

Amphiregulin and epiregulin expression in neoplastic and inflammatory lesions in the colon

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Abstract. Amphiregulin and epiregulin belong to the epidermal growth factor family and mediate the biological functions of epithelial and mesenchymal cells through epidermal growth factor receptors. In this study, we evaluated the amphiregulin and epiregulin expression in neoplastic and inflammatory lesions from the human colon. Surgically-obtained specimens were stained using standard immunohistochemical procedures. Amphiregulin and epiregulin were not expressed in the normal colonic mucosa, but were clearly detectable in adenomas and carcinomas. Weak immunostaining was also detected in mesenchymal cells from the tumor tissues. In the active mucosa of patients with ulcerative colitis and Crohn's disease, amphiregulin was mainly expressed by the epithelial cells. In addition, positive immunostaining was also detectable in the surrounding mesenchymal cells. In conclusion, amphiregulin and epiregulin may play important roles in colonic tumor growth and mucosal repair in the inflamed mucosa of inflammatory bowel disease.

Introduction

Amphiregulin and epiregulin belong to the epidermal growth factor (EGF) family which includes EGF, transforming growth factor (TGF)- α , heparin-binding (HB)-EGF, betacellulin and various heregulins. These factors mediate the biological functions of epithelial and mesenchymal cells through EGF receptors (EGFRs) (1). Several studies have demonstrated that the EGF family and the EGFR signalling pathway play a crucial role in the regenerative response of the mucosal damage in the gastrointestinal tract (2-4).

Amphiregulin is a 252 amino acid transmembrane glycoprotein and was originally isolated from the human breast carcinoma cell line MCF-7 (5). The mRNA expression

for amphiregulin can be detected in a variety of carcinoma cell lines and in non-transformed epithelial and mesenchymal cells from the colon, liver, stomach, lung, breast, ovary and kidney (6,7). Amphiregulin stimulates the proliferation of keratinocytes, fibroblasts and epithelial cells (8-10). In some carcinoma cell lines, amphiregulin stimulates their proliferation through autocrine mechanisms (11,12). Beales demonstrated that amphiregulin stimulates the proliferation of the gastric mucosa (13).

Epiregulin is a 46 amino acid active protein and was initially purified from a conditioned medium from the fibroblast-like cell line NIH3T3/T7 (14). Epiregulin stimulates the proliferation of non-transformed fibroblasts, hepatocytes, smooth muscle cells and keratinocytes, but inhibits the growth of several tumor-derived epithelial cell lines (15-17). Lee *et al* reported that epiregulin null transgenic mice are highly susceptible to intestinal damage caused by the oral administration of dextran sulfate sodium (17).

These studies suggest functional roles for amphiregulin and epiregulin in neoplastic growth and/or mucosal repair, but precise studies for the *in situ* expression of these factors have yet to be performed in the colon. In particular, the amphiregulin and epiregulin expression in the inflamed mucosa, such as in IBD, remains unclear. In this study, we immunohistochemically evaluated the amphiregulin and epiregulin expression in neoplastic and inflammatory lesions from the human colon.

Materials and methods

Tissue samples. The diagnoses of ulcerative colitis (UC) and Crohn's disease (CD) were based on conventional clinical, endoscopic and histopathologic criteria. Surgically-obtained specimens from 5 patients with UC and 5 patients with CD were used after informed consent. All patients were active as defined by the colitis activity index for UC (18) and the Crohn's disease activity index (19). Surgically-resected samples of colonic adenoma (n=5) and adenocarcinoma [well-differentiated (n=3) and moderately-differentiated (n=2)] were used. Normal colorectal tissues were obtained by surgical resection of the colon cancers at sites distant from the tumors (n=5).

Immunohistochemistry. Immunohistochemical analyses were performed according to the method described in our previous

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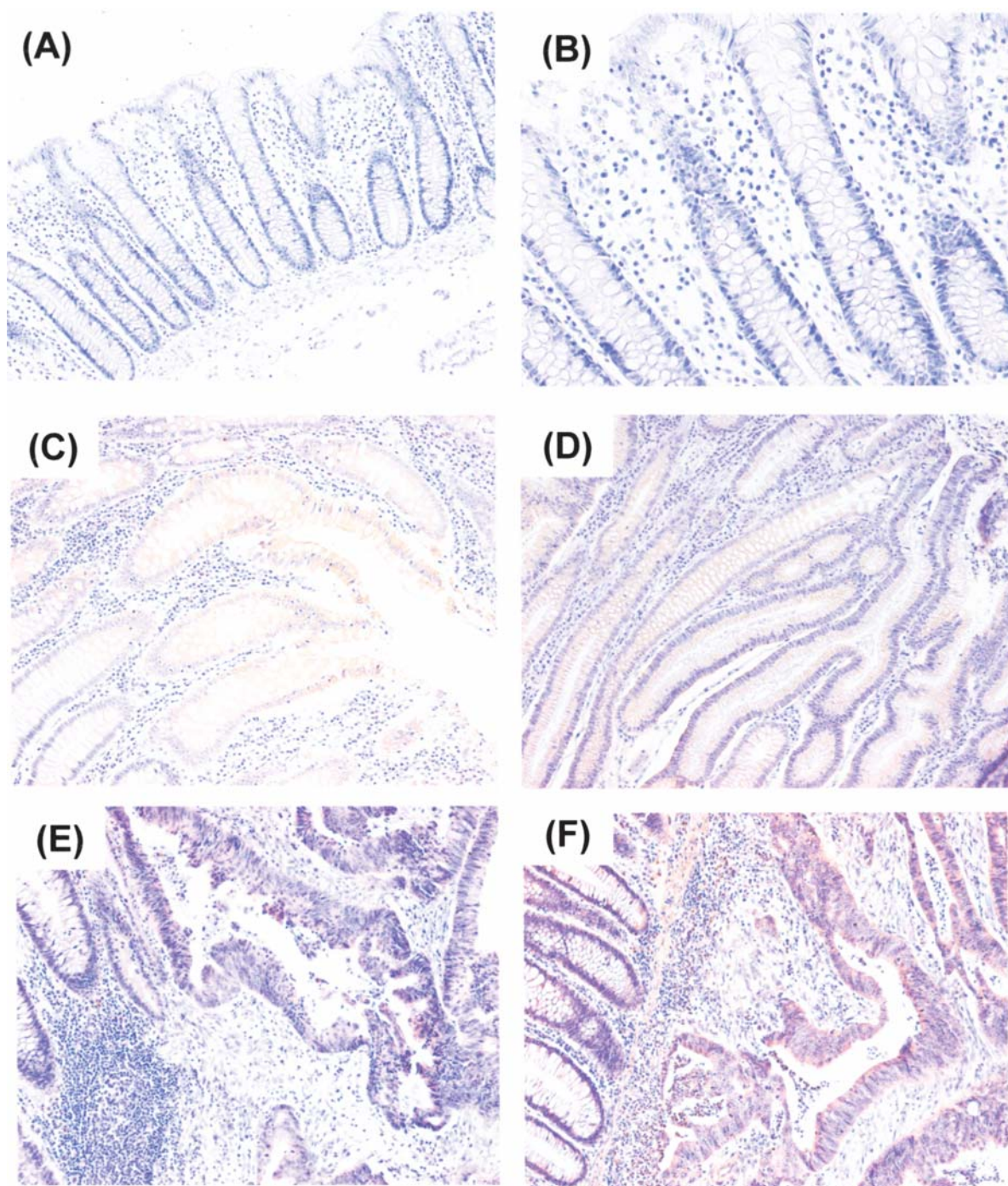


Figure 1. Amphiregulin expression in normal colonic mucosa, adenomas and carcinomas. (A) and (B), normal human colonic mucosa; (C) and (D), adenomas; and (E) and (F), carcinomas. Original magnification, except (B), x100. (B), x200.

report (20). Goat anti-human amphiregulin antibodies (R&D Systems Inc., Minneapolis, MN) and goat anti-human epiregulin antibodies (R&D Systems Inc.) were used as the primary antibodies. After incubation with the primary antibody, the sections were treated with a biotin-conjugated rabbit anti-goat IgG (Vector, Burlingame, CA) and avidin-biotin-peroxidase complex (ABC, Vector).

Results

Amphiregulin expression was investigated in the normal colonic mucosa, adenomas and carcinomas (Fig. 1A-F). As

shown in Fig. 1A and B, amphiregulin expression was not detected in the normal colonic mucosa. However, the amphiregulin expression was clearly detected in neoplastic cells from adenomas (Fig. 1C and D) and carcinomas (Fig. 1E and F). Weak immunostaining was also detected in mesenchymal cells from the carcinoma tissues and these immunostaining patterns were detectable in all adenoma and carcinoma samples.

Epiregulin was immunostained in the adenomas and carcinomas, but the epiregulin expression was not detected in the normal colonic mucosa (Fig. 2A). Similar to the amphiregulin expression, epiregulin was detected in adenomas (Fig. 2B-D) and carcinomas (Fig. 2E and F). In the carcinoma

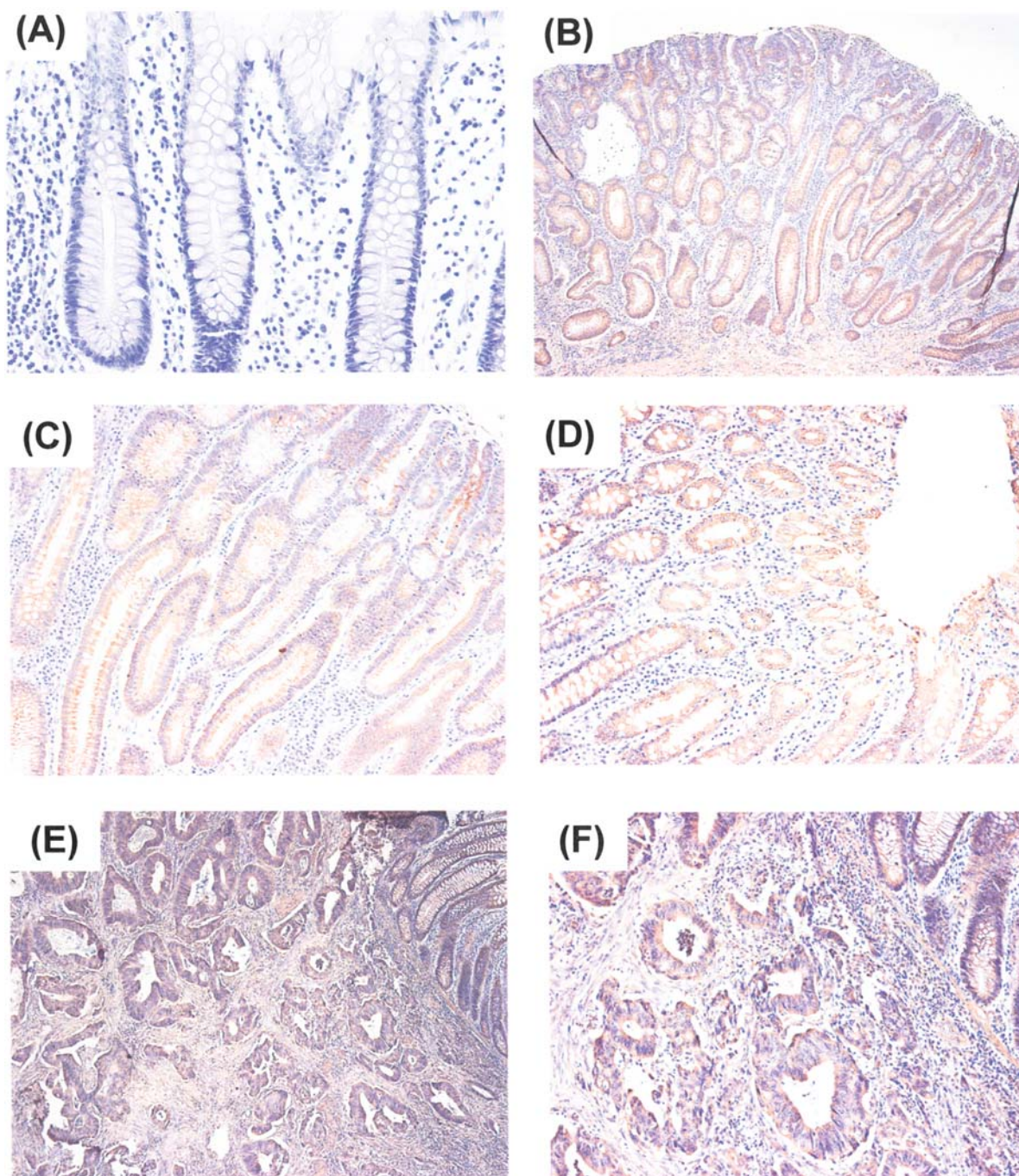


Figure 2. Epiregulin expression in normal colonic mucosa, adenomas and carcinomas. (A) Normal human colonic mucosa; (B) to (D), adenomas; and (E) and (F), carcinomas. Original magnification, except (A), x100. (A), x200.

samples, the mesenchymal cells were also immunopositive for the epiregulin expression. These immunostaining patterns were detected in all adenoma and carcinoma samples.

The amphiregulin expression was evaluated in the active IBD mucosa. In the active mucosa of UC patients (Fig. 3A and B), amphiregulin was mainly expressed by the epithelial cells. In addition, positive immunostaining was detected in the mesenchymal cells. Similar immunostaining patterns were detected in the active mucosa of patients with Crohn's disease (Fig. 3C and D). The amphiregulin and epiregulin expression was similarly detectable in all samples from the UC and CD patients.

Epiregulin was immunostained in the active IBD mucosa. As with amphiregulin immunostaining, epiregulin was expressed by the epithelial cells in the active mucosa of UC (Fig. 4A and B) and CD patients (Fig. 4C and D). The mesenchymal cells were also immunopositive for epiregulin expression. Similar immunostaining patterns were observed in all samples from the IBD patients.

Discussion

This study demonstrated the amphiregulin and epiregulin expression in neoplastic lesions from the colon and in the

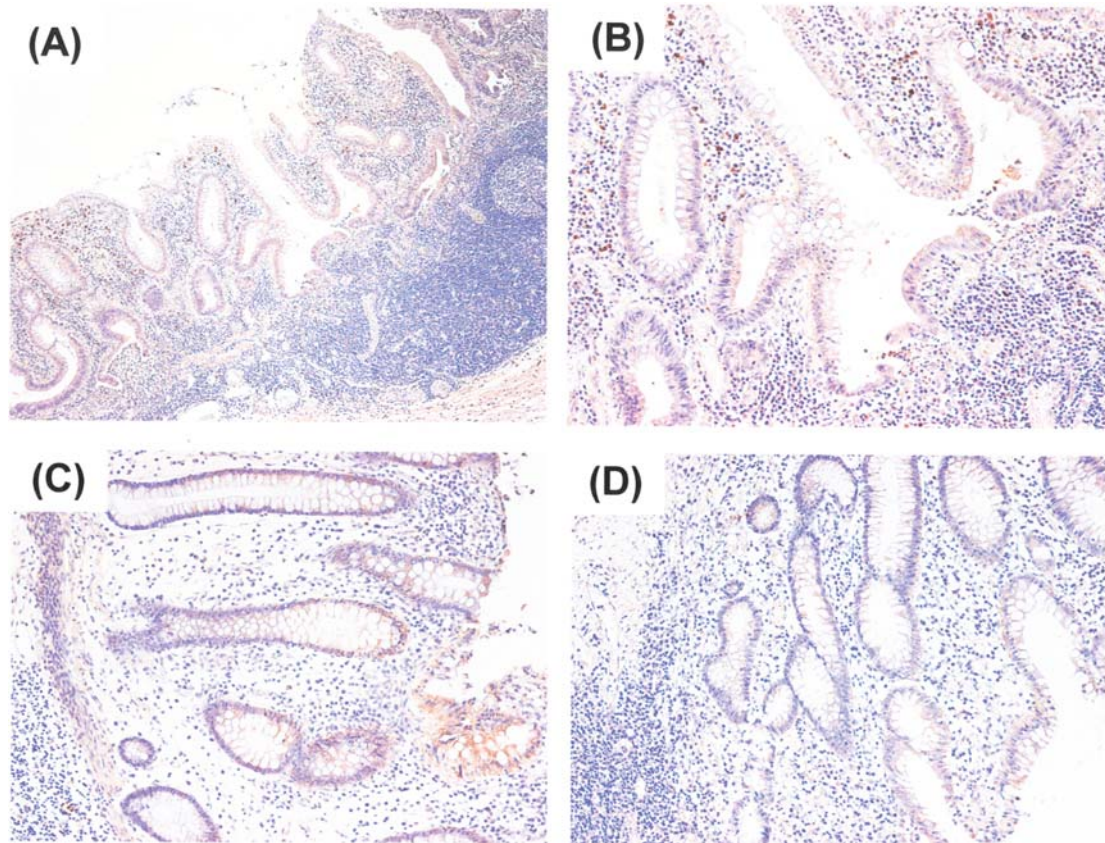


Figure 3. Amphiregulin expression in IBD mucosa. (A) and (B), ulcerative colitis; and (C) and (D), Crohn's disease. Original magnification (A) x100 and (B) to (D) x200.

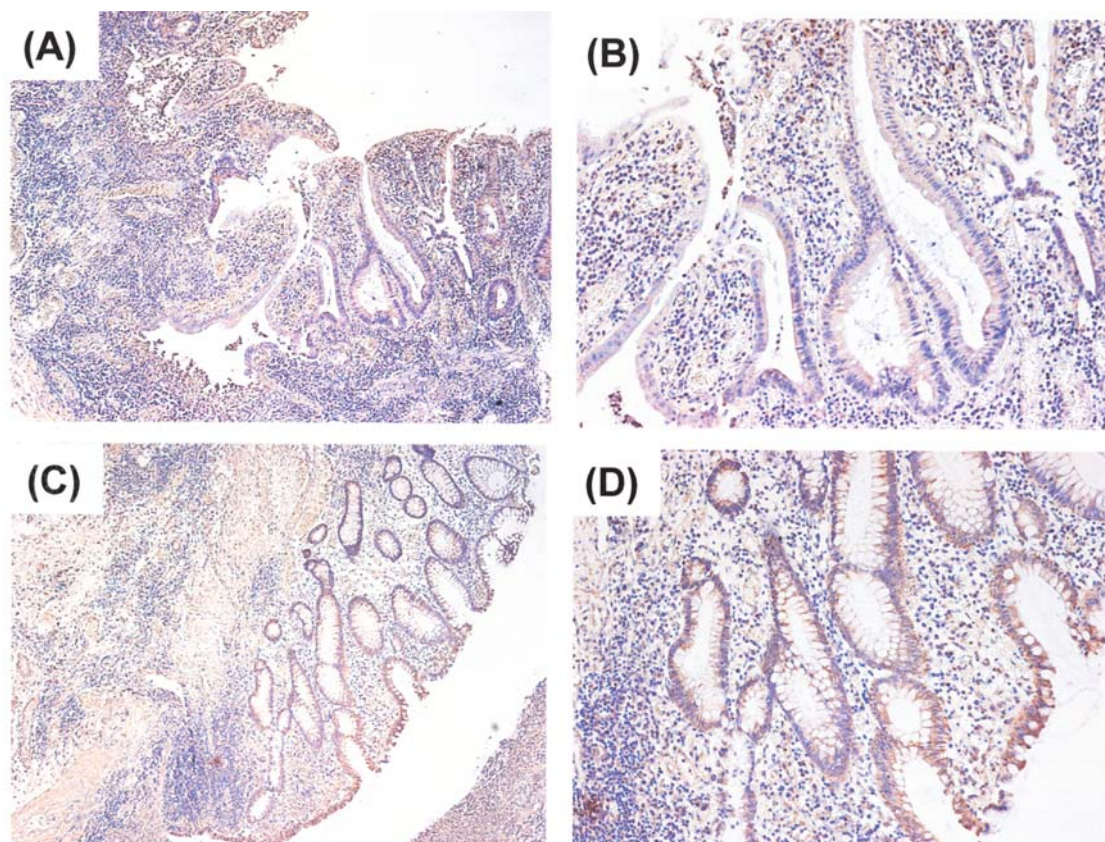


Figure 4. Epieregulin expression in IBD mucosa. (A) and (B), ulcerative colitis; and (C) and (D), Crohn's disease. Original magnification (A) x100.

inflamed mucosa of IBD patients. Amphiregulin and epiregulin were not expressed in the normal colonic mucosa, but were detectable in the neoplastic lesions and in the IBD mucosa. To our knowledge, this is the first report on the amphiregulin expression in the inflamed mucosa of IBD patients. Furthermore, this is the first immunohistochemical analysis of the epiregulin expression in neoplastic lesions in the colon and IBD mucosa. These observations suggest that amphiregulin and epiregulin play a role in tumor growth and mucosal repair in the colon.

In various cells, amphiregulin and epiregulin are bifunctional growth factors. Recently, we demonstrated that recombinant amphiregulin and epiregulin stimulated the proliferation of human colonic myofibroblasts in a dose- and time-dependent manner (21). Previous studies also showed that amphiregulin and epiregulin stimulate the proliferation of colonic epithelial cells (11,12,17,22). This suggests that amphiregulin and epiregulin play a crucial role in the process of wound healing through their action on both epithelial and mesenchymal cells in the intestine. This hypothesis may be supported by previous reports which indicate that mice deficient in EGFRs show an increased susceptibility to DSS-induced colitis (17,23).

In this study, we found that the amphiregulin and epiregulin expression was increased in epithelial and mesenchymal cells from the IBD mucosa. We have recently reported that IL-1 β and TNF- α induced the amphiregulin and epiregulin expression in colonic myofibroblasts (21). The proinflammatory cytokines IL-1 β and TNF- α promote inflammatory responses and regulate aspects of cellular immunity which are important for host defence against infection. The expression of IL-1 β and TNF- α was reported to be elevated in the inflamed mucosa of inflammatory bowel disease. These findings suggest that proliferative responses in inflamed colonic mucosa are mediated by proinflammatory stimuli-induced amphiregulin and epiregulin in both autocrine and paracrine fashions. It is likely that proinflammatory cytokines such as IL-1 β and TNF- α may play an important role in the process of tissue remodelling and wound healing through the induction of amphiregulin and epiregulin in the intestinal mucosa. EGF may also coordinate with these proinflammatory cytokines to promote tissue repair processes via the induction of amphiregulin and epiregulin.

Colonic carcinogenesis involves a stepwise accumulation of mutations in tumor-suppressor genes and proto-oncogenes (24). These mutations in turn deregulate the mechanisms controlling crypt cell proliferation, maturation and apoptosis that are normally controlled by multiple homeostatic mechanisms, including signals from the EGFR. Targets of these signalling pathways include cyclin D1 and cyclooxygenase-2 (COX-2). Cyclin D1 is an important EGFR target that controls G1-S cell cycle progression. COX-2 is the prevalent and rate-limiting enzyme required for prostaglandin biosynthesis. In human colon cancers, alterations in ErbB receptors, such as the up-regulation of EGFR and ErbB2 and increases in EGFR ligands including transforming growth TGF- α , have been described (24). Increases in the EGFR or ErbB2 expression portend a greater invasiveness of these tumors and a worse prognosis. Our observations of the amphiregulin and epiregulin expression in adenomas and

carcinomas indicate that autocrine and paracrine secretion mechanisms of these factors are involved in tumor growth in the colon.

In conclusion, this study demonstrated the amphiregulin and epiregulin expression in neoplastic and inflammatory lesions in the colon. The findings suggest that amphiregulin and epiregulin may play an important role in tumor growth and mucosal repair in the colon.

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