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Both epidermal growth factor and insulin-like growth factor receptors are dispensable for structural intestinal adaptation

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Abstract

Purpose—Intestinal adaptation structurally represents increases in crypt depth and villus height in response to small bowel resection (SBR). Previously, we found that neither epidermal growth factor receptor (EGFR) nor insulin-like growth factor 1 receptor (IGF1R) function was individually required for normal adaptation. In this study, we sought to determine the effect of disrupting *both* EGFR and IGF1R expression on resection-induced adaptation.

Methods—Intestinal-specific EGFR and IGF1R double knockout mice (EGFR/IGF1R-IKO) (n=6) and wild-type (WT) control mice (n=7) underwent 50% proximal SBR. On postoperative day (POD) 7, structural adaptation was scored by measuring crypt depth and villus height. Rates of crypt cell proliferation, apoptosis, and submucosal capillary density were also compared.

Results—After 50% SBR, normal adaptation occurred in both WT and EGFR/IGF1R-IKO. Rates of proliferation and apoptosis were no different between the two groups. The angiogenic response was less in the EGFR/IGF1R-IKO compared to WT mice.

Conclusion—Disrupted expression of EGFR and IGF1R in the intestinal epithelial cells does not affect resection-induced structural adaptation but attenuates angiogenesis after SBR. These findings suggest that villus growth is driven by receptors and pathways that occur outside the epithelial cell component, while angiogenic responses may be influenced by epithelial-endothelial crosstalk.

Keywords

short gut syndrome; intestinal adaptation; epidermal growth factor receptor; insulin-like growth factor 1 receptor

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INTRODUCTION

Intestinal adaptation is defined by structural increases in crypt depth and villus height resulting in increased mucosal surface area in response to massive small bowel resection (SBR) [1]. Epidermal growth factor (EGF), insulin-like growth factor 1 (IGF1) [2, 3] and 2 (IGF2) [4] are recognized intestinotrophic factors which bind and signal through their respective receptors, epidermal growth factor receptor (EGFR) and insulin-like growth factor 1 receptor (IGF1R). Infusion of EGF [5, 6] and IGF1 [2, 4, 7–10] are both associated with an exaggerated adaptation response to SBR.

Previously, we have demonstrated that resection induced adaptation was attenuated when EGFR was inhibited in mutant (waved-2) mice with globally perturbed EGFR activity [11, 12]. However, more recently, with the use of intestinal specific EGFR [13] and IGF1R [14] –null mice, we have found that normal adaptation occurred after SBR. These findings suggest that EGFR and IGF1R receptor activity in the stromal (non-epithelial) compartments of the small bowel may be more important for structural adaptation. Alternatively, one major receptor may be capable of fully compensating for the other in the process of regulating adaptive intestinal growth. Furthermore, enterocyte receptor signaling is likely relevant since crypt cell proliferation is significantly elevated after SBR. The purpose of this study was to directly test the hypothesis that both EGFR and IGF1R signaling in the epithelium are required for normal adaptation responses to SBR.

MATERIALS AND METHODS

Animals and experimental design

All protocols and experiments were approved by the Washington University Animals Studies Committee (Protocol #20130038) and followed National Institutes of Health (NIH) animal care guidelines.

Intestinal epithelial-specific EGFR and IGF1R double knockout mice (EGFR/IGF1R-IKO) (n=6) were generated using a tamoxifen inducible Villin-Cre (VC-ER (+)) recombinant system [15] which disrupts genes between tagged (floxed; (f/f)) regions. Wild type littermates VC-ER (–); EGFR (f/f); IGF1R (f/f) (n=7) were used as control mice. Both VC-ER (+) and VC-ER (–); EGFR (f/f); IGF1R (f/f) mice received intraperitoneal injections of tamoxifen (0.5mg/day; Sigma, St. Louis, MO) for 3 consecutive days prior to SBR. Mice were kept in the animal holding area with a 12 hour light-dark schedule and given rodent chow ad lib after weaning.

EGFR-IKO/IGF1R-IKO mice (n=7) and their wild type (WT) littermates (n=6) underwent 50% proximal SBR between 8 to 10 weeks of age. All mice were fed standard rodent liquid diet (Micro-Stabilized Rodent Liquid Diet LD 101; Purina Mills, St Louis, MO) 1 day prior to SBR. Post-operatively, mice were given water ad lib and resumed rodent liquid diet on post-operative day (POD) 1. All mice were weighed before resection and at the time of harvest on POD 7.

Ileal tissue was collected at the time of SBR as a baseline control. On POD 7, the remnant ileum was removed and histology was collected to compare adaptive structural changes within the crypts and villi with preoperative baseline measurements. Enterocytes were isolated and used for protein analysis to confirm gene deletion and to analyze downstream markers of receptor-mediated phosphorylation pathways. Rates of crypt cell proliferation, apoptosis, and submucosal capillary density were also recorded.

Small bowel resection

EGFR/IGF1R-IKO and their WT littermates underwent a 50% proximal SBR as previously described [1]. The intestinal resections were performed by transecting the bowel 1 to 2 cm distal from the ligament of Treitz and at 12 cm proximal to the ileocecal junction followed by removal of the intervening segment. Intestinal continuity was re-established by an end-to-end primary anastomosis using interrupted 9-0 monofilament sutures. A 2 cm distal segment of the resected ileum was fixed in 10% neutral-buffered formalin for baseline histology.

Tissue isolation

All mice were sacrificed on POD 7 as previously described [13]. Briefly, a midline laparotomy was performed and the entire small intestine was flushed with ice-cold phosphate buffered saline containing protease inhibitors (0.2nM phenylmethanesulfonyl fluoride, 5µg/mL aprotinin, 1µM benzamidine, 1mM sodium orthovanadate, and 2µM cantharidin; EMD, Gibbstown, NJ). A 2 cm segment of bowel distal to the anastomosis was fixed in 10% neutral-buffered formalin to compare with intraoperative histology. The remainder of the distal segment of bowel was used to isolate crypt and villus enterocyte using our laboratory published protocol [16]. Protein from enterocytes was used to verify the efficiency of deletion of EGFR and IGF1R.

Histology

Intestinal tissue was cut into two sections each, 50µm apart followed by H&E staining. Crypt depth and villus height were measured using MetaMorph computer program (Molecular Devices, Downingtown, PA). At least 20 villi and crypts were measured per mouse. Post-operative and intra-operative crypt depth and villus height were compared to calculate the magnitude of the structural adaptation.

Cell proliferation and apoptosis

Paraffin-embedded tissue sections were immunohistochemically stained with p-histone 3 as previously described [17]. Rates of proliferation were determined by counting the number of cells staining positive with p-histone 3 divided by the total number of cells in each crypt. A minimum of 20 crypts were counted per mouse.

H&E stained slides were analyzed for apoptotic bodies (pyknotic nuclei, condensed chromatin, and nuclear fragmentation) [18]. An apoptosis index was determined by counting the number apoptotic bodies found within 50 well-oriented crypts.

Submucosal capillary density

Small intestine tissues were immunohistochemically stained with cd31 to determine the capillary density in the submucosal layer. Immunohistochemistry staining with cd31 was performed using our previously published protocol [19]. The number of cd31-stained vessels was counted in the submucosal layer per high power field (40x). Ten well oriented power fields per mouse were counted and averaged.

Western blotting

Crypts were isolated and then lysed with sodium dodecyl sulfate (SDS) lysing buffer (50mM Tris-HCL pH 6.8, 2% sodium dodecyl sulfate, 10% glycerol, and 5% mercaptoethanol). The cell samples were sonicated and heated in 100°C for 5 minutes. Protein quantification was performed using a RC DC kit (Bio-Rad, Hercules, CA). Equal amounts of protein were separated in 10% SDS-PAGE gel and probed with their respective antibodies. (EGFR, IGF1R, p-ERK(Thr202/Tyr204), p-AKT(Ser473), Actin (Cell Signaling Technology, Danvers, MA); Tubulin (EMD Millipore, Darmstadt, Germany)).

Statistical analysis

All values are reported as mean $\pm$ standard error of the mean. Statistical analysis was performed using Student's t-test to compare the two experimental groups. A p-value of less than 0.05 was considered significant.

RESULTS

EGFR/IGF1R-IKO mice demonstrate normal structural adaptation after SBR

The successful deletion of EGFR and IGF1R protein within enterocytes of the small intestine was confirmed by Western blot (Figure 1a). Both intestine-specific single EGFR (EGFR-IKO) and IGF1R knockout mice (IGF1R-IKO) have been previously characterized and neither baseline crypt depth or villus height was significantly different from their respective wild type littermate [13, 14, 20]. Interestingly, disruption of both EGFR and IGF1R in enterocytes similarly had little effect on baseline small intestine structure (data not shown).

Both EGFR/IGF1R-IKO and WT mice had nearly identical percent increases in villus height and crypt deepening 7 days after SBR. EGFR/IGF1R-IKO mice had an average of $29.6.0 \pm 9.6\%$ increase in crypt depth and an average of $45.7 \pm 8.0\%$ increase in villus height after SBR while the WT littermates had an average of $30.3 \pm 4.8\%$ increase in crypt depth and $45.6 \pm 5.5\%$ in villus height after SBR (Figure 1b).

Post-operative weight change

Mice were weighed before resection and after resection at the time of harvest on POD 7. EGFR/IGF1R-IKO mice had an average of $13.7 \pm 2.6\%$ weight loss versus WT mice had an average $17.8 \pm 2.5\%$ weight loss however, there was no statistical significant difference between the two groups for the percent of weight loss after bowel resection.

Resection-induced enterocyte proliferation and apoptosis are not affected in EGFR/IGF1R-IKO mice

Postoperative proliferation and apoptosis in both EGFR/IGF1R-IKO and WT mice were not different following SBR. EGFR/IGF1R-IKO had an average proliferative index of 0.16 ± 0.01 and the WT mice had an average of 0.15 ± 0.01 (Figure 2). At baseline, EGFR/IGF1R-IKO mice had an average proliferative index of 0.08 ± 0.01 compared to the WT control with an average of 0.09 ± 0.01 (Figure 2). These proliferative indices increased after SBR to the same extent as we have previously shown [1, 13].

The apoptosis indexes in EGFR/IGF1R-IKO were no different at baseline or after SBR when compared with WT mice (data not shown).

Epithelial expression of EGFR/IGF1R is involved in submucosal capillary growth

To evaluate the angiogenic response after SBR, the number of cd31-stained vessels in the submucosal area was compared between ileal tissue taken at baseline and at 7 days following SBR. Both EGFR/IGF1R-IKO and WT mice had elevated submucosal capillary density after SBR. However, there was a greater increase in capillary density in the WT mice (9.0 ± 0.1 vessels per high power field) versus EGFR/IGF1R-IKO mice (7.76 ± 0.2 vessels per high power field; $p=0.002$) (Figure 3) after SBR.

EGFR/IGF1R expression does not alter p-ERK and p-AKT activity in adapting enterocytes

Since p-ERK and p-AKT are known to be important in the transmission of EGFR and IGF1R signals to the nucleus, we sought to look at these common downstream markers in resected WT and EGFR/IGF1R-IKO enterocytes. Surprisingly, simultaneous ablation of EGFR and IGF1R had no effects on p-ERK and p-AKT phosphorylation when compared with WT mice (Figure 4).

DISCUSSION

EGF [21], IGF1 [3], and IGF2 [22] are known intestinotrophic factors. Systemic administration of these growth factors has shown to magnify adaptive intestinal mucosal growth after SBR [3, 8]. EGF, IGF1/IGF2 binds and signals through EGFR and IGF1R respectively. When EGFR [13] and IGF1R [14] are individually deleted within the enterocytes, structural adaptation is not affected. Meanwhile, both of these tyrosine kinase receptors have similar structural homology [23] and share converging downstream pathways which may explain why adaptation is not attenuated after disrupting only one receptor. This background provided the rationale for testing resection-induced adaptation after simultaneously deleting EGFR and IGF1R within the enterocyte cell compartment. Furthermore, this would provide more insight into the significance of growth factor signaling within epithelial versus non-epithelial compartments of the small bowel. In this study, we found that deletion of both EGFR and IGF1R within the gut epithelium did not affect structural adaptation, rates of proliferation and apoptosis, and had no measurable effect on the signature of downstream signaling within the enterocyte cellular compartment after SBR.

EGFR and IGF1R are major tyrosine kinase receptors, which are often overexpressed in many human cancers [24–27]. These structural homologous receptors are recognized for tumor cell regulation of proliferation, survival, apoptosis, and angiogenesis [28, 29] making these two receptors favorable targets for cancer therapy [24, 29, 30]. Recent studies have shown IGF1R mainly mediates via the PI3K/AKT pathway while the EGFR mediates through the Ras/MAP kinase pathway [31, 32]. Multiple studies have shown selective inhibition of one receptor will lead to compensatory and enhanced activation of the other receptor axis [29, 33, 34]. These cellular and molecular mechanistic observations have led to research on specific targeted therapy for dual inhibition of EGFR and IGF1R [28–30, 35].

Since EGFR and IGF1R have converging and overlapping downstream pathways, we performed Western blots to evaluate the expression of downstream activation via both Ras/MAP kinase and PI3/AKT pathways in WT and double-knockout mice after SBR. Despite lacking functional EGFR and IGF1R in the epithelium, Ras/MAP kinase and PI3/AKT pathways did not differ between the two groups of mice. This would suggest that the phosphorylation and activation of these pathways occurs independent of these receptors or there are additional structural homologous receptors such as insulin receptor [36, 37] that may compensate for the deletion of these two major receptors.

Alternatively, it must be considered that EGFR and IGF1R signaling functions to regulate adaptation within the underlying submucosal and muscularis compartments of the intestine. Previously, we have observed resection-induced adaptation when intestinal epithelial EGFR was selectively deleted [13], but not when EGFR was globally inhibited [11, 38]. Regarding IGF1R, it is plausible that the IGF system stimulates adaptation via non-epithelial cells within the intestine. Since IGF1 is known to be mitogenic to subepithelial myofibroblasts, it is reasonable to consider that IGF1R activity within the subepithelial mesenchyme drives intestinal mucosal growth after SBR [39].

In future experiments, exogenous EGF and IGF1/2 could be provided to the EGFR/IGF1R-IKO mice to evaluate whether the expected magnified adaptation response is attenuated. Enhanced adaptation after SBR in the background of deficient EGFR and IGF1R expression would further support the notion that receptors within enterocytes have a minimal role in intestinal adaptation.

Angiogenesis is observed in the small intestine of normal mice after SBR in both the lamina propria and the submucosal compartment [40]. The EGFR/IGF1R-IKO mice had an impaired angiogenic response following SBR, but this did not appear to alter the structural features of adaptation. Similarly, we have previously found that impaired angiogenesis does not affect structural adaptation, however, intestinal function is impaired [19, 41]. This observation confirms previous suggestions that structural and functional intestinal adaptation are independent processes [42].

In summary, EGF and IGF1/2 are two major recognized intestinotrophic factors that have shown to increase intestinal mucosal growth and provide an added degree of intestinal adaptation after SBR. We have learned that deletion of each receptor individually and both receptors simultaneously within the enterocytes do not affect structural adaptation or crypt

cell proliferation after SBR. Meanwhile, there is evidence of sustained activity of downstream intestinotrophic signaling pathways despite deletion of EGFR and IGF1R in the crypts. Despite having this compensatory mechanism, the angiogenic response is impaired after SBR in EGFR/IGF1RKO mice. These observations lead us to conclude that crypt cell proliferation and structural adaptation after SBR may depend on receptors and signaling from within the submucosal and mesenchymal compartments instead of the intestinal epithelium. Secondly, our study provides further evidence to support that structural and functional adaptation are independent processes. Further investigation is needed to explore other signaling pathways and dissect into the submucosal cell compartments to better understand the compensatory adaptive response after SBR.

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References

1. Helmrath MA, VanderKolk WE, Can G, et al. Intestinal adaptation following massive small bowel resection in the mouse. *J Am Coll Surg*. 1996; 183:441–449. [PubMed: 8912612]
2. Conlon MA, Tomas FM, Owens PC, et al. Long R3 insulin-like growth factor-I (IGF-I) infusion stimulates organ growth but reduces plasma IGF-I, IGF-II and IGF binding protein concentrations in the guinea pig. *J Endocrinol*. 1995; 146:247–253. [PubMed: 7561636]
3. Bortvedt SF, Lund PK. Insulin-like growth factor 1: common mediator of multiple enterotrophic hormones and growth factors. *Curr Opin Gastroenterol*. 2012; 28:89–98. [PubMed: 22241077]
4. Conlon MA, Francis GL, Tomas FM, et al. Continuous 14 day infusion of IGF-II increases the growth of normal female rats, but exhibits a lower potency than IGF-I. *J Endocrinol*. 1995; 144:91–98. [PubMed: 7534330]
5. Shin CE, Helmrath MA, Falcone RA Jr, et al. Epidermal growth factor augments adaptation following small bowel resection: optimal dosage, route, and timing of administration. *J Surg Res*. 1998; 77:11–16. [PubMed: 9698525]
6. Chaet MS, Arya G, Ziegler MM, et al. Epidermal growth factor enhances intestinal adaptation after massive small bowel resection. *J Pediatr Surg*. 1994; 29:1035–1038. discussion 1038–1039. [PubMed: 7965502]
7. Burrin DG, Wester TJ, Davis TA, et al. Orally administered IGF-I increases intestinal mucosal growth in formula-fed neonatal pigs. *Am J Physiol*. 1996; 270:R1085–1091. [PubMed: 8928910]
8. Dahly EM, Guo Z, Ney DM. IGF-I augments resection-induced mucosal hyperplasia by altering enterocyte kinetics. *Am J Physiol Regul Integr Comp Physiol*. 2003; 285:R800–808. [PubMed: 12763742]
9. Lemmey AB, Ballard FJ, Martin AA, et al. Treatment with IGF-I peptides improves function of the remnant gut following small bowel resection in rats. *Growth Factors*. 1994; 10:243–252. [PubMed: 7803042]
10. Lemmey AB, Martin AA, Read LC, et al. IGF-I and the truncated analogue des-(1-3)IGF-I enhance growth in rats after gut resection. *Am J Physiol*. 1991; 260:E213–219. [PubMed: 1996625]
11. Helmrath MA, Erwin CR, Warner BW. A defective EGF-receptor in waved-2 mice attenuates intestinal adaptation. *J Surg Res*. 1997; 69:76–80. [PubMed: 9202650]
12. O'Brien DP, Nelson LA, Williams JL, et al. Selective inhibition of the epidermal growth factor receptor impairs intestinal adaptation after small bowel resection. *J Surg Res*. 2002; 105:25–30. [PubMed: 12069497]

13. Rowland KJ, McMellen ME, Wakeman D, et al. Enterocyte expression of epidermal growth factor receptor is not required for intestinal adaptation in response to massive small bowel resection. *J Pediatr Surg.* 2012; 47:1748–1753. [PubMed: 22974617]
14. Sun RC, Choi PM, Guo J, et al. Insulin-like growth factor 2 and its enterocyte receptor are not required for adaptation in response to massive small bowel resection. *J Pediatr Surg.* 2014; 49:966–970. discussion 970. [PubMed: 24888844]
15. el Marjou F, Janssen KP, Chang BH, et al. Tissue-specific and inducible Cre-mediated recombination in the gut epithelium. *Genesis.* 2004; 39:186–193. [PubMed: 15282745]
16. Guo J, Longshore S, Nair R, et al. Retinoblastoma protein (pRb), but not p107 or p130, is required for maintenance of enterocyte quiescence and differentiation in small intestine. *J Biol Chem.* 2009; 284:134–140. [PubMed: 18981186]
17. Choi PM, Sun RC, Sommovilla J, et al. IGF-2 Is Necessary for Retinoblastoma-Mediated Enhanced Adaptation after Small-Bowel Resection. *J Gastrointest Surg.* 2014
18. Wakeman D, Guo J, Santos JA, et al. p38 MAPK regulates Bax activity and apoptosis in enterocytes at baseline and after intestinal resection. *Am J Physiol Gastrointest Liver Physiol.* 2012; 302:G997–1005. [PubMed: 22383494]
19. Rowland KJ, Diaz-Miron J, Guo J, et al. CXCL5 is required for angiogenesis, but not structural adaptation after small bowel resection. *J Pediatr Surg.* 2014; 49:976–980. discussion 980. [PubMed: 24888846]
20. Rowland KJ, Trivedi S, Lee D, et al. Loss of glucagon-like peptide-2-induced proliferation following intestinal epithelial insulin-like growth factor-1-receptor deletion. *Gastroenterology.* 2011; 141:2166–2175. e2167. [PubMed: 21925122]
21. Rowland KJ, Choi PM, Warner BW. The role of growth factors in intestinal regeneration and repair in necrotizing enterocolitis. *Semin Pediatr Surg.* 2013; 22:101–111. [PubMed: 23611614]
22. Lund PK. Molecular basis of intestinal adaptation: the role of the insulin-like growth factor system. *Ann N Y Acad Sci.* 1998; 859:18–36. [PubMed: 9928367]
23. Ward CW, Garrett TP. Structural relationships between the insulin receptor and epidermal growth factor receptor families and other proteins. *Curr Opin Drug Discov Devel.* 2004; 7:630–638.
24. Gallardo A, Lerma E, Escuin D, et al. Increased signalling of EGFR and IGF1R, and deregulation of PTEN/PI3K/Akt pathway are related with trastuzumab resistance in HER2 breast carcinomas. *Br J Cancer.* 2012; 106:1367–1373. [PubMed: 22454081]
25. Hellawell GO, Turner GD, Davies DR, et al. Expression of the type 1 insulin-like growth factor receptor is up-regulated in primary prostate cancer and commonly persists in metastatic disease. *Cancer Res.* 2002; 62:2942–2950. [PubMed: 12019176]
26. Ryan CJ, Haqq CM, Simko J, et al. Expression of insulin-like growth factor-1 receptor in local and metastatic prostate cancer. *Urol Oncol.* 2007; 25:134–140. [PubMed: 17349528]
27. Schlomm T, Kirstein P, Iwers L, et al. Clinical significance of epidermal growth factor receptor protein overexpression and gene copy number gains in prostate cancer. *Clin Cancer Res.* 2007; 13:6579–6584. [PubMed: 18006757]
28. Wang Y, Yuan JL, Zhang YT, et al. Inhibition of both EGFR and IGF1R sensitized prostate cancer cells to radiation by synergistic suppression of DNA homologous recombination repair. *PLoS One.* 2013; 8:e68784. [PubMed: 23950876]
29. van der Meel R, Oliveira S, Altintas I, et al. Inhibition of tumor growth by targeted anti-EGFR/IGF-1R nanobullets depends on efficient blocking of cell survival pathways. *Mol Pharm.* 2013; 10:3717–3727. [PubMed: 23889133]
30. Fidler MJ, Shersher DD, Borgia JA, et al. Targeting the insulin-like growth factor receptor pathway in lung cancer: problems and pitfalls. *Ther Adv Med Oncol.* 2012; 4:51–60. [PubMed: 22423264]
31. Riedemann J, Takiguchi M, Sohail M, et al. The EGF receptor interacts with the type 1 IGF receptor and regulates its stability. *Biochem Biophys Res Commun.* 2007; 355:707–714. [PubMed: 17307140]
32. Ueda S, Hatsuse K, Tsuda H, et al. Potential crosstalk between insulin-like growth factor receptor type 1 and epidermal growth factor receptor in progression and metastasis of pancreatic cancer. *Mod Pathol.* 2006; 19:788–796. [PubMed: 16575403]

33. Riedemann J, Sohail M, Macaulay VM. Dual silencing of the EGF and type 1 IGF receptors suggests dominance of IGF signaling in human breast cancer cells. *Biochem Biophys Res Commun.* 2007; 355:700–706. [PubMed: 17320820]
34. Chakravarti A, Loeffler JS, Dyson NJ. Insulin-like growth factor receptor I mediates resistance to anti-epidermal growth factor receptor therapy in primary human glioblastoma cells through continued activation of phosphoinositide 3-kinase signaling. *Cancer Res.* 2002; 62:200–207. [PubMed: 11782378]
35. Li P, Veldwijk MR, Zhang Q, et al. Co-inhibition of epidermal growth factor receptor and insulin-like growth factor receptor 1 enhances radiosensitivity in human breast cancer cells. *BMC Cancer.* 2013; 13:297. [PubMed: 23777562]
36. Sukhotnik I, Shehadeh N, Shamir R, et al. Oral insulin enhances intestinal regrowth following massive small bowel resection in rat. *Dig Dis Sci.* 2005; 50:2379–2385. [PubMed: 16416194]
37. Pollak M. The insulin receptor/insulin-like growth factor receptor family as a therapeutic target in oncology. *Clin Cancer Res.* 2012; 18:40–50. [PubMed: 22215905]
38. Warner BW, Erwin CR. Critical roles for EGF receptor signaling during resection-induced intestinal adaptation. *J Pediatr Gastroenterol Nutr.* 2006; 43 (Suppl 1):S68–73. [PubMed: 16819405]
39. Simmons JG, Pucilowska JB, Keku TO, et al. IGF-I and TGF-beta1 have distinct effects on phenotype and proliferation of intestinal fibroblasts. *Am J Physiol Gastrointest Liver Physiol.* 2002; 283:G809–818. [PubMed: 12181198]
40. Martin CA, Perrone EE, Longshore SW, et al. Intestinal resection induces angiogenesis within adapting intestinal villi. *J Pediatr Surg.* 2009; 44:1077–1082. discussion 1083. [PubMed: 19524720]
41. Diaz-Miron J, Sun RC, Choi P, et al. The effect of impaired angiogenesis on intestinal function following massive small bowel resection. *Journal of Pediatric Surgery.* 2014 submitted.
42. Choi PM, Guo J, Erwin CR, et al. Disruption of retinoblastoma protein expression in the intestinal epithelium impairs lipid absorption. *Am J Physiol Gastrointest Liver Physiol.* 2014; 306:G909–915. [PubMed: 24742992]

Fig. 1a

Fig. 1b

Figure 1.

A) Western blot confirming deletion of EGFR and IGF1R protein expression in crypt enterocytes. Both EGFR and IGF1R expression were knocked out in the intestinal epithelium in adult mice (EGFR/IGF1R-IKO; n=6) following the injection of tamoxifen. Both the knockout mice and wild-type (n=7) controls were administered tamoxifen for three days prior to resection. Tubulin was used as loading control. Successful deletion of EGFR and IGF1R protein was confirmed with all Villin Cre-ER(+); EGFR (f/f), IGF1R (f/f) mice.

B) Percentage increase in crypt depth and villus height for EGFR/IGF1R-IKO (n=6) and WT (n=7) mice after small bowel resection. There were no statistical differences in postoperative villus or crypt growth between groups.

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Figure 2.

Rates of crypt cell proliferation at baseline and 7 days after 50% proximal small bowel resection (SBR) in EGFR/IGF1R-IKO (n=6) and wild-type (WT; n=7) littermates. P-histone 3 immunostaining was performed on tissue sections and positively stained cells were counted in each crypt. A ratio was calculated as number of cells stained positive divided by total number of cells in the crypts. A minimum of 20 crypts were counted per mouse.

Figure 3.

Angiogenesis at baseline and 7 days after 50% proximal small bowel resection (SBR) as measured by counting the number of cd31 positive-stained vessels per high power field. Ten high power fields were counted and averaged. Both EGFR/IGF1R-IKO (n=6) and wild-type (WT; n=7) mice showed an increase in cd31 positive stained vessels per high power field after SBR. However, EGFR/IGF1R-IKO mice had an impaired angiogenic response compared with the WT group (p-value<0.01).

Figure 4. Western blot demonstrates phosphorylation of ERK (Thr 202/Tyr 204) and AKT (Ser 473) pathways in both EGFR/IGF1R-IKO and WT mice in crypt enterocytes. Actin was used as a loading control.