Bugging the intestinal response to radiation

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A n article by Crawford and Gordon (1) in a recent issue of PNAS describes a new relationship between the gut microbial flora and the host that has importance for understanding how radiation damage is processed by the small intestine. Trillions of diverse microbes colonize the intestine that provide essential functions for host survival (2). In addition to digestion, the gut microbiota regulate caloric storage, metabolize xenobiotics, modify the immune system, and regulate postnatal gut development including a network of capillaries in the mesenchyme of the small intestinal villi (3–8). Understanding of the microbiota–host relationship has been increased by studies on germ-free (GF) mice compared with animals that acquire a microbiota from birth (conventionally raised (CONV-R)) or are initially germ-free and then colonized at various stages of postnatal development or during adulthood with microflora from CONV-R donors (designated as CONV-D, or “conventionalized”) (3, 5). Crawford and Gordon apply these models to investigate whether the intestinal flora modifies the tissue response of the small bowel to ionizing radiation (IR). IR has been used in cancer therapy for >100 years. Approximately 50% of all cancer patients receive radiotherapy. However, radiation therapy is limited by effects of IR on normal tissues as well as the resistance of tumors to this treatment. Radiation effects on the small intestine limit potentially curative radiation doses from being delivered in whole-abdomen radiotherapy for ovarian cancer and also limit the target volume and dose that can be delivered for other abdominal malignancies such as pancreatic, stomach, and colon cancer (9).

Gut Microbiota Influence Radiosensitivity

The authors initially studied whether GF mice were more resistant to death from radiation-induced small intestine damage compared with CONV-R controls. Total body radiation was delivered, and bone marrow transplantation was necessary to ensure that the effects of a gastrointestinal death dominated in the experiments. The data convincingly demonstrated that GF animals have improved survival after 16 Gy compared with CONV-R animals at 7 days after irradiation. The authors next colonized the GF mice with cecal microbes from CONV-R donors. Conventionalized mice (CON-D) demonstrated similar survival characteristics to the radiosensitive CONV-R controls after 16 Gy. These findings were supported by histological examination of the small bowel that demonstrated an increase in radiation injury in the CONV-R mice but not the GF mice. The authors infected the GF mice with obligate and facultative anaerobes, which did not modify the radiosensitive phenotype. These data suggested that microbial specificity conferred relative radiosensitivity. Based on results that demonstrated no difference in the mean number of aerobic or anaerobic bacteria in the spleens of CONV-R or CON-D mice at sublethal or lethal doses of radiation, the authors ruled out systemic infection as a possible mechanism to explain the differences in radiation-induced small intestine damage. The authors next studied possible mechanisms by which the small bowels of GF mice are (relatively) radioresistant. After 16 Gy, cells populating the crypts of Lieberkühn, which contain the multipotent progenitor cells of the small bowel, exhibited extensive apoptosis in all experimental groups, whereas the mesenchymal (endothelial) cells of the GF mice demonstrated significantly less apoptosis than the CON-V-D or CONV-R animals. However, when the dose of radiation was increased to 22 Gy, radiation enteritis developed in GF mice in the absence of a significant increase in endothelial apoptosis, suggesting that epithelial cell death dominates GI death at higher radiation doses. The authors used immunohistochemical techniques and transmission electron microscopy that identified the endothelial cells as a subset of the apoptotic villus mesenchymal population that mediates radiation effects. Interestingly, lymphocyte populations in the bowel stroma were also resistant to radiation-induced apoptosis in GF mice. However, mature lymphoid cells are not required for gut radio-protection in GF mice as demonstrated by data that showed that GF rag−/− mice were also protected from 16 Gy. The authors hypothesized that fasting-induced adipose factor (Fiaf) mediated radioprotection of the bowel endothelium in GF mice. Fiaf, produced by the small intestine in mice is a circulating inhibitor of lipoprotein lipase and mediates the microbiota’s ability to promote energy storage extracted from indigestible polysaccharides in adipocytes. Intriguingly, Fiaf is also known as angiopoietin like 4 protein and is reported to support endothelial survival and vascular sprouting through as yet undefined mechanisms (4, 10–12). The investigators demonstrated a lack of radioprotection as measured by intestinal endothelial apoptosis in GF Fiaf−/− mice. Also, up-regulation of Fiaf after radiation in GF mice was suppressed by conventionalization. Therefore, Fiaf mediated radioprotection in GF mice but not CON-D or CONV-R mice in part because the small bowel microbiota suppressed Fiaf in colonized animals. The model is complex because GF Fiaf−/− animals did not demonstrate an increase in lethal radiation enteritis when compared with GF littermates. Therefore, other factors likely interact with Fiaf or perhaps act independently of Fiaf gut radioprotection.

Endothelia Influence Radiation Effects on Tumors and Tissues

The acute effects of radiation on normal tissues and tumors are historically associated with damage to stem cells. Recently, the effects of supporting tissue structures on epithelia in normal tissues and the analogous role of the interaction of the tumor stroma with tumor cells has received much attention in understanding radiation effects. Reports that combined treatment with radiation and specific anti-angiogenic compounds demonstrated superior antitumor effects compared with radiation alone. These data suggested that differential tumor radio sensitization could be achieved by selectively destroying the endothelial component of the tumor stroma (13, 14). Genetic evidence of effects of endothelia on normal tissue responses to IR was demonstrated by the observation...
that asmase/– (acid sphingomyelinase) mice, characterized by radiation-resistant endothelia, are more resistant to gastrointestinal death induced by IR compared with control "wild-type" mice. These results were supported by experiments which demonstrated that bFGF, a specific endothelial radio protector, also increased the survival of "wild-type" mice after total-body irradiation (15). Confirmation of the potential importance of the tumor-associated vasculature was demonstrated by a report from the same group which noted that tumors implanted in radioresistant asmase/– or Bax/– mice (which also have radioresistant endothelia) were more radioresistant than mice with "wild type vasculature" (16). Recent experiments in asmase/– mice suggest a hierarchy for thresholds of normal tissue radiation damage in the small bowel whereby relatively lower doses of radiation damage the small intestine by targeting endothelia, whereas higher doses (<18 Gy) destroyed the intestinal stem cells in an endothelial independent mechanism (17). Crawford and Gordon report that GF animals are preferentially protected at 16 Gy compared with CONV-D or CONV-R mice but not at higher doses of 22 Gy, thereby supporting the hypothesis that normal tissues and perhaps tumors have differentially radiosensitive targets (1, 17). The concept of differentially radiosensitive components of normal tissues or tumors may be important in the development of radioprotectors or radio sensitizers, sensitizing the most "vulnerable" component of the tumor, e.g., the tumor vasculature. The interaction between the various components of normal tissues and tumors that mediate the response to radiation is likely to be complex. This is especially true because tumors secrete factors that mediate endothelial cell radio resistance, and it is likely that endothelial cells secrete factors that mediate the survival of tumor cells after IR (18, 19). A similar reciprocal paracrine relationship is likely to be true in normal tissues as well. The new findings reported by Crawford and Gordon add a new dimension to these complex relationships in that the microbial organisms may suppress (or add) factors that mediate tissue radiosensitivity. The data presented in their article suggest that alteration of the microbia of the gut may be clinically useful to increase the resistance of the small bowel to radiotherapy. Also, it is unknown how or whether the microbia of the oral cavity, pharynx, and large bowel influence normal tissue radiosensitivity of these organs. Some tumors, especially of the head and neck region, may be accompanied by localized infections that could influence tumor radiosensitivity. Experimental studies that alter the microbia the small bowel may provide a new method to identify radioprotective or radiosensitizing compounds.

As in many important studies that introduce new concepts, caution is required in the potential application of the results. For example, radiotherapy is most commonly delivered as multiple relatively small daily doses (1.8–3.0 Gy/day) (20). Doses used in this and most of the related studies are large by comparison. Therefore, applications of concepts that alter the small bowel microbia to increase normal-tissue radio resistance should be tested in experimental models that employ doses used clinically. Interestingly, the use of very large single doses to sterilize tumors is an emerging concept in radiotherapy that is currently undergoing clinical investigation (20). GF Fiaf/– mice did not have an increase in lethal enteritis when compared with GF mice, suggesting that achieving Fiaf-mediated radio protection may be very complex. Nonetheless, the work by Crawford and Gordon opens a new chapter for the study of radiation effects.

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