Reduction of spontaneous and irradiation-induced apoptosis in small intestine of IGF-I transgenic mice

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INSULIN-LIKE GROWTH FACTOR I (IGF-I) regulates somatic growth in pre- and postnatal life (1, 2). The small intestinal epithelium, one of the most rapidly proliferating systems in the body, is a target organ for IGF-I action (20, 31, 32). Systemically administered IGF-I acts in an endocrine manner to increase the mass of small bowel mucosa and crypt cell proliferation (11, 18, 31, 32, 37, 43, 48). In addition, IGF-I is locally expressed throughout the gastrointestinal tract (12, 21). Changes in local IGF-I expression correlate with factors, such as IGF-I, that prevent apoptosis and induce intestinal growth could therefore, increase cancer risk (4). Indeed, epidemiological data indicate a positive correlation between circulating levels of IGF-I or the structurally homologous IGF-II and the risk of colorectal cancer (15, 38, 39). Although antiapoptotic actions of IGF-I have been noted (19, 29) in colon cancer cells in culture, to date the effect of IGF-I on spontaneous or irradiation-induced apoptosis

Evidence is accumulating (28, 30, 41, 44) that IGF-I not only stimulates cell proliferation but can also protect against apoptosis in many cell types in vitro. In cultured cells, IGF-I protects against apoptosis due to cytotoxic agents (41) or serum deprivation (28, 30, 44). Systemically administered IGF-I decreases apoptosis in rat liver after cutaneous thermal injury (13). There are fewer reports that address the action of IGF-I on apoptosis in vivo. However, it is known that systemically administered IGF-I decreases apoptosis in rat liver after cutaneous thermal injury (13). In addition, transgenic (TG) mice that overexpress IGF-I in the brain exhibit decreases in apoptosis of cerebellar neurons (6), and IGF-I null mice have increased apoptosis of cochlear neurons (5).

The antiapoptotic actions of IGF-I have been shown to occur through several mechanisms. IGF-I is known to decrease expression of the mitochondrial-associated cell death protein Bax as well as to increase expression of the antiapoptotic proteins Bcl-xL and Bcl-2 (6, 26, 28, 30). In cultured cells, IGF-I protects against apoptosis due to cytotoxic agents (41) or serum deprivation (28, 30, 44). Inhibition of apoptosis by IGF-I has also been shown to occur through suppression of caspases, a family of cell death-promoting enzymes (14), as well as IGF-I-induced phosphorylation and inactivation of the proapoptotic protein Bad (6, 9, 10, 30).

Irradiation and cytotoxic drugs dramatically increase the low level of spontaneous apoptosis in intestinal crypts (33–36). Spontaneous apoptosis of intestinal crypt cells is believed to protect against the survival and expansion of genetically damaged cells (33–36). Irradiation increases apoptosis and is also known to cause cell cycle arrest in intestinal crypts (7), an effect that limits proliferation of genetically damaged cells. Factors, such as IGF-I, that prevent apoptosis and induce intestinal growth could therefore, increase cancer risk (4). Indeed, epidemiological data indicate a positive correlation between circulating levels of IGF-I or the structurally homologous IGF-II and the risk of colorectal cancer (15, 38, 39). Although antiapoptotic actions of IGF-I have been noted (19, 29) in colon cancer cells in culture, to date the effect of IGF-I on spontaneous or irradiation-induced apoptosis


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of intestinal crypt cells in vivo has not been examined. Determining whether IGF-I alters crypt cell apoptosis in vivo is relevant to the potential risks of therapeutic strategies aimed at promoting increased intestinal mucosal growth and/or elevating levels of circulating or locally expressed IGF-I (18, 20, 22, 31, 32, 37, 48, 49).

Although some of the downstream mediators of spontaneous and irradiation-induced apoptosis have been identified in the small intestine, the in vivo effects of IGF-I on these pathways have not been determined. Several studies (8, 25) have demonstrated that p53, a tumor-suppressor gene product, is required for irradiation-induced crypt cell apoptosis. Other research (16, 17) has indicated that the expression of Bax increases after irradiation, suggesting that Bax plays an important role in the regulation of spontaneous or irradiation-induced apoptosis in the small intestine.

The goals of our study were to assess whether IGF-I alters spontaneous or irradiation-induced apoptosis and cell cycle arrest in the small intestine in vivo and if the effects of IGF-I are associated with altered expression of Bax mRNA or protein. TG mice, which express a metalloathioneen promoter-driven human IGF-I transgene, and wild-type (WT) littermates have been used for the study. Because TG mice eat more than WT mice when food is unrestricted (27), the daily food intake of TG mice was adjusted to that of WT mice. This was done by the guanidine isothiocyanate-CsCl method. Aliquots of total RNA (15 μg) were denatured with glyoxal and dimethyl sulfoxide, size fractionated on 1% agarose gels, and transferred to Gene Screen (New England Nuclear, Boston, MA).

The proximal jejunum (defined as one-third of the small bowel beginning at the ligament of Treitz) was collected for extraction of RNA and rapidly frozen in liquid nitrogen and stored at −80°C until analysis. An adjacent 1-cm piece of jejenum was processed for protein extraction; the mucosa was scraped and placed in lysis buffer (50 mM HEPES, 150 mM NaCl, 20 mM sodium pyrophosphate, 100 mM NaF, 1.5% Triton X-100, and 10 mM EDTA). Three pieces of adjacent jejunum (each 0.5-cm long) were collected for histological analyses. Tissue for morphological analyses of apoptotic or mitotic cells was fixed in Carnoy’s fixative for 30 min followed by dehydration in 70% ethanol and paraffin embedding. Tissue used for terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) or immunohistochemistry was fixed in 10% formalin for 4 h, dehydrated in 70% ethanol, and embedded in paraffin. The tissue for in situ hybridization was embedded in OCT compound (Miles, Elkhart, IN), frozen in isopentane at −40°C, and stored at −80°C until sectioning.

Analysis of apoptosis and mitosis. Paraffin-embedded jejunal tissue was cut transversely at 4 μm and stained with Mayer’s hematoxylin and eosin. Analyses of apoptosis were performed on hematoxylin and eosin-stained sections and restricted to well-oriented crypts that contained Paneth cells at the base, a lumen, and clearly defined cells aligned with the base of adjacent villi. The morphological identification and quantification of apoptotic crypt cells were based on distinctive morphological features described elsewhere (see Ref. 46 for review). Morphological features of apoptosis include nuclear margination, chromatin and cytoplasmic condensation, shrinkage from neighboring cells, and the formation of apoptotic bodies due to nuclear and cytoplasmic fragmentation. Small apoptotic bodies clustered at a single position were regarded as one apoptotic cell. The position of apoptotic cells along the depth of the intestinal crypt was recorded as described previously (34) with cells at the base designated as position 1. Mitotic figures were counted within the same crypt sections used for morphological scoring of apoptosis. All scoring was performed twice by a single investigator unaware of the mouse genotype or treatment. Scoring was performed on two sections from the same specimen that were more than 50 μm apart to avoid counting the same apoptotic or mitotic cell twice. Scoring was also verified by a second investigator.

TUNEL labeling of apoptotic cells. The TUNEL method was used on segments of irradiated jejunum to verify quantitation of apoptosis by morphological methods. Jejunal tissue specimens fixed in 10% formalin and embedded in paraffin were cut transversely at 8 μm. Detection of DNA fragmentation in apoptotic cells was determined by the TUNEL method as described in the handbook from Intergen (Purchase, NY). Briefly, slides were deparaffinized, treated with proteinase K and 3.0% hydrogen peroxide, and incubated with TdT and digoxigenin-labeled nucleotides for 1 h at room temperature. Slides were washed and then treated with a peroxidase-conjugated digoxigenin antibody (Intergen). Visualization was accomplished using diaminobenzidine (DAB). Slides were counterstained with hematoxylin, dehydrated in ethanol, and coverslipped. As a positive control, sections were incubated with DNAse I before the TdT reaction. Negative controls were processed in the same manner as test slides except that TdT was omitted. Cells labeled by the TUNEL assay were quantified using the same method as described for morphological analysis of apoptotic and mitotic cells.

RNA isolation and Northern blot hybridization assay of Bax mRNA. Total RNA was isolated from proximal jejunum by the guanidine isothiocyanate-CsCl method. Aliquots of total RNA (15 μg) were denatured with glyoxal and dimethyl sulfoxide, size fractionated on 1% agarose gels, and transferred to Gene Screen (New England Nuclear, Boston, MA).
Hybridization was performed using standard conditions and an antisense Bax RNA probe labeled with \([^{35}S]UTP\). The antisense probe was synthesized by in vitro transcription from linearized plasmid template comprising rat Bax cDNA subcloned into pGEM4Z. The Bax plasmid was a generous gift from Dr. J. L. Tilly (Johns Hopkins University, Baltimore, MD) (42). After washing and exposure to PhosphorImager screens, each blot was stripped and rehybridized with a rat \(\beta\)-actin complementary RNA probe (Ambion, Austin, TX). Abundance of mRNA was calculated on a scanning densitometer using NIH Image software (National Technical Information Service, Springfield, VA). Abundance of Bax mRNA in each sample was normalized to the abundance of invariant \(\beta\)-actin mRNA to control for minor differences in RNA loading.

In situ hybridization histochemistry. In situ hybridization was performed using frozen sections (10 \(\mu\)m) as previously described (27). Briefly, sections were fixed with 4% paraformaldehyde, treated with proteinase K, and dehydrated before hybridization. Hybridization was performed at 55°C for 18 h in buffer containing \([^{35}S]UTP\)-labeled rat Bax antisense RNA probe (2 \(\times\) 10\(^6\) cpm/slide) prepared from the same template cDNA used for Northern blot. All sections were treated with RNase A (200 mg/ml) after hybridization and washed with 0.5\% SSC (75 mM NaCl and 7.5 mM sodium citrate, pH 7.0) at 55°C. The sections were dehydrated, air dried, and exposed to Kodak NTB-2 emulsion at 4°C for 14 days. After developing, sections were counterstained with hematoxylin. Sections prepared as negative controls were treated with RNase A before hybridization or hybridized with a control Bax sense RNA probe.

Western blot analysis. Protein was isolated from jejunal mucosa. Aliquots of total protein (30 \(\mu\)g) were separated on a 14\% acrylamide gel and transferred to an Immobilon-P membrane (Millipore). Immunoblots were blocked in 4\% milk in Tris-buffered saline (TBS) overnight at 4°C. After washing in TBS with 0.05\% Tween, primary antibody (Bax, Santa Cruz Biotech; N-20 or actin, Jackson Immunoresearch) was added in TBS for 2 h at room temperature. Blots were then washed and treated with a horseradish peroxidase-conjugated donkey anti-rabbit antibody (Jackson Immunoresearch) for 30 min and placed in enhanced chemoiluminescence mix.

Localization of protein using immunohistochemistry. Formalin-fixed paraffin sections of jejunum were cut at 8 \(\mu\)m for immunohistochemistry. Coded sections from TG and WT littermate pairs were mounted on the same slides and processed simultaneously to eliminate interslide variability. Slides were deparaffinized, blocked with 3\% hydrogen peroxide followed by 10\% normal goat serum, and washed in PBS. Primary antibody (Bax, Santa Cruz Biotech; N-20) was added to sections in PBS containing 1\% normal goat serum and Triton X-100 overnight for 18 h. Slides were then washed in PBS with Triton X-100 and treated with a biotinylated goat anti-rabbit antibody (Vectastain) for 60 min. Slides were incubated with avidin-biotin complex (Vectastain) for 60 min, and labeling was visualized using DAB. Slides were counterstained with hematoxylin, dehydrated in ethanol, coverslipped, and analyzed using light microscopy. Positively stained cells within the crypt epithelia were quantified as described for apoptotic and mitotic cells in Analysis of apoptosis and mitosis.

Statistical analyses. Values are expressed as means \(\pm\) SE. Statistical comparisons of means were performed using the Mann-Whitney \(U\)-test. \(P < 0.05\) was considered to be statistically significant. A two-factor ANOVA was also used to determine whether there was significant interaction between irradiation and IGF-I overexpression on Bax mRNA and protein expression.

RESULTS

Apoptotic indexes. In both nonirradiated and irradiated WT mice, apoptosis assessed by morphological criteria or TUNEL was confined to the crypts with no detectable apoptosis in villus enterocytes. The mean apoptotic index (the sum of apoptotic cells in all crypts counted per total number of cells in all small intestinal crypts counted) was calculated from both hematoxylin-stained and TUNEL-labeled tissue from all TG and WT animals (Table 1). The mean apoptotic index in nonirradiated IGF-I TG mice was significantly lower than that of WT littermates (\(P < 0.05\)), indicating that the frequency of spontaneous apoptosis was decreased in IGF-I TG mice. At 4 h after exposure to 5 Gy, apoptotic indexes were markedly increased in both WT and IGF-I TG mice; however, there were significantly fewer apoptotic cells per total cells counted in irradiated IGF-I TG mice than in WT littermates (Fig. 1 and Table 1). To determine whether the decreased apoptotic index in TG mice was partially due to the fact that the number of cells per crypt was greater in IGF-I TG mice, we also compared the absolute number of apoptotic cells per crypt. There were significantly fewer apoptotic cells per crypt in IGF-I TG mice than in WT littermates in both nonirradiated and irradiated groups (Table 1, \(P < 0.05\)), confirming that both spontaneous and irradiation-induced apoptosis occurred less frequently in IGF-I TG mice than in WT littermates.

To assess whether the reduction of apoptosis in IGF-I TG mice occurred in a specific cell population, the frequency of apoptosis at each cell position within intestinal crypts was determined in hematoxylin-stained tissue from irradiated WT and TG mice (Fig. 2A). In WT mice, the maximum number of apoptotic cells was observed at the third and fourth cell positions from the crypt base and less apoptosis was observed at higher positions. Similarly, even though the overall level of apoptosis was decreased in IGF-I TG mice compared with WT mice, the maximum number of apoptotic cells was observed at position 4 from the crypt base.

Table 1. Apoptosis in jejunum of nonirradiated and irradiated TG and WT mice

<table>
<thead>
<tr>
<th>Method</th>
<th>Genotype</th>
<th>Treatment</th>
<th>Mean Apoptotic Cells/Crypt, No.</th>
<th>Apoptotic Cells/Total Cells, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morph</td>
<td>WT</td>
<td>Non</td>
<td>0.22 (\pm) 0.018</td>
<td>0.72 (\pm) 0.064</td>
</tr>
<tr>
<td>Morph</td>
<td>TG</td>
<td>Non</td>
<td>0.06 (\pm) 0.024*</td>
<td>0.20 (\pm) 0.058*</td>
</tr>
<tr>
<td>Morph</td>
<td>WT</td>
<td>Irr</td>
<td>2.70 (\pm) 0.225</td>
<td>9.35 (\pm) 1.117</td>
</tr>
<tr>
<td>Morph</td>
<td>TG</td>
<td>Irr</td>
<td>1.00 (\pm) 0.479*</td>
<td>3.05 (\pm) 1.581*</td>
</tr>
<tr>
<td>TUNEL</td>
<td>WT</td>
<td>Irr</td>
<td>1.41 (\pm) 0.159</td>
<td>8.66 (\pm) 1.233</td>
</tr>
<tr>
<td>TUNEL</td>
<td>TG</td>
<td>Irr</td>
<td>0.77 (\pm) 0.132*</td>
<td>4.44 (\pm) 0.733*</td>
</tr>
</tbody>
</table>

Values are means \(\pm\) SE; \(n = 4\) for each group. Morph, apoptotic cells identified based on morphology in hematoxylin-stained sections; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling; WT, wild-type mice; TG, insulin-like growth factor I (IGF-I) transgenic littermates; Non, nonirradiated; Irr, irradiated. *\(P < 0.05\) for all WT vs. TG groups.
crypt base in IGF-I TG mice. The antiapoptotic effect of IGF-I was more dramatic in cells positioned at the base (positions 3 and 4) of the crypts in IGF-I TG mice (Fig. 2B). The difference in the percentage of apoptotic cells located at higher positions between IGF-I TG and WT mice was not as great as in cells located at the crypt base. Because the putative stem cells reside at the lower positions of the crypt (33, 36), this provides evidence that IGF-I preferentially protects stem cells from apoptosis.

Mitotic indexes. The mitotic index (the sum of mitotic cells counted in all crypts per total number of cells in all crypts counted) was significantly greater in nonirradiated IGF-I TG mice than in WT littermates (Table 2, P < 0.05). At 4 h after 5 Gy of irradiation, no mitotic figures were observed within the crypts in any sections of jejunum from WT mice. In contrast, irradiation did not completely eliminate mitotic figures in IGF-I TG mice. Irradiated TG mice maintained a higher number of mitotic cells per crypt than nonirradiated WT mice (Table 2). The greatest percentage (63%) of mitotic cells was located in the bottom third (positions 1–5) of the crypts in irradiated TG mice with peaks at the third (19%) and fifth (19%) cell positions. The remainder of mitotic cells within irradiated TG mouse crypts were located in the middle (33%) and upper third (4%) of the crypts.

Expression and localization of Bax mRNA. To determine whether IGF-I overexpression had an effect on expression of mitochondrial-associated apoptotic proteins, the amount of Bax mRNA was quantified in jejunum from each group of mice (Fig. 3). The expression of Bax mRNA was significantly increased after irradiation in both IGF-I TG and WT mice. The abundance of jejunal Bax mRNA did not differ between IGF-I TG and WT littermates in the nonirradiated groups and was significantly greater in IGF-I TG mice than in WT mice after irradiation. A two-factor ANOVA revealed that irradiation and genotype have a significant effect (P < 0.05) on Bax mRNA expression. However, there was no significant interaction between these two variables.

To assess the localization of Bax mRNA in small intestine, we performed in situ hybridization histochemistry (Fig. 4). In WT mice, Bax mRNA was expressed at high levels in the crypt region and at lower levels in the lower part of the villi. The intensity of hybridization signals for Bax mRNA was markedly stronger in jejunum of irradiated mice than nonirradiated mice. Consistent with the results of Northern blot levels in the lower part of the villi. The intensity of hybridization signals for Bax mRNA was markedly stronger in jejunum of irradiated mice than nonirradiated mice. Consistent with the results of Northern blot

Table 2. Mean mitotic cells in hematoxylin-stained jejunal crypts

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Treatment</th>
<th>Mean Mitotic Cells/Crypt, No.</th>
<th>Mitotic Cells/Total Cells, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT 5</td>
<td>Non</td>
<td>0.22 ± 0.039</td>
<td>1.72 ± 0.318</td>
<td></td>
</tr>
<tr>
<td>TG 5</td>
<td>Non</td>
<td>0.69 ± 0.118*</td>
<td>5.53 ± 0.938*</td>
<td></td>
</tr>
<tr>
<td>WT 4</td>
<td>Irr</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>TG 4</td>
<td>Irr</td>
<td>0.35 ± 0.238*</td>
<td>1.00 ± 0.667*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 for all WT vs. TG groups as determined by Mann-Whitney U-test.
analyses, the intensity of the hybridization signal for Bax mRNA was stronger in irradiated IGF-I TG mice than in irradiated WT mice. The location of jejunal Bax mRNA did not differ between IGF-I TG mice and WT littermates in either the nonirradiated or irradiated groups. No signal was detected on the sections treated with RNase before hybridization or on the sections hybridized with the sense Bax RNA probe (data not shown).

Expression and localization of Bax protein. The abundance of Bax protein in the entire mucosa from IGF-I TG and WT mice is shown in Fig. 5. Although there was no significant difference in protein levels, Bax was found in TG and WT mice both before and after irradiation.

To determine whether IGF-I overexpression had an effect on the localization of mitochondrial-associated cell death proteins, the expression pattern of Bax protein was determined using immunohistochemistry on jejunal tissue collected from irradiated IGF-I TG and WT littermates. As shown in Fig. 6, Bax protein was localized to cells throughout the crypts of irradiated WT mice, whereas in IGF-I TG mice Bax protein was localized primarily to cells within the middle and upper portions of the crypts. Semiquantitative scoring of Bax expression within jejunal crypt epithelia indicated that the number of Bax-expressing cells per total cells counted throughout the intestinal crypts was 26.6% lower in IGF-I TG mice than in WT littermates, and in fact, very few Bax-positive cells were observed at the crypt base in irradiated IGF-I TG mice (Fig. 7).

DISCUSSION
The results of this study demonstrate that chronic IGF-I excess results in lower levels of spontaneous and irradiation-induced apoptosis in small intestinal crypts in vivo. Although IGF-I is known to reduce apoptosis in a number of tissues in vivo (3, 6, 13), this has not previously been demonstrated in the bowel. While spontaneous levels of apoptosis in WT mice were low, as reported previously (33), they were even lower in TG mice, and apoptotic cells in TG mice were located primarily at the base of the crypts where putative stem cells are located.

Apoptosis induced by γ-irradiation in the small intestine has been extensively studied (8, 16, 25, 33, 35). Consistent with previous observations (33), irradiation in WT mice resulted in a more than 10-fold increase in apoptosis with peak apoptosis occurring at positions 3 and 4, the likely position of crypt stem cells. Irradiation also induced a 10-fold increase in apoptotic cells over...
spontaneous levels in IGF-I TG mice, demonstrating that there is a population of cells not protected from apoptosis by IGF-I. However, the overall number of apoptotic cells or apoptotic cells per crypt in IGF-I TG mice was 30–40% of the number observed in WT mice, as assessed by morphological criteria. Independent evaluation of apoptosis by TUNEL validated the lower levels of irradiation-induced apoptosis in TG mice (50% of WT mice), although the relative level of apoptotic cells was somewhat different. Given that apoptotic cells exhibiting morphological features of apoptosis may not contain sufficient DNA capable of TUNEL labeling, it is not surprising that there is a difference in apoptotic indexes derived from quantitation using these two methods. The similarity of our results in WT mice by both TUNEL and morphological criteria to previous results (33) validates the scoring system used in this study to compare IGF-I TG and WT mice.

Intriguingly, the difference in the percentage of apoptotic cells undergoing apoptosis between IGF-I TG and WT mice was much greater at positions 1 through 4 than at higher positions within the crypt. This indicates that IGF-I preferentially promotes survival of cells located at the base of the crypts. Because the lower positions in the crypts correspond to the putative stem cell population (33), IGF-I-mediated survival of stem cells after γ-irradiation might facilitate crypt regeneration and at the same time enhance clonal expansion of genetically damaged stem cells and predispose to neoplasia. The mechanism whereby IGF-I may preferentially affect stem cells is not defined, but it has been reported that IGF-I receptor abundance may be higher in the crypts than in the villi (see Ref. 22 for review).

Induction of the proapoptotic protein Bax appears to be a key mediator of irradiation-induced crypt cell apoptosis (16, 17). As expected, irradiation induced Bax mRNA expression in both WT and IGF-I TG mice. Despite the fact that there were significantly fewer apoptotic cells in crypts from IGF-I TG mice compared...
with their WT littermates, the abundance of Bax mRNA was greater in IGF-I TG mice than WT mice as indicated by Northern blot analysis. Semiquantitative in situ hybridization histochemistry also indicates that Bax mRNA was induced at higher levels in IGF-I TG mice in all crypt compartments, including cells at the base of the crypts. Thus irradiation induces Bax mRNA in basal crypt cells despite the presence of elevated IGF-I. IGF-I therefore, appears to exert antiapoptotic actions by mechanisms downstream of transcription of the Bax gene or independent of Bax expression.

IGF-I is known (6, 41, 44) to exert its protective action on several cell types in culture by repressing the expression of Bax protein. Semiquantitative analysis of Bax protein using immunocytochemistry indicated that irradiated IGF-I TG mice had significantly less Bax protein in cells at the base of jejunal crypts compared with WT mice. The basal area of jejunal crypts contains stem cells and is also where the most potent protective effects of IGF-I on apoptosis were observed. Therefore, our findings raise the intriguing possibility that IGF-I acts to protect these stem cells in part by decreasing the accumulation of Bax protein expression in this selected cell population. Together, our results indicate that IGF-I may decrease apoptosis of crypt stem cells through posttranscriptional effects on Bax expression such as decreased translation or increased degradation of protein. Other studies (24, 47) have also indicated that protection from apoptosis is sometimes associated with a decrease in Bax protein expression despite an increase in expression of Bax mRNA. The results of our study are strikingly similar to results from Chrysis et al. (6) who showed that chronic overexpression of IGF-I in the cerebellum results in protection from apoptosis, associated with decreased Bax protein abundance despite elevated Bax mRNA.

Exposure to irradiation or DNA-damaging agents causes mammalian cells to arrest at the G1/S boundary, that is the G1 phase of the cell cycle (7). G1 arrest results in a decrease in the mitotic index. In the small intestine, temporal G2 arrest, also referred to as mitotic delay, occurs at 1 h/Gy in the midcrypt and 2.5 h/Gy in the crypt base (7). Our observation that no mitotic figures were present in crypts from WT mice 4 h after 5 Gy of irradiation are consistent with complete G2 phase arrest. In contrast, IGF-I TG mice exhibited incomplete arrest at the G2 stage of the cell cycle because their crypts exhibited significant numbers of mitotic cells. Interestingly, most of the mitotic cells observed in IGF-I TG mice were found in the lower third of the crypt compartment in the stem cell region. This supports the hypothesis that IGF-I not only preferentially promotes survival of this cell population after exposure to irradiation but also permits continued proliferation.

Our current findings do not exclude other proapoptotic or antiapoptotic proteins as mediators of IGF-I protection against crypt cell apoptosis. In fact, it seems likely that all the mediators of IGF-I antiapoptotic action have not yet been defined. Evaluation of other potential mediators is worthy of future investigation.

In summary, chronic overexpression of IGF-I resulted in 1) decreased spontaneous apoptosis, 2) decreased irradiation-induced apoptosis, 3) decreased expression of Bax protein within the stem cell region, 4) reduced irradiation-induced mitotic delay in small intestinal crypts, and 5) preferential protection of the putative stem cell population.

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REFERENCES


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