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p21$^{waf1/cip1}$ deficiency does not perturb the intestinal crypt stem cell population after massive small bowel resection☆

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Abstract

Background: After small bowel resection (SBR), adaptation is initiated in intestinal crypts where stem cells reside. Prior studies revealed SBR-induced enterocyte proliferation requires the expression of p21$^{waf1/cip1}$. As deficient expression of p21$^{waf1/cip1}$ has been shown to result in reduced numbers of hematopoietic stem cells. We sought to test the hypothesis that p21$^{waf1/cip1}$deficiency similarly perturbs the intestinal stem cell population after SBR.

Methods: Control (n = 21; C57Bl/6) and p21$^{waf1/cip1}$-null mice (n = 30) underwent 50% proximal SBR or sham operation. After 3 days, the ileum was harvested and the crypt stem cell population evaluated by counting crypt base columnar cells on histologic sections, determining the expression of Musashi-1 and Lgr5, and profiling the transcriptional expression of 84 known stem cell genes.

Results: There were no significant differences in crypt base columnar cells, expression of Musashi-1 or Lgr5, or in stem cell gene expression after SBR in control mice. Furthermore, there were no differences in these markers between controls and p21$^{waf1/cip1}$-null mice.

Conclusion: In contrast with bone marrow stem cells, the stem cell population of the gut is unaffected by deficient expression of p21$^{waf1/cip1}$. Additional mechanisms for the role of p21$^{waf1/cip1}$ in small bowel proliferation and adaptation after massive SBR must be considered.

Small intestine adaptation is a phenomenon that occurs after a massive amount of intestinal length is removed. Through this process the remaining intestine compensates for the loss of absorptive area, either macroscopically through increased villus height and crypt depth, and intestinal lengthening, or microscopically through increased cellular protein and DNA content per unit length [1].

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Patients with short bowel syndrome are in a state of malnutrition and malabsorption, and are destined to remain on intravenous sources of nutrition if small bowel adaptation is incomplete [2].

Increased proliferation of the intestinal stem cells into enterocytes is critical and necessary for adaptation [3]. Intestinal stem cells can give rise to any of the 4 types of intestinal epithelial cells (enterocytes, enteroendocrine cells, Paneth cells, goblet cells) and also replenish their own population. Under normal conditions, it is believed this occurs by asymmetric division: each stem cell divides and gives rise to one stem cell and one progenitor cell [4]. Under perturbed conditions, such as chemotherapy and radiation, the intestinal stem cells undergo symmetric division and replenish their own population by dividing and giving rise to two daughter stem cells [5].

In mice that are deficient in the expression of the cell-cycle inhibitor $p21^{waf1/cip1}$, we have previously demonstrated that resection-induced adaptation does not occur [6,7]. In addition, baseline rates of proliferation rate are slightly less. In hematopoietic progenitor cells from $p21^{waf1/cip1}$-null mice, the ability to mount a proliferative response was prevented, and there were overall decreased numbers of hematopoietic progenitor cells [8]. This was postulated to be caused by the requirement of $p21^{waf1/cip1}$ for asymmetric stem cell division. In the absence of this protein, stem cells might divide symmetrically, leaving fewer remaining stem cells. In the small intestine, it has been suggested that intestinal stem cells undergo expansion after massive bowel resection in normal mice [9]. The purpose of this study therefore was to test the hypothesis that, similar to hematopoietic stem cells, the mechanism for the lack of resection-induced adaptation in $p21^{waf1/cip1}$-null mice is caused by the diminished numbers of crypt stem cells.

2. Methods

2.1. Animals

The protocol for this study was approved by the Washington University Institutional Animal Care and Use Committee (Protocol 20070145; Washington University School of Medicine, St Louis, MO). Control (C57/Blk6) and homozygous breeding pairs for $p21^{waf1/cip1}$-null mice (developed on a C57/Bl6 background) were obtained from Jackson Laboratories (Bar Harbor, ME). Male mice aged 8 to 13 weeks were used in this study with a weight range of 20 to 25 g. Mice were kept on a 12-hour light-dark schedule and were housed in a standard facility and allowed to acclimate to their environment for at least 7 days.

2.2. Experimental design

Both control ($n = 21$) and $p21^{waf1/cip1}$-null ($n = 30$) mice were randomly assigned to either 50% proximal small bowel resection (SBR) or sham operation. On postoperative day 3, the remnant small bowel was removed to measure markers for intestinal stem cells and histologic parameters of adaptation (villus height and crypt depth). Protein and RNA were extracted from the isolated crypt cells to detect the expression of $p21^{waf1/cip1}$ and Musashi-1 protein [10] by Western blotting. For transcriptional studies of gene expression, reverse transcriptase–polymerase chain reaction was used to measure the expression of leucine-rich repeat-containing G-protein–coupled receptor 5 (Lgr5), a putative marker of intestinal stem cells [11]. We also used a commercially available stem cell polymerase chain reaction (PCR) array for the simultaneous determination of expression of 84 known genes to be linked to stem cells.

2.3. Operative procedure

Specific details of this procedure have been described previously [12]. Briefly, a 50% proximal SBR was performed by transecting the small bowel 12 cm proximal to the cecum and removing approximately 12 cm of proximal small intestine. Intestinal continuity was then restored with an end-to-end, single layered, interrupted anastomosis using a 9-0 monofilament suture. A sham operation was performed by transecting greater than 50% of the circumference of the bowel 12 cm proximal to the cecum and immediately restoring intestinal continuity with the same anastomosis. All mice were placed on a preoperative liquid diet (Micro-stabilized Rodent Liquid Diet LD101; Purina Mills, St Louis, MO) 1 day before their operation. After their operation, the animals received water only for the first 24 hours, followed by the same liquid diet until sacrifice. Animals that died, appeared ill (unkempt fur, lethargy), or had signs of intestinal obstruction at the time of sacrifice were excluded from further analyses.

2.4. Small bowel harvest and enterocyte isolation

On the third postoperative day, the mice were sacrificed with a subcutaneous injection of ketamine, xylazine, and acepromazine (4:1:1 proportion) followed by cervical dislocation. This time point was chosen as we have already established greater rates of proliferation in normal mice [12] as well as lack of proliferation in p21-null mice at this postoperative interval [6,7]. The abdominal cavity was opened, the intestinal anastomosis identified, and the remaining distal bowel excised from the mesentery and cecum. The intestine was immediately flushed with and placed in ice-cold phosphate-buffered saline. The first centimeter of the segment distal to the anastomosis was discarded, the next 2 cm was fixed for histology in 10% neutral-buffered formalin, and the subsequent 5 cm was cut open and transferred into tubes containing 5 mL of ice-cold PBS with protease inhibitors (0.2 mmol/L phenylmethylsulfonyl fluoride, 5 μg/mL aprotinin, 1 mmol/L benzamidine, 1