatsa et al., 2017
<i>ErbB2</i>	<i>ErbB2</i> null embryos die before E11 due to abnormal cardiac and neural development.	Lee et al., 1995
<i>ErbB2</i>	<i>ErbB2</i> KO embryos die on E10.5 and display cardiac and neural malfunction.	Erickson et al., 1997
<i>ErbB2</i> (i)	Mice harboring intestinal specific loss of <i>ErbB2</i> are viable and display no obvious gut abnormalities. However, <i>ErbB2</i> is required for tissue regeneration following DSS mediated injury.	Zhang et al., 2012
<i>ErbB3</i>	<i>ErbB3</i> null embryos die at E10.5 due to neural defect. No developmental defects in epithelia of <i>ErbB3</i> mutant embryos.	Riethmacher et al., 1997
<i>ErbB3</i>	<i>ErbB3</i> loss is embryonically lethal at E13.5. Mice display cardiac, neural and gastrointestinal defects.	Erickson et al., 1997
<i>ErbB3</i>	<i>ErbB3</i> KO results in embryonic lethality.	Lee et al., 2009
<i>ErbB3</i> (i)	The intestinal epithelium of mice with intestine-specific genetic ablation of <i>ErbB3</i> exhibits no cytological abnormalities. However, <i>ErbB3</i> KO mice display more severe intestinal injury mediated by DSS.	Lee et al., 2009
<i>ErbB3</i> (i)	Deletion of intestinal epithelial <i>ErbB3</i> in adult mice do not cause defects in architecture of the small intestine or colon. However, <i>ErbB3</i> is required for tissue regeneration following DSS-mediated injury.	Zhang et al., 2012
<i>ErbB3</i> (i)	Intestinal epithelial <i>ErbB3</i> KO causes early appearance of Paneth cells. <i>ErbB3</i> KO mice are more sensitive to intestinal damage mediated by DSS.	Almohazey et al., 2017
<i>ErbB4</i>	<i>ErbB4</i> loss is embryonically lethal. Mice display cardiac and neural defects.	Gassmann et al., 1995
<i>ErbB4</i> (i)	Deletion of intestinal epithelial <i>ErbB4</i> in adult mice do not cause intestinal defects.	Almohazey et al., 2017

(i) indicates intestinal specific deletion.

epithelial monolayer is exposed to an extremely harsh chemical and mechanical environment, it is highly vulnerable to damage. This is partially compensated for by the daily dynamic renewal

of the epithelial layer, with differentiated cells being replaced every few days via the activity and neutral competition of a small population of stem cells (Snippert et al., 2010). Damage

induced by pathogenic bacteria that primarily impacts the villi is rapidly repaired, but severe infection can also compromise the function of stem cells deep within crypts and result in more extensive damage (Mileto et al., 2020). Inflammation and treatments such as chemotherapy and radiotherapy also impact the integrity of cells within crypts. Recovery from these insults involves a repair and regeneration process that involves extensive remodeling of both cells within the epithelium and the surrounding niche cells (Hageman et al., 2020). Following injury, macrophages are recruited and secrete WNT ligands and IL6 (Taniguchi et al., 2015), innate lymphoid cells secrete IL22 (Aparicio-Domingo et al., 2015), stromal cells are remodeled (Kinchen et al., 2018) and there are distinct changes in the extracellular matrix that generate the mechanical cues to activate YAP/TAZ signaling in the epithelium (Yui et al., 2018). Strikingly, despite the widely reported observation of EGF stimulating proliferation of intestinal cells, there is little change in the expression of EGF during the regenerative response following injury in the intestinal epithelium (Jardé et al., 2020; **Figure 2**). In contrast, NRG1 is robustly up-regulated following injury in macrophages, endothelial cells and in PDGFR $\alpha$ + stromal cells (**Figure 2**). The effect of NRG1 on the epithelium induces both a strong proliferative response and induction of stem cell characteristics in regenerating crypts (Jardé et al., 2020). AREG and EREG have also been observed to be induced in epithelial cells following injury, with knockout animals displaying a significant decrease in the number of regenerating crypt domains and a more significant weight loss following injury, respectively (Lee et al., 2004; Shao and Sheng, 2010; **Table 1**). The expression of ErbB3 is up-regulated during regeneration (Jardé et al., 2020) and the requirement for ErbB receptor function during tissue regeneration has been clearly demonstrated using knockout models. Indeed, loss of epithelial ErbB2 or ErbB3 decreases the ability of the intestine to efficiently regenerate following DSS-mediated injury (Lee et al., 2009; Zhang et al., 2012; Almohazey et al., 2017; **Table 1**).

## UTILIZING ORGANOID CULTURES TO INTERROGATE CONTRIBUTIONS OF LIGANDS AND DIFFERENTIAL ACTIVATION OF SIGNALING PATHWAYS

It is clear that coordination of stem cell maintenance, progenitor proliferation and differentiation of mature cell types in the intestinal epithelium is orchestrated by gradients of active growth factors, agonists and antagonists *in vivo* (Tian et al., 2015; Basak et al., 2017; McCarthy et al., 2020b; Beumer and Clevers, 2021). The behavior of cells can change depending on the threshold and length of active signals and crosstalk between signaling pathways. Intestinal organoid culture was developed based on substitution of key *in vivo* niche signals (Sato et al., 2009, 2011) with Matrigel providing the appropriate stiffness to mimic the extracellular matrix. Under these conditions, the epithelium has the capacity to self organize, with many different epithelial cell types forming from single stem cells (Sato et al., 2009). Although

this system has clear limitations, it provides an opportunity to study the intestinal epithelial population in isolation. Complex cellular interactions can be replicated by performing co-cultures with fibroblasts, nerve cells and immune cells (Kabiri et al., 2014; Rogoz et al., 2015; Pastula et al., 2016), in which genetic deletion of specific ligands can be performed and the impact on epithelial cells characterized. The effect of the microbiome and its by-products can also be evaluated (Mileto et al., 2020). However, and as opposed to applying the ligand-mediated stimulation in an unspecific manner in the current organoid technology, new systems that fully replicate the growth factor gradients observed *in vivo*, including WNT and EGF enrichment at the bottom of intestinal crypts, are required. Indeed, recent technological advances have allowed preservation of such complex tissue systems and gradients in culture using intestine- and organoid-on-a-chip models (Wang et al., 2018; Nikolaev et al., 2020). These systems will expand our understanding of complexity of the cellular microenvironment in which the cellular gradients of EGF family of ligands can be tested and assessed.

The relative proportion of different epithelial cell types can be manipulated in organoids by altering the culture conditions. For example, addition of CHIR99021 to elevate WNT signaling can enrich for Lgr5+ stem cells and inhibition of Notch induces secretory cells (Yin et al., 2014). Therefore, the organoid culture system permits the function of different environmental signals and signaling components to be interrogated by addition of proteins, toxins and chemical inhibitors (Clevers and Tuveson, 2019; Hageman et al., 2020). The function of the EGF family of ligands and receptors have been investigated using this methodology. EGF was included in the medium utilized for the first intestinal organoid cultures based on observations that EGF could promote proliferation of intestinal epithelial cells (McKenna et al., 1994; Dignass and Sturm, 2001; Abud et al., 2005; Sato et al., 2009). Along with R-spondin 1, EGF is required to maintain organoid crypt growth. Inhibition of signaling through the EGFR by addition of gefitinib and/or withdrawal of EGF from mouse small intestinal organoid cultures dramatically reduces proliferation within organoids and induces quiescence and an enteroendocrine molecular signature in Lgr5+ cells (Basak et al., 2017). However, one of the limitations in studying EGF signaling in intestinal organoids is the production by niche epithelial cells of EGF itself, which makes the analysis of exogenous supplementation vs. endogenous challenging.

It is important to keep in mind that the composition of the extracellular matrix surrounding epithelial cells in adult tissues, which is not fully replicated in organoid culture, might also affect the downstream molecular response to EGFR/ErbB activation (Yarwood and Woodgett, 2001). Other ligands from the family have also been tested in organoids. NRG1 can substitute for EGF and robustly induces proliferation and budding of mouse small intestinal organoids through prolonged activation of MAPK and AKT signaling that is dramatically more effective than EGF (Jardé et al., 2020). In addition, HB-EGF supports the growth of human adult intestinal organoids in a similar fashion to EGF, which contrasts with the decreased ability of EREG to sustain organoid growth (Fujii et al., 2018). Human fetal enteroid cultures established in either NRG1 or EGF also display

different phenotypes. EGF promotes proliferation and intestinal lineage identity while NRG1 supports cellular diversity and intestinal epithelial stem cell maturation (Holloway et al., 2021; Yu et al., 2021).

## CONCLUSION

A striking feature of the intestinal epithelium is the high turnover of cells which occurs on a daily basis, the dramatic expansion of tissue during embryonic development and the rapid remodeling observed in response to injury. Members of the EGF family of ligands and their receptors contribute substantially to these processes where the current evidence suggests there are distinct functions for particular ligands but also some functional redundancy (Gregorieff et al., 2015; Jardé et al., 2020; Holloway et al., 2021). The downstream signaling pathways that mediate these processes and the crosstalk that may occur with other pathways are less defined. Lineage tracing studies have revealed the high level of plasticity present within intestinal crypts with progeny of Lgr5+ cells having the capacity to de-differentiate following injury to replace the stem cell pool (Tian et al., 2011; Metcalfe et al., 2014; Schmitt et al., 2018; Yu et al., 2018; Ayyaz et al., 2019; Jones et al., 2019; Murata et al., 2020). Although several of the intrinsic signals required for this process have

been identified, the influence of signals in the microenvironment that control this are less clear. It is likely that ligands such as NRG1, which is significantly upregulated in stromal cells during regeneration, play a role (Jardé et al., 2020). Organoid cultures offer a resource to further define these activities, especially for human tissues, and future studies incorporating co-cultures of specific niche cell types will further clarify cellular mechanisms. Defining these signals could ultimately inform strategies to improve epithelial repair in conditions such as inflammatory bowel disease, necrotizing enterocolitis and short gut syndrome.

## AUTHOR CONTRIBUTIONS

HA and TJ wrote the manuscript. WC prepared the figures. HA, TJ, and WC edited and approved the final manuscript. All authors contributed to the article and approved the submitted version.

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## REFERENCES

- Abud, H. E., Watson, N., and Heath, J. K. (2005). Growth of intestinal epithelium in organ culture is dependent on EGF signalling. *Exp. Cell Res.* 303, 252–262. doi: 10.1016/j.yexcr.2004.10.006
- Almohazey, D., Lo, Y. H., Vossler, C. V., Simmons, A. J., Hsieh, J. J., Bucar, E. B., et al. (2017). The ErbB3 receptor tyrosine kinase negatively regulates Paneth cells by PI3K-dependent suppression of Atoh1. *Cell Death Differ.* 24, 855–865. doi: 10.1038/cdd.2017.27
- Aoki, R., Shoshkes-Carmel, M., Gao, N., Shin, S., May, C. L., Golson, M. L., et al. (2016). Foxl1-expressing mesenchymal cells constitute the intestinal stem cell niche. *Cell. Mol. Gastroenterol. Hepatol.* 2, 175–188. doi: 10.1016/j.jcmgh.2015.12.004
- Aparicio-Domingo, P., Romera-Hernandez, M., Karrich, J. J., Cornelissen, F., Papazian, N., Lindenbergh-Kortleve, D. J., et al. (2015). Type 3 innate lymphoid cells maintain intestinal epithelial stem cells after tissue damage. *J. Exp. Med.* 212, 1783–1791. doi: 10.1084/jem.20150318
- Ayyaz, A., Kumar, S., Sangiorgi, B., Ghoshal, B., Gosio, J., Ouladan, S., et al. (2019). Single-cell transcriptomes of the regenerating intestine reveal a revival stem cell. *Nature* 569, 121–125. doi: 10.1038/s41586-019-1154-y
- Basak, O., Beumer, J., Wiebrands, K., Seno, H., van Oudenaarden, A., and Clevers, H. (2017). Induced Quiescence of Lgr5+ Stem Cells in Intestinal Organoids Enables Differentiation of Hormone-Producing Enteroendocrine Cells. *Cell Stem Cell* 20, 177–190.e4.
- Beumer, J., and Clevers, H. (2021). Cell fate specification and differentiation in the adult mammalian intestine. *Nat. Rev. Mol. Cell Biol.* 22, 39–53. doi: 10.1038/s41580-020-0278-0
- Britto, J. M., Lukehurst, S., Weller, R., Fraser, C., Qiu, Y., Hertzog, P., et al. (2004). Generation and characterization of neuregulin-2-deficient mice. *Mol. Cell Biol.* 24, 8221–8226. doi: 10.1128/MCB.24.18.8221-8226.2004
- Brugger, M. D., Valenta, T., Fazilat, H., Hausmann, G., and Basler, K. (2020). Distinct populations of crypt-associated fibroblasts act as signaling hubs to control colon homeostasis. *PLoS Biol* 18:e3001032. doi: 10.1371/journal.pbio.3001032
- Busslinger, G. A., Weusten, B. L. A., Bogte, A., Begthel, H., Brosens, L. A. A., and Clevers, H. (2021). Human gastrointestinal epithelia of the esophagus, stomach, and duodenum resolved at single-cell resolution. *Cell Rep.* 34:108819. doi: 10.1016/j.celrep.2021.108819
- Chin, A. M., Hill, D. R., Aurora, M., and Spence, J. R. (2017). Morphogenesis and maturation of the embryonic and postnatal intestine. *Semin. Cell Dev. Biol.* 66, 81–93. doi: 10.1016/j.semcdb.2017.01.011
- Clevers, H., and Tuveson, D. A. (2019). Organoid Models for Cancer Research. *Annu. Rev. Cancer Biol.* 3, 223–234. doi: 10.1146/annurev-cancerbio-030518-055702
- Dahlhoff, M., Schäfer, M., Wolf, E., and Schneider, M. R. (2013). Genetic deletion of the EGFR ligand epigen does not affect mouse embryonic development and tissue homeostasis. *Exp. Cell Res.* 319, 529–535. doi: 10.1016/j.yexcr.2012.11.001
- De Schepper, S., Verheijden, S., Aguilera-Lizarraga, J., Viola, M. F., Boesmans, W., Stakenborg, N., et al. (2018). Self-Maintaining Gut Macrophages Are Essential for Intestinal Homeostasis. *Cell* 175, 400–415.e13.
- Degirmenci, B., Valenta, T., Dimitrieva, S., Hausmann, G., and Basler, K. (2018). GLI1-expressing mesenchymal cells form the essential Wnt-secreting niche for colon stem cells. *Nature* 558, 449–453. doi: 10.1038/s41586-018-0190-3
- Dignass, A. U., and Sturm, A. (2001). Peptide growth factors in the intestine. *Eur. J. Gastroenterol. Hepatol.* 13, 763–770. doi: 10.1097/00042737-200107000-00002
- Downward, J., Yarden, Y., Mayes, E., Scrace, G., Totty, N., Stockwell, P., et al. (1984). Close similarity of epidermal growth factor receptor and v-erb-B oncogene protein sequences. *Nature* 307, 521–527. doi: 10.1038/307521a0
- Durand, A., Donahue, B., Peignon, G., Letourneur, F., Cagnard, N., Slomianny, C., et al. (2012). Functional intestinal stem cells after Paneth cell ablation induced by the loss of transcription factor Math1 (Atoh1). *Proc. Natl. Acad. Sci. U. S. A.* 109, 8965–8970. doi: 10.1073/pnas.1201652109
- Erickson, S. L., O'Shea, K. S., Ghaboosi, N., Loverro, L., Frantz, G., Bauer, M., et al. (1997). ErbB3 is required for normal cerebellar and cardiac development: a comparison with ErbB2- and heregulin-deficient mice. *Development* 124, 4999–5011. doi: 10.1242/dev.124.24.4999
- Farin, H. F., Van Es, J. H., and Clevers, H. (2012). Redundant sources of Wnt regulate intestinal stem cells and promote formation of Paneth cells. *Gastroenterology* 143, 1518–1529.e7.

- Fujii, M., Matano, M., Toshimitsu, K., Takano, A., Mikami, Y., Nishikori, S., et al. (2018). Human Intestinal Organoids Maintain Self-Renewal Capacity and Cellular Diversity in Niche-Inspired Culture Condition. *Cell Stem Cell* 23, 787–793.e6.
- Gassmann, M., Casagrande, F., Orioli, D., Simon, H., Lai, C., Klein, R., et al. (1995). Aberrant neural and cardiac development in mice lacking the ErbB4 neuregulin receptor. *Nature* 378, 390–394. doi: 10.1038/378390a0
- Gregorieff, A., Liu, Y., Inanlou, M. R., Khomchuk, Y., and Wrana, J. L. (2015). Yap-dependent reprogramming of Lgr5(+) stem cells drives intestinal regeneration and cancer. *Nature* 526, 715–718. doi: 10.1038/nature15382
- Greicius, G., Kabiri, Z., Sigmundsson, K., Liang, C., Bunte, R., Singh, M. K., et al. (2018). PDGFRalpha(+) pericryptal stromal cells are the critical source of Wnts and RSP03 for murine intestinal stem cells in vivo. *Proc. Natl. Acad. Sci. U. S. A.* 115, E3173–E3181.
- Hageman, J. H., Heinz, M. C., Kretzschmar, K., van der Vaart, J., Clevers, H., and Snippert, H. J. G. (2020). Intestinal regeneration: regulation by the microenvironment. *Dev. Cell* 54, 435–446. doi: 10.1016/j.devcel.2020.07.009
- Hayes, L. N., Shevelkin, A., Zeledon, M., Steel, G., Chen, P. L., Obie, C., et al. (2016). Neuregulin 3 knockout mice exhibit behaviors consistent with psychotic disorders. *Mol. Neuropsychiatry* 2, 79–87. doi: 10.1159/000445836
- Holik, A. Z., Krzystyniak, J., Young, M., Richardson, K., Jarde, T., Chambon, P., et al. (2013). Brg1 is required for stem cell maintenance in the murine intestinal epithelium in a tissue-specific manner. *Stem Cells* 31, 2457–2466.
- Holloway, E. M., Czerwinski, M., Tsai, Y. H., Wu, J. H., Wu, A., Childs, C. J., et al. (2021). Mapping Development of the Human Intestinal Niche at Single-Cell Resolution. *Cell Stem Cell* 28, 568–580.e4.
- Horvay, K., and Abud, H. E. (2013). Regulation of intestinal stem cells by Wnt and Notch signalling. *Adv. Exp. Med. Biol.* 786, 175–186. doi: 10.1007/978-94-007-6621-1\_10
- Inatomi, O., Andoh, A., Yagi, Y., Bamba, S., Tsujikawa, T., and Fujiyama, Y. (2006). Regulation of amphiregulin and epi-regulin expression in human colonic subepithelial myofibroblasts. *Int. J. Mol. Med.* 18, 497–503.
- Iwakura, Y., and Nawa, H. (2013). ErbB1-4-dependent EGF/neuregulin signals and their cross talk in the central nervous system: pathological implications in schizophrenia and Parkinson's disease. *Front. Cell Neurosci.* 7:4. doi: 10.3389/fncel.2013.00004
- Iwamoto, R., Yamazaki, S., Asakura, M., Takashima, S., Hasuwa, H., Miyado, K., et al. (2003). Heparin-binding EGF-like growth factor and ErbB signaling is essential for heart function. *Proc. Natl. Acad. Sci. U.S.A.* 100, 3221–3226. doi: 10.1073/pnas.0537588100
- Jackson, L. F., Qiu, T. H., Sunnarborg, S. W., Chang, A., Zhang, C., Patterson, C., et al. (2003). Defective valvulogenesis in HB-EGF and TACE-null mice is associated with aberrant BMP signaling. *EMBO J.* 22, 2704–2716. doi: 10.1093/emboj/cdg264
- Jardé, T., Chan, W. H., Rossello, F. J., Kaur Kahlon, T., Theocharous, M., Kurian Arackal, T., et al. (2020). Mesenchymal Niche-Derived Neuregulin-1 Drives Intestinal Stem Cell Proliferation and Regeneration of Damaged Epithelium. *Cell Stem Cell* 27, 646–662.e7.
- Jones, J. C., Brindley, C. D., Elder, N. H., Myers, M. G. Jr., Rajala, M. W., Dekaney, C. M., et al. (2019). Cellular Plasticity of Defa4(Cre)-Expressing Paneth Cells in Response to Notch Activation and Intestinal Injury. *Cell. Mol. Gastroenterol. Hepatol.* 7, 533–554. doi: 10.1016/j.jcmgh.2018.11.004
- Kabiri, Z., Greicius, G., Madan, B., Biechele, S., Zhong, Z., Zaribafzadeh, H., et al. (2014). Stroma provides an intestinal stem cell niche in the absence of epithelial Wnts. *Development* 141, 2206–2215. doi: 10.1242/dev.104976
- Kallincos, N. C., Xian, C. J., Dunbar, A. J., Couper, R. T., and Read, L. C. (2000). Cloning of rat betacellulin and characterization of its expression in the gastrointestinal tract. *Growth Factors* 18, 203–213. doi: 10.3109/08977190009003245
- Kim, J. E., Fei, L., Yin, W. C., Coquenlorge, S., Rao-Bhatia, A., Zhang, X., et al. (2020). Single cell and genetic analyses reveal conserved populations and signaling mechanisms of gastrointestinal stromal niches. *Nat. Commun.* 11:334.
- Kinchen, J., Chen, H. H., Parikh, K., Antanaviciute, A., Jagielowicz, M., Fawcner-Corbett, D., et al. (2018). Structural Remodeling of the Human Colonic Mesenchyme in Inflammatory Bowel Disease. *Cell* 175, 372–386.e17.
- Kramer, R., Bucay, N., Kane, D. J., Martin, L. E., Tarpley, J. E., and Theill, L. E. (1996). Neuregulins with an Ig-like domain are essential for mouse myocardial and neuronal development. *Proc. Natl. Acad. Sci. U.S.A.* 93, 4833–4838. doi: 10.1073/pnas.93.10.4833
- Kraus, M. H., Issing, W., Miki, T., Popescu, N. C., and Aaronson, S. A. (1989). Isolation and characterization of ERBB3, a third member of the ERBB/epidermal growth factor receptor family: evidence for overexpression in a subset of human mammary tumors. *Proc. Natl. Acad. Sci. U. S. A.* 86, 9193–9197. doi: 10.1073/pnas.86.23.9193
- Lee, D., Pearsall, R. S., Das, S., Dey, S. K., Godfrey, V. L., and Threadgill, D. W. (2004). Epi-regulin is not essential for development of intestinal tumors but is required for protection from intestinal damage. *Mol. Cell Biol.* 24, 8907–8916. doi: 10.1128/mcb.24.20.8907-8916.2004
- Lee, D., Yu, M., Lee, E., Kim, H., Yang, Y., Kim, K., et al. (2009). Tumor-specific apoptosis caused by deletion of the ERBB3 pseudo-kinase in mouse intestinal epithelium. *J. Clin. Invest.* 119, 2702–2713. doi: 10.1172/jci36435
- Lee, K. F., Simon, H., Chen, H., Bates, B., Hung, M. C., and Hauser, C. (1995). Requirement for neuregulin receptor erbB2 in neural and cardiac development. *Nature* 378, 394–398. doi: 10.1038/378394a0
- Leemmon, M. A., Schlessinger, J., and Ferguson, K. M. (2014). The EGFR family: not so prototypical receptor tyrosine kinases. *Cold Spring Harb. Perspect. Biol.* 6:a020768. doi: 10.1101/cshperspect.a020768
- Luetteke, N. C., Qiu, T. H., Fenton, S. E., Troyer, K. L., Riedel, R. F., Chang, A., et al. (1999). Targeted inactivation of the EGF and amphiregulin genes reveals distinct roles for EGF receptor ligands in mouse mammary gland development. *Development* 126, 2739–2750. doi: 10.1242/dev.126.12.2739
- Luetteke, N. C., Qiu, T. H., Peiffer, R. L., Oliver, P., Smithies, O., and Lee, D. C. (1993). TGF alpha deficiency results in hair follicle and eye abnormalities in targeted and waved-1 mice. *Cell* 73, 263–278. doi: 10.1016/0092-8674(93)90228-i
- McCarthy, N., Kraiczky, J., and Shivdasani, R. A. (2020a). Cellular and molecular architecture of the intestinal stem cell niche. *Nat. Cell Biol.* 22, 1033–1041. doi: 10.1038/s41556-020-0567-z
- McCarthy, N., Manieri, E., Storm, E. E., Saadatpour, A., Luoma, A. M., Kapoor, V. N., et al. (2020b). Distinct Mesenchymal Cell Populations Generate the Essential Intestinal BMP Signaling Gradient. *Cell Stem Cell* 26, 391–402.e5.
- McKenna, K. J., Ligato, S., Kauffman, G. L. Jr., Abt, A. B., Stryker, J. A., and Conter, R. L. (1994). Epidermal growth factor enhances intestinal mitotic activity and DNA content after acute abdominal radiation. *Surgery* 115, 626–632.
- Metcalfe, C., Kljavin, N. M., Ybarra, R., and de Sauvage, F. J. (2014). Lgr5+ stem cells are indispensable for radiation-induced intestinal regeneration. *Cell Stem Cell* 14, 149–159. doi: 10.1016/j.stem.2013.11.008
- Meyer, D., and Birchmeier, C. (1995). Multiple essential functions of neuregulin in development. *Nature* 378, 386–390. doi: 10.1038/378386a0
- Miettinen, P. J., Berger, J. E., Meneses, J., Phung, Y., Pedersen, R. A., Werb, Z., et al. (1995). Epithelial immaturity and multiorgan failure in mice lacking epidermal growth factor receptor. *Nature* 376, 337–341. doi: 10.1038/376337a0
- Mileto, S. J., Jarde, T., Childress, K. O., Jensen, J. L., Rogers, A. P., Kerr, G., et al. (2020). Clostridioides difficile infection damages colonic stem cells via TcdB, impairing epithelial repair and recovery from disease. *Proc. Natl. Acad. Sci. U. S. A.* 117, 8064–8073. doi: 10.1073/pnas.1915255117
- Murata, K., Jadhav, U., Madha, S., van Es, J., Dean, J., Cavazza, A., et al. (2020). Ascl2-Dependent Cell Dedifferentiation Drives Regeneration of Ablated Intestinal Stem Cells. *Cell Stem Cell* 26, 377–390.e6.
- Nikolaev, M., Mitrofanova, O., Broguiere, N., Geraldo, S., Dutta, D., Tabata, Y., et al. (2020). Homeostatic mini-intestines through scaffold-guided organoid morphogenesis. *Nature* 585, 574–578. doi: 10.1038/s41586-020-2724-8
- Pastula, A., Middelhoff, M., Brandtner, A., Tobiasch, M., Hohl, B., Nuber, A. H., et al. (2016). Three-Dimensional gastrointestinal organoid culture in combination with nerves or fibroblasts: a method to characterize the gastrointestinal stem cell niche. *Stem Cells Int.* 2016:3710836.
- Plowman, G. D., Culouscou, J. M., Whitney, G. S., Green, J. M., Carlton, G. W., Foy, L., et al. (1993). Ligand-specific activation of HER4/p180erbB4, a fourth member of the epidermal growth factor receptor family. *Proc. Natl. Acad. Sci. U. S. A.* 90, 1746–1750. doi: 10.1073/pnas.90.5.1746
- Rayego-Mateos, S., Rodrigues-Diez, R., Morgado-Pascual, J. L., Valentijn, F., Valdivielso, J. M., Goldschmeding, R., et al. (2018). Role of Epidermal Growth Factor Receptor (EGFR) and Its Ligands in Kidney Inflammation and Damage. *Mediators Inflamm.* 2018:8739473.



- Riethmacher, D., Sonnenberg-Riethmacher, E., Brinkmann, V., Yamaai, T., Lewin, G. R., and Birchmeier, C. (1997). Severe neuropathies in mice with targeted mutations in the ErbB3 receptor. *Nature* 389, 725–730. doi: 10.1038/39593
- Rogoz, A., Reis, B. S., Karssemeijer, R. A., and Mucida, D. (2015). A 3-D enteroid-based model to study T-cell and epithelial cell interaction. *J. Immunol. Methods* 421, 89–95. doi: 10.1016/j.jim.2015.03.014
- Sato, T., van Es, J. H., Snippert, H. J., Stange, D. E., Vries, R. G., van den Born, M., et al. (2011). Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. *Nature* 469, 415–418. doi: 10.1038/nature09637
- Sato, T., Vries, R. G., Snippert, H. J., van de Wetering, M., Barker, N., Stange, D. E., et al. (2009). Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* 459, 262–265. doi: 10.1038/nature07935
- Schechter, A. L., Stern, D. F., Vaidyanathan, L., Decker, S. J., Drebin, J. A., Greene, M. I., et al. (1984). The neu oncogene: an erb-B-related gene encoding a 185,000-Mr tumour antigen. *Nature* 312, 513–516. doi: 10.1038/312513a0
- Schmitt, M., Schewe, M., Sacchetti, A., Feijtel, D., van de Geer, W. S., Teeuwssen, M., et al. (2018). Paneth Cells Respond to Inflammation and Contribute to Tissue Regeneration by Acquiring Stem-like Features through SCF/c-Kit Signaling. *Cell Rep.* 24, 2312–2328.e7.
- Sehgal, A., Donaldson, D. S., Pridans, C., Sauter, K. A., Hume, D. A., and Mabbott, N. A. (2018). The role of CSF1R-dependent macrophages in control of the intestinal stem-cell niche. *Nat. Commun.* 9:1272.
- Semba, K., Kamata, N., Toyoshima, K., and Yamamoto, T. (1985). A v-erbB-related protooncogene, c-erbB-2, is distinct from the c-erbB-1/epidermal growth factor-receptor gene and is amplified in a human salivary gland adenocarcinoma. *Proc. Natl. Acad. Sci. U. S. A.* 82, 6497–6501. doi: 10.1073/pnas.82.19.6497
- Shao, J., and Sheng, H. (2010). Amphiregulin promotes intestinal epithelial regeneration: roles of intestinal subepithelial myofibroblasts. *Endocrinology* 151, 3728–3737. doi: 10.1210/en.2010-0319
- Shirasawa, S., Sugiyama, S., Baba, I., Inokuchi, J., Sekine, S., Ogino, K., et al. (2004). Dermatitis due to epiregulin deficiency and a critical role of epiregulin in immune-related responses of keratinocyte and macrophage. *Proc. Natl. Acad. Sci. U.S.A.* 101, 13921–13926. doi: 10.1073/pnas.0404217101
- Shoshkes-Carmel, M., Wang, Y. J., Wangenstein, K. J., Toth, B., Kondo, A., Massasa, E. E., et al. (2018). Subepithelial telocytes are an important source of Wnts that supports intestinal crypts. *Nature* 557, 242–246. doi: 10.1038/s41586-018-0084-4
- Sibilia, M., and Wagner, E. F. (1995). Strain-dependent epithelial defects in mice lacking the EGF receptor. *Science* 269, 234–238. doi: 10.1126/science.7618085
- Singh, A. B., and Harris, R. C. (2005). Autocrine, paracrine and juxtacrine signaling by EGFR ligands. *Cell Signal.* 17, 1183–1193. doi: 10.1016/j.cellsig.2005.03.026
- Snippert, H. J., van der Flier, L. G., Sato, T., van Es, J. H., van den Born, M., Kroon-Veenboer, C., et al. (2010). Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. *Cell* 143, 134–144. doi: 10.1016/j.cell.2010.09.016
- Sphyris, N., Hodder, M. C., and Sansom, O. J. (2021). Subversion of niche-signalling pathways in colorectal cancer: what makes and breaks the intestinal stem cell. *Cancers* 13:1000. doi: 10.3390/cancers13051000
- Srivatsa, S., Paul, M. C., Cardone, C., Holmann, M., Amberg, N., Pathria, P., et al. (2017). EGFR in Tumor-Associated Myeloid Cells Promotes Development of Colorectal Cancer in Mice and Associates With Outcomes of Patients. *Gastroenterology* 153, 178–190.e10.
- Suzuki, A., Sekiya, S., Gunshima, E., Fujii, S., and Taniguchi, H. (2010). EGF signaling activates proliferation and blocks apoptosis of mouse and human intestinal stem/progenitor cells in long-term monolayer cell culture. *Lab. Invest.* 90, 1425–1436. doi: 10.1038/labinvest.2010.150
- Talbot, J., Hahn, P., Kroehling, L., Nguyen, H., Li, D., and Littman, D. R. (2020). Feeding-dependent VIP neuron-ILC3 circuit regulates the intestinal barrier. *Nature* 579, 575–580. doi: 10.1038/s41586-020-2039-9
- Taniguchi, K., Wu, L. W., Grivnikov, S. I., de Jong, P. R., Lian, I., Yu, F. X., et al. (2015). A gp130-Src-YAP module links inflammation to epithelial regeneration. *Nature* 519, 57–62. doi: 10.1038/nature14228
- Tetteh, P. W., Basak, O., Farin, H. F., Wiebrands, K., Kretschmar, K., Begthel, H., et al. (2016). Replacement of Lost Lgr5-Positive Stem Cells through Plasticity of Their Enterocyte-Lineage Daughters. *Cell Stem Cell* 18, 203–213. doi: 10.1016/j.stem.2016.01.001
- Threadgill, D. W., Dlugosz, A. A., Hansen, L. A., Tennenbaum, T., Licht, U., Yee, D., et al. (1995). Targeted disruption of mouse EGF receptor: effect of genetic background on mutant phenotype. *Science* 269, 230–234. doi: 10.1126/science.7618084
- Tian, H., Biehs, B., Chiu, C., Siebel, C. W., Wu, Y., Costa, M., et al. (2015). Opposing activities of Notch and Wnt signaling regulate intestinal stem cells and gut homeostasis. *Cell Rep.* 11, 33–42. doi: 10.1016/j.celrep.2015.03.007
- Tian, H., Biehs, B., Warming, S., Leong, K. G., Rangell, L., Klein, O. D., et al. (2011). A reserve stem cell population in small intestine renders Lgr5-positive cells dispensable. *Nature* 478, 255–259. doi: 10.1038/nature10408
- Van Landeghem, L., Chevalier, J., Mahe, M. M., Wedel, T., Urvil, P., Derkinderen, P., et al. (2011). Enteric glia promote intestinal mucosal healing via activation of focal adhesion kinase and release of proEGF. *Am. J. Physiol. Gastrointest. Liver Physiol.* 300, G976–G987. doi: 10.1016/j.jss.2005.11.269
- Wang, G. X., Zhao, X. Y., Meng, Z. X., Kern, M., Dietrich, A., Chen, Z., et al. (2014). The brown fat-enriched secreted factor Nrg4 preserves metabolic homeostasis through attenuation of hepatic lipogenesis. *Nat. Med.* 20, 1436–1443. doi: 10.1038/nm.3713
- Wang, Y., Kim, R., Gunasekara, D. B., Reed, M. I., DiSalvo, M., Nguyen, D. L., et al. (2018). Formation of Human Colonic Crypt Array by Application of Chemical Gradients Across a Shaped Epithelial Monolayer. *Cell. Mol. Gastroenterol. Hepatol.* 5, 113–130. doi: 10.1016/j.jcmgh.2017.10.007
- Wu, N., Sun, H., Zhao, X., Zhang, Y., Tan, J., Qi, Y., et al. (2021). MAP3K2-regulated intestinal stromal cells define a distinct stem cell niche. *Nature* 592, 606–610. doi: 10.1038/s41586-021-03283-y
- Xia, G., Rachfal, A. W., Martin, A. E., and Besner, G. E. (2003). Upregulation of endogenous heparin-binding EGF-like growth factor (HB-EGF) expression after intestinal ischemia/reperfusion injury. *J. Invest. Surg.* 16, 57–63. doi: 10.1080/713829588
- Xian, C. J., Mardell, C. E., and Read, L. C. (1999). Specificity of the localization of transforming growth factor-alpha immunoreactivity in colon mucosa. *J. Histochem. Cytochem.* 47, 949–958. doi: 10.1177/002215549904700712
- Yan, L., Shamir, A., Skirzewski, M., Leiva-Salcedo, E., Kwon, O. B., Karavanova, I., et al. (2018). Neuregulin-2 ablation results in dopamine dysregulation and severe behavioral phenotypes relevant to psychiatric disorders. *Mol. Psychiatry* 23, 1233–1243. doi: 10.1038/mp.2017.22
- Yang, Y. P., Ma, H., Starchenko, A., Huh, W. J., Li, W., Hickman, F. E., et al. (2017). A Chimeric Egfr Protein Reporter Mouse Reveals Egfr Localization and Trafficking In Vivo. *Cell Rep.* 19, 1257–1267. doi: 10.1016/j.celrep.2017.04.048
- Yarwood, S. J., and Woodgett, J. R. (2001). Extracellular matrix composition determines the transcriptional response to epidermal growth factor receptor activation. *Proc. Natl. Acad. Sci. U. S. A.* 98, 4472–4477. doi: 10.1073/pnas.081069098
- Yin, X., Farin, H. F., van Es, J. H., Clevers, H., Langer, R., and Karp, J. M. (2014). Niche-independent high-purity cultures of Lgr5+ intestinal stem cells and their progeny. *Nat. Methods* 11, 106–112. doi: 10.1038/nmeth.2737
- Yu, Q., Kilik, U., Holloway, E. M., Tsai, Y. H., Harmel, C., Wu, A., et al. (2021). Charting human development using a multi-endodermal organ atlas and organoid models. *Cell* 184, 3281–3298.e22.
- Yu, S., Tong, K., Zhao, Y., Balasubramanian, I., Yap, G. S., Ferraris, R. P., et al. (2018). Paneth Cell Multipotency Induced by Notch Activation following Injury. *Cell Stem Cell* 23, 46–59.e5.
- Yui, S., Azzolin, L., Maimets, M., Pedersen, M. T., Fordham, R. P., Hansen, S. L., et al. (2018). YAP/TAZ-Dependent Reprogramming of Colonic Epithelium Links ECM Remodeling to Tissue Regeneration. *Cell Stem Cell* 22, 35–49.e7.
- Zhang, Y., Dube, P. E., Washington, M. K., Yan, F., and Polk, D. B. (2012). ErbB2 and ErbB3 regulate recovery from dextran sulfate sodium-induced colitis by promoting mouse colon epithelial cell survival. *Lab. Invest.* 92, 437–450. doi: 10.1038/labinvest.2011.192

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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