FGF-2 enhances intestinal stem cell survival and its expression is induced after radiation injury

COURTNEY W. HOUCHEN, ROBERT J. GEORGE, MARK A. STURMOSKI, AND STEVEN M. COHN
Department of Medicine, Washington University School of Medicine, St. Louis, Missouri 63110

Fibroblast growth factors (FGFs) have mitogenic activity toward a wide variety of cells of mesenchymal, neuronal, and epithelial origin and regulate events in normal embryonic development, angiogenesis, wound repair, and neoplasia. FGF-2 is expressed in many normal adult tissues and can regulate migration and replication of intestinal epithelial cells in culture. However, little is known about the effects of FGF-2 on intestinal epithelial stem cells during either normal epithelial renewal or regeneration of a functional epithelium after injury. In this study, we investigated the expression of FGF-2 in the mouse small intestine after irradiation and determined the effect of exogenous FGF-2 on crypt stem cell survival after radiation injury. Expression of FGF-2 mRNA and protein began to increase at 12 h after γ-irradiation, and peak levels were observed from 48 to 120 h after irradiation. At all times after irradiation, the higher molecular mass isoform (~24 kDa) of FGF-2 was the predominant form expressed in the small intestine. Immunohistochemical analysis of FGF-2 expression after radiation injury demonstrated that FGF-2 was predominantly found in the mesenchyme surrounding regenerating crypts. Exogenous recombinant human FGF-2 (rhFGF-2) markedly enhanced crypt stem cell survival when given before irradiation. We conclude that expression of FGF-2 is induced by radiation injury and that rhFGF-2 can enhance crypt stem cell survival after subsequent injury.

ionizing radiation; basic fibroblast growth factors; injury repair

Houchen, Courtney W., Robert J. George, Mark A. Sturmoski, and Steven M. Cohn. FGF-2 enhances intestinal stem cell survival and its expression is induced after radiation injury. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G249–G258, 1999.—Fibroblast growth factors (FGFs) have mitogenic activity toward a wide variety of cells of mesenchymal, neuronal, and epithelial origin and regulate events in normal embryonic development, angiogenesis, wound repair, and neoplasia. FGF-2 is expressed in many normal adult tissues and can regulate migration and replication of intestinal epithelial cells in culture. However, little is known about the effects of FGF-2 on intestinal epithelial stem cells during either normal epithelial renewal or regeneration of a functional epithelium after injury. In this study, we investigated the expression of FGF-2 in the mouse small intestine after irradiation and determined the effect of exogenous FGF-2 on crypt stem cell survival after radiation injury. Expression of FGF-2 mRNA and protein began to increase at 12 h after γ-irradiation, and peak levels were observed from 48 to 120 h after irradiation. At all times after irradiation, the higher molecular mass isoform (~24 kDa) of FGF-2 was the predominant form expressed in the small intestine. Immunohistochemical analysis of FGF-2 expression after radiation injury demonstrated that FGF-2 was predominantly found in the mesenchyme surrounding regenerating crypts. Exogenous recombinant human FGF-2 (rhFGF-2) markedly enhanced crypt stem cell survival when given before irradiation. We conclude that expression of FGF-2 is induced by radiation injury and that rhFGF-2 can enhance crypt stem cell survival after subsequent injury.
injury, levels are elevated in the small intestine after radiation.

MATERIALS AND METHODS

We report that during radiation injury repair, using the microcolony response of the epithelial stem cell population to FGF-2. The biological effects of FGF-2 are mediated by binding to and activating cell surface receptor tyrosine kinases encoded by a family of four related genes, FGFFR-1, -2, -3, and -4 (32). The ligand-binding specificity of each of these receptors is determined by sequences present in Ig-like domains II and III in the extracellular portion of the receptor molecule. Additionally, multiple receptor isoforms of FGFFR-1, -2, and -3 with differing ligand-binding specificities can be expressed as a result of alternative mRNA splicing events that alter the sequence encoding the carboxy-terminal region of Ig-like domain III of each of these receptors. Binding of FGFs to their receptors induces receptor dimerization, transphosphorylation of their cytoplasmic domains, and activation of downstream signaling cascades.

In this study, we examine the expression of FGF-2 in the mouse small intestine after irradiation and the response of the epithelial stem cell population to FGF-2 during radiation injury repair, using the microcolony assay. We report that 1) FGF-2 mRNA and protein levels are elevated in the small intestine after radiation injury. 2) FGF-2 is localized to the mesenchyme surrounding regenerative crypts in irradiated mice. 3) exogenous recombinant FGF-2 enhances crypt stem cell survival when administered before irradiation but has no effect on epithelial stem cell survival when given only after radiation injury.

MATERIALS AND METHODS

Animals. FVB/N female mice (Taconic, Germantown, NY) were maintained on a 12:12-h light-dark cycle and fed standard laboratory mouse chow ad libitum. Mice were irradiated at age 8–12 wk in a Gamacel 40 cesium irradiator at 0.96 cGy/min. Some animals received recombinant human FGF-2 (rhFGF-2; 1–4 µg/g body wt; gift of Dr. J. Judith Abraham, Scios-Nova, Mountainview, CA) and/or heparin sulfate (1–4 µg/g body wt; Sigma-Aldrich, St. Louis, MO) at various times either before or after γ-irradiation. Animals were killed at various times after irradiation and rapidly dissected, as previously described (9). Some mice received 120 mg/kg 5-bromo-2-deoxyuridine (BrDU) (Sigma-Aldrich) and 12 mg/kg 5-fluoro-2-deoxyuridine (Sigma-Aldrich) 2 h before death to permit identification of replicating S phase cells by immunohistochemistry. The proximal jejunum was fixed in Bouin’s solution and divided into 5-µm segments before paraffin embedding and immunohistochemical analysis. The distal jejunum was snap frozen in liquid nitrogen, and total cellular RNA was prepared for RNase protection analysis from the frozen tissue using TRIzol (GIBCO BRL, Bethesda, MD), according to the manufacturer’s suggested protocol.

Measurement of FGF-2 mRNA levels. Jejunal RNA was prepared as described above. Samples (20 µg) of total RNA were hybridized with a 32P-labeled antisense RNA probe corresponding to mouse FGF-2 (24). To quantitate FGF-2 mRNA expression, we also hybridized the 32P-labeled anti-sense FGF-2 RNA probe to samples containing known amounts of in vitro-transcribed FGF-2 sense-strand RNA. Using the RPA II RNase protection kit (Ambion, Austin, TX) according to the manufacturer’s suggested protocol, we hybridized the FGF-2 probe to a 479-nt fragment encompassing the entire coding region of the FGF-2 mRNA. The RNA hybrids were digested with RNase A and T1 at 37°C. The protected RNA fragments were separated by electrophoresis in 7.5 M urea-8% acrylamide sequencing gels. To control for differences in preparation and loading of individual samples, we also performed RNase protection analysis on duplicate samples using a mouse glyceraldehyde 3-phosphate dehydrogenase antisense probe. Gels were dried, and autoradiography was performed using Kodak BioMax MR film.

Western blot analysis. Intestines from treated and control mice were rapidly dissected and frozen in liquid nitrogen. Tissues were then homogenized with a Tekmar Tissueemizer in 1 ml of proteinase inhibitor cocktail (25 µg/ml antipain, 25 µg/ml aprotinin, 25 µg/ml leupeptin, 25 µg/ml chymostatin, 50 µM o-phenanthroline, 10 µg/ml pepstatin A, and 23 mM diithiothreitol) in 20 mM TES, pH 7.4). Homogenates were centrifuged at 4°C at 8000 g. The supernatant was frozen on dry ice. The protein concentration of the homogenates was determined using the Bradford method (Bio-Rad, Richmond, CA). One half volume of 3X Laemmli SDS sample buffer was added to samples (final sample buffer concn, 2% SDS, 100 mM diithiothreitol, and 60 mM Tris, pH 6.8) before PAGE. Typically, 50 µg of protein were resolved on 15% polyacrylamide gels containing 0.1% SDS and transferred to polyvinylidene difluoride membrane by semidry blotting, according to the manufacturer’s instructions (Bio-Rad). Membranes were blocked with a 5% nonfat dry milk solution in Tris-buffered saline (TBS), pH 7.6, containing 0.1% Tween 20. FGF-2 protein bands were detected by enhanced chemiluminescence (Amersham, Arlington Heights, IL), using a primary antibody to FGF-2 (Ab-2, Oncogene Research Products, Cambridge, MA) and anti-rabbit-horseradish peroxidase secondary antibody (Amersham). Immunohistochemical techniques. For immunohistochemical localization of mouse FGF-2, we incubated deparaffinized sections of Bouin’s fixed tissue with a 1:2,000 to 1:8,000 dilution of a polyclonal rabbit anti-rat FGF-2 prepared against amino acids 1–23 of rat FGF-2 (Chemicon International, Temecula, CA). The specificity of this antibody and its use for immunohistochemistry have been previously described (17, 18). After quenching of endogenous peroxidase activity with 3% hydrogen peroxide, we washed sections with TBS. Bound
rabbit anti-FGF-2 was detected by fluorescein-conjugated tyramide signal amplification (TSA direct, Du Pont NEN Life Science Products, Boston, MA), according to the manufacturer’s suggested protocol, after incubation with biotin-labeled donkey anti-rabbit IgG (Jackson ImmuResearch Laboratories, West Grove, PA) and subsequent incubation with streptavidin-horseradish peroxidase. To identify replicating cells, we detected BrdU incorporated into S phase cells in deparaffinized sections, using a goat anti-BrdU antibody as previously described (7, 8). Bound anti-BrdU was subsequently visualized with either gold-labeled rabbit anti-goat IgG with silver enhancement (Amersham) or by fluorescence with Texas red-labeled donkey anti-goat IgG (Jackson ImmunoResearch Laboratories).

Crypt survival. Crypt survival was measured in young adult (8- to 12-wk-old) mice killed 3.5 days after irradiation, using a modification of the microcolony assay (8). Each mouse received 120 mg/kg BrdU (Sigma-Aldrich) and 12 mg/kg 5-fluoro-2-deoxyuridine (Sigma-Aldrich) 2 h before death to label the S phase cells. Five-micrometer paraffin sections were prepared from proximal jejunum oriented so that the sections were cut perpendicular to the long axis of the small intestine. For purposes of the microcolony assay, a regenerative crypt was determined to have survived irradiation based on its histological appearance. The viability of each surviving crypt was confirmed by immunohistochemical detection of BrdU incorporation into five or more epithelial cells within each regenerative crypt. A minimum of 12 complete cross sections were scored for each mouse. Because the size of the regenerating crypt may not be the same for each treatment group, the number of surviving crypts per cross section was corrected for crypt size to control for the effect of treatment on the probability of observing a regenerative crypt within a section (37, 38). The width of 15 representative crypts for each animal was measured in longitudinal sections of proximal jejunum at the widest point in each crypt, and the mean surviving crypts per circumference was corrected for the variation in crypt size, as previously described by Potten et al. (38).

RESULTS

Levels of basic FGF mRNA and protein are increased after radiation injury. The effect of radiation injury on the expression of FGF-2 mRNA in normal adult mice was examined by RNase protection analysis at various times after γ-irradiation (Fig. 1). In the proximal jejunum of unirradiated mice, FGF-2 mRNA was detectable at low levels (~8 fg/µg total cellular RNA). Levels of FGF-2 mRNA began to increase at 12 h after γ-irradiation with 13 Gy and rose progressively, reaching peak levels at 72 through 96 h after irradiation that were ~11-fold higher (87 fg/µg total RNA) than observed in unirradiated control mice (Fig. 1). FGF-2 mRNA levels subsequently fell from 96 to 144 h after irradiation to the low levels that were present in unirradiated control mouse small intestine. The effect of radiation dose on the expression of FGF-2 mRNA was also determined in the proximal jejunum of mice 72 h after γ-irradiation, a time point at which regenerating crypts first become apparent (Fig. 2). At doses of 8 Gy or less there were no significant differences in FGF-2 mRNA levels compared with unirradiated control mice. However, at doses of γ-irradiation above 8 Gy, FGF-2 mRNA levels increased markedly with increasing doses. At 14 Gy, levels of FGF-2 mRNA were ~12-fold higher than present in the proximal jejunum of unirradiated mice.

Several isoforms of FGF-2 with different apparent molecular masses have been previously reported (31, 40, 41). The higher molecular mass isoforms of FGF-2 are expressed in many different cell lines in vitro (40). Furthermore, there appears to be tissue-specific differential expression of FGF-2 isoforms in vivo (5). To examine expression of the various FGF-2 protein isoforms in the mouse proximal jejunum after radiation injury, we performed Western blot analysis with anti-FGF-2 directed at an epitope common to all of the reported FGF-2 isoforms (Fig. 3). Low levels of the ~24-kDa isoform were observed in unirradiated control mice. Levels of the ~24-kDa FGF-2 isoform progressively increased after γ-irradiation (13 Gy), reaching a
maximum at 96 h after irradiation. Expression of the
~24-kDa isoform subsequently fell to an intermediate
level by 168 h after irradiation. A second fainter
~21.5-kDa band was also observed on Western blots
from 72 to 120 h after 13-Gy γ-irradiation. The 18-kDa
FGF-2 isoform resulting from initiation at the AUG
codon was not detectable in the proximal jejunum at
any time point after irradiation.

Localization of FGF-2 within small intestine after
radiation injury. Immunohistochemical analysis of
FGF-2 in the proximal jejunum of unirradiated mice
demonstrated scant immunoreactive FGF-2 associated
with the basement membrane underlying the crypt and
lower portion of the villus epithelium, a pattern similar
to that previously found in the developing rat intestine
(Fig. 4A) (18). After 13-Gy γ-irradiation, abundant
FGF-2 was observed throughout the lamina propria
underlying the lower villus epithelium and beneath
the base of the villi (Fig. 4, B, C, E, and G). FGF-2 was also
present in the lamina propria beneath the villus base in
regions in which regenerating crypts were not present.
FGF-2 was not observed in crypt epithelial cells or in
the lamina propria associated with the middle or upper
villus epithelium. At higher magnification, the immuno-
reactive FGF-2 appeared to be distributed in a fibrilar
pattern, associated with the extracellular matrix sur-
rounding mesenchymal cells within the lamina propria
and adjacent to the basilar surface of epithelial cells
within regenerating crypts (Fig. 4C). Dual localization
of FGF-2 and BrdU confirmed that immunoreactive
FGF-2 was predominantly located in the lamina pro-
pria surrounding viable actively regenerating crypts
containing numerous S phase cells (Fig. 4, E–G).
Staining was undetectable in an adjacent control sec-
tion in which normal preimmune rabbit serum was
substituted for the rabbit anti-FGF-2 antibody (Fig.
4D). After irradiation, abundant immunoreactive FGF-2
was also seen surrounding small blood vessels in the
submucosa (data not shown).

Recombinant FGF-2 enhances intestinal crypt stem
cell survival after irradiation. In unirradiated mice,
exogenous rhFGF-2 (4 µg/g) given 25 h before analysis
had no effect on the number of crypts per cross section
or on the fraction of crypt epithelial cells in S phase
(data not shown). To determine whether FGF-2 might
regulate crypt stem cell survival in response to injury,
we treated adult FVB/N mice with rhFGF-2 either
before or after γ-irradiation and crypt stem cell sur-
vival was measured using the microcolony assay (Figs.
5 and 6) (8). When FGF-2 was administered intraperi-
toneally before irradiation, crypt survival increased with
increasing duration of FGF-2 administration (Fig. 5).
Maximal enhancement of crypt survival was observed
when treatment with recombinant FGF-2 was begun 25
h before irradiation. A similar enhancement of crypt
survival was observed when mice received either a
single dose of rhFGF-2 (4 µg/g) 25 h before irradiation
or were given rhFGF-2 (1 µg/g) every 8 h beginning 25 h
before irradiation (Fig. 5B). Heparin sulfate, which was
coadministered with FGF-2, had no effect on crypt
survival when given alone. rhFGF-2 (4 µg/g) did not
affect crypt survival when it was given daily beginning
immediately after irradiation through the time of death
for the microcolony assay (Fig. 6). As has been previ-
ously reported by Potten et al. (37, 38), the size of regenerating crypts after γ-irradiation was significantly larger than the size of crypts in unirradiated control mice. Treatment with rhFGF-2, either 25 h before 13.8-Gy γ-irradiation or daily beginning immediately postirradiation, did not significantly alter the size of regenerating crypts compared with mice receiving only 13.8-Gy γ-irradiation (Table 1).

The effect of radiation dose on crypt stem cell survival in the jejunum was investigated in mice treated with FGF-2 (4 µg/g) 25 h before irradiation and in mice treated with the heparin sulfate vehicle alone (Fig. 7). At doses of 10 Gy or less, pretreatment with FGF-2 did not significantly affect crypt survival. However, at doses above 10 Gy, crypt survival decreased with increasing radiation dose. The number of surviving crypts per jejunal cross section at 16 Gy in the heparin sulfate control mice was 2.8-fold higher at 12 Gy, 5.3-fold higher at 14 Gy, and 11-fold higher at 16 Gy than observed in mice treated with the heparin sulfate vehicle alone.

**DISCUSSION**

In this study we show that radiation injury results in increased expression of FGF-2 mRNA and protein. Levels of FGF-2 mRNA and protein began to rise at 12 h postirradiation when crypt stem cells may be recovering from any cell cycle arrest and are beginning the process of epithelial regeneration. Peak levels of FGF-2 expression occurred from 72 to 96 h after irradiation, a time period when the appearance of regenerative crypts and disruption of epithelial integrity are the predominant morphological features of the reparative process. Increased FGF-2 expression was dependent on the extent of radiation injury. Increased levels of FGF-2 mRNA were only observed at doses above 8 Gy that produce moderate to severe injury to the gastrointestinal epithelium, resulting in progressive loss of viable regenerative crypts and compromise of the epithelial barrier (compare Figs. 2 and 7). Lower doses of radiation injury that suppress replication in the transit cell population but do not impair crypt survival or compromise epithelial barrier function had no effect on levels of FGF-2 mRNA or protein.

The marked increase in FGF-2 mRNA and protein levels that we observed in the small intestine taken...
together with our immunohistochemical findings suggest that ionizing radiation induces FGF-2 gene expression locally within mesenchymal cells in the lamina propria of the intestine. It is unlikely that FGF-2 is expressed in regenerating crypt epithelial cells or that these epithelial cells induce local production of FGF-2 since FGF-2 was not detectable within epithelial cells after irradiation and because abundant FGF-2 was present both in regions containing regenerating crypts and in areas where regenerating crypts were not present (Fig. 4B). Much of the immunoreactive FGF-2 appeared to be associated with extracellular matrix beneath the base of the villi rather than localized to a particular cell type within the lamina propria. These data are consistent with the known affinity of FGF-2 for heparin sulfate like proteoglycans present in the extracellular matrix.

**Table 1. Effect of irradiation and FGF treatment schedule on crypt dimensions in proximal jejunum**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Crypt Width, µm</th>
<th>±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>48.8</td>
<td>0.8</td>
</tr>
<tr>
<td>13.8 Gy alone</td>
<td>78.1*</td>
<td>2.9</td>
</tr>
<tr>
<td>FGF-2 (24 h preirradiation) × 13.8 Gy</td>
<td>80.7††</td>
<td>4.6</td>
</tr>
<tr>
<td>13.8 Gy + FGF-2 (0, 24, and 48 h postirradiation)</td>
<td>80.6††</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Fibroblast growth factor-2 (FGF-2) (4 µg/g) was administered by intraperitoneal injection at indicated times. Crypt width was determined in longitudinal sections of proximal jejunum from 5 mice per treatment group. Longitudinal crypt width was measured in 15 representative crypts from each mouse, and results were averaged. Control, no treatment. *P < 0.001 compared with control. †Not significant compared with 13.8 Gy alone.
and nuclear factor-
radiation-induced increase in FGF-2 expression may
be observed after irradiation in vivo. Alternatively, the
mediators of the increase in FGF-2 expression that we
any of these transcription factors are the proximate
most pronounced (see Fig. 1). Thus it is unlikely that
increased expression of FGF-2 was falling back to baseline levels before the time period
the first few hours after irradiation and is transient,
induction of these transcription factors occurs within
factors in mammalian cells, such as c-
induce the expression of a number of transcription
agents that result in cellular DNA damage can also
lines in vitro (21, 28). Ionizing radiation and other
lines, including some endothelial and epithelial cell
induce expression of FGF-2 directly in several cell
after irradiation. Ionizing radiation has been shown to
other signaling molecules elaborated by other cell types
functions as crypt cell replication, epithelial migration,
are immediately adjacent to regenerative crypts. It is
clear that mesenchymal cells, such as pericytrophic
blasts, are important in regulating such epithelial
functions as crypt cell replication, epithelial migration,
and regional differentiation events (19).

The increased expression of FGF-2 in the small
intestine induced by irradiation could be due to a direct
effect of radiation injury on the transcription of the
FGF-2 gene, to a series of sequential events occurring
within the cells that express FGF-2, or to a response of
FGF-2-expressing cells to cytokines, growth factors, or
other signaling molecules elaborated by other cell types
after irradiation. Ionizing radiation has been shown to
induce expression of FGF-2 directly in several cell
lines, including some endothelial and epithelial cell
lines in vitro (21, 28). Increasing radiation and other
agents that result in cellular DNA damage can also
induce the expression of a number of transcription
factors in mammalian cells, such as c-jun, c-fos, EGR-1,
and nuclear factor-κB (2, 3, 10, 14, 28, 29). However,
the induction of these transcription factors occurs within
the first few hours after irradiation and is transient,
falling back to baseline levels before the time period
(48–120 h) when increased expression of FGF-2 was
most pronounced (see Fig. 1). Thus it is unlikely that
any of these transcription factors are the proximate
mediators of the increase in FGF-2 expression that we
observe after irradiation in vivo. Alternatively, the
radiation-induced increase in FGF-2 expression may
result from a cascade of cellular events occurring
within FGF-2-expressing cells that is initiated by radia-
tion injury. Another possibility is that the increase in
FGF-2 expression after irradiation results from effects
of a cytokine or other mediator produced by another cell
type on FGF-2-expressing cells in the intestine. For
example, expression of cyclooxygenase-1 (COX-1) and
production of PGE₂ are induced by radiation injury
with kinetics (8) that are remarkably similar to the
time course of FGF-2 expression after irradiation that
we observed in this study. Several other growth factors
and cytokines, including tumor necrosis factor-α (TNF-
α), transforming growth factor-beta1 (TGF-β1), and interleukin-1β (IL-1β), are also produced by endothelial
cells, myofibroblasts, and other cell types in response to
radiation injury, suggesting the possibility that FGF-2
expression might be regulated through such extracellu-
lar signaling mechanisms (21, 46).

The predominant isoform of FGF-2 that we observe
after irradiation has an apparent molecular mass of
~24 kDa (Fig. 3). Several investigators (31, 40) have
observed that multiple higher molecular mass isoforms
of FGF-2 can result from utilization of alternative
translation initiation sites at in-frame CUG codons
within the FGF-2 mRNA located 5’ to the AUG, which
is the initiation site for the ~18-kDa isoform. NH₂-
terminally extended forms of FGF-2 have been isolated
from colonic mucosa as well as from other cell types and
are mitogenic for intestinal epithelial cell lines in
culture (12, 31). Experiments using transgenic mice
expressing a single human FGF-2 mRNA suggest that
tissue-specific expression patterns of the different mo-
lecular mass FGF-2 isoforms are regulated at the
translational level (5). The higher molecular mass
isoforms of FGF-2 contain a nuclear translocation
signal that results in nuclear accumulation of these
higher molecular mass isoforms in cultured cells (41).
The 18-kDa isoform of FGF-2 lacks this sequence and is
primarily found in the cytosol of cells in culture. We find
that after irradiation much of the immunoreactive
FGF-2 is associated with the extracellular matrix
(Fig. 4). Nuclear localization of immunoreactive FGF-2
was not observed. These data taken together with the
results of the Western blot analysis showing that only
the higher molecular mass ~24- and ~21.5-kDa iso-
forms are detectable in the mouse small intestine (Fig.
3) suggest that, in contrast to cells in culture, the
high-molecular-mass isoforms of FGF-2 are synthe-
size and released from cells in response to radiation
injury in vivo. In experiments using transfected cell
lines that express either the 18-kDa isoform or the
high-molecular-mass isoform of FGF-2 exclusively, the
different isoforms of FGF-2 had distinct biological
effects on motility, integrin expression, serum growth
requirements, and FGF receptor expression in addition
to their shared biological activities (13, 26). The high-
molecular-mass isoforms of FGF-2 are thought to medi-
ate at least some of their effects on cellular function
independent of activation of cell surface receptor tyro-
sine kinases. Thus it is possible that the expression of
endogenous 24-kDa FGF-2 after irradiation can affect

![Fig. 7. Effect of radiation dose on crypt survival in control and rhFGF-2-treated mice.](image)
the response of stem cells to radiation injury through mechanisms that are different from the effects that we observe on crypt stem cell survival with the exogenously administered rhFGF-2.

It is likely that exogenous rhFGF-2 alters the sensitivity of epithelial stem cells to subsequent radiation-induced damage since rhFGF-2 maximally enhanced crypt stem cell survival when it was given 25 h before irradiation. A number of mechanisms could account for this radioprotective effect of rhFGF-2 on intestinal epithelial stem cells. 1) FGF-2 might reduce radiation-induced damage by increasing cellular levels of substances that prevent formation or increase breakdown of reactive oxygen intermediates that are thought to mediate damage of DNA and other macromolecules within the cell. However, studies (15) on cell lines in culture have found no effect of FGF-2 on the spectrum or amount of DNA damage produced by γ-irradiation. 2) FGF-2 could directly enhance injury-repair mechanisms in epithelial stem cells. 3) FGF-2 could cause cell-cycle arrest, allowing time for repair to occur in otherwise replicating cell populations. Ionizing radiation can induce transient arrest of the cell cycle at checkpoints in G1 and/or G2 in a variety of intestinal and nonintestinal cell lines (6, 27). Such cell cycle checkpoints have been shown to play an important role in the repair of DNA damage and enhancement of survival of cells exposed to genotoxic agents (6, 27).

Several studies (6, 15, 16) have shown that treatment of cell lines with exogenous FGF-2 and transfection of cell lines with plasmids expressing FGF-2 enhance cell survival and repair of DNA damage after irradiation. Furthermore, radioresistance induced by the high-molecular-mass isoforms of FGF-2 was associated with hypophosphorylation of the cell cycle regulatory protein p34cdc2 and prolongation of G2 in Hela cells (6).

4) FGF-2 might enhance crypt survival by protecting stem cells from undergoing programmed cell death after irradiation. Apoptosis is thought to be the primary mechanism for regulating the number of intestinal epithelial stem cells in the crypt during normal epithelial renewal and to be the predominant biological response of crypt epithelial cells to low levels of damage that occur chronically in the small intestine and colon (39). However, this may not be an appropriate biological response in epithelial stem cells when injury is more severe and intestinal barrier function is compromised.

Although expression of FGF-2 is markedly increased after irradiation, exogenous rhFGF-2 had no effect on crypt stem cell survival when administration was begun immediately after radiation injury. It is possible that FGF-2 is not able to prevent stem cell death when radiation injury has already occurred or that the effects of endogenously produced FGF-2 on crypt stem cells during epithelial regeneration may already be maximal. Another possibility is that the pharmacological effects of exogenously administered rhFGF-2 may be different from the effects of endogenous FGF-2 present in the gut after injury.

A variety of cytokines and other intracellular regulatory molecules, including TGF-β3, KGF, TNF-α, IL-1, IL-11, and the prostaglandin derivatives dimethyl-PGE2 and misoprostol, when given before injury, have shown to protect epithelial stem cells within the intestine from cell death induced by radiation or other cytotoxic agents (22, 25, 30, 35, 37). Here, we show that exogenous rhFGF-2 given before irradiation also enhances endogenous cell survival in response to subsequent injury. It is interesting that the expression of COX-1 and the production of intestinal PGE2 are increased after radiation injury (8) with a time course that is nearly identical to the kinetics of endogenous FGF-2 expression that we report in this study. Furthermore, inhibition of PGE-2 synthesis through COX-1 resulted in decreased stem cell survival after γ-irradiation. Additionally, the radiation dose response of FGF-2 expression was remarkably similar to the dose-dependent increase for COX-1 and PGE2 after irradiation that we previously reported (8).

Increased expression of FGF-2, COX-1, and PGE2 were only observed at radiation doses that cause a significant decrease in the number of regenerating crypts within the intestinal epithelium and result in significant damage to or loss of the epithelial barrier. Thus increased expression of endogenous FGF-2 appears to be a component of a coordinated response to radiation injury in the intestine.

A variety of biological functions have been suggested for FGF-2 in injury repair, including stimulation of angiogenesis, enhanced remodeling of the extracellular matrix, regulation of fibroblast and smooth muscle proliferation, and stimulation of epithelial migration (11, 12). Although the biological consequences of increased endogenous FGF-2 expression in the small intestine after radiation injury have not yet been determined, our data raise the possibility that, in addition to its other activities in injury repair, a potential function of FGF-2 may be to enhance survival of intestinal epithelial stem cells after genotoxic or cytotoxic damage.

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Address for reprint requests: S. M. Cohn, Division of Gastroenterology and Hepatology, Univ. of Virginia Health Sciences Center, PO Box 10013, Charlottesville, VA 22906.

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REFERENCES


4. Cheng, H., and C. P. Leblond. Origin, differentiation and renewal of the four main epithelial cell types in the mouse small
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17. 18.


