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Two- and Three-Dimensional Models for Risk Assessment of Radiation-Enhanced Colorectal Tumorigenesis

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Abstract

Astronauts may be at an increased risk for developing colorectal cancer after a prolonged interplanetary mission given the potential for greater carcinogenic effects of radiation to the colon. In addition, with an increase in age, there is a greater incidence of premalignant colon adenomas with age. In the present study, we have compared the effects of radiation on human colon epithelial cells in two-dimensional (2D) monolayer culture, in three-dimensional (3D) culture, and in intact human colon tissue biopsies. Immortalized colon epithelial cells were irradiated at the NASA Space Radiation Laboratory (NSRL) with either 1 Gy 1 GeV/nucleon ⁵⁶Fe particles or 1 Gy 1 GeV/nucleon protons and were stained at various times to assess DNA damage and repair responses. The results show more persisting damage at 24 h with iron-particle radiation compared to protons. Similar results were seen in 3D colon epithelial cell cultures in which ⁵⁶Fe-particle-irradiated specimens show more persisting damage at 24 h than those irradiated with low-LET γ rays. We compared these results to those obtained from human colon tissue biopsies irradiated with 1 Gy γ rays or 1 Gy 1 GeV ⁵⁶Fe particles. Observations of radiation-induced DNA damage and repair in γ -irradiated specimens revealed more pronounced early DNA damage responses in the epithelial cell compartment compared to the stromal cell compartment. After low-LET irradiation, the damage foci mostly disappeared at 24 h. Antibodies to more than one type of DNA repair factor display this pattern of DNA damage, and staining of nonirradiated cells with non-phosphorylated DNA-PKcs shows a predominance of epithelial staining over stromal cells. Biopsy specimens irradiated with high-LET radiations also show a pattern of predominance of the DNA damage response in the highly proliferative epithelial cell compartment. Persistent unrepaired DNA damage in colon epithelial cells and the differing repair responses between the epithelial and mesenchymal compartments in tissues may enhance tumorigenesis by both stem cell transformation and alterations in the radiation-induced permissive tissue microenvironment that may potentiate cancer progression.

INTRODUCTION

Exposure to highly energetic charged particles such as galactic cosmic radiation (GCR) and solar proton events is a significant concern for astronauts embarking on long-term space missions. Previous studies using statistical model systems have demonstrated a significant probability of developing cancer after a Mars mission secondary to chronic radiation exposure (1). Despite these recent findings, large uncertainties still exist when making risk projections, mainly because there are few biological data sets describing the effects of protons and GCR on human tissues (2). One of the cancers of high concern is colorectal cancer. In the United States colorectal cancer is the third most prevalent cancer and the third leading cause of cancer-related death (American Cancer Society, <http://www.cancer.org>). Approximately 90 to 95% of all colorectal cancer patients do not have a familial predisposition, and thus the cancers are

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sporadic in nature. It is well established that sporadic colorectal cancer arises from the progressive accumulation of genetic (and perhaps epigenetic) aberrations that over time lead normal tissue to progress to a premalignant adenoma, intermediate adenoma and then adenocarcinoma (3). The presence of premalignant colon adenomas is the most important risk factor for the development of colorectal cancer (4), with the incidence of adenomas increasing with age (5). More recent studies identify non-adenomatous lesions, such as the sessile serrated polyps, as potentially having a higher risk to transform to cancer (6). The overall incidence of polyploid lesions in persons of age 40 to 49 in some recent studies has been stated to be 22% (7), while other studies have determined the prevalence to be as high as 30 to 40% in persons of this same age group (8). Given these numbers, it is critical that we study the biological effects of space radiation on human colon tissues since the average astronaut going into space is 42 years old.

In space, the radiation types most likely to contribute to colorectal cancer progression are high mass and energy (HZE) particles (e.g., iron ions) (9). This type of radiation causes complex DNA double-strand breaks, the most detrimental type of DNA lesion produced by ionizing radiation. On a trip to Mars, it is estimated that all cell nuclei in the human body would be traversed every few days by protons and about once a month by HZE particles (10). The accumulated effect of heavy-ion damage in addition to possible detrimental effects caused by protons may result in additional mutations in a cancer-initiated cell, thus enhancing the progression of benign lesions to frank carcinoma.

The tumorigenic effect of space radiation exposure on nontransformed human colon epithelial cells has never been explored. Up to now, radiation studies on normal nontransformed human colon epithelial cells have not been performed because of the difficulty of passaging these cells in conventional monolayer culture and as a result of their short life span (approximately 48–72 h). We recently developed a two-dimensional (2D) model of extended life-span nontransformed human colon epithelial cells that are capable of dividing continuously in long-term cell culture and are able to undergo terminal differentiation (manuscript in review). These cells, termed HCEC CT, have been immortalized by ectopic expression of Cdk4 [to bypass the elevated p16 levels that frequently lead to premature senescence as a result of tissue culture conditions (11)] and hTERT [to maintain telomere length and prevent replicative senescence (12)]. This technique has been described previously in the immortalization process of other epithelial cells such as human bronchial epithelial cells (13). In the present series of experiments, we focused on describing the DNA damage and kinetics of repair in HCEC CT cells after exposure to low- or high-linear energy transfer (LET) radiation to determine whether high-LET radiation causes more persisting damage at 24 h compared to lower-LET radiation as has been seen previously in other cells such as skin fibroblasts (14). We then compared the findings in the 2D model to similarly irradiated 3D colon organotypic cultures and to intact human colon biopsies to identify any similarities and differences in the kinetics of DNA repair between the cell and tissue levels. Although high-LET radiation damage is more persistent than low-LET radiation damage in 2D cell culture, before the present study it was not known whether the same applies to human cells in a tissue setting where cells are closer to each other, multiple cell types co-exist, and cells may reside in or out of the cell cycle (quiescent, replicating or differentiated cells). Observing overall increased persisting damage after exposure to low- or high-LET radiation in a 3D setting compared to a 2D setting would have implications for cancer progression since this would mean that cells in an intact tissue may have more difficulty repairing damage. In addition, stem cells and early progenitor cells would have higher probabilities of accumulating mutations. Finally, significant differences in the DNA damage response of various cell types in a 3D or tissue setting after exposure to low- or high-LET radiation would imply further risk for cancer progression resulting from alterations in the tissue microenvironment.

METHODS

Colon Tissues

Multiple random colon biopsies (20 to 30 samples, $\sim 0.5 \text{ cm}^3$) from tissue not involved with endoscopically visible adenomas or masses were obtained from healthy patients undergoing routine screening colonoscopy after obtaining informed consent and following institutional review board-approved protocols. Colon biopsies were used to extract epithelial cells either for immortalization or for whole tissue biopsy irradiation.

Growth Media and Tissue Culture Substrate

HCEC CT cells were grown in basal medium consisting of four parts high-glucose DMEM and one part medium 199 (HyClone, Logan, UT; referred to hereafter as X medium) supplemented with EGF (100 ng/ml) (PreproTech, Inc., Rocky Hill, NJ), hydrocortisone (1 $\mu\text{g/ml}$), insulin (10 $\mu\text{g/ml}$), transferrin (2 $\mu\text{g/ml}$), sodium selenite (5 nm) (all from Sigma Chemical, St. Louis, MO), and gentamicin sulfate (50 $\mu\text{g/ml}$). HCEC CT cells were cultured in Primaria® (BD Biosciences, San Jose, CA) tissue culture flasks for optimal of cell attachment and growth. Immortalized human colon fibroblast cells [C26Ci (15), PD 150] and CaCo2 cells were maintained in X medium supplemented with 10% cosmic calf serum (HyClone). C26Ci cells were treated with 10 $\mu\text{g/ml}$ mitomycin C (Sigma) for 2 h and then used as feeder layers from the point of initial crypt attachment until the first passage. All cultures were grown in atmosphere consisting of 3–5% oxygen and 5% carbon dioxide.

Human Colon Epithelial Cell Isolation and Immortalization

Briefly, colon biopsies were immersed in cold X medium containing antibiotic/antimycotic solution and brought to the laboratory within 40–60 min after colonoscopy. Specimens were washed copiously with phosphate-buffered saline (PBS) containing antibiotic/antimycotic agents, cut with apposing blades into multiple small pieces ($\sim 1 \text{ mm}$ in size), and exposed to collagenase (Worthington Biochemical, Lakewood, NJ), dispase (Roche, Germany), and 2.5% cosmic calf serum for digestion at 37°C for a total of 2.5 h. After enzymatic digestion, the crypts were resuspended in basal medium with growth supplements including 2% serum and seeded into a Primaria® vessel of the desired size seeded 48 h previously with 50% confluent colon fibroblast feeder layers. Primary cultures arising from attached cells were allowed to expand in growth medium under low oxygen conditions. During the first 10 days after attachment, cells were fed every 5 days, reducing the serum by 1% at each change until it reached 0% to prevent growth of unwanted cells such as pericryptal fibroblasts and endothelial cells. Once nests of expanding epithelial cells were easily observed, cells were transduced with a retroviral vector containing *Cdk4* [retroviral parent vector pSR α MSU (G418⁺) expressing mouse *Cdk4* (Charles J. Sherr, St. Jude Children's Research Hospital, Memphis, TN)] in the presence of 2 $\mu\text{g/ml}$ Polybrene (Sigma). This was followed 48 h later by a retroviral infection with the catalytic component of human telomerase (*hTERT*). Cells were routinely subcultured approximately every 7 days or when confluent using trypsin/EDTA (0.025% and 0.01%, respectively; Cascade Biologics, Portland, OR).

3D Colon Organotypic Cultures

Cultures were established as described previously for skin (16) and bronchial (17) equivalents. CaCo2 cells (cells derived from colon cancer cells that retain their ability to differentiate in monolayer culture) were used as the epithelial portion of the 3D culture. Briefly, collagen gels were allowed to polymerize after mixing type I collagen (BD Biosciences) and C26Ci fibroblasts. The gels were released and incubated for 4 days to allow the fibroblasts to contract the gels. Eight-millimeter cloning rings were then placed atop the gels, and 6×10^4 CaCo2 cells in a volume of 50 μl were seeded inside the cloning ring. Cells were allowed to attach

overnight, the rings were removed, and organotypic cultures were brought to an air–liquid interface in X medium with 10% cosmic calf serum for up to 10 days in culture (medium change every 3 days), after which the cultures were used for radiation experiments.

Irradiation Schemes

A total of 1×10^4 HCEC CT cells were seeded on to Deckgläser cover slips (Germany) and irradiated 48 h later at the NSRL with either 1 Gy 1 GeV/nucleon ^{56}Fe particles or 1 Gy 1 GeV/nucleon of protons. Cells were then fixed with cold methanol (-20°C) for 10 min at various times (30 min, 2 h, 24 h) and stained for DNA damage. 3D organotypic cultures and colon biopsies were irradiated at UT Southwestern with low-LET ^{137}Cs γ radiation or at the NSRL with 1 Gy 1 GeV/nucleon ^{56}Fe particles and fixed at 30 min, 2 h, 4 h and 24 h (3D cultures) or 30 min (colon biopsy) with neutral buffered formalin overnight. Colon biopsies were obtained from the patient, placed in medium, transported by air to New York at 4°C , and irradiated at the NSRL. The total time from obtaining tissue from the patient to irradiation was 24 h.

Immunofluorescence, Quantification of DNA Damage and Microscopy 2D Cultures

Briefly, cells were washed with PBS after fixation, permeabilized with cold 0.05% Triton X-100 for 10 min, and blocked with 5% BSA (Sigma) at room temperature. Samples were exposed to primary antibodies for assessment of DNA damage and repair [rabbit polyclonal phosphorylated DNA-PKcs (pT2609, ab4194; Abcam, Cambridge, MA) and mouse monoclonal anti-histone H2AX (phospho S139, ab22551; Abcam)] for 1 h at room temperature, followed by washing and exposure to species-specific fluorochrome-labeled secondary antibodies (Invitrogen) for 30 min at room temperature. DAPI (Vector Laboratories) was used for nuclear counterstaining. Cover slips were then affixed to glass slides with Mowiol (Calbiochem). An epifluorescence microscope (Zeiss Axiovert 200M) was used to take images of cells at $40\times$ magnification. Co-localized yellow dots were counted per individual cell nucleus at each time. Foci on more than 50 cell nuclei were counted by eye for each time from one experiment, and the average percentage of foci remaining per nucleus was calculated relative to the number at 30 min, the time with the largest amount of DNA damage foci. Values were corrected relative to those for nonirradiated controls. The average percentages of foci remaining for the respective times were graphed using GraphPad Prism 5 software. Standard errors were calculated after the percentage of DNA damage foci remaining per nucleus for each time was averaged ($n > 50$ nuclei).

3D Cultures and Colon Biopsies

After fixation for 24 h in neutral buffered formalin, 3D cultures and biopsies were processed and embedded in paraffin blocks and sectioned at $5\text{-}\mu\text{m}$ intervals onto charged glass slides. Specimens were then depar-affinized with four consecutive 10-min xylene immersions and followed by washing with ethanol and deionized water. Antigen retrieval was performed by placing slides in a sodium citrate buffer (10 mM, pH 6.5) and heating for 25 min of total boiling time in a conventional microwave oven. Specimens were then left to cool for 5 min in the hot sodium citrate and transferred to room-temperature PBS for an additional 20 min. Deionized water washes were performed again followed by immersion in 3% hydrogen peroxide in methanol for 15 min at room temperature to reduce background (18). Tissue sections on slides were then boxed with a PAP pen followed by a 1% BSA/PBS block for 30 min in a humidified chamber at 37°C . Specimens were washed with deionized water, then exposed to primary antibodies [pT2609, γ -H2AX, 53BP1 (Abcam) and non-phosphorylated DNA-PKcs (Abcam) at 1:400 dilution for each] in 1% BSA/PBS solution for 2 h at room temperature. Water washes were performed again followed by incubation with fluorochrome-labeled secondary antibodies in 1% BSA/PBS solution for 30 min (1:800 dilution). Slides were then washed with PBS and

subjected to an ethanol dehydration series. DAPI was used for nuclear counterstaining. Specimens were visualized through the epifluorescence microscope. In the case of the 3D cultures, DNA damage foci were counted in over 50 nuclei per section, and the average percentage of foci remaining relative to 30 min was calculated for each time. In the case of the colon biopsies, foci were counted in over 50 nuclei in both epithelial cells (cells contained in the crypt structures) and stromal cells (cells between crypts). The average percentage of foci remaining was calculated for both epithelial and stromal cells relative to the 30-min value for epithelial cells. All values were corrected using the values for nonirradiated controls. Standard errors were calculated after averaging the percentage of DNA damage foci remaining per nucleus for each time ($n > 50$ nuclei).

RESULTS

Damage Induced by High-LET Radiation Persists Longer than Damage Induced by Lower-LET Radiation in Logarithmically Growing Cells in 2D Culture

The extended life-span nontransformed human colon epithelial cells (HCEC CT, where C and T represent ectopic expression of CDK4 and hTERT, respectively) are able to divide continuously in long-term cell culture and still be capable of differentiation in monolayer culture (unpublished observations). Logarithmically growing cells were irradiated at the NASA Space Radiation Laboratory (NSRL) (Brookhaven National Laboratory, Upton, NY) with either 1 Gy 1 GeV/nucleon ^{56}Fe particles or 1 Gy 1 GeV/nucleon protons. Cells were fixed 30 min, 2 h and 24 h after irradiation and stained for DNA damage proteins. The average numbers of foci for each respective time were corrected to the values for control cells and normalized to the value at 30 min. More persisting damage in the form of co-localized γ -H2AX and phosphorylated DNA-PKcs was observed in ^{56}Fe -particle-irradiated cells at 24 h compared to proton irradiation (Fig. 1a). Twenty-four hours after irradiation, approximately 20% of the foci remained with ^{56}Fe particles compared to protons (Fig. 1b). These results demonstrate that in nondifferentiated proliferating HCEC CT cells, high-LET ^{56}Fe particles impart DNA damage that is more complex and more difficult to repair than that induced by lower-LET protons.

Damage Induced by High-LET Radiation Persists Longer than Damage Induced by Low-LET Radiation in Colon Epithelial Cells in 3D Culture

To determine whether the DNA damage and repair kinetics observed in 2D cultures is similar or different in a more physiological environment in which cells are close to each other and in which subsets of cells may be replicating more slowly and may in fact be differentiating, we constructed 3D colon epithelial cell organotypic cultures using Caco2 cells. After 10 days of culture, Caco2 cells in this 3D arrangement develop tube-like structures that when cut in cross section appear similar to cross-sectioned circular crypts seen in intact human colon tissues (Fig. 2a).

3D cultures were fixed at 30 min, 4 h and 24 h after irradiation and stained with an antibody to phosphorylated DNA-PKcs (pT2609) and γ -H2AX. Co-localization was observed between the γ -H2AX and pT2609 foci (Supplementary Fig. 1), supporting prior observations that DNA damage proteins exist in proximity at DNA damage sites (14, 19). Given the limitations of γ -H2AX staining in paraffin-embedded tissues (incidence of high background and lack of uniform discrete foci visualized in stained samples compared to pT2609), pT2609 foci were then counted on irradiated cell nuclei, and the average number of foci for each time was corrected to the control values and normalized to the value for 30 min. At 24 h, ^{56}Fe -particle-irradiated cells had more persisting pT2609 foci than those exposed to γ rays (Fig. 2b and c). In γ -irradiated 3D cultures, the majority of the foci disappeared, similar to what was seen in 2D cultures, implying that foci disappear in all cell types encountered in the 3D cultures, such as replicating and differentiating cells (Fig. 2d). These findings demonstrate that the kinetics

of DNA damage and repair in epithelial cells after exposure to low- or high-LET radiation is similar in the 2D and 3D environment.

Epithelial Cell DNA Damage Foci in Intact Human Colon Biopsies after γ Irradiation Diminish with Time while Stromal Cells Show a Markedly Decreased Amount of DNA Damage Foci at all Times

Human colon biopsies are composed of epithelial cells surrounded by lamina propria containing the stromal cells. Stromal cells include pericryptal and interstitial fibroblasts, which are important for epithelial cell differentiation and tissue integrity, respectively (20). Colon biopsies from non-diseased colons therefore represent an ideal system for studying the immediate DNA damage and repair responses in different types of neighboring cells as well as cells that may be in and out of the cell cycle (replicating, differentiated and quiescent cells). Thirty minutes was the earliest time studied after γ irradiation (Fig. 3a). At this time there was a noticeable difference in the DNA damage response between the epithelial and stromal compartments. Qualitatively, the epithelial cells had much more DNA damage in the form of pT2609 foci than the stromal cells (Fig. 3a). A similar pattern of DNA damage response was observed when 53BP1 antibody was used (Fig. 3b and inset). Staining of nonirradiated control biopsies with nonphosphorylated DNA-PKcs showed predominant nuclear staining in crypt epithelial cells and significantly less staining in stromal cells (Fig. 3c). At later times, the number of DNA pT2609 foci diminished significantly in the epithelial cells, while the number in stromal cells remained relatively constant, with on average less than one focus per nucleus per section of tissue (Fig. 4a and b). Tissue biopsies irradiated with ^{56}Fe particles also showed a predominant damage pattern in the epithelial compartments, as shown by pT2609 (Fig. 4c) and 53BP1 (Fig. 4d) staining at 30 min.

DISCUSSION

Much uncertainty underlies risk estimates for developing colorectal cancer after chronic exposure to HZE particles during a prolonged deep space mission. We initially studied the acute effects of radiation on 2D and 3D tissue culture systems to determine whether there were any significant differences in the DNA damage response and repair between cells in an artificial monolayer system and cells in the more physiological tissue environment. Persisting DNA damage foci at 24 h in ^{56}Fe -particle-irradiated human colon epithelial cells compared to the levels after exposure to lower-LET particles such as protons may have implications in cancer risk. Previous studies have shown that HZE particles have a higher relative biological effectiveness (RBE) (21) that is thought to be a result of the densely ionizing effect of HZE particles and the consequent production of clustered DNA damage that is more difficult to repair (22). In the case of colon stem cells, the rarely dividing and quiescent cells thought to remain in specialized niches for most of an individual's lifetime, nonlethal yet difficult-to-repair damage can initiate or promote accrual of genetic and chromosomal aberrations that can later manifest as malignant disease.

Few studies have attempted to determine whether the observation that HZE-particle damage is more difficult to repair translates to tissue structures in which various cell types and cells in different stages of the cell cycle co-exist. Would DNA damage persist even longer in a 3D setting in quiescent and differentiated cells compared to replicating cells exposed to low- or high-LET radiation? Would quiescent cells display a DNA damage response similar to that of replicating cells? What would be the effect on the microenvironment with respect to cancer permissiveness?

The 3D colon epithelial cell culture did not show any differential persisting damage in the circular (crypt-like) structures compared to the other cells in the 3D culture, suggesting that these more differentiated crypt-like cell structures have repair mechanisms similar to those of

the proliferating cells. As in the human colon epithelial cell 2D culture system, HZE-particle-induced damage persists longer in 3D organotypic cultures compared to 2D cultures. This implies that HZE-particle-induced damage also persists longer in tissues and that the complexity of the damage may later contribute to tumorigenesis.

The 3D organotypic cell cultures do not have significant numbers of stromal cells neighboring the epithelial cells, so we also irradiated intact human colon biopsies with low- and high-LET radiation to observe the patterns of DNA damage in different types of cells within a tissue. There was a prominent difference in the DNA damage repair protein response between the epithelial and stromal compartments in the biopsy specimens exposed to low- and high-LET radiation, as seen with both pT2609 and 53BP1 staining 30 min after irradiation. This was unexpected because stromal fibroblast cells in previous studies display prominent foci after irradiation in a 2D culture setting, as seen with γ -H2AX focus formation (23) as well as phosphorylated DNA-PKcs (19) and 53BP1 (24).

It is likely that the stromal cells experience DNA double-strand breaks, but there are several reasons why there may be decreased 53BP1 and pT2609 focus formation in the stromal compartment after irradiation. One possibility is that in a non-proliferative quiescent cell there may be reduced DNA damage repair in transcriptionally inactive genes (25,26). In previous studies, Bielas and Heddle have shown that embryonic mouse cells exposed to DNA-damaging agents had fewer genetic mutations when exposure was done in a quiescent rather than a proliferative state, though when cells were induced to proliferate, mutations and DNA repair were initiated (27,28). Another possibility may be that quiescent cells in tissues may not have the levels of repair proteins present in actively replicating cells, as seen by the diminished DNA-PKcs staining in the control nonirradiated biopsy stromal cells (Fig. 3c). A final and more plausible possibility is that the diminished radiation damage in quiescent stromal cells compared to replicating epithelial cells may be a result of radiation-induced foci constrained by chromatin domains. It has been suggested that radiation-induced foci not only reflect individual damage events but also accumulate at junctions between heterochromatin and euchromatin (S. V. Costes and M. H. Barcellos-Hoff, personal communication). Thus, while double-strand breaks are occurring in stromal cells, they may not display a similar intensity of focus formation as epithelial cells, due to chromatin effects. It is possible that stromal cells are less euchromatic than epithelial cells, allowing access of DNA damage proteins or antibodies to local double-strand breaks or poor access of the antibody to the DNA repair enzymes.

It is intriguing to think that quiescent cells displaying fewer foci after irradiation may signify potentially unrepaired DNA damage. This has implications for cancer progression. An abnormal communication between fibroblasts and epithelial cells might result from stromal cells not repairing their DNA damage. This might lead to alterations in the tissue microenvironment important for maintaining the stem cell niche homeostasis that may contribute to tumorigenesis. More importantly, unrepaired DNA damage in other quiescent cells, such as epithelial stem cells, would promote accrual of key genetic mutations that would drive tumorigenesis. This overall carcinogenic effect may be magnified in HZE-particle-induced DNA damage in that this damage is more difficult to repair in both the 2D and 3D environment.

More tissue studies and progress with the development of 3D colon cultures will allow for further characterization of DNA damage and repair of tissues. Further studies are needed to identify the colon stem cells and to understand their repair response to DNA damage. Knowledge gained from 3D systems and from 2D culture cancer progression studies will assist in making risk assessment calculations as well as in developing countermeasures that would allow the safe, long-term presence of humans in space.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIG. 1.

Panel a: HCEC CT cells in 2D culture irradiated with protons or ^{56}Fe particles at the NSRL and stained for DNA damage response proteins. Panel b: More damage persists at 24 h in the form of co-localized DNA-PKcs pT2609 and γ -H2AX (yellow foci) in iron-particle-irradiated specimens compared to those irradiated with protons (panel a). Standard errors were calculated after averaging the percentage of DNA damage foci remaining per nucleus for each time ($n > 50$ nuclei). Error bars are smaller than the symbols at 24 h.

FIG. 2.

Panel a: DNA damage and repair response in CaCo2 cell organotypic cultures after low- or high-LET irradiation. Epithelial cells in these 3D cultures make circular structures (arrows) resembling the crypt openings observed in human colon tissues. 3D cultures irradiated with (panel b) γ rays and (panel c) ^{56}Fe particles. Panel d: More damage persists in the form of DNA-PKcs pT2609 foci (green) in specimens exposed to high-LET radiation compared to those exposed to low-LET radiation. Standard errors were calculated after averaging the percentage of DNA damage foci remaining per nucleus for each time ($n > 50$ nuclei). Error bars are smaller than the symbols at 24 h for the proton-irradiated specimens.

FIG. 3.

Panel a: DNA damage and repair response in intact normal colon tissues after irradiation with 1 Gy γ rays. Human colon biopsies have more DNA damage foci per nucleus (in the form of phosphorylated DNA PKcs pT2609) in the epithelial cells (arrow) compared to the stromal cells (arrowhead), as seen in this specimen fixed 30 min after irradiation. Panel b: This pattern is also present in specimens fixed 30 min after irradiation and stained with 53BP1. Panel c: Nonirradiated colon biopsies stained with nonphosphorylated DNA-PKcs show predominance of nuclear staining in the crypt epithelial cells (arrow) compared to stromal cells (arrowhead). Green staining outside of the nucleus is a result of autofluorescence of red blood cells and collagen.

FIG. 4.

Panels a and b: DNA damage repair in colon biopsies shows that damage is highest at 30 min and decreases at 2 h, with most of the damage disappearing by 24 h. Note that the pT2609 damage foci in the stromal compartment at 30 min is less than half of that observed in the epithelial cell compartment, with the percentage of damage mostly staying stable up to 24 h (panel c). Colon biopsy fixed at 30 min after irradiation with 1 Gy 1 GeV ^{56}Fe particles and stained with pT2609 or 53BP1 (panel d). Note that the HZE-particle tracks are present at the crypts containing the epithelial cells but are not contiguous with the adjacent stromal cells. Green staining outside of the nucleus is secondary to autofluorescent red blood cell and collagen.