Phosphatidylinositol 3-kinase/Akt signaling stimulates colonic mucosal cell survival during aging

Adhip P. N. Majumdar and Jianhua Du
Veterans Affairs Medical Center, Karmanos Cancer Institute, Department of Internal Medicine, Wayne State University, Detroit, Michigan

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Majumdar, Adhip P. N., and Jianhua Du. Phosphatidylinositol 3-kinase/Akt signaling stimulates colonic mucosal cell survival during aging. Am J Physiol Gastrointest Liver Physiol 290: G49–G55, 2006. —Although aging is shown to be associated with decreased apoptosis and increased survival of cells in the colonic mucosa of Fischer 344 rats, the regulatory mechanisms are poorly understood. The current investigation examines the involvement of phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway in mediating the events of colonic mucosal cell survival during aging. We have observed that aging is associated with activation of PI3K/Akt signaling, as evidenced by the higher levels of phosphorylated forms of p85, the regulatory subunit of PI3K and of Akt in the proximal and distal colonic mucosa, of aged (21–23 mo) than in young (4–7 mo) rats. These increases are accompanied by a concomitant rise in phosphorylation of proapoptotic protein Bad, which is sequestered by the 14-3-3 family of proteins following phosphorylation by Akt, resulting in a reduction in nonphosphorylated Bad. The amount of antiapoptotic Bcl-xL bound to nonphosphorylated Bad in the colonic mucosa is found to be substantially lower in aged than in young rats, resulting in a marked rise in the unbound/free form of Bcl-xL in the aging colon. The age-related activation of PI3K and the reduction in caspase-3 activity could be reversed by wortmannin, a specific inhibitor of PI3K. Increased levels of Bcl-xL and phosphorylated forms of Akt and Bad and reduction in caspase-3 activity were observed throughout the entire length of the colonic crypt of aged rats. We conclude that the constitutive activation of PI3K/Akt-signaling pathway is partly responsible for the age-related increase in colonic mucosal cell survival. This is evident throughout the entire length of the colonic crypt.

First published August 25, 2005; doi:10.1152/ajpgi.00106.2005.—Although aging is shown to be associated with decreased apoptosis and increased survival of cells in the colonic mucosa of Fischer 344 rats, the regulatory mechanisms are poorly understood. The current investigation examines the involvement of phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway in mediating the events of colonic mucosal cell survival during aging. We have observed that aging is associated with activation of PI3K/Akt signaling, as evidenced by the higher levels of phosphorylated forms of p85, the regulatory subunit of PI3K and of Akt in the proximal and distal colonic mucosa, of aged (21–23 mo) than in young (4–7 mo) rats. These increases are accompanied by a concomitant rise in phosphorylation of proapoptotic protein Bad, which is sequestered by the 14-3-3 family of proteins following phosphorylation by Akt, resulting in a reduction in nonphosphorylated Bad. The amount of antiapoptotic Bcl-xL bound to nonphosphorylated Bad in the colonic mucosa is found to be substantially lower in aged than in young rats, resulting in a marked rise in the unbound/free form of Bcl-xL in the aging colon. The age-related activation of PI3K and the reduction in caspase-3 activity could be reversed by wortmannin, a specific inhibitor of PI3K. Increased levels of Bcl-xL and phosphorylated forms of Akt and Bad and reduction in caspase-3 activity were observed throughout the entire length of the colonic crypt of aged rats. We conclude that the constitutive activation of PI3K/Akt-signaling pathway is partly responsible for the age-related increase in colonic mucosal cell survival. This is evident throughout the entire length of the colonic crypt.

HOMEOSTASIS OF THE GASTROINTESTINAL mucosa is maintained by sustained proliferation of precursor cells and exfoliation of surface cells (19). A detailed study of these events is, therefore, essential for a better understanding of the normal aging process as well as many gastrointestinal diseases, including malignancy, that represent disorders of tissue growth (19). In recent years, studies from this and other laboratories have demonstrated that in Fischer 344 rats, aging is associated with increased mucosal proliferative activity in much of the gastrointestinal tract, including the colon (1, 9, 10–12, 20, 21, 35). In contrast, the numbers of cells undergoing apoptosis, as determined by TdT-mediated dUTP nick end-labeling (TUNEL) assay, were found to decrease in the gastric and colonic mucosa during aging in Fischer 344 rats (22, 35). Further support for the age-related decline in apoptosis in the colonic mucosa was provided by the observation that aging also resulted in decreased levels of the proapoptotic protein Bak, diminished activity of caspases, and increased levels of antiapoptotic Bcl-xL protein (35). Because induction of proliferation and inhibition of apoptosis are associated with the development and progression of carcinogenesis, it has been suggested that aging may predispose the gastrointestinal tract to neoplasia (1, 12, 21, 22).

Although the precise responsible mechanisms for age-related changes in gastrointestinal mucosal proliferation and apoptosis are still not fully understood, we have observed that these events are accompanied by increased expression and activation of EGFR receptor (EGFR)/ErbB1 and its family member ErbB2/HER-2 (28, 31, 32). Activation of EGFR and/or ErbB2 leads to stimulation of a number of downstream signaling pathways, including Ras/Raf/MAPK kinase (MEK)/MAPK and phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathways that regulate the growth-related processes (15, 23, 33). However, whereas induction of MAPK signaling is shown to augment the proliferative potential, stimulation of the PI3K/Akt pathway has been linked to increased cell survival (5, 7, 23–25).

PI3K, which catalyzes the phosphorylation of the inositol ring at the D3 position in a variety of phosphoinositol substrates forming 3-phosphorylated phosphoinositides (18), is composed of an Src homology 2 domain containing an 85-kDa regulatory subunit (p85) and a 110-kDa catalytic subunit (p110) (3, 15). Induction of PI3K, which leads to subsequent activation of Akt, promotes cell survival directly by means of its ability to phosphorylate and inactivate several proapoptotic targets, including Bad and the forkhead transcription factors (5, 7). Akt also promotes cell survival through its indirect effect on NF-κB and p53 (5, 7). Interestingly, aging has been shown to be associated with increased activation of NF-κB in the gastric mucosa (34). However, it remains to be determined whether the age-related increase in cell survival in the gastrointestinal tract could partly be mediated by PI3K/Akt signaling, which is the subject of the current investigation.

Intestinal cell renewal is highly regulated and position dependent such that absorptive cells generated within intestinal crypt migrate upward until they either die by apoptosis or are shed into the gut lumen (26). Additional experiments were performed to determine whether and to what extent survival of cells along the colonic crypt is affected by aging and the involvement of PI3K/Akt signaling in regulating this event.

METHODS

Reagents. Monoclonal antibodies to phospho-Bad (Ser\textsuperscript{73}) and polyclonal antibodies to Bad, Bcl-xL, and EGFR were obtained from
Santa Cruz Biotechnology (Santa Cruz, CA). Monoclonal antibodies to phospho-Akt (Ser^473) and β-actin were obtained from Cell Signaling Technology (Beverly, MA) and Chemicon International (Temecula, CA), respectively. Polyclonal antibodies to p85 subunit of PI3K were the product of Upstate Biotechnology (Lake Placid, NY), and caspase-3/CPP32 colorimetric assay kit was purchased from Bio Vision (Mountain View, CA). Polyclonal antibodies to rabbit alkaline phosphatase were the product of Fitzgerald Industries International (Concord, MA), and Wortmannin was obtained from Calbiochem (San Diego, CA).

Animals and collection of tissues. Male Fischer 344 rats, aged 4–7 (young) and 21–23 (old) mo, were used. The animals were purchased from the National Institute on Aging (Bethesda, MD) at least 2 mo before the experiment. They were housed two per cage and had access to Purina chow and water ad libitum. The reasons for using Fischer 344 rats for aging studies are because of purity of breeding, low susceptibility to spontaneous colorectal cancer, and their ability to maintain body weight. All animals were fasted overnight before being killed. The overnight-fasted animals were either used without any intervention or injected intraperitoneally with wortmannin [0.1 mg/kg body wt in 15% DMSO (16)] or vehicle 6 h before being killed. All overnight-fasted animals, except those treated with wortmannin or vehicle, were killed between 8 and 10 AM. The animals injected with either wortmannin or vehicle were killed in the afternoon, between 2 and 3 PM. The entire colon (~18 cm) was removed, cut open along the longitudinal median, and rinsed thoroughly in cold normal saline. The distal (~8 cm proximal to the rectum) and proximal (~8 cm distal to the cecum) colon were cut open, and mucosa from each region was obtained by scraping with glass slides. Mucosal aliquots were either processed immediately or stored at −80°C. In some experiments, the entire colon was used immediately to isolate cells from the mucosa, as described below.

Isolation of colonic mucosal epithelial cells. Cells were isolated from the entire colon by a slight modification of the procedure described for isolation of gastric mucosal cells (33). Briefly, the contents of the colon were washed with PBS. The colon was everted and ligated at both ends after being filled with a 3–5 ml protease solution [5 mg/ml in buffer A composed of (in mM) 0.5 NaH2PO4, 1 Na2HPO4, 70 NaCl, 5 KCl, 11 glucose, 50 HEPES, 20 NaHCO3, and 2 EDTA with 2% BSA]. The colon was placed in pronase-free buffer A and incubated for 30 min at 37°C and gassed with O2. The colonic bags were then transferred into 50 ml buffer B containing 1.0 mM CaCl2 and 1.5 mM MgCl2 instead of EDTA in buffer A and gently agitated by a magnetic stirrer at room temperature for 30 min. The dispersed cells were discarded by centrifugation. The colonic bag was transferred into 50 ml buffer B and incubated initially for 60 min at room temperature to obtain the cells from the upper part of the colonic crypt (referred to as fraction 1). The colonic tissue was then incubated at room temperature for another 45 min to obtain the cells from the middle region of the crypt (referred to as fraction 2). After cells were obtained in fraction 2, the colon was incubated further for 45 min at room temperature to obtain the cells from the lower part of the crypt (referred to as fraction 3). The dispersed mucosal cells were collected by centrifugation at 500 g for 10 min, washed with PBS, and used either immediately or stored at −90°C.

Immunoprecipitation and Western blot analysis. Mucosal scrapings or the isolated colonic cells were homogenized in lysis buffer (50 mM Tris; 100 mM NaCl; 2.5 mM EDTA; 1% Triton X-100; 1% Nonidet P-40; 2.5 mM Na3VO4; 25 μg/μl aprotinin; 25 μg/μl leupeptin; 25 μg/l pepstatin A; and 1 mM phenylmethylsulfonyl fluoride). The samples were rotated for 30 min at 4°C and subsequently clarified by centrifuging at 10,000 g for 10 min. The supernatant was used for all analyses. For immunoprecipitation and Western blot analysis, protein concentration, determined by the Bio-Rad Protein Assay kit (Bio-Rad, Hercules, CA), was standardized among the samples. In experiments where proteins were immunoprecipitated, 0.5 mg protein from each sample were incubated with appropriate antibodies for 16 h at 4°C as described previously (33).

In all Western blot analyses, immunoprecipitates or cell lysates containing the same amount of protein (50 μg) were separated by SDS-PAGE. After electrophoresis, protein was transferred electrophoretically onto supported nitrocellulose membranes (Osmonics, Gloucester, MA). Membranes were incubated for 1 h at room temperature with blocking buffer [20 mM Tris, pH 7.6, 100 mM NaCl, and 0.1% Tween 20 (TBS-T)] and 5% nonfat dry milk with gentle agitation. After the membranes were washed with TBS-T, they were incubated overnight at 4°C in TBS-T buffer containing 5% milk and with appropriate antibodies (1:1,000 final dilution). The membranes were washed three times with TBS-T and subsequently incubated with the appropriate secondary antibodies (IgG from Upstate, Cell Signaling, or Chemicon) in TBS-T containing 5% milk for 2 h at room temperature with gentle agitation. The membranes were washed again with TBS-T, and the protein bands were visualized by enhanced chemiluminescence detection system (Amersham). Membranes were stripped +2% for 15 min at 55°C in stripping buffer containing 100 mM 2-mercaptoethanol, 2% SDS, and 62.5 mM Tris-HCl (pH 6.7). The membranes were then reprobed for total (nonphosphorylated) EGFR (Upstate), ERKs (Cell Signaling), or Akt as well as for α-tubulin (Oncogene, San Diego, CA) using corresponding antibodies. α-Tubulin was used as an internal control. All Western blot analyses were performed at least three times for each experiment.

Immunoblots were scanned by HP Precisionscan Pro 3.13 (Hewlett-Packard, Palo Alto, CA). Densitometric measurements of the scanned bands were performed using the digitized scientific software program UN-SCNA-IT (Silk Scientific, Orem, UT). Data were normalized to either β-actin or α-tubulin.

Caspase-3 activity. The activity of caspase-3, an enzyme that initiates apoptosis, was determined in mucosal cell lysates using the colorimetric assay from the BioVision Research Products (Mountain View, CA) according to the manufacturer’s instruction. The assay is based on spectrophotometric detection of the chromophore p-nitroanilide (pNA) after cleavage from the labeled substrate DEVD-pNA.

Statistical analysis. Unless otherwise stated, data are expressed as means ± SD. Where applicable, the results were analyzed using a two-tailed Student’s t-test taking P < 0.05 as the level of significance.

RESULTS

Previously, we reported that aging is associated with activation of EGFR in the colonic mucosa, as demonstrated by a higher tyrosine phosphorylation of the receptor in the proximal and distal colonic mucosa of 24-mo-old Fischer 344 rats, compared with their 12- and 4-mo-old counterparts (28).

Activation of EGFR leads to induction of many downstream signaling pathways that play critical roles in regulating proliferation, differentiation, and/or cell survival. One of the downstream pathways activated by EGFR is PI3K/Akt signaling, which is known to be involved in regulating the processes of cell survival. Promotion of cell survival by the activation of PI3K/Akt occurs by inhibition of apoptotic signals and stimulation of survival signals (5, 7).

PI3K is composed of a regulatory subunit (p85) and a 110-kDa catalytic unit. To determine whether aging affects PI3K activity in the colonic mucosa during aging, we examined the levels of total as well as the tyrosine phosphorylated form of p85 subunit in the proximal and distal colonic mucosa of overnight-fasted 4-(young)- and 21-mo-old (aged) Fischer 344 rats. We observed that whereas there were no significant differences in the levels of total p85 subunit of PI3K in the proximal or the distal colonic mucosa between 4- and 21-mo-old rats, the levels of activated form (tyrosine phosphorylated)
of p85 were 40–50% higher in both parts of the colon of aged than in young rats (Fig. 1).

To determine whether the age-related increase in PI3K activation in the colonic mucosa would result in a parallel rise in Akt activation, mucosa from the proximal and distal colon of 6- and 21-mo-old rats was analyzed for the levels of the phosphorylated form of Akt. Akt may be activated by phosphorylation of threonine (Thr308) and serine (Ser473) in response to the growth factor-stimulated kinases, PDK1 and PDK2. We observed that the levels of phosphorylated form of Akt (Thr308/Ser473) were two- to threefold higher in the proximal and distal colonic mucosa of aged than in their corresponding levels in young rats (Fig. 2).

Activation of Akt leads to induction of downstream signaling events that promote cell survival (5, 7). One of the downstream molecules of Akt signaling is Bad, a proapoptotic member of the Bcl-2 family of proteins. Akt phosphorylates Bad at Ser136, causing it to be sequestered by the 14-3-3 proteins resulting in inactivation of Bad. To determine whether the age-related rise in Akt activation in the colonic mucosa would be associated with increased phosphorylation of Bad, the levels of the phosphorylated form of Bad were determined in the proximal and distal colonic mucosae of 6- and 21-mo-old rats. As expected, the levels of the phosphorylated form of Bad were 60–80% higher in the proximal and distal colonic mucosa of aged than in their corresponding levels in young rats (Fig. 3). The relative levels of phosphorylated Bad were expressed as a ratio of phosphorylated Bad to total Bad.

Because nonphosphorylated Bad binds the antiapoptotic protein Bcl-xL to induce apoptosis, we examined the amount of Bcl-xL bound to Bad in the proximal and distal colon of aged rats was considerably lower (30–50%) than in their younger counterparts (Fig. 4A). In contrast, the levels of unbound or free form of Bcl-xL in both parts of colonic mucosa were higher (40–60%) in aged than in young rats (Fig. 4B). This suggests that the increased levels of unbound or free form of Bcl-xL may partly be responsible for the age-associated increase in survival of colonic mucosal cells.

If the age-related increase in survival of colonic mucosal cells is the result of constitutive activation of PI3K signaling, then it is reasonable to assume that inhibition of PI3K activation should reverse the situation. This was tested by examining whether and to what extent treatment of young and aged rats with wortmannin, a specific inhibitor of PI3K, affected the events of mucosal cell survival. We observed that although the amount of Bcl-xL bound to Bad in the proximal and distal colon of aged rats was considerably lower (30–50%) than in their younger counterparts (Fig. 4A). In contrast, the levels of unbound or free form of Bcl-xL in both parts of colonic mucosa were higher (40–60%) in aged than in young rats (Fig. 4B). This suggests that the increased levels of unbound or free form of Bcl-xL may partly be responsible for the age-associated increase in survival of colonic mucosal cells.

Fig. 1. Western blot analysis showing changes in the levels of tyrosine-phosphorylated and nonphosphorylated (total) forms of the p85 subunit of phosphotyidylinositol 3-kinase (PI3K) in the mucosa of proximal and distal colon of 4- and 21-mo-old Fischer 344 rats. Changes in the levels of phosphorylated and total forms of p85 in young and aged animals, as determined by densitometric analysis, are shown in the histogram. Values are means ± SE of 4 experiments. *P < 0.01 compared with the corresponding levels in 4-mo-old rats. IP, immunoprecipitated.

Fig. 2. Western blot analysis showing changes in the levels of the phosphorylated form of Akt in the mucosa of proximal and distal colon from 6- and 21-mo-old Fischer 344 rats. Changes in the levels of the phosphorylated form of Akt in young and aged animals (expressed as ratios of phospho-Akt to total Akt), as determined by densitometric analysis, are shown in the histogram. Values are means ± SE of 4–5 experiments. *P < 0.01 compared with the corresponding levels in 6-mo-old rats.

Fig. 3. Western blot analysis showing changes in the levels of the phosphorylated and nonphosphorylated (total) forms of Bad in the mucosa of proximal and distal colon from 6- and 21-mo-old Fischer 344 rats. Relative changes in the levels of the phosphorylated form of Bad (expressed as ratios of phospho-Bad to total Bad) in young and aged animals, as determined by densitometric analysis, are shown in the histogram. Values are means ± SE of 4 experiments. *P < 0.01 compared with the corresponding levels in 6-mo-old rats.
levels of the phosphorylated (active) form of p85 subunit of PI3K in both proximal and distal colonic mucosa was higher in vehicle-injected (controls) aged than in young rats, they decreased substantially 6 h after a single injection (intraperitoneally) of wortmannin (Fig. 5). In fact, after wortmannin treatment, the levels of the phosphorylated form of p85 in the proximal and distal colonic mucosa in aged rats were 50–60% lower, compared with the corresponding vehicle-treated controls (Fig. 5). Wortmannin-mediated inhibition of activation of PI3K in the proximal and distal colonic mucosa of aged rats also resulted in a significant 75–150% increase in caspase-3 activity in these tissues over the corresponding vehicle-injected controls (Fig. 6). Interestingly, in young rats, wortmannin caused an increase in caspase-3 activity in the distal, but not in the proximal, colonic mucosa, when compared with the corresponding control (Fig. 6).

Renewal of mucosal cells in the intestine is highly regulated and position dependent. Absorptive cells generated within intestinal crypts migrate upward until they either die by apoptosis or are shed into the gut lumen (26). The next set of experiments was undertaken to examine whether survival of different mucosal cells along the entire length of the colonic crypt is affected by aging. To conduct this investigation, we isolated mucosal cells from the upper (fraction 1), middle (fraction 2), and the lower regions of the colonic crypt (fraction 3) of young (5–7 mo old) and aged (21–23 mo old) Fischer 344 rats.

Because mature absorptive intestinal mucosal cells exhibit alkaline phosphatase, we measured the levels of alkaline phosphatase using rabbit anti-alkaline phosphatase antibodies, as an indicator of differentiation/maturation, in freshly isolated cells from different regions of the colonic crypt. Western blot analysis revealed that, in both young and aged rats, the levels of alkaline phosphatase protein in cells from fractions 1 and 2 of colonic crypt were considerably higher, compared with the levels in cells from fraction 3 (Fig. 7A). The observation of comparatively higher levels of alkaline phosphatase protein in cells from fractions 1 and 2 suggests that most of the mature or absorptive cells are present in these two fractions.

To determine whether aging may affect cell survival along the entire length of the colonic crypt, we examined the levels and activity of cleaved caspase-3 in colonic mucosal cells isolated from the upper, middle, and lower regions of the colonic crypt of young and aged Fischer 344 rats. The levels of cleaved caspase-3, as an indicator of apoptosis, were found to be significantly lower in cells isolated from all three regions of the colonic crypt of aged than in young rats, suggesting an age-related increase in survival of colonic mucosal cells throughout the entire length of the colonic crypt (Fig. 7B). In both young and aged rats, the highest caspase activity was
observed in mucosal cells from the upper region (fraction 1) of the colonic crypt, suggesting that cells in this part of the colonic crypt are more sensitive to apoptosis (Fig. 7C). However, caspase-3 activity in mucosal cells from fraction 1 of aged rats was found to be \( \sim \)60% lower, compared with young animals (Fig. 7C). A small but significant 25–30% reduction in caspase-3 activity was also observed in mucosal cells from the middle (fraction 2) and lower (fraction 3) parts of the colonic crypt of aged rats compared with young animals (Fig. 7C). Taken together, the results show that with aging, there is a reduction in apoptosis (or increase in survival) of mucosal cells along the entire length of the colonic crypt. The reduction in caspase-3 activity in the upper, middle, and lower parts of the colonic crypt in aged rats was accompanied by a concomitant increase in Bcl-xL levels (Fig. 8).

To determine whether the increased survival of mucosal cells along the entire length of the colonic crypt of aged rats could be attributed to activation of Akt and subsequent phosphorylation of Bad, we examined the levels of phosphorylated forms of Akt and Bad in mucosal cells from the upper, middle, and lower regions of the colonic crypt of young (7 mo) and aged (22 mo) Fischer 344 rats. The levels of the phosphorylated form of Akt, expressed as ratios of phospho-Akt to total Akt, in mucosal cells from all three regions of the colonic crypt of aged rats were found to be 50–140% higher when compared with the corresponding levels in young rats (Fig. 9). The maximal age-related increase in Akt phosphorylation was observed in cells from the upper part of the colonic crypt (Fig. 9). No significant change in total Akt levels was observed among the samples (Fig. 9).

As have been observed for the levels of phosphorylated form of Akt, the levels of phospho-Bad in mucosal cells from all...
higher compared with the corresponding vehicle-treated controls.

It is becoming increasingly clear that the PI3K pathway is activated in response to the activation of a number of growth factor receptors, including EGFR (14, 23, 33). Because aging has been shown to be associated with increased expression and activation of EGFR in the gastric and colonic mucosa (28, 31, 32), our current observation of age-related increases in PI3K/Akt signaling could partly be the consequence of constitutive activation of EGFR in the colonic mucosa.

The major component of the survival signal provided by activation of PI3K is mediated by the Akt family of serine/threonine kinases (4, 6). Akt phosphorylates the Bcl-2 family of proapoptotic protein Bad on serine 136 (15, 36). Unphosphorylated Bad promotes apoptosis by binding to and neutralizing antiapoptotic Bcl-xL protein. As a result of phosphorylation, Bad interacts with 14–3–3 proteins and is prevented from blocking the protective function of Bcl-xL. Our current observation of the markedly elevated levels of phosphorylated Bad in the proximal and distal colonic mucosa of aged rats suggests that a greater amount of Bad is available for binding to 14–3–3 protein in aged colonic mucosa than in young rats, thereby reducing the proapoptotic function of Bad in aged colonic mucosa. Moreover, the fact that the amount of unbound or free form of Bcl-xL is higher in the proximal and distal colonic mucosa in aged than in young rats further suggests that the elevated levels of Bcl-xL contribute to the age-related increase in survival of colonic mucosal cells.

Renewal of cells within the intestinal epithelium is highly regulated and position dependent. Absorptive cells generated within the intestinal crypts migrate upward until they either die by apoptosis or are shed into the gut lumen, a process that takes 3–5 days (26). Our current observation that the levels of alkaline phosphatase in both age groups are higher in cells in fractions 1 and 2 compared with the corresponding values in fraction 3 indicates the presence of more mature and differentiated cells in the first two fractions that represent mucosal cells from the upper and middle regions of the colonic crypt. The fact that, in both young and aged rats, caspase-3 activity is substantially higher in cells isolated from the upper than those from lower region of the colonic crypt suggests that, in both age groups, cells in the upper part of colonic crypts are more sensitive to apoptosis than in other regions. This is in agreement with observations by others who noted increased expression of Bax, a proapoptotic protein, in the upper part of the colonic crypts of rats (17). Interestingly, we have observed that the levels of alkaline phosphatase in mucosal cells present in fractions 1 and 2 from aged rats are significantly higher than the corresponding fractions from young colonic crypts. Although the reasons for the age-related increase in alkaline phosphatase in mucosal cells from the upper and middle regions of the colonic crypt are not fully understood, it is plausible that increased survival of colonic cells in aged rats leads to a greater maturation with a concomitant increase in alkaline phosphatase.

Aging appears to suppress apoptosis and increase survival of cells throughout the entire length of the colonic crypt in Fischer 344 rats as evidenced by a reduction in caspase activity, an increase in Bcl-xL levels, and stimulation of phosphorylation of p85 of PI3K as well as Akt and Bad. However, the differences in these parameters between the aged and young rats are
substantially greater in cells from the upper part of the colonic crypt than those from the middle or lower part of the crypt, indicating a comparatively greater degree of suppression of apoptosis of cells in the upper than in other regions of the colonic crypt of aged rats.

Alterations in apoptosis are now recognized to be an important event in development, normal cell turnover, hormone-induced tissue atrophy, and in many pathological conditions including cancer (8, 27, 29). It has been demonstrated that cells derived from a variety of cancers, including colorectal cancer, show decreased apoptosis in response to physiological stimuli (2, 8, 30). Additional evidence supporting the involvement of apoptosis in the development of colorectal neoplasia comes from the observation that transformation of colorectal epithelium to carcinoma is associated with progressive inhibition of apoptosis (2). Our earlier observation of the age-related decrease in apoptosis in the colonic mucosa together with the current observation that aging is associated with the induction of the PI3K/Akt signaling pathway, with resultant activation of the downstream effector molecules that are involved in stimulating cell survival, further strengthens the postulation that aging is associated with increased survival of colonic mucosal cells. This, together with the fact that aging is also associated with increased proliferation of colonic mucosa, suggests that aging may predispose the colon to neoplastic transformation.

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