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GASTROINTESTINAL MUCOSAL REGENERATION: ROLE OF GROWTH FACTORS

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1. ABSTRACT

Growth factors and their receptors play important roles in cell proliferation, migration, tissue injury repair and ulcer healing. In gastric mucosa, transforming growth factor alpha (TGF-alpha) and epidermal growth factor (EGF) by activating their common receptor, control cell proliferation. TGF-alpha predominantly plays this role under normal conditions and after acute injury, while EGF exerts its actions mainly during healing of chronic ulcers. During regeneration of injured gastric mucosa, these growth factors serve predominantly to restore the epithelial component. Other growth factors, basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) serve to promote restoration of the connective tissue and microvessels (angiogenesis) in injured mucosa. During healing of chronic ulcers, a new epithelial lineage secreting EGF and other growth peptides develops and the majority of cells lining the ulcer margin overexpress the EGF receptor. Activation of the EGF receptor induces dramatic increases in MAP (Erk -1 and -2) kinase activity and phosphorylation levels. Inhibition of this signaling pathway dramatically delays ulcer healing. Granulation connective tissue, which grows under the stimulation of bFGF and VEGF is the major source for regeneration of connective tissue lamina propria and microvessels within the ulcer scar. Other growth factors such as insulin – like growth factor, keratinocyte growth factor, hepatocyte growth factor and trefoil peptides have been implicated in gastrointestinal (gastric ulcers, colitis) regeneration following injury.

This paper is intended to provide an overview of the role of growth factors in gastrointestinal mucosal regeneration.

2. INTRODUCTION

Cells of the gastrointestinal tract have a rapid turnover rate, which makes the gastrointestinal mucosa one of the most rapidly proliferating tissues in the body, second only to the skin (1). Under normal conditions, cell populations within the gastrointestinal tract are maintained at a dynamic steady state because the cell loss through exfoliation of surface cells (resulting from frequent exposure of the gastric mucosa to substances, with a wide range of pH, osmolarity, and temperature) is balanced by a continuous cell renewal (2). Undifferentiated precursor or stem cells within the crypts of the small intestine and colon, and the mucous cells of the glandular neck of the gastric mucosa are responsible for continuous cell renewal in the gastrointestinal tract (2). The balance between cell loss and cell renewal must be tightly regulated since excessive cell loss can result in atrophy or ulceration while excessive proliferation or prolonged cell life span can lead to hyperplasia (3).

Important in this regulation are several growth factor peptides including those of the epidermal growth factor (EGF) family, the transforming growth factor beta

(TGF-beta) family, the insulin-like growth factor (IGF) family, and the fibroblast growth factor (FGF) family (3, 4). In general, growth factor peptides function as mediators of cell proliferation and/or differentiation although some growth factors (eg TGF-beta) have been shown to inhibit the proliferation of certain cell types. The binding of these peptides to specific transmembrane receptors on the surface of target cells initiates signal transduction cascades which culminate in the activation of specific genes within the nucleus leading to cell division or differentiation (for a comprehensive review, please see ref 5). The peptide growth factors within a particular family either share structural similarities and/or share a common receptor or family of receptors. There are also other growth factors present in the gastrointestinal tract, which do not share similarities with any other growth factors identified to date (4). These include hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF). The latter of which has multiple forms arising from transcriptional splice variants of the same gene. In addition to their function in cell proliferation and differentiation, some growth factor peptides in the gastrointestinal tract (e.g. EGF and TGF-alpha) elicit other independent actions such as inhibition of gastric acid secretion and stimulation of mucus production (6, 7). Moreover, while the expression of most, if not all, of the growth factors identified in the gastrointestinal tract is not limited to specific sites, the levels of expression of specific growth factors vary widely depending on the state of the tissue (eg normal state vs injured state) (8-10). This review is intended as a summary of current knowledge regarding the involvement of growth factors in gastrointestinal mucosal regeneration.

3. NORMAL MUCOSAL CELL RENEWAL

Immunohistochemical studies have identified the epidermal growth factor receptor (EGF-R), which is shared by both EGF and TGF-alpha in some of the gastric mucous neck cells (8). Correspondingly, in the gastric mucosa, the highest levels of its ligand - TGF-alpha (but not EGF) are present in the mucous neck cells. In the small intestine, the highest concentrations of TGF-alpha are found in the differentiated villus cell compartments (11, 12). TGF-alpha is also found in colonic epithelial cells (13). While EGF appears to be involved in maturation of the gastrointestinal tract (14-16), the levels of TGF-alpha in the gut far exceed those of EGF (6, 7). The normal expression of EGF in the gastrointestinal tract is restricted to the salivary glands, pancreas and Brunner's glands of the proximal duodenum (4). Luminal EGF is most likely primarily derived from the saliva, duodenal and pancreatic secretions. However, since the (EGF-Rs) are confined to the basolateral compartment of polarized epithelial cells (17-19), TGF-alpha, rather than EGF, would be more likely to utilize the EGF-R under normal circumstances, as luminal EGF would not be able to encounter the basolateral EGF-R in the intact mucosa. This may not be the case in the gastrointestinal tract of young mammals since experimental data suggest that EGF is rapidly transported across the jejunal epithelium in suckling rats (20). It is possible that EGF serves functions in the maturing gastrointestinal tract that are distinct from those in the adult gastric mucosa. Acute gastric mucosal injury is associated with overexpression of EGF-R and TGF-alpha but not EGF (9). In contrast, chronic mucosal injury results in the appearance of a novel cell lineage that produces EGF (21). Once the gastrointestinal mucosa has been breached by injury, this source of EGF, as well as luminal EGF, may access the basolateral EGF-Rs and thus participate in reparative events. On the basis of these observations, Playford et al have suggested that EGF should be considered a luminal surveillance peptide and TGF-alpha as a mucosal integrity peptide (19).

4. ACUTE MUCOSAL INJURY

Gastric mucosa, upon exposure to ulcerogenic and/or necrotizing agents, such as aspirin, indomethacin, bile acids, alcohol and ischemia, develops characteristic morphologic, ultrastructural and functional changes reflecting injury. Acute gastric mucosal injury is associated with 1) disruption of the unstirred layer and surface hydrophobicity, 2) injury and exfoliation of the surface epithelium with loss of its barrier and electrical function, 3) injury of the deeper gastric mucosal layers including: a) microvascular endothelial cells, b) proliferative zone cells and c) the parietal and chief cells. Damage to the microvascular endothelium leads to microvascular stasis, cessation of oxygen delivery and transport of nutrients. All these events result in formation of mucosal erosions or ulcerations. The difference between an erosion and an ulcer is that the former is confined to the mucosa, while an ulcer also penetrates to muscularis mucosae.

Once the mucosal necrosis has occurred, all mucosal components, including microvessels, are destroyed within such lesions. Healing of deep mucosal erosions requires reconstruction of the surface epithelium (re-epithelialization) glandular epithelial structures, restoration of the lamina propria including the mucosal microvascular network, nerves and connective tissue cells.

5. MUCOSAL REGENERATION FOLLOWING ACUTE INJURY

5.1. Restitution (re-epithelialization) and cell proliferation

The gastrointestinal mucosa has a remarkable ability to repair damage. When the integrity of the superficial mucosa is breached, repair is dependent on the ability of the epithelial cells to migrate and proliferate. Restitution is the process in which viable cells bordering the damaged area migrate to cover the denuded basal membrane. This process is rapid and in vivo can be accomplished within 15-60 min. (22). It appears to be independent of proliferation or differentiation but is dependent on uninterrupted blood flow (23, 24). In these regards, several growth factors including EGF, TGF-alpha and bFGF have been shown to stimulate cell migration and to increase blood flow (5, 25-28). In addition to re-epithelialization, repair of deeper injury (erosions) requires epithelial cells to fill the mucosal defect. TGF-alpha and

EGF which are mitogenic for progenitor cell populations, increase the release of gastric mucin, attenuate gastric acid secretion and, in epithelial cell monolayers, have been shown to stimulate cell migration (29). Expression of both EGF-R and TGF-alpha rapidly increase following mucosal damage by taurocholate, hydrochloric acid or stress (9, 30). These factors (but not PDGF or FGF) may inhibit gastric acid secretion by paracrine or autocrine mechanisms thereby reducing the extent of mucosal damage (31).

The majority of receptors for peptide growth factors found in gastrointestinal tract possess intrinsic tyrosine kinase activity in their intracellular domains (32). Relan and co-workers and Majumdar et al have shown that tyrosine kinases associated with several growth factors may play important roles in regulating gastric mucosal cell proliferation after injury (32-34). Their studies demonstrated that tyrosine kinase activity associated with EGF-R was significantly (more than 200%) elevated within 30 minutes after gastric mucosal injury, suggesting that activation of this enzyme may be an important event in the initiation of the reparative process (32, 33). The phosphorylation of phospholipase C-gamma1 (PLC-gamma1) has been shown to increase its catalytic activity. PLC-gamma1 is a substrate for EGF-R tyrosine kinase and its phosphorylation subsequent to EGF-R tyrosine kinase activation has been demonstrated in the early phase of gastric mucosal injury repair. Subsequent studies by Majumdar and co-workers (33) demonstrated that 24 hrs after gastric mucosal injury by hypertonic saline, EGF-R levels were increased by 36-fold and this increase closely correlated with mucosal regeneration. These studies clearly indicate that activation of EGF-R is an important event in gastric mucosal regeneration following acute injury (32-34).

5.2. Angiogenesis

Repair of injury also requires angiogenesis - the formation of new microvessels (35). This facilitates nutrient and oxygen delivery to the injured area, thus enabling cell proliferation and migration. Angiogenesis is important for repair of both acute gastric mucosal injury and chronic gastroduodenal ulcer healing (see below). VEGF and bFGF are both strong angiogenic factors for vascular endothelial cells. We have demonstrated that, following acute ethanol-induced injury to the gastric mucosa, the expression of bFGF and its receptors, and VEGF increases, in the mucosa bordering necrosis and that these increases are followed by the angiogenic response (36, 37).

6. HEALING OF CHRONIC GASTRIC ULCERS

6.1. Epithelial component – mucosa of the ulcer margin

An ulcer is a deep defect in the gastric wall involving the entire mucosal thickness and penetrating through the muscularis mucosae (38-41). Healing of an ulcer requires not only cell migration (re-epithelialization) but also intense cell proliferation to fill this deep mucosal defect and to reconstruct gastric glands within the scar (38-41). It is accomplished by proliferation of cells from the ulcer margin and their migration onto the granulation tissue to cover (re-epithelialize) the ulcer base. In addition, the poorly differentiated cells from the base of the ulcer margin sprout into the granulation tissue forming tubules, which undergo transformation into gastric glands (21, 4). HGF and TGF-beta appear to be involved in tubulgenesis and cell differentiation (42). The stimulus for increased epithelial cell proliferation in the mucosa of the ulcer margin is most likely initiated by EGF and/or TGF-alpha (43). There is evidence that endogenous luminal EGF is important in the ulcer healing process (23, 27, 30). In the rat model, sialoadenectomy - removal of the salivary glands (a major source of endogenous EGF in the gastric lumen) - markedly delays healing of experimental gastric and duodenal ulcers (30). Oral administration of exogenous EGF to sialoadenectomized rats restores the healing rate to that of control rats with intact salivary glands (30). In humans, as previously mentioned, EGF is normally present in large amounts in the salivary, duodenal, and pancreatic glands and is released into saliva and duodenal and pancreatic secretions (44). A study has shown that patients with gastric or duodenal ulcers have a significantly lower salivary content of EGF compared to healthy subjects suggesting that decreased luminal EGF may predispose these patients to ulcers (23). Moreover, smoking has been shown to suppress the release of EGF in both salivary and duodenal secretions, and to delay ulcer healing (23). Although expression of EGF in the healthy gastrointestinal tract appears to be limited to salivary duodenal Brunner's glands and the pancreas, expression of EGF and EGF-R are dramatically upregulated at the edge of gastrointestinal ulcerations. Immunohistochemical studies demonstrated that cells lining dilated gastric glands at the ulcer margin display an enormous increase in expression of EGF and EGFR at the initial stage (1-7 days) after ulcer induction (10, 21, 38). Ulceration or other damage to the mucosa may allow luminal EGF, which normally is not absorbed when the mucosa is intact, to penetrate below the mucosal surface and stimulate proliferation of connective tissue cells and epithelial cells bordering the damaged area (21, 39). Moreover, gastrointestinal ulceration can induce the development of a novel cell lineage, which grows from the bases of existing crypts or glands to form new glands (21, 45). These cells produce neutral mucin and secrete abundant EGF, as well as several other peptides (45).

Studies from our laboratory have shown increased expression of EGF-R in the epithelial cells of gastric ulcer margins (10) and increased EGF-R phosphorylation levels leading to a dramatic increase in MAP-ERK-1 and ERK-2 kinase phosphorylation levels and activities (by more than 440% and 880%, respectively) during early stages of experimental gastric ulcer healing (46). Moreover, tyrphostin A46, an inhibitor of EGF-R kinase and EGF-R kinase – dependent cell proliferation, significantly inhibited the above signaling pathways and ulcer healing (46).

6.2. *Helicobacter pylori* interference with EGF-R - activated signal transduction pathway: Key mechanism to *H. pylori* inhibition of EGF - induced cell proliferation and ulcer healing?

Helicobacter pylori, a spiral, gram – negative bacterium that colonizes gastric mucosa, is the predominant cause of chronic gastritis and peptic ulcer disease in

humans (47, 48). *H. pylori* infection of the gastric mucosa results in delayed ulcer healing and is the main cause of ulcer recurrence (47, 48). This is clearly indicated by the fact that eradication of *H. pylori* infection dramatically reduces the ulcer recurrence (48). Although *H. pylori* - induced gastric pathology has been intensively studied during the past 16 years, the mechanisms by which *H. pylori* infection induces peptic ulcer disease and/or interferes with ulcer healing are not fully understood. Our previous studies have demonstrated that VacA(+) *H. pylori* culture supernatant containing vacuolating cytotoxin: 1) delays healing of experimental gastric ulcers in rats and worsens the quality of the mucosal scar (49), 2) significantly inhibits epithelial cell proliferation in the mucosa of the ulcer margin (49), 3) reduces specific binding of EGF to its receptor (50), and 4) significantly diminishes EGF – stimulated proliferation of human gastric epithelial Kato III cells (50). In a recent study, we have demonstrated that *H. pylori* culture supernatant containing vacuolating cytotoxin interferes with the EGF – triggered signal transduction cascade in human gastric epithelial Kato III cells (51). These data provide direct evidence for *H. pylori* interference with EGF – activated signal transduction pathways in gastric epithelial cells, which, as discussed above, are essential for cell proliferation and ulcer healing.

6.3.Connective (granulation) tissue component of the ulcer healing - angiogenesis

Angiogenesis is essential for the healing of chronic gastroduodenal ulcers (38-41). By forming the capillary network, angiogenesis in granulation tissue enables nutrient and oxygen delivery to the ulcer base and thus facilitates the healing process (38-41). It has been shown that stimulation of angiogenesis in granulation tissue by administration of bFGF dramatically accelerates healing of experimental (cysteamine-induced) duodenal ulcers in rats (52, 53), while chronic administration of indomethacin inhibits angiogenesis in granulation tissue and delays healing of experimental gastric ulcers in rats (54).

The review of above events clearly indicates that growth factors are intimately involved in both components of the ulcer healing process: the epithelial component from the "healing zone" at the ulcer margin where epithelial cells are under the control of EGF, TGF-alpha and other growth factors and cytokines; and, the connective tissue component (including microvessels), originating from granulation tissue, under the control of fibroblast growth factors and VEGF (38-41, 52, 53). It should be noted that topically active ulcer healing drugs such as antacid – Talcid, and Rebamipide have been shown to activate in the gastric mucosa, the genes encoding EGF, EGF-R and bFGF and its receptors, 1 and 2 (55, 56). This activation leads to increased synthesis of the respective growth factor peptides and their receptors which, in turn, activate cell proliferation, migration, re-epithelialization and angiogenesis. These findings provide a molecular basis for the ulcer healing action of these drugs (55, 56).

7. OTHER GROWTH FACTORS IMPLICATED IN MUCOSAL REGENERATION

Several other growth factors have also been implicated in mucosal healing within the gastrointestinal tract. Some evidence indicates that insulin-like growth factor (IGF) and keratinocyte growth factor (KGF) play roles in gastrointestinal healing. Activation of IGF receptors expressed on crypt cells, for example, may be important to the regeneration of the intestinal mucosa following injury (57). Increased expression of the gene encoding KGF has been associated with mucosal ulcerations in inflammatory bowel disease (58, 59). Administration of exogenous KGF has also been shown to significantly improve intestinal mucosal damage in an experimental model of colitis (59). Hepatocyte growth factor (HGF) has been shown to induce the proliferation of rabbit gastric epithelial cells in primary culture (60) and to stimulate the migration of gastric epithelial cells. It has also been implicated in playing an important role in the morphogenesis of gastric mucosal cells (61-63). The findings that the expression of HGF is greatly increased at the margins of healing gastric ulcers and that expression of the HGF-specific receptor, c-met, was increased in an experimental model of acute gastric injury strongly suggest that HGF plays an important role in the healing of gastrointestinal injury (64, 65). Another class of proteins which are most likely important in maintaining the integrity of the gastrointestinal tract and in the healing of gastric mucosal injury are the trefoil peptides. There are three known trefoil peptides: pS2 (TFF-1), human intestinal trefoil factor (hITF/TFF-2) and human spasmolytic polypeptide (hSP/TFF-3). All three proteins are predominantly expressed in the gastrointestinal tract; however, while pS2 and hSP are mainly expressed in the stomach, hITF is mainly expressed in the duodenum and colon (66, 67). The primary effect of these peptides appears to be on cell migration and all three proteins have been shown to be motogenic (promoting cell migration) but not mitogenic (68-70).

8. ROLE OF GROWTH FACTORS IN COLITIS

Both EGF and TGF-alpha, not only play crucial roles in gastroduodenal mucosal regeneration but also play important roles in colonic mucosa during colitis as seen in several animal and human models (71-75).

Several studies demonstrated EGF and TGF-alpha involvement in mucosal repair in colitis (71-75). Sottili et al found increase in TGF-alpha binding sites in experimental colitis in rabbits (71). They hypothesized that TGF-alpha is the locally expressed member of the EGF family that maintains mucosal integrity and accelerates restitution after injury (71). The hypothesis is further supported by work of Egger et al who found that knock-out mice lacking TGF-alpha had significantly increased colonic mucosal damage following injury by oral dextran sulfate compared to controls (73). EGF has also been shown to be protective for colonic mucosa in experimental colitis models (74, 75).

Colonic mucosal protection by EGF was shown in a model in which colitis was induced in rats by 2, 4, 6-trinitrobenzenesulfonic acid plus ethanol enemas (74). This study revealed that systemic EGF administration reduced mucosal damage and inflammation as demonstrated microscopically by a > 74% reduction of mucosal erosions in the EGF treated group compared to controls. Macroscopically, a reduction in tissue injury of > 60% was clearly evident in the EGF treated group and persisted for up to a week (74). Myeloperoxidase activity, a quantitative assay for acute inflammation was also reduced in the EGF treated group. These data were independently confirmed by Bass and Luck who found that EGF administration before the induction of colitis, and daily thereafter, significantly accelerated healing of colonic ulcerations (75). The mechanisms of these protective actions of EGF are not entirely clear. It has been postulated that they may be due to the ability of EGF to stimulate the synthesis and secretion of mucin glycoprotein, leading to an enhancement of host natural defenses against mucosal injury by creating a dilutional barrier that restricts injury from caustic agents and potentially scavenges toxic oxygen metabolites. Other possible mechanisms include induction of TGF-beta expression leading to cell migration and epithelial cell restitution.

The importance of the EGF/TGF-alpha peptide family has not only been shown in animal models, but also in human conditions as well. A prospective multicenter study performed on 126 preterm infants, demonstrated that necrotizing enterocolitis in formula-fed infants was 6 – 10 times more frequent than in those fed with breast milk alone and three times more common than in those fed with formula plus breast milk (76). This finding was thought to be due to absence of EGF in commercial formula. Based on this rationale, Sullivan et al gave continuous infusion of EGF for 6 days to a 9-month old girl with necrotizing enterocolitis. Following this treatment they found on biopsy a significant increase in crypt-cell mitotic activity with a rapid recovery of the villous architecture (77).

EGF was also used to treat three children with congenital microvillous atrophy, a defect associated with intractable diarrhea and characteristic microvillous involutions of the proximal small intestine (78, 79). EGF treatment caused an increase in crypt cell production rate accompanied by an increase in crypt-cell proliferation (78, 79).

9. PERSPECTIVES

From the above review it is evident that growth factors are intimately involved in gastrointestinal mucosal regeneration and healing. Future directions will include clinical application of growth factors and/or local in situ transfection of wounded mucosa with genes encoding for respective growth factors.

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