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Enterocyte expression of epidermal growth factor receptor is not required for intestinal adaptation in response to massive small bowel resection☆

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Abstract

Purpose: Intestinal adaptation after massive small bowel resection (SBR) permits improved absorption of enteral nutrition despite significant loss of bowel length. Epidermal growth factor (EGF) and its receptor (EGFR) have previously been established to play major roles in the pathogenesis of adaptation. This study tested the hypothesis that EGFR signaling within the epithelial cell compartment (enterocytes) is required for intestinal adaptation.

Methods: We developed a tamoxifen-inducible Villin-Cre/LoxP recombinant system for enterocyte-directed EGFR deletion using EGFR-floxed mice. Epidermal growth factor receptor–null mice and wild-type littermates underwent either 50% proximal SBR or sham operation. Ileal tissue was harvested on postoperative day 7. To assess for adaptation, villus height and crypt depth as well as rates of crypt cell proliferation and apoptosis were measured.

Results: Adaptation after SBR occurred normally, as demonstrated by significant increases in villus height, crypt depth, and crypt proliferative and apoptotic index in both the wild-type and EGFR-null mice.

Conclusion: Enterocyte EGFR expression is not required for the adaptation response to massive SBR. This novel finding suggests that enterocyte proliferation during adaptation is regulated by EGFR signaling in cells other than enterocytes, perhaps within the mesenchymal cell compartment of the bowel wall via factor(s) that are presently unknown.

Intestinal adaptation is a compensatory response after massive small bowel resection (SBR) and allows for adequate absorption of enteral nutrition despite significant loss of bowel length. This phenomenon is characterized by significant increases in villus height and crypt depth, resulting in increased absorptive mucosal surface area [1].

Epidermal growth factor (EGF) and its receptor (EGFR) have previously been established to play major roles in the pathogenesis of adaptation. Systemic EGF stimulation

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enhances adaptation. If EGF is given exogenously after SBR or overexpressed in transgenic mice, a magnified adaptation response occurs [2-4]. Perturbed EGFR activity inhibits this important response. Removal of the major endogenous source of EGF via sialoadenectomy attenuates adaptation, an effect that is partially reversible with either systemic or oral administration of EGF [5]. Furthermore, resection-induced adaptation after SBR is inhibited by systemic administration of EGFR inhibitors and in mutant mice (waved-2) that have generalized perturbed EGFR activity [6,7].

Although these experimental manipulations confirm an important role for EGFR signaling during adaptation, the exact cellular compartment within the intestinal wall (enterocyte vs underlying mesenchyme) that is governed by EGFR signaling to direct adaptation is presently unknown. The purpose of the present study, therefore, was to test the hypothesis that enterocyte-specific EGFR signaling is required for intestinal adaptation.

1. Materials and methods

1.1. Experimental design

Protocols for this study were approved by the Washington University Animal Studies Committee (Protocol 20100103) and were in accordance with the National Institute of Health laboratory animal care and use guidelines. Baseline intestinal morphological data were collected from enterocyte EGFR-null mice (n = 5) and wild-type (WT) littermates (n = 5) at 7 and 30 days after mucosal EGFR deletion. Four experimental groups were studied: WT mice that underwent sham operation (n = 9) or 50% proximal SBR (n = 10) and EGFR-null mice that underwent sham operation (n = 8) or 50% proximal SBR (n = 11). Ileal tissue was harvested on postoperative day 7. Epidermal growth factor receptor deletion was confirmed via Western blotting and reverse transcription–polymerase chain reaction (RT-PCR) of the purified epithelial cells. To assess for adaptation, villus height and crypt depth were measured via H&E-stained histology. Rates of crypt cell proliferation and apoptosis were also recorded.

1.2. Animals

Because disrupted EGFR expression in enterocytes has been shown to be embryonic lethal, we used a tamoxifen (TAM)-inducible Villin-Cre/LoxP recombinant system for enterocyte-directed EGFR deletion. Epidermal growth factor receptor–floxed mice [8] (*Egfr*^{tm1Dwt} generously provided by David Threadgill, University of North Carolina, Chapel Hill) were crossed with mice harboring a TAM-inducible Cre-fusion protein under control of the villin promoter [9] (*vil-Cre ERT2*; obtained via generous donation from Sylvie Robine, Curie Institute, Paris, France). Both lines were on a C57BL/6 background. The construct is such that TAM administration causes the deletion of exon 3 in the EGFR gene in VC=Villin-Cre(+) mice. Mice with both the Cre fusion protein and floxed EGFR alleles (*VC(+)/ EGFR(f/f)*) were used for the knockout group (VC(+)). Littermates lacking Cre (*VC(−)/EGFR(f/f)*) were used as WT controls. Male and female mice aged 10 to 12 weeks were used in this study with a weight range of 19.5 to 27.5 g. Mice were kept on a 12-hour light-dark schedule and were housed in a standard facility. Both WT and EGFR-floxed mice were injected with intraperitoneal TAM (0.5 mg/d; Sigma, St. Louis, MO) for 3 days in a row starting 4 days before surgery. The mice were given a liquid rodent diet (Micro-Stabilized Rodent Liquid Diet LD101; Purina Mills, St Louis, MO) 1 day before surgery.

1.3. Operative technique

Mice underwent 50% proximal SBR or sham operation (transection and reanastomosis only) as previously described [1]. Briefly, mice that underwent SBR had transection of the bowel at a point 12 cm proximal to the ileal-cecal junction and also at a point 1 to 2 cm distal to the ligament of Treitz. The mesentery was ligated, and the intervening bowel was removed. Intestinal continuity was restored with an end-to-end anastomosis using 9-0 monofilament suture. In mice undergoing sham operation, the bowel was transected at a point 12 cm proximal to the ileal-cecal junction, and intestinal continuity was restored with an end-to-end reanastomosis. After the operation, mice were provided free access to water for the first 24 hours and then given a liquid rodent diet until sacrifice.

1.4. Tissue harvest and isolation of epithelial cells

On the seventh postoperative day, the mice were anesthetized with a subcutaneous injection of ketamine, xylazine, and acepromazine (4:1:1). A midline laparotomy was performed, and the small bowel was flushed with ice-cold phosphate-buffered saline with protease inhibitors (0.2 mM phenylmethylsulfonyl fluoride, 5 μg/mL aprotinin, 1 mM benzamidine, 1 mM sodium orthovanadate, and 2 μM cantharidin; EMD, Gibbstown, NJ) and excised. The first 1-cm segment of bowel distal to the anastomosis was discarded. The next 2-cm segment of bowel was fixed in 10% neutral-buffered formalin for histology. The subsequent 5 cm of bowel was cut longitudinally and transferred into tubes containing 5 mL of ice-cold PBS with protease inhibitors for 1 hour at 4°C. Crypt and villus enterocytes were isolated using a calcium chelation, mechanical vibration, and cell-straining protocol that we have beforehand described [10].

1.5. Reverse transcription–polymerase chain reaction confirmation of EGFR messenger RNA knockout in enterocytes

Total RNA was extracted from frozen isolated crypt and villus enteroocytes and underlying mesenchymal/muscularis