Peptide Growth Factors and Intestinal Adaptation in Short Bowel Syndrome

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Abstract

Intestinal adaptation is the term applied to progressive recovery from intestinal failure following a loss of intestinal length. The regulation of intestinal adaptation is maintained through a complex interaction of many different factors. These include nutrients and other luminal constituents, hormones, and peptide growth factors. The current paper discusses the role of peptide growth factors in intestinal adaptation following massive small bowel resection. This review focuses on the mechanisms of action of peptide growth factors in intestinal cell proliferation, and summarizes the effects of these factors on intestinal regrowth in an animal model of short bowel syndrome.

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Short bowel syndrome is a condition characterized by a diminished ability to digest and absorb a regular diet following massive loss of intestinal length [1,2]. The short-gut syndrome is a particularly important complication that occurs in newborns and infants suffering from necrotizing enterocolitis, intestinal atresia, and volvulus requiring massive intestinal resection [3]. SBS is a common problem in pediatric surgery and a significant cause of infant morbidity and mortality. Despite the availability of total parenteral nutrition, advances in resuscitation, availability of potent antibiotics, and modern techniques of organ support, the morbidity from SBS remains strikingly high [4]. The loss of functional small bowel surface area occasionally requires long-term parenteral nutrition. However, the key to survival after massive small bowel resection is the ability of the residual bowel to adapt. In the early 20th century it was first observed that the residual intestine can undergo structural changes that result in increased surface area and enhanced nutrient absorptive capacity. In 1957 Piling and Cresson [5] described the first successful extensive resection in two infants who survived with only 26 and 28 cm of remaining small bowel. Subsequently, many series of patients have documented survival in infants with even shorter small bowel remnants. A review of 50 infants with significant small bowel resection showed a good probability of survival with 15 cm or more of residual gut when ileocecal valve is preserved; a loss of ileocecal valve, however, requires at least 40 cm of residual small bowel for a reasonable chance of survival [6].

SBS = short bowel syndrome

"Adaptation" is the term applied to the progressive recovery from the intestinal failure following a loss of intestinal length in an attempt to compensate for the loss of mucosal absorptive surface area. Intestinal adaptation begins within 48 hours following resection and includes various structural and functional changes of the residual bowel. Morphologic changes are characterized by increased villus height and crypt depth due to increasing rates of enterocyte proliferation. Functional changes result in elevated nutrient uptake by isolated enterocytes. Several studies have shown that the functional integrity of the remaining intestine is much more important than the outward appearance of the bowel [4].

The dynamic process of intestinal cell turnover is a function of crypt cell proliferation rates, migration along the crypt-villus axis, enterocyte differentiation, and cell death via apoptosis. The regulation of proliferation and the balance between these processes are maintained through the complex interaction of many different factors including nutrients, hormones and secretory products of the gastrointestinal tract. Over the past decades several proteins derived from a variety of animal cells and tissues, designated peptide growth factors, have been reported to play an important role in stimulating enterocyte proliferation. Our understanding of the structure and function of the peptide growth factors has advanced rapidly in recent years. Peptide growth factors appear to mediate many of the processes required for normal intestinal growth and differentiation. Every growth factor modulates growth via autocrine, juxtacrine or paracrine mechanisms and acts usually as a mitogen through the stimulation of specific cell surface receptors. It has been reported that growth factors stimulate cell proliferation through the alteration of transcription of various genes [7].

Peptide growth factors are often divided into several groups based on their structure and mode of induction. They include the epidermal growth factor family, the transforming growth factor-beta family, the insulin-like growth factor family and the fibroblast growth factor family. In addition, a smaller number of peptide growth factors having different structural properties as compared to main families have also been identified within the gastrointestinal tract. They include hepatocyte growth factor, platelet-derived growth factor, trefoil peptides, hematopoietic stem cell factors and many more [7]. The mechanisms of action of the different growth factors within the gastrointestinal tract have been described in detail by several investigators. A number of cytokines have been

presumed to have some trophic effects in the gastrointestinal tract of animals [8] and in primary cultures of small intestinal epithelium [9]. The effects of epidermal growth factor, insulin-like growth factor, transforming growth factor-alpha, and hepatocyte growth factor have been evaluated for their role in modifying cell proliferation and in stimulating the enterocyte functional activities in animal models of short bowel syndrome. The effect of other factors has been studied in normal gastrointestinal tract or in primary cultures of small intestinal epithelium, but has not been evaluated following bowel resection. Further research is required to study their effect on intestinal adaptation. Instead of providing an overview of all these factors, we will focus on those peptide growth factors that stimulate bowel growth and stimulate intestinal adaptation in short bowel syndrome.

Epidermal growth factor family

EGF was initially isolated by Cohen [10] in 1962 from mouse submandibular salivary glands as the factor responsible for promoting premature eyelid opening in neonatal mice. EGF is a multifunctional, 53 amino acid peptide that acts through stimulation of specific cell surface receptors [11]. EGF exerts a variety of biological influences in numerous cell populations. Among these, it regulates proliferation of gastrointestinal epithelium through interaction with the enterocytes at the luminal surface, and increases functional capacity of the gastrointestinal tract mucosa [12]. EGF has a number of important roles in gastrointestinal homeostasis, including cytoprotection, inhibition of gastric acid secretion, trophic effect on the gastrointestinal tract after ulceration, modulation of intestinal enzyme activity, and autocrine stimulation of tumor cell proliferation [13]. The most potent effect of EGF is related to the control of bowel growth and development and to stimulation of enterocyte cell proliferation. Additionally, EGF reverses the intestinal mucosal atrophy associated with fasting and total parenteral nutrition [14].

EGF augments the intestinal adaptation in an animal model of SBS. Multiple studies have suggested a positive effect of EGF on both structural [15] and functional [16] parameters of intestinal adaptation. However, not all investigators support this concept. Lukish and colleagues [17] showed that EGF has minimal effect on microscopic and ultrastructural features of the rat small intestinal mucosa following massive small bowel resection.

The second member of the EGF family, transforming growth factor-alpha, is a 50 amino acid polypeptide that was first identified in non-transformed fibroblast indicator cells in soft agar and was found to promote anchorage-independent growth of these cells [18]. TGFα shares many structural homologies with EGF and appears to act through the same receptor. Since its isolation from transformed cell lines, TGFα has been identified in many epithelial cell populations, including epithelium of gastrointestinal tract. Increased intestinal expression of TGFα in suckling animals during the period of significant structural and functional changes within the small intestinal mucosa suggests a role of this factor in maturation and remodeling of the neonatal mucosa [19]. TGFα has been demonstrated to directly promote cell proliferation and to exert a trophic effect on intact gastric, intestinal and colonic mucosa. TGFα as well as EGF increased thymidine incorporation in the intestinal epithelial cells, activated mitogen-activated protein kinase and its substrates in gut epithelium, and increased cell proliferation [20]. TGFα with glutamine restored mucosal architecture within 72 hours of severe ischemia of intestine [21].

Recently, the effect of TGFα on intestinal adaptation was evaluated in a mouse model of SBS. Falcone et al. [22] reported that intestinal adaptation occurs in mice with SBS despite absent TGFα expression in remaining bowel; however, exogenous TGFα stimulated enterocyte proliferation and intestinal adaptation. The role of the other EGF family members (amphiregulin, heparin-binding EGF, poxvirus growth factor, cripto, and heregulin) has not been examined during intestinal adaptation, and future studies will be needed to evaluate the role of these alternative EGF ligands during the adaptive response.

Transforming growth factor-beta family

In the intestinal mucosa, numerous cytokines affect epithelial cell differentiation and proliferation through epithelial-mesenchymal and epithelial-immune cell interaction [23]. The TGFβ family includes TGFβ1 and several peptides exhibiting various degrees of homology to this prototypic member. TGFβ1 was first isolated from human platelets as a large propeptide of 391 amino acids with the characteristics of secretory polypeptide. TGFβ is secreted usually as a biologically inactive complex. The physiologic processes regulating its bioactivation from the secreted latent form are poorly understood. TGFβ1 binds to at least five specific cell surface molecules (designated types I-V) [24] and modulates expression of a wide variety of genes.

TGFβ inhibits proliferation in all epithelial cell populations through prolongation of the gastrointestinal phase. TGFβ also overrides the effects of direct mitogens such as EGF, TGFα [8], and IGF [25], including their effect on intestinal epithelium. Evidently, no published studies have explored the effect of TGFβ on intestinal adaptation following massive small bowel resection. The most interesting possibility, but one that is speculative at present, is that TGFβ in conjunction with TGFα can contribute to the regulation of the dynamic turnover of the intestinal epithelium in the adapting gut. Additionally, TGFβ may affect bowel growth through its stimulating effect on the extracellular matrix. Homeostasis of the gut epithelium depends on interactions with the underlying connective tissue. The intestinal epithelium in the crypt-villus axis represents a continuous developmental system in which the role of fibroblast-epithelial interactions is important.

TGFβ may have a positive effect on bowel remodeling and growth following extensive resection. The possible mechanism of this effect may include its stimulating effect on syntheses of DNA by isolated fibroblast, fibroblast differentiation, and modulation of epithelial-stroma interaction. TGFβ induces intestinal site-specific

EGF = epidermal growth factor
TGFα = transforming growth factor-alpha

TGFβ = transforming growth factor-beta
IGF = insulin growth factor

tissue remodeling [26] and regulates the function and organization of the overlying intestinal epithelium [27]. Having reviewed the evidence for and against the role of TGFβ as a hypothetical pro-adaptive agent, we should also mention the recent work of Mowat et al. [28] suggesting that TGFβ plays a critical role in active mucosal immunity and regulates interaction between local immune cells, epithelial tissues and antigen-presenting cells, discriminating between harmful pathogens and antigens that are beneficial, such as food proteins and commensal bacteria [28].

Insulin-like growth factor family

The IGF family includes three peptides: insulin, IGF1, and IGF2. The evidence that both IGF1 and 2 are involved in modulation of growth and differentiation of normal small bowel comes from experiments by Laburthe and colleagues [29]. Olanrewaju and co-workers [30] showed that infusion of IGF1 into the rodent ileum resulted in a twofold increase in mucosal weight and other parameters of bowel growth. IGF1 and insulin stimulated the proliferation of intestinal epithelial cells lines; however, TGFβ antagonized the IGF1-induced cell proliferation [25]. As mentioned above, IGF1 is produced in the liver in response to growth hormone and may stimulate cell proliferation and increase villus height and nutrient absorptive capacity in an animal model of SBS. Increased IGF2 content was observed in ileal mucosa in a rat following massive small bowel resection. Additional evidence that IGF1 is involved in intestinal adaptation comes from experiments by Ziegler and colleagues [31]. They showed that in rats with SBS, the ileal IGF1 mRNA expression rose nearly twofold during intestinal adaptation after bowel resection, which was augmented with IGF1 administration. Lukish and associates [32] demonstrated that EGF and IGF1 increase substrate absorption after small bowel resection in rats, and this increase in absorption persists after cessation of administration of these growth factors.

Hepatocyte growth factor

Hepatocyte growth factor is a distinctive growth-modulating peptide that was identified in primary hepatocytes and is also expressed in stomach, small intestine and colon. HGF was found early in all human fetal digestive tissues, suggesting its morphogenic role in digestive system development during embryogenesis [33]. It was reported that intestinal mesenchyme secretes HGF, which stimulates the growth of attaching epithelial cells by a paracrine mechanism. Comparing the effect of TGFα, TGFβ, keratinocyte growth factor and HGF on restitution of intestinal epithelial cells, Nishimura and collaborators [34] found that HGF was the most potent of the cytokines in accelerating repair of the damaged monolayer of intestinal epithelium. Further experiments demonstrated that HGF can increase intestinal epithelial cell mass and function *in vivo*. After reviewing the evidence for the role of HGF as a pro-adapting agent after bowel resection, it should be mentioned that recent work by Schwartz and colleagues [35] demonstrated a dramatic response in mucosal mass and enterocyte

functional capacities following bowel resection in rats exposed to HGF. Other research showed that HGF up-regulates intestine epithelial glucose transporter gene expression, which is responsible for the enhanced carbohydrate absorption in rats with SBS after HGF administration [36].

Fibroblast growth factor family

Fibroblast growth factors play key roles in controlling tissue growth, morphogenesis, and repair in animals. FGF2 was responsible for 60% of the fibroblast-stimulating activity of small intestinal submucosa, which is primarily an acellular extracellular matrix material that may modulate wound healing and tissue remodeling [37]. Additionally, basic FGF provides protective effects in liver and gut in rats following ischemia and reperfusion injury. The other member of the FGF family, FGF18, was recently found to stimulate proliferation and increase organ weight in liver and small intestine in normal mice [38].

Platelet-derived growth factor family

PDGF is highly expressed in mesenchyme supporting an epithelium in many developing organs such as the trachea and intestine. Additionally, PDGF is involved in growth and development of blood vessels [39]. PDGF may activate and proliferate intestinal sub-epithelial myofibroblasts. Growth factors and cytokines secreted by these cells promote epithelial restitution and proliferation and play an important role in the organogenesis of the intestine [40].

The effects of the FGF family, TGFβ family, and PDGF family on bowel growth have been examined in normal intestine, but have not been evaluated in an animal model of short bowel syndrome. Future experiments will therefore be needed to examine the role of these factors and to elucidate the potential mechanisms by which they affect the adaptive response. More work will be required to resolve questions concerning enterocyte gene regulation and protein expression responsible for the enhancement of substrate absorption by several peptide growth factors beyond that which occurs with intestinal adaptation. Finally, much is still to be learned about the ability of peptide growth factors to enhance intestinal adaptation in clinical trials, which will suggest new therapeutic strategies for augmenting these processes in humans.

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HGF = hepatocyte growth factor

FGF = fibroblast growth factor
PDGF = platelet-derived growth factor

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