PDGF-α stimulates intestinal epithelial cell turnover after massive small bowel resection in a rat

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Sukhotnik I, Mogilner JG, Pollak Y, Blumenfeld S, Bejar J, Coran AG. PDGF-α stimulates intestinal epithelial cell turnover after massive small bowel resection in a rat. Am J Physiol Gastrointest Liver Physiol 302: G1274–G1281, 2012. First published March 29, 2012; doi:10.1152/ajpgi.00532.2011.—Numerous cytokines have been shown to affect epithelial cell differentiation and proliferation through epithelial-mesenchymal interaction. Growing evidence suggests that platelet-derived growth factor (PDGF) signaling is an important mediator of these interactions. The purpose of this study was to evaluate the effect of PDGF-α on enterocyte turnover in a rat model of short bowel syndrome (SBS). Male rats were divided into four groups: Sham rats underwent bowel transection, Sham-PDGF-α rats underwent bowel transection and were treated with PDGF-α, SBS rats underwent a 75% bowel resection, and SBS-PDGF-α rats underwent bowel resection and were treated with PDGF-α. Parameters of intestinal adaptation, enterocyte proliferation and apoptosis were determined at euthanasia. Illumina’s Digital Gene Expression analysis was used to determine PDGF-related gene expression profiling. PDGF-α and PDGF-α receptor (PDGFR-α) expression was determined by real-time PCR. Western blotting was used to determine p-ERK, Akt1/2/3, bax, and bcl-2 protein levels. SBS-PDGF-α rats demonstrated a significant increase in PDGF-α and PDGF-α receptor (PDGFR-α) expression in jejunum and ileum compared with sham animals. SBS-PDGF-α rats demonstrated a significant increase in bowel and mucosal weight, villus height, and crypt depth in jejunum and ileum compared with SBS animals. PDGF-α receptor expression in crypts increased in SBS rats (vs. sham) and was accompanied by an increased cell proliferation following PDGF-α administration. A significant decrease in cell apoptosis in this group was correlated with lower bax protein levels. In conclusion, in a rat model of SBS, PDGF-α stimulates enterocyte turnover, which is correlated with upregulated PDGF-α receptor expression in the remaining small intestine.

intestinal adaptation; platelet-derived growth factor; enterocyte proliferation; enterocyte apoptosis; short bowel syndrome

INTESTINAL GROWTH, GUT CELL proliferation, cell differentiation, and cell death via apoptosis after massive bowel resection are regulated by reciprocal interactions between the epithelium and the underlying mesenchymal stroma. Growing evidence suggests that platelet-derived growth factor (PDGF) is an important mediator of these interactions. The formation of intestinal villi is normally preceded by the condensation of mesenchymal cells adjacent to the epithelium at the presumptive sites of new villus formation. Proliferating cells are restricted to crypts that are deeply embedded in the submucosal mesenchyme. As cells begin to differentiate, they migrate toward the lumen and are eventually shed, either from the tips of the intestinal villi or from the surface of the intestinal epithelium (13, 20). Changes in the stromal environment may indirectly contribute to changes in the number and size of the crypts as well as allow their progressive invasion of villus tissue. PDGF-α and its receptor (PDGFR-α) are positive regulators of stromal proliferation in the gastrointestinal tract and are normally expressed throughout the villus epithelium (1). Mice lacking PDGF-α and its receptor were found to develop an abnormal intestinal mucosal lining, including fewer and misshapen villi and loss of pericryptal mesenchyme, presumably as a result of loss of a PDGF-α target cell population in the mesenchyme of the developing submucosa (16).

The purpose of this study was to evaluate the effects of PDGF-α on intestinal regrowth following massive small bowel resection in rat and to determine the mechanisms by which PDGF-α influences mucosal hyperplasia as well as its effect on enterocyte proliferation and cell death via apoptosis. Additionally, the present study was conducted to determine whether effects of PDGF-α on enterocyte turnover are correlated with PDGF-α receptor (PDGFR-α) expression along the villus-crypt axis.

METHODS

This study was approved by the Institutional (Rappaport Faculty of Medicine, Technion, Haifa, Israel) Animal Care and Use Committee, and all experiments were carried out in accordance with the guidelines established by the “Guide for the Care and Use of Laboratory Animals.” Adult male Sprague-Dawley rats weighing ~250–260 g were maintained in a 12-h light-dark cycle (6 AM lights on, 6 PM lights off) and were allowed free access to water and standard rat chow for 5–7 days to acclimatize to the housing conditions.

Design

In the first experiment, the role of PDGF signaling after massive small bowel resection was investigated. Animals were divided randomly into two experimental groups of 8 rats each. Group A rats underwent bowel transection and reanastomosis (Sham); group B animals underwent 75% bowel resection (SBS). Illumina’s Digital Gene Expression analysis using Illumina Rat Quad BeadChips was used to determine PDGF-related gene expression profiling in intestinal mucosa from jejunum and ileum (7). MIAME (Minimum Information About a Microarray Experiment) was used to enable the interpretation of the results of the experiment. Data were submitted to the GenBank database (GEO accession GSE 36122). PDGF-α, PDGFR-α, PDGFR-β, and PDGFR-β mRNA throughout gastrointesti-
nal tract was determined using real-time PCR. Immunohistochemistry for PDGFR-α was used to investigate PDGFR-α expression along the villus-crypt axis. In the second experiment, the effect of exogenous PDGF-α on intestinal adaptation after massive small bowel resection was evaluated. Thirty-two rats were randomly assigned to one of four groups: group A, Sham rats underwent bowel transection (n = 8); group B, Sham-PDGF-α rats (n = 8) underwent bowel transection and were treated with PDGF-α given at a dose of 50 mg/kg ip from day 4 to day 14 as previously described (26); group C, SBS animals underwent 75% bowel resection (n = 8), and group D, SBS-PDGF-α (n = 8) rats underwent bowel resection and were treated with PDGF-α similar to group B.

Operative Technique

Following an overnight fast, the animals were anesthetized with an intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg). The abdomen was opened through a midline incision. In resected animals, a point 5 cm from the ligament of Treitz as well as a point 10 cm proximal to the ileocecal junction were used to determine the extent of bowel resection. The remaining bowel was reanastomosed with a single layer of interrupted 6-0 silk sutures, ensuring patency of the lumen. A sham group of animals underwent distal small bowel transection (at a point 10 cm proximal to the ileocecal junction) and reanastomosis without resection. In all animals, the abdomen was closed with interrupted 3-0 Dexon sutures. Following surgery, the rats were fasted for 24 h and then were pair-fed a standard chow diet ad libitum.

Genome Sequencing Library Preparations

Microarray expression profiling was performed in the Genomics Core Facility (BioRap Technologies, Rappaport Research Institute, Technion). Total RNA was isolated from mucosa of remaining ileum by using a TRIzol (Invitrogen, Carlsbad, CA) extraction protocol with chloroform interphase separation, isopropanol precipitation, and ethanol wash steps. Precipitated total RNA was resuspended in RNAase-free water. Spectrophotometric determination of purified total RNA yield was performed using the Nano-Drop ND-1000 (Thermo Scientific, Wilmington, DE). Total RNA quality was checked by using an Experion RNA StdSens Chip (Bio-Rad). The RNA was amplified into cRNA and biotinylated by in vitro transcription by using the TargetAmp Nano-g Biotin-aRNA labeling kit for the Illumina system (Epicentre Biotechnologies) according to the manufacturer’s protocol, with 200 ng of total RNA as input material. Biotinylated cRNA was purified, fragmented, and subsequently hybridized to an Illumina RatRef-12 Expression BeadChip V1 for genome-wide expression analysis containing 21,910 probes selected primarily from the NCBI RefSeq database (Release 16) according to the Direct Hybridization assay (Illumina). The hybridized chip capable of querying 12 samples in parallel was stained with streptavidin-Cy3 (Amersham) and scanned with an Illumina BeadArray 500GX Reader. The scanned images were imported into GenomeStudio (Illumina) for extraction and quality control, generating an output file for statistical analysis.

Pathway analysis was performed using Ingenuity Pathway Analysis (IPA) to identify canonical (i.e., cell signaling and metabolic) pathways and gene-gene interaction networks potentially involved in SBS within our dataset. Differentially expressed transcripts in the SBS group (P < 0.05) were imported into IPA (v7.5; Ingenuity Systems, Redwood City, CA; www.ingenuity.com). Transcripts’ gene identifiers were mapped to their corresponding gene object in the Ingenuity Pathways Knowledge Base (17).

Real-Time PCR

RNA was isolated by using TRizol (Invitrogen) reagent according to the manufacturer’s instructions, and quantification of RNA was performed by 260/280 nm spectrophotometry. The method was extended by using reverse transcriptase (PrimeScript RT reagent Kit TaKaRa) to convert 500 ng of total RNA into complementary DNA (cDNA), which was then amplified by PCR-Thermal Cycler (2720 Thermal Cycler, ABI). Gene expression of PDGF-α, PDGFR-α, PDGF-β, and PDGFR-β was determined by quantitative real-time PCR ABI-PRISM 7000 (Applied Biosystems, Foster City, CA) on cDNA samples by use of Cyber Green Master Mix (Takara) with the exception of template and primers.

PDGF-α Receptor Expression Along the Villus-Crypt Axis

Immunohistochemistry for PDGF-α receptor (PDGFR-α polyclonal antibody; dilution 1:100, ab61219, Abcam, Cambridge, UK) was performed to identify PDGF-α expression, by using a combination of streptavidin-biotin-peroxidase method according to the manufacturers’ protocols. The paraffin-embedded sections were dewaxed and rehydrated with xylene and graded alcohol. Tissue sections were microwave pretreated in 10 mM EDTA buffer and incubated with an endogenous peroxidase (3%) in methanol for 10 min. After incubation with blocking solution at room temperature for 10 min, the sections were then incubated with PDGF-α receptor concentrated polyclonal antibody (dilution 1:50) for 60 min and second human-absorbed, biotinylated, affinity-purified antibody for 20 min. PDGF-α receptor-positive color development was obtained by incubating the sections with DAB (dioxynaminobenzidine) substrate (Zymed Laboratories). PDGFR-α receptor expression and enterocytes apoptosis along the villi were differentiated between the lower one-third of the villi (lateral villi), upper one-third of the villi (villi tips), and crypt compartment. ImageLab was used to quantify the intensity of brownish-color immunostaining. Twelve randomized labeled cytoplasmic regions from different cells were indicated, with the same-sized square (tool of ImageLab system). The average optical density (OD) of these areas was automatically calculated and represents the average of red, green, and blue color composition per area of cytoplasm analyzed, expressed in optical units per micrometer squared.

Enterocyte Proliferation and Apoptosis

Cryp cell proliferation was assessed with 5-bromodeoxyuridine (5-BrdU). BrdU labeling reagent (Sigma B5002) was injected intraperitoneally at a concentration of 100 mg/100 g body wt 2 h before euthanasia. Tissue slices were stained with a biotinylated monoclonal anti-BrdU antibody system provided in a kit form (Zymed Laboratories, San Francisco, CA). An index of proliferation was determined as the ratio of crypt cells staining positively for BrdU per 10 crypts. Additional 5-μm-thick sections were prepared to establish the degree of enterocyte apoptosis. Immunohistochemistry for caspase-3 (caspase-3 cleaved polyclonal antibody, dilution 1:100, CP229B, Biocare Medical) was performed for identification of apoptotic cells using a combination of the streptavidin-biotin-peroxidase method and microwave antigen retrieval on formalin-fixed, paraffin-embedded tissues according to the manufacturer’s protocols. A qualified pathologist blinded as to the source of intestinal tissue performed all measurements.

Western Blotting

Tissue was homogenized in RIPA lysis buffer containing 50 mM Tris·HCl (pH 7.4), 150 mM NaCl, 1% Nonidet P-40, and 2 mM EDTA, supplemented with a cocktail of protease (Roche Diagnostic) and phosphatase cocktail inhibitors (Sigma). Protein concentrations were determined by Bradford reagent according to the manufacturer’s instructions. Samples containing equal amounts of total protein (30 μg) were resolved by SDS-PAGE under reducing conditions. After electrophoresis, proteins were transferred to polyvinylidene difluoride membrane and probed with various primary antibodies anti-Bcl-2 antibody (1:1,000 dilution, sc-7382), anti-Bax antibody (1:2,000 dilution, sc-493), anti-phospho-ERK (p-ERK) antibody (1:2,500 dilution, sc-493), anti-β-actin antibody (1:12,000 dilution, sc-37000). Future 1:2,000 dilution of DAB (3,3′-diaminobenzidine substrate, Zymed Laboratories) was applied to visualize the protein expression.

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tion, sc-7383), and anti-Akt 1/2/3 antibody (1:500 dilution, sc-8312) and two other antibodies, anti-ERK2 (1:2,000 dilution, Cell Signaling no. 9108) and anti-β/tubulin (1:5,000 dilution, Sigma T6557), and used for the protein normalization. Horseradish peroxidase-conjugated secondary antibody was purchased from Jackson ImmunoResearch Laboratories (West Grove, PA) and an enhanced chemiluminescent substrate from Biological Industries (Kibbutz Beth HaEmek, Israel). The optical density of the specific protein bands was quantified by using a densitometer (Vilber Lourmat, Lyon, France).

Statistical Analysis

The data are expressed as the means ± SE. A one-way ANOVA for comparison, followed by Tukey’s test for pairwise comparison, was used for statistical analysis. Prism software was used (GraphPad Software, San Diego, CA), and statistical significance was defined as \( P < 0.05 \). Microarray data were analyzed by using the Partek Genomics Suite software program. To evaluate the magnitude of differential gene expression the displacement of each detected transcript’s mean expression value was measured between the two groups. A standard regression analysis was performed on the SBS group to test whether the mean transcription level differed from that of the Sham group.

RESULTS

First Experiment

Microarray expression profiling. One hundred sixty-five genes were differentially expressed in rat intestine after massive small bowel resection compared with controls (fold change > 1.2, \( P < 0.001 \)). From this pool of genes, 10 genes were more than twofold regulated. In jejunum, 76 genes were dorsally enriched and 89 genes were ventrally enriched; in ileum, 74 genes were dorsally enriched and 91 genes were ventrally enriched. Forty-three genes were differently enriched in jejunum and ileum. Functional clustering analysis revealed increased expression in signaling pathways involved in tryptophan, ascorbate and aldarate metabolism, biosynthesis of steroids, propanoate metabolism, glycine, serine and threonine metabolism, and fatty acid metabolism. The less significantly changed functions were purine metabolism, pyrimidine metabolism, and ephrine receptor signaling. From the total amount of...
20,000 probes, 16 genes related to PDGF signaling were investigated. From these genes, five genes remained unchanged and one gene (regulator of G-protein signaling 14) was downregulated. Ten genes were found to be upregulated in SBS vs. sham animals with a relative change in gene expression level of 20% or more in five genes: Jun D proto-oncogene (Gene ID ILMN_70319), GTP binding protein (ILMN_58213), c-fos oncogene (ILMN_64186), CCAT enhancer binding protein (ILMN_53454), and transforming growth factor-β-induced (ILMN_67786). Moreover, cluster analysis based on all genes represented on the microarray chip showed a clear differentiation.

**PDGF-α, PDGFR-α, PDGF-β, and PDGFR-β mRNA expression**. The effect of bowel resection on PDGF-α, PDGFR-α, PDGF-β, and PDGFR-β mRNA throughout the gastrointestinal tract was determined by real-time PCR (Fig. 1). As expected, bowel resection (group B) resulted in a significant increase in PDGF-α mRNA expression in jejunum (twofold increase, \( P < 0.05 \)) and ileum (twofold increase, \( P < 0.05 \)) as well as in PDGFR-α receptor expression in jejunum (twofold increase, \( P < 0.05 \)) and ileum (not significant) compared with sham animals (group A). Evaluation of PDGF-β and PDGFR-β mRNA showed a strong increase in PDGF-β mRNA expression in jejunum (fivefold increase, \( P < 0.05 \)) and ileum (threefold increase, \( P < 0.05 \)) without changes in PDGF-β receptor mRNA expression.

**PDGF-α receptor expression along the villus-crypt axis**. PDGF-α receptor-positive cells are normally dispersed in the subepithelial layer of the small intestine at both peripheral mesenchyme and subepithelial mesenchyme. Our data suggest that in sham animals PDGFR-α was expressed in the submucosal mesenchyme and in the mesenchymal cells between the muscle layers. Additionally, a weak staining was observed in a single cell layer beneath the epithelium of crypts and in the intervillus epithelium (Fig. 2). After massive bowel resection, a strong PDGFR-α expression was seen in clusters of mesenchymal cells (villus clusters) associated with the growing villus. Strong PDGFR-α expression was also seen in a discontinuous layer of mesenchymal cells beneath the intervillus epithelium and in the base of the crypts. The average OD increased significantly in SBS rats compared with sham animals (16%, \( P = 0.005 \)).

**Second Experiment**

**Parameters of intestinal adaptation**. Massive small bowel resection resulted in a significant decrease in body weight. SBS rats (groups C and D) had a significantly lower final body weight compared with Sham rats (98 ± 4 and 96 ± 2 vs. 120 ± 2% initial weight, \( P < 0.001 \)). Treatment with PDGF-α of sham and SBS animals (groups B and D) did not significantly change final body weight compared with non-treated rats (groups A and C). SBS rats (group D) demonstrated a significant increase in overall bowel and mucosal weight in jejunum (fourfold increase, \( P < 0.001 \)) and in ileum (twofold increase, \( P < 0.001 \)) (Fig. 3), mucosal DNA and protein in jejunum (sevenfold and fourfold, respectively, \( P < 0.001 \)) and in ileum (twofold and threefold, \( P < 0.001 \)) (Fig. 4), villus height and crypt depth in jejunum (1,042 ± 64 \( \mu \)m and 440 ± 64 vs. 599 ± 43 \( \mu \)m and 292 ± 20 \( \mu \)m, correspondingly, \( P < 0.05 \)) and ileum (906 ± 73 \( \mu \)m and 371 ± 65 vs. 577 ± 35 \( \mu \)m and 234 ± 27 \( \mu \)m, correspondingly, \( P < 0.05 \)) (Fig. 5) compared with sham animals (group A). Sham-PDGF-α animals (group B) demonstrated a significant increase in villus height and crypt depth in jejunum (890 ± 146 and 422 ± 29 \( \mu \)m vs. 599 ± 43 and 292 ± 20 \( \mu \)m, correspondingly, \( P < 0.05 \)) and ileum (815 ± 88 and 392 ± 35 \( \mu \)m vs. 577 ± 35 and 234 ± 27 \( \mu \)m, correspondingly, \( P < 0.05 \)) compared with sham animals (group A) (Fig. 5). Treatment of SBS animals with PDGF-α resulted in additional bowel growth. SBS-PDGF-α rats (group D) demonstrated an additional increase in overall bowel weight in jejunum (52%, \( P = 0.008 \)), mucosal weight in jejunum and ileum (24 and 21%, \( P < 0.05 \), respectively) (Fig. 3), mucosal protein in jejunum (31%, \( P < 0.05 \)) (Fig. 4), villus height and crypt depth in jejunum (42 and 39%, respectively, \( P < 0.05 \)) and ileum (41 and 55%, respectively, \( P < 0.05 \)) (Fig. 5) compared with SBS-ununtreated animals (group C).

**Enterocyte proliferation and apoptosis**. Treatment of sham animals with PDGF-α (group B) resulted in a significant increase in cell proliferation rates in jejunum (146 ± 1 vs. 128 ± 3 BrdU-positive cells/10 crypts, \( P < 0.001 \)) and ileum (154 ± 1 vs. 126 ± 3 BrdU-positive cells/10 crypts, \( P < 0.001 \)) compared with sham animals (group A). SBS rats (group C) demonstrated a significant increase in cell proliferation in jejunum (171 ± 4 vs. 128 ± 3 BrdU-positive
cells/10 crypts, \( P < 0.001 \) and ileum (167 \( \pm \) 6 vs. 126 \( \pm \) 3 BrdU-positive cells/10 crypts, \( P < 0.001 \)) and concomitant increase in cell apoptosis in jejunum (2.6 \( \pm \) 0.4 vs. 0.87 \( \pm \) 0.2 apoptotic cells/10 villi, \( P < 0.001 \)) and ileum (2.4 \( \pm \) 0.2 vs. 1.2 \( \pm \) 0.3 apoptotic cells/10 villi, \( P < 0.001 \)) compared with Sham rats (group A). Treatment with PDGF-\( \alpha \) (group D) resulted in an additional increase in cell proliferation rate in jejunum (24\%, \( P = 0.001 \)) and ileum (24\%, \( P < 0.001 \)) as well as in a significant decrease in cell apoptosis in jejunum (1 \( \pm \) 0.3 vs. 2.6 \( \pm \) 0.4 apoptotic cells/10 villi, \( P < 0.001 \)) and ileum (1.3 \( \pm \) 0.3 vs. 2.4 \( \pm \) 0.2 apoptotic cells/10 villi, \( P < 0.001 \)) compared with SBS rats (group C).

**Western blot.** Elevated cell proliferation rates in SBS animals (group C) (vs. sham) were accompanied by elevated p-ERK protein levels and unchanged Akt 1/2/3 levels (Fig. 7). Treatment of both sham and SBS animals with PDGF-\( \alpha \) (groups B and D) resulted in a significant upregulation of Akt 1/2/3 levels and concomitant increase in p-ERK levels compared with nontreated animals. A significant increase in cell apoptosis in SBS rats (group B) (vs. Sham) was accompanied by a significant increase in proapoptotic Bax gene expression (\( P < 0.05 \)) and a concomitant decrease in Bcl-2 gene expression (\( P < 0.05 \)) compared with Sham rats (group A) (Fig. 3). Treatment with PDGF-\( \alpha \) (group D) did not significantly change Bcl-2 gene expression, but led to significant downregulation in Bax mRNA expression compared with SBS animals (\( P < 0.05 \)) that was in agreement with decreased cell apoptosis.

**DISCUSSION**

PDGF was originally isolated from blood platelets as a growth factor for connective tissue and glial cells (11) and consists of a family of A, B, C, and D polypeptides that promote cell migration, proliferation, and survival. PDGFs exert their effect by binding to their cognate homo- or heterodimeric tyrosine kinase receptors, PDGFR-\( \alpha \) and PDGFR-\( \beta \) (5). Several experiments have demonstrated that PDGF-\( \beta \) and PDGFR-\( \beta \) are essential for the development of support cells in the vasculature (12), whereas PDGF-\( \alpha \) and PDGFR-\( \alpha \) are more broadly required during embryogenesis (6), with essential roles in the development of central nervous system (18), lung (3), kidney (8), testis (4), skeleton (21), and intestine (16). PDGF actions are mediated through a dimeric transmembrane receptor containing a tyrosine kinase in its intracellular domain. In addition, PDGF has been proven to bind intracellular PDGF receptors present on intracellular vesicles in some transformed fibroblast cell lines (11). Although the frequency of this intracellular ligand-receptor binding mechanism (called intracrine regulation) is unknown, it seems likely to be relatively uncommon. Recent evidence suggests that PDGF-\( \alpha \) and its receptor, PDGFR-\( \alpha \), are necessary for the correct structuring of the mucosal lining of the gastrointestinal tract. Lack of PDGF-\( \alpha \) or PDGFR-\( \alpha \) in knockout mice resulted in progressive depletion of PDGFR-\( \alpha \)-positive mesenchymal cells at the tip of the mesenchymal core of the growing villus, the formation of fewer villus clusters, and premature expression of smooth muscle actin in the villus mesenchyme (16).
Since PDGF signaling has been implicated in tissue remodeling and cellular differentiation, and in inductive events involved in patterning and morphogenesis, we hypothesized in the present study that PDGF signaling may be involved in intestinal tissue regrowth after massive bowel resection and the present study that PDGF signaling may be involved in patterning and morphogenesis, we hypothesized in regrowth and cellular differentiation, and in inductive events in intestinal mucosal samples. Values are means ± SE. *P < 0.05 all groups vs. Sham rats, †P < 0.05 SBS, PDGF-α vs. SBS rats.

Next, we investigated whether exogenous PDGF-α may stimulate intestinal regrowth following massive small bowel resection. Our results show that massive bowel resection in a rat results in apparent stimulation of structural intestinal adaptation. This is evident from increased bowel and mucosal weight, mucosal DNA and protein content, and villus height and crypt depth, reflecting a promoted adaptive response. Comparison of morphometric parameters revealed intestinal hyperplasia to be a predominant feature of mucosal adaptation. Increase in mucosal DNA and protein along with hypertrophy of the individual cells, which we have demonstrated morphometrically, is characteristic of tissues undergoing increased cell proliferation or repair. Compared with the sham-transected group, villus height and crypt depth were significantly increased in SBS animals by 2 wk after resection, suggesting increased absorptive surface area. Mucosal response to massive resection in our experiment is comparable to the changes previously observed in our laboratory (2, 23, 24). Via BrdU immunostaining, rates of enterocyte proliferation were found to be increased in SBS rats compared with sham animals and was accompanied by increased p-ERK protein levels. ERK is one of the MAPK signaling pathways triggered by cytokines or growth factors and regulates various cellular activities, such as gene expression, mitosis, cell proliferation, differentiation, and apoptosis (19). Elevated cell proliferation in the present study was accompanied by increased rates of enterocyte apoptosis, suggesting accelerated cell turnover. In the rapidly proliferating intestinal epithelium the ratio between enterocyte proliferation and apoptosis is responsible for maintenance and enhancement or loss of intestinal absorptive function. Growing evidence suggests that the postresection increases in enterocyte proliferation are matched by greater rates of enterocyte apoptosis to maintain mucosal homeostasis. An increased cell apoptosis may be considered the mechanism that counterbalances the increased enterocyte proliferation to reach a new homeostatic set during intestinal adaptation. In addition, increased apoptosis promotes disposal of genetically aberrant stem cells and prevents tumorigenesis (9). Bax protein level was upregulated whereas bcl-2 mRNA was downregulated during intestinal adaptation. As a result, the bax/bcl-2 ratio increased in SBS rats, which correlates with the enhanced enterocyte apoptosis in this group. Extensive studies in various experimental models of SBS have established that Bax is the major protein that drives increased programmed cell death after small bowel resection (9, 22).

Administration of PDGF-α significantly enhanced structural intestinal adaptation. The observed changes in weight loss, and particularly the lower degree of weight loss in the SBS-PDGF-α group, suggest an improvement in SBS adaptation in the PDGF-α treated group, although specific absorption studies would need to be performed in future research to prove this. PDGF-α-treated rats demonstrated an additional increase in bowel and mucosal weight, mucosal DNA, and protein in jejenum. Increased villus height and crypt depth are the result of increased proliferation and accelerated migration along the villus and are a marker of increased absorptive surface area. Observed increase in enterocyte proliferation rate may be considered as a main...
mechanism responsible for compensatory hyperplasia and is responsible for increased intestinal cell mass. Our findings demonstrate that massive bowel resection appears to be considerably more effective in activating the MAPK pathway than PI3K/Akt signaling. On the other hand, treatment of sham and resected animals with PDGF-α was more efficient in transducing cell signaling through the PI3K pathway. Our data are consistent with recent evidence that the PDGF and PDGF receptors are critical for the PI3K/Akt activation and are negatively regulated by mTOR signaling (25). Enterocyte apoptosis decreased significantly in SBS-PDGF-α rats compared with SBS animals, which was consistent with decreased expression of Bax protein.

Next, we investigated whether the effects of exogenous PDGF-α on enterocyte turnover (proliferation and apoptosis) are correlated with PDGF-α receptor expression along the villus-crypt axis. Several experimental studies have demonstrated that different growth factors act differentially along the crypt-villus axis in accordance with differential expressions of their receptor. In recent studies, we have shown that TGF-α (23), leptin (24), and insulin (2) stimulate intestinal epithelial cell turnover in conjunction with their receptors’ expression along the villus-crypt axis. In the present experiment, we have shown that the proliferative and antiapoptotic effect of PDGF-α on enterocyte turnover was correlated with PDGF-α receptor expression along the villus-crypt axis. In the crypt compartment, expression of the PDGF-α receptor increased significantly after bowel resection compared with control animals (first experiment). This receptor expression was observed in mesenchymal cells beneath the intervillus epithelium and in the base of the crypts (close to the location of intestinal stem cells) and coincided with increased cell proliferation following PDGF-α administration (second experiment). Intestinal growth, gut cell proliferation, cell differentiation, and cell death via apoptosis after massive bowel resection are regulated by reciprocal interactions between the epithelium and the underlying mesenchymal stroma (10). The formation of intestinal villi is normally preceded by the condensation of mesenchymal cells adjacent to the epithelium at the presumptive sites of new villus formation. Proliferating cells are restricted to crypts that are deeply embedded in the submucosal mesenchyme. As cells begin to differentiate, they migrate toward the lumen and are eventually shed, either from the tips of the intestinal villi or from the surface of the intestinal epithelium. The upregulation of PDGF-α receptor expression in the submucosal mesenchyme near the intestinal stem cells emphasizes the possible effect of PDGF-α in the control of stem cell activity after bowel resection. In the villus, cell apoptosis was upregulated in resected rats compared with Sham rats. Similar to the crypt compartment, PDGF-α receptor expression was upregulated in clusters of villus mesenchymal cells (villus clusters) associated with the growing villus. Since PDGF-α exerts antiapoptotic effects, this increase in PDGF-α receptor coincides with decreased cell apoptosis in the villi following PDGF-α administration. Morphologically, this decline in cell apoptosis was reflected in a significant increase in total villus length, suggesting increased surface area.

In conclusion, our data indicate that 1) bowel resection in a rat results in activation of PDGF signaling; 2) exogenous PDGF-α stimulates intestinal regrowth after massive small bowel resection; and 3) the stimulating effect of PDGF-α on enterocyte turnover (proliferation and apoptosis) is correlated with PDGF-α receptor expression along the villus-crypt axis.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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