Roles for \textsuperscript{p}21\textsuperscript{waf1/cip1} and \textsuperscript{p}27\textsuperscript{kip1} during the adaptation response to massive intestinal resection

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SHORT BOWEL SYNDROME is a critical health concern affecting the lives of patients who experience the consequences of massive small bowel loss and varying degrees of intestinal failure. Many such patients require lifelong parenteral nutrition and are plagued by serious associated comorbidities (17, 33). Although p21\textsuperscript{waf1/cip1} and p27\textsuperscript{kip1} are generally considered to be inhibitors of the cell cycle (10, 11, 36), these proteins have also been shown to promote the assembly of cyclin-CDK complexes (5, 16), which are necessary for cell cycle progression during proliferation. This dual function may be related to the p21\textsuperscript{waf1/cip1} and p27\textsuperscript{kip1} abundance, because high levels of these Cip/Kip family members inhibit cyclin-CDK complex formation, whereas low levels are required to chaperone their assembly. Thus the mechanism of the failure of adaptation in the p21\textsuperscript{waf1/cip1}\textsuperscript{-null} mice could be due to either the requirement for p21\textsuperscript{waf1/cip1} to assemble cyclin-CDK complexes and initiate enterocyte proliferation or as a consequence of altered expression of p27\textsuperscript{kip1}.

In light of these questions, the purpose of the present study was to gain insight into specific roles for p21\textsuperscript{waf1/cip1} and p27\textsuperscript{kip1} during the adaptive intestinal response to massive SBR. We specifically tested the hypothesis that p21\textsuperscript{waf1/cip1}, not p27\textsuperscript{kip1}, is necessary for enterocyte proliferation during adaptation.

**MATERIALS AND METHODS**

**Animals.** The protocol for this study was approved by the Children’s Hospital Research Foundation Institutional Animal Care and Use Committee.

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Use Committee (Cincinnati Children’s Hospital Medical Center, Cincinnati, OH). Control (C57BL/6), p21wa1f1/cip1-null, and p27kip1-null mice were obtained from The Jackson Laboratory (Bar Harbor, ME). p21wa1f1/cip1/p27kip1 double-null mice were generated by cross-breeding homozygous male p21wa1f1/cip1-null mice with heterozygous female p27kip1-null mice. The p21wa1f1/cip1-null and heterozygous p27kip1-null mice were then again cross-bred to obtain p21wa1f1/cip1/p27kip1 double-null offspring. Genotyping was performed by analysis of DNA extracted from tail clippings of each animal according to the protocol provided by the manufacturer (available online at http://www.jax.org/imr/tail_nonorg.html). Absent p21wa1f1/cip1 and p27kip1 protein expression also was confirmed using Western blot analysis of protein derived from intestinal homogenates of each animal (see below).

**Experimental design.** Initially, baseline protein and mRNA expression for p21wa1f1/cip1 and p27kip1 in the small bowels of nonoperated animals was measured in control, p21wa1f1/cip1-null, and p27kip1-null mice. Four animals from each strain were analyzed for protein expression, and two animals from each strain were analyzed for cell compartment-specific mRNA expression using laser capture microdissection (LCM) microscopy.

In the first series of experiments, a temporal expression profile was developed for p21wa1f1/cip1, p27kip1, and other cell-cycle-regulatory proteins (cyclin D1, cyclin E, CDK2, CDK4, and CDK6) in the adapting bowel of normal mice that had undergone either a 50% proximal SBR or a sham operation. Animals from both groups were killed at 1, 6, 12, or 24 h or 3 or 7 days after surgery.

In the second series of experiments, a regional profile for enterocyte expression along the crypt-villus axis for p21wa1f1/cip1 and p27kip1 mRNA was determined using LCM microscopy in control mice after the mice underwent SBR or sham operations. From the isolated cells, extracted mRNA was reverse transcribed to cDNA and then quantitated using real-time PCR. Enterocytes were extracted on the third day after surgery because this time coincided with the protein changes observed in the first series of experiments as well as with the finding that enterocyte proliferation and adaptation are well under way after this postoperative interval in our murine resection model (12).

In the third set of experiments, the effect of absent p27kip1 expression on the magnitude of intestinal adaptation was determined by performing SBR or sham operations in p27kip1-null mice and control mice. Adaptation parameters were recorded in the adapting bowel on the third postoperative day and included changes in morphology (villus height, crypt depth, and wet weight) as well as the kinetics of enterocyte turnover (i.e., rates of enterocyte proliferation and apoptosis).

In the final set of studies, we determined the effect of absent expression of both p21wa1f1/cip1 and p27kip1 on the capacity for adaptation by performing SBR or sham operations on p21wa1f1/cip1/p27kip1 double-null mice. Parameters of adaptation were measured in the remnant ileum and compared with those in control mice on the third postoperative day.

**Operative procedure and feeding protocol.** All animals underwent surgery at 6–10 wk of age and weighed between 22 and 30 g. The details of this procedure have been described previously (12). All procedures were performed under sterile conditions with the aid of an operating microscope. In mice undergoing SBR, the small bowel was transected 12 cm proximal to the ileocecal valve. Approximately 12 cm of the proximal intestine were removed (50% enterectomy), and reanastomosis was performed. Mice that had sham operations underwent division of the intestine and reanastomosis alone at a point 12 cm proximal to the ileocecal valve. Mice were fed a standard liquid rodent diet for 1 day before surgery (Test Diet, Richmond, IN). After surgery, the animals were provided only water for the first 24 h, followed by the liquid diet until they died. Although animals were not specifically pair fed, prior studies from our laboratory revealed that food intake differences are negligible between various mouse strains after either sham operations or SBRs. Animals that appeared ill (e.g., unkempt fur, lethargic), were dehydrated, or had signs of intestinal obstruction at the time of death were excluded from further analysis.

**Tissue harvest.** After animals were killed by cervical dislocation, intestinal anastomosis was identified and the first 1-cm segment of bowel distal to this point was discarded. The next 1-cm segment was fixed with 10% neutral buffered formalin for histological examination. The luminal content of the next 5-cm segment was gently expressed with cotton swabs, and the segment was weighed for determination of ideal wet weight. For protein analysis, this segment of bowel was flushed with normal saline solution and snap frozen in liquid nitrogen. For LCM, this 5-cm segment was immediately removed, flushed, covered with optimal cutting temperature embedding medium (Tissue-Tec; Sakura Finetek USA, Torrance, CA), and frozen on dry ice (38).

**Histological examination.** All histological measurements were performed independently by two investigators who were blinded to the specific mouse strain, the operative procedure, and the time of tissue harvest. Formalin-fixed specimens of ileum were embedded in paraffin and oriented to display longitudinal sections of the bowel. Five-micrometer tissue sections were mounted on glass slides and used for analysis of adaptive parameters and scoring rates of proliferation and apoptosis.

**Morphological parameters of intestinal adaptation.** Hematoxylin and eosin-stained sections were used to measure villus height and crypt depth using a video-assisted integrated software program (Meta morph software; Universal Imaging, Downingtown, PA). A minimum of 20 villi and crypts were counted per sample. Only villi in which the central lymphatic channel could be visualized were measured. Only intact crypts extending from the crypt-villus junction to the basement membrane were measured.

**Rates of enterocyte proliferation.** Ninety minutes before tissues were harvested, the mice were subjected to subcutaneous injection with 1 ml/100 g body wt of 5-bromodeoxyuridine (BrDU) labeling agent (3 mg BrdU/ml; Zymed Laboratories, San Francisco, CA). BrDU incorporation into proliferating (S phase) enterocytes was detected using immunohistochemistry with an anti-BrDU MAb provided in kit form (Zymed Laboratories). BrDU detection methods have been reported previously (12). A proliferative index was derived by counting the number of BrDU-labeled cells in 15 full crypts divided by the total number of cells per crypt.

**Rates of enterocyte apoptosis.** Rates of apoptosis were measured using blinded scoring of immunohistochemically labeled DNA strand breaks (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling, or TUNEL) in crypt enterocytes (ApopTag Kit; Chemicon International, Temecula, CA) and confirmed using morphometric analysis of hematoxylin and eosin-stained tissue sections. A quantitative index of apoptosis was derived by counting the percentage of crypt cells that were both nick-end labeled and had abnormal morphology (pyknotic nuclei, condensed chromatin, and nuclear fragmentation). A total of 20 full crypts/animal were counted within each group.

**Western blot analysis and immunoprecipitation.** Segments of frozen remnant ileum were homogenized in a buffer containing 250 mM sucrose, 20 mM HEPES, pH 7.4, 1 mM EDTA, and a protease inhibitor tablet (Complete Mini, EDTA-free; Roche Diagnostics, Indianapolis IN). The samples were processed on ice using a Tissue Tearor (Biospec, Bartlesville, OK) for 30 s and subsequently centrifuged at 1,000 g for 10 min at 4°C. The protein concentration in the supernatant was determined using the Better Bradford Assay (Pierce Biotechnology, Rockford, IL). For Western blot analysis, total protein was detected on polyacrylamide gel (Page-One; Oval Separation Systems, Portsmouth, RI) and transferred onto a PVDF-Plus membrane (Micron Separations, Westboro, MA). After being blocked in 5% nonfat milk, the membrane was exposed for 1 h at room temperature to a 1:500 dilution of the following antibodies: p21wa1f1/cip1 (BD Pharmingen, San Diego, CA), p27kip1, CDK2, CDK4, CDK6, and cyclin D1 (Cell Signaling Technology, Beverly, MA), and cyclin E.
Baseline p21<sup>waf1/cip1</sup> and p27<sup>kip1</sup> protein and mRNA expression in ileum. In the ilea of unoperated mice, the expression of p21<sup>waf1/cip1</sup> protein was unaffected in the background of deficient p27<sup>kip1</sup> expression. On the other hand, the expression of p27<sup>kip1</sup> protein was decreased in p21<sup>waf1/cip1</sup>-null mice compared with controls (Fig. 1, A and B).

The expression of mRNA for p21<sup>waf1/cip1</sup> and p27<sup>kip1</sup> varied within enterocytes along the crypt-villus axis but increased as enterocytes migrated from the crypt to the villus tip in all three mouse strains. Expression of p21<sup>waf1/cip1</sup> mRNA levels in p27-null mice was significantly lower in all three enterocyte locations (Fig. 2A). Consistent with our observations regarding protein expression, p27<sup>kip1</sup> mRNA expression in the villus tips of p21<sup>waf1/cip1</sup>-null mice was significantly reduced (Fig. 2B). No mRNA of the deleted gene was found to be expressed in the respective null mouse strains.

Ileal expression of p21<sup>waf1/cip1</sup> and p27<sup>kip1</sup> mRNA after SBR. Using LCM microscopy, a greater abundance of p21<sup>waf1/cip1</sup> transcript was measured in the villi than in the crypts. In the crypt compartment, a significant increase in p21<sup>waf1/cip1</sup> mRNA expression occurred after SBR (Fig. 3). In the villus tip, the reverse was true, because SBR was associated with a significant decrease in p21<sup>waf1/cip1</sup> mRNA copy number. Resection-induced changes in p27<sup>kip1</sup> transcript in the crypt compartment were not significant (Fig. 3). On the other hand, SBR resulted in a significant increase in p27<sup>kip1</sup> mRNA copy number in enterocytes adjacent to the villus tips.

Ileal expression of cell cycle-regulatory proteins after SBR. Of the 160 animals that underwent surgery, 128 survived, appeared healthy, and had no evidence of ileal obstruction at the time of harvest. Eight animals (4 sham operations and 4 SBRs) were harvested at each of the six time points for histological analysis. The ilea of the remaining mice were used for protein analysis after adaptation was verified histologically. No differences in ileal wet weight, villus height, apoptotic index, or proliferative index were detected between animals.
that had undergone sham operations or SBRs at the 1-, 6-, 12-, or 24-h postoperative time points (Fig. 4, A–D). However, at 3 and 7 days postoperatively, mice that had undergone SBRs showed significant increases in each of these adaptive parameters compared with sham-operated animals.

Similarly to the timing of significant changes in adaptive histology, Western blot analysis of whole bowel homogenates failed to identify significant differences in p21\(^{\text{waf1/cip1}}\) or p27\(^{\text{kip1}}\) protein expression after SBR at the early time points (Fig. 5). However, after 3 days, significantly increased expression of p21\(^{\text{waf1/cip1}}\) was detected. Although the trend at 7 days remained higher, this finding was not statistically significant compared with the data regarding sham-operated animals. In contrast, the expression of p27\(^{\text{kip1}}\) protein was slightly lower (but statistically insignificant) at the 3-day time point in animals that had undergone SBRs. Western blot analysis of other cell cycle-regulatory proteins, including CDK2, CDK4, CDK6, and cyclin D\(_1\), revealed no major differences in expression between the two groups at any postoperative time point studied (Fig. 6). Cyclin E protein levels varied after SBR, showed increased expression at 1 h, had a slight decrease at 6 h, increase again at 12 h, and changed minimally at later time points. In other experiments, we did not detect differences in the protein expression of other CDKIs such as p57\(^{\text{kip2}}\) or of members of the kinase inhibitor family of proteins, p15, p16, p18, or p19, in either control mice or p21\(^{\text{waf1/cip1}}\)-null mice after SBRs (data not shown).

To detect the association of specific cell cycle-regulatory proteins with one another as opposed to detecting simple changes in protein expression, we performed IP for cyclin D\(_1\) and then probed for p21\(^{\text{waf1/cip1}}\), p27\(^{\text{kip1}}\), and CDK6 (Fig. 7). Because of several factors, such as lack of suitable antibodies for IP as well as the abundance of protein recovered during IP, we were unsuccessful in detecting a signal for CDK2 or CDK4 in any combination of IP or probing antibody. Nonetheless, the cyclin D\(_1\) IP revealed greater association with p27\(^{\text{kip1}}\) at 7 days, although less was associated in the sham-operated group at this time point compared with all other groups. CDK6 was associated with cyclin D\(_1\) in greater abundance at all time points after SBR. There was no obvious change in the association of p21\(^{\text{waf1/cip1}}\) protein with cyclin D1 at any time point studied after SBR. In other experiments, we were unable to demon-

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**Fig. 2.** Expression of p21\(^{\text{waf1/cip1}}\) (A) and p27\(^{\text{kip1}}\) (B) mRNA in enterocytes isolated using laser capture microdissection (LCM) microscopy of tissues from unoperated control mice (C57/BL6), p21\(^{\text{waf1/cip1}}\)-null mice, and p27\(^{\text{kip1}}\)-null mice (n = 2 per strain). Using LCM microscopy, cell compartment-specific enterocytes were harvested from the crypt, the crypt-villus junction (cvj), and the villus tip. \(*P < 0.05\), significant difference in gene expression between villus tip vs. crypt (using ANOVA). \(#P < 0.05\), significant differences between villus tip among different strains (using ANOVA).

**Fig. 3.** Expression of p21\(^{\text{waf1/cip1}}\) (left) and p27\(^{\text{kip1}}\) (right) mRNA in villus (top) and crypt (bottom) enterocytes from the ilea of control mice (C57/BL6) harvested 3 days after 50% proximal small bowel resection (SBR) or sham operation (bowel transection with reanastomosis alone). Cell compartment-specific mRNA expression was quantified in enterocytes harvested using LCM microscopy and analyzed using quantitative real-time PCR. \(*P < 0.05\), SBR vs. sham; \(#P < 0.05\), sham villus vs. sham crypt (ANOVA).
Intestinal adaptation in p27kip1-null mice. At 3 days after surgery, 12 of the 16 p27kip1-null (sham, n = 6; SBR, n = 6) and 13 of the 15 control mice (sham, n = 6; SBR, n = 7) were examined. Adaptation occurred normally in the p27kip1-null mice after SBR as revealed by significant increases in ileal wet weight (data not shown). In addition, p27kip1-null mice demonstrated significant increases in both parameters of adaptive morphology, including villus height and crypt depth, as well as in rates of enterocyte turnover (Fig. 8, A–D). There was no significant difference in the magnitude of adaptation between the p27kip1-null mice and the control mice.

Intestinal adaptation in p21waf1/cip1/p27kip1 double-null mice. At 3 days after surgery, 5 of 6 p21waf1/cip1/p27kip1 double-null mice (sham, n = 2; SBR, n = 3) and 13 of 15 control mice met the inclusion criteria (sham, n = 6; SBR, n = 7). No significant changes had occurred in ileal wet weight after SBR (data not shown). Resection-induced adaptation resulted in taller villi and deeper crypts in control mice, but no such changes occurred in the double-null mice. (Fig. 9, A and B). There was a significant increase in the rate of enterocyte apoptosis after SBR in both control and double-null mice (Fig. 9C); however, the normal induction of proliferation after SBR observed in the control mice and p27kip1-null mice (described above) was blocked in the p21waf1/cip1/p27kip1 double-null mice (Fig. 9D).

DISCUSSION

In the present study, we sought to determine a mechanism for the failure of adaptation to occur after massive intestinal resection in p21waf1/cip1-deficient mice (27). Specifically, we tested the hypothesis that deficient expression of p21waf1/cip1 affects the expression of the related CDKI p27kip1 to block adaptation. Initially, we identified a lower expression of p27kip1 protein in the ilea of p21waf1/cip1-deficient mice. In normal mice, we used LCM microscopy to determine that the expression of p21waf1/cip1 and p27kip1 mRNA both increased as enterocytes migrated upward along the crypt-villus axis. Furthermore, we found that deficient expression of one CDKI attenuated this progression and reduced overall expression of the other CDKI. After massive SBR, we failed to identify significant alterations in the expression of p27kip1 or other cell cycle-regulatory proteins in the ileum at various postoperative time points. Only p21waf1/cip1 protein expression was increased and corresponded with the time point at which enterocyte proliferation was significantly elevated. In addition, the mRNA expression for p21waf1/cip1 was elevated in the crypts that correlated with the zone of enterocyte proliferation. Finally, we confirmed that p27kip1-null mice adapt normally after SBR, an effect that is nullified in the context of p21waf1/cip1 deficiency. Altogether, these results suggest that the expression of p21waf1/cip1, independently of p27kip1, is fundamentally required for a normal proliferative and adaptive response of the enterocyte to massive intestinal loss.

The possibility of absent expression of one Cip/Kip family CDKI affecting the expression of another was evaluated previously. In a study of p27kip1-deficient mice, the expression of p21waf1/cip1 was found to be elevated in several tissues, including the liver, kidney, brain, heart, lung, and spleen (15). Alternatively, in p21waf1/cip1-deficient mice, the expression of p27kip1 was found to be unaffected in the liver (2). More recently, Yang et al. (39) reported that p21waf1/cip1-deficient mice in the background of Muc2 gene inactivation had reduced expression of p27kip1 in the colon. This effect therefore could be unique to the colon or a result of combined Muc2 gene inactivation and p21waf1/cip1 deficiency. In contrast to the present study, none of these prior reports specifically measured expression of these CDKIs in the small intestine.

Fig. 4. Time course of adaptation parameters in remnant ilea of control mice (C57/BL6) after 50% proximal SBR or sham operation. A: villus height. B: ileal wet weight. C: rates of enterocyte apoptosis. Apoptosis index was calculated as the number of terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL)-positive cells per crypt and verified using hematoxylin and eosin staining. D: rates of enterocyte proliferation. A proliferation index was calculated as the percentage of 5-bromodeoxyuridine (BrdU)-labeled cells per total number of cells per crypt. *P < 0.05, SBR vs. sham (ANOVA).
We previously reported that p27kip1 mRNA was elevated in the intestine of p21waf1/cip1-deficient mice (27), which contradicts our current findings. This might have been due to the fact that qualitative PCR was used to determine mRNA expression within homogenates of whole bowel in earlier experiments. In the current study, we specifically used quantitative real-time PCR in LCM-extracted enterocytes. Thus the increased expression observed in our prior work could have been a result of the use of different PCR methods and conditions as well as of the possibility that confounding mRNA from other cells within whole bowel homogenates interfered with the ability to determine accurate expression within enterocytes. The reduced mRNA expression of p27kip1 observed in the present study coincides appropriately with the same trend observed for protein expression, thus supporting the validity of our present data.

Our inability to identify changes in the expression or activity of other cell cycle-regulatory proteins at multiple time points might have occurred because we measured protein changes in whole bowel homogenates. Nonetheless, because adaptation is a proliferative signal to all layers of the intestinal wall, we were a bit surprised that we could not detect more significant alterations in protein expression. These negative findings endorse the value of the use of methods such as LCM microscopy for the determination of expression within individual cell populations in animal models. While LCM technology has been helpful in providing information regarding protein expression within select intestinal cell populations (24), this technology is still evolving.

Because proliferative signals may involve the association of preformed proteins as opposed to de novo production, we performed IP experiments using antibodies to cyclin D1, followed by immunoblot analysis for several cell cycle-regulatory proteins. We were encouraged to find a greater association of CDK6 with cyclin D1 at all postoperative time points in the ilea from mice that had undergone SBR. Although an increased association of p21waf1/cip1 with cyclin D1 would have been expected, given the increased expression of p21waf1/cip1, it is possible that our inability to measure this association was due to the capacity for p21waf1/cip1 and cyclin D1 association to exceed a maximal threshold. Alternatively, the true association of these proteins may have been obscured by confounding proteins from nonenterocyte cells within the whole bowel homogenates.
SBR resulted in significant increases in the rates of enterocyte proliferation coupled to enhanced p21waf1/cip1 protein and crypt enterocyte mRNA expression. Although the literature predominantly supports the concept that expression of p21waf1/cip1 is typically associated with cell cycle arrest, increased expression of this CDKI has been reported in other models in which proliferation is induced. For example, after partial hepatectomy, p21waf1/cip1 expression was found to be elevated within the regenerating liver (1, 2). Increased expression of this CDKI has been linked directly to invasiveness and prognosis in tumors of the oral pharynx (19) and larynx (21). In vascular smooth muscle cells, increased expression of cytosolic p21waf1/cip1 was associated with increased cell cycle progression (8). Alternatively, in cell cultures of model crypt enterocytes, decreased expression of p21waf1/cip1 was observed as cells underwent density-dependent growth arrest (3). In addition, the induction of enterocyte proliferation after massive intestinal resection was abolished in p21waf1/cip1-deficient mice (27). Altogether, these data endorse the notion that p21waf1/cip1 either stimulates or inhibits cell proliferation, depending on the specific mitogenic stimulus, the intracellular location, and the type of tissue or cell studied.

Intestinal adaptation in p27kip1-null mice after SBR was not different from that in control animals when evaluating villus height, crypt depth, or rates of enterocyte apoptosis and pro-
liferation. In other studies, adaptation in these mice was no different even at later postoperative times (7 days; data not shown). These findings confirm that p27kip1 expression does not play a significant role in the proliferative intestinal adaptation response in these animals. Although the expression of p21waf1/cip1 and p27kip1 were both induced under normal conditions as crypt enterocytes migrated upward along the crypt-villus axis, our observations of increased crypt expression of p21waf1/cip1 and reduced expression of p27kip1 after SBR suggest that these two highly related proteins possess specific biological activities and are not functionally interchangeable. Distinctive and opposing roles for these related CDK1 family members during adaptation were recently revealed on the basis of the finding that the differentiation status of villus enterocytes was reduced significantly in p27kip1-null mice compared with both p21waf1/cip1-null mice and control mice (25). We found reduced expression and activity of several markers of enterocyte differentiation, such as intestinal alkaline phosphatase, sucrase, villin, and intestinal fatty acid binding protein. In addition, rates of enterocyte proliferation, migration, and villus height were not different in p27kip1-null mice from those in wild-type mice. In additional studies, rates of enterocyte apoptosis were also similar (data not shown). These in vivo findings are consistent with in vitro studies conducted by Deschênes et al. (7) confirming the main role for p27kip1 during the program of postmitotic enterocyte differentiation. Divergent functions of p21waf1/cip1 and p27kip1 in cell cycle regulation and differentiation also were recently described in a leukemia cell line (18).

Mice deficient in both p21waf1/cip1 and p27kip1 demonstrated reactions identical to those of p21waf1/cip1-null animals in that adaptation after SBR was completely prevented. These experiments confirm that the expression of p21waf1/cip1 is the most important CDK1 involved in the adaptation response to massive SBR. The specific mechanism for increased expression of p21waf1/cip1 during intestinal adaptation is presently unknown. Because enterocyte EGFR activity appears to be required for normal adaptation to occur, it is possible that signaling through this receptor acts as a transcriptional regulator of p21waf1/cip1 expression. In intestinal epithelial cells, EGFR has been shown to induce the phosphorylation of the signal transducer and activator of transcription (STAT) factor and to increase its localization to the nucleus and the Golgi apparatus (13). This protein has been shown to be a direct transcriptional inducer of p21waf1/cip1 expression with defined STAT1 binding sites within the p21waf1/cip1 promoter region (6). In addition to STAT proteins, EGFR activation of the MAPK pathway also must be considered. Although its role in the activation of proliferation is well established, MAPK has been demonstrated to induce cell cycle withdrawal and inhibition of cell cycle regulators, depending on signaling intensity or duration. This latter effect has been shown to be mediated via MAPK induction of p21waf1/cip1 expression (22, 23, 37). Experiments are presently under way in our laboratory to investigate which specific pathway (STAT or MAPK) is most relevant for the induction of p21waf1/cip1 expression.

Because proliferation and apoptosis are both elevated in the crypts of mice after SBR and because p21waf1/cip1 is usually associated with cell cycle withdrawal, the notion that increased p21waf1/cip1 expression might be involved in the apoptotic response should be considered. Several lines of experimental evidence argue against this hypothesis. In resection models of p21waf1/cip1 deficiency, such as p21waf1/cip1-null mice (27) or p21waf1/cip1/p27kip1 double-null mice (as in the present study), the apoptotic response is preserved while the proliferative response is blocked. Alternatively, in mice deficient in the expression of the proapoptotic Bcl-2 family member Bax, the apoptotic response during resection-induced adaptation is prevented completely (28, 29), despite normal induction of proliferation. Collectively, these data support the concept that resection-induced apoptosis and proliferation are regulated independently and that p21waf1/cip1 is critically involved in the latter.

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