Chapter XVII: New Radiobiology Methods

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How Do New Advances in Basic Science Methodologies Apply to Radiation Biology?

Current advances in Computational Biology, Computer Science, and Data Management have extended both the robustness and rate of data collection and processing in nearly all aspects of Modern Science. Other chapters in the web-based textbook describe exciting new areas in Radiation Biology and how focused research in these areas would be an advantage to new investigators. Yet other chapters deal with the recent advances in the study of DNA repair, transcriptomics, proteomics, protein networks, and irradiation-induced cellular death pathway. This chapter will address, which new methods and resources should be particularly applicable to radiobiology.

The mission of the Center for Medical Countermeasures Against Radiation Consortium (CMCRC) Program of the National Institutes of Allergy and Infectious Diseases (NIAID) is important to understand, if one seeks to apply new methodologies and scientific tools with a focused effort. New methodologies are needed to safely, rapidly, and accurately determine radiation dose sustained by exposed experimental animals and humans (dosimetric and metabolomics methodologies). New radiation countermeasures are needed, which can be delivered to exposed humans 24 hours or later after irradiation exposure. These agents must be safe, effective, and deliverable to mass casualties of all ages, irrespective of gender, combined injury, and in the setting of polypharmacy.

This chapter will divide the current topic of methodologies into those, which apply to acute radiation effects and late effects.

Approaches to Study Acute Radiation Effects

Since the first demonstration of ionizing irradiation effects, which produce ionization of oxygen and water within fractions of a second; there has been a gap in understanding how rapidly these radiochemical changes translate to detectable biological effects.

A classic study by Bakkenist and Kastan (1) demonstrated the detection of phosphorylation of the ataxia telangiectasia mutant (ATM) protein, within minutes of irradiation exposure to very low doses of less than one cGy, (or essentially, as fast as the assay could be carried out). This publication established that ATM phosphorylation was the first biological event to follow irradiation-induced DNA double strand breaks, and upon which an entire cascade of DNA repair gene products appeared at the break, formed a scaffold for repair enzymes, and established a sequence for subsequent steps in the activation of DNA repair enzymes. Thus, one huge advance in Modern Radiobiology is the speed with, which molecular biologic changes can be detected.

Acute radiation effects depend upon the ionizing irradiation dose sustained. A definite advance in this area has been with techniques available for physical dosimetry. The chapter on Radiation Dosimetry (physics) by Ke Sheng describes the creation of phantoms (biological analogs of an experimental animal – mouse or rat), which can be used to quantitate the conformity of radiation dose delivered by a given beam. Radiation dosimetry techniques are now applicable to Cesium gamma cell irradiators, which emit gamma radiation from a radioisotope. orthovoltage x-ray

machines, modern high dose rate linear accelerators, as well as machine, that produce protons, neutrons, or alpha particles. One example, is the TrueBeam linear accelerator, which has multiple x-ray energies and multiple dose rates. Radiobiology experiments using this machine demonstrated the unexpected differing biological effects of the highest dose rate of 2400 cGy per minute and beam energy of 10 MV inducing mRNA for radiation repair genes (2).

Methodologies for precise measurement of x-ray dose in single cell, or subcellular components are also now available (3). Animal irradiator for focused clinical beams are also available (4) for subtotal body irradiation.

Radiobiology experiments using animals and cell culture systems always include non-irradiated controls; however, fluctuation in the baseline control levels for studies using experimental animals have now been a focus of investigation. There are new efforts to understand different biological effects of a standard does of irradiation detected at different facilities. When the calculation of dose and dose rate have been standardized, there are still reported differences. Prior explanations allowed the assumption that differences related to "uncontrollable" physical structural modifications between facilities, difference in animal diet, difference in the physical health of animal handlers, differences in institutional oversight for opportunistic pathogens, different protocols for use of sentinel animals, quarantine facilities, and making certain of pathogen free environments. New attention has been directed to the animal microbiome and how change relate to an animal response to ionizing irradiation. New high throughput programs are available for measuring bacterial taxa by I6SRNA (5), and how specific strains of the major categories of bacteria in both the intestinal and the pulmonary system differ between groups of experimental rodents. Furthermore, the genetics of the background bacterial strains is now a focus of research, given the availability of high throughput sequencing technology for detection of changes in bacterial DNA.

Acute responses to irradiation (Defined as those measurable during the so-called acute reaction and usually within 1 – 21 days of exposure) now range from precise study of DNA, RNA, protein, and lipid messenger pathways of radiation response to specific cellular changes, tissue changes with respect to interaction of different cell phenotypes, and animal responses to irradiation based not only on the animal genetics, but that of the microbiome.

There has developed a new appreciation of potential role of all pharmaceutical agents in the irradiation response. With the respect to humans, the role of polypharmacy in outcomes analysis (What drugs or agents is an individual consuming at the time of irradiation exposure). With respect to experimental animals, drug administration is critical to document Are animals receiving prophylactic antibiotics or antifungal agents, and are animals experiencing combined injury in the form of physical injury (male mice may display skin wounds from fighting) to psychological stress (from cage-confinement, absence of appropriate bedding, absent exercise wheel, and lack of definition of other measures by which to standardize animal housing conditions). Most institutional animal care committee programs (IACUC) recognize the importance of these concerns for animal health. However, the radiobiology of combined injury now extends to these areas including psychological stress, and degree of or susceptibility to combined injury.

The Inflammasome, and Sterile Inflammation

Biomarkers for measurement of inflammation are now available in a robust series of assays for the biomarkers of inflammation. Does radiation induces sterile inflammation in locally irradiated tissues and after total body irradiation? The response to cellular damage from ionizing irradiation overlaps with respect to pathways with that induced by chemical toxin, hyperthermia, ultraviolet irradiation, and physical trauma including concussion and bone fracture. In the management of acute radiation injury, administration of corticosteroids or non-steroidal anti-inflammatory drugs can interrupt these pathways, but there is now appreciation for the effects of these medications on the radiation response. There has been a revolution in understanding interactions between inflammatory cell subsets. Subdivisions of T-lymphocytes (Originally described as those originating from the thymus) and B-lymphocytes (Originally associated with the Bursa of Fibritious, and formally associated with those lymphocytes that produce immunoglobulins.) now expanded to multiple subcategories of each: With T-cells that are so-called helper or suppressor T-lymphocytes and multiple categories of B-lymphocytes including those with short and long-term memory for responses to antigens by specific antibody production. The role of these cellular subsets in the inflammasome is well established. New areas of investigation in cellular immunology include: study of dendritic cells or so-called "antigen presenting cells" of both lymphocyte and marrow myeloid cell origin. Most interesting, has been the expanded understanding of the role of neutrophils in the immune response. Polymorphonuclear leukocytes (neutrophils) were originally described as those responding to bacteria) in a setting of sterile inflammation, and the ionizing irradiation response. Neutrophils rapidly traffic to locations in which there is expression of chemokines by irradiated cells. These molecular moieties call neutrophils to the site of damage. Neutrophil tracking is increased by Interleukin 8 (IL-8) leukotriene B4 and Hepoxillin-A3 among others. The arrival of neutrophils in tissue results in their interaction with endothelial cells, and other cells in the microenvironment tissues that display upregulated adhesion molecules. Activation of neutrophils results in not only degranulation with release of cytotoxic enzymes such as myeloid peroxidase, but also signaling to multiple other cell phenotypes in the region or attracted through the circulation.

There is recent appreciation for the role of neutrophils crossing the blood/brain barrier into the irradiated brain and spinal cord, where there interaction with neurons and astrocytes leads to secondary effects on differentiation to microglial cells (6). Microglial cells of bone marrow origin can both increase inflammation within the central nervous system (M1 microglial cells) or suppress inflammation by releasing neurotrophic, beneficial cytokines (M2 microglial cells). These changes can occur in the absence of a detectable infection at the site.

In the acute radiation response, sterile inflammation activates multiple intracellular and extracellular pathways including those for mitophagy, autophagy, apoptosis, necroptosis (7-8), ferroptosis, parthanatosis, and once bacterial or viral opportunistic infections ensue, pyroptosis (9). In the study of sterile inflammation, which is a major component of the acute radiation response, investigators should familiarize themselves with these cellular signaling pathways, but also with the overlapping cellular death pathways (9).

New Techniques by Which to Study the Late Radiation Response

Research with the late radiation effects require understanding of the concept of time. Cells in culture, organ explant systems studied in culture (including organoids), as well as, animal tissues, organs, and total body responses in vivo change over time after irradiation (10). There is a new appreciation for the role of a particular radiation source, beam quality, dose rate, and total or subtotal body irradiation exposure in understanding late effects. In vitro systems for studying irradiation late effects relate to cell culture experiments. Radiation induction of mutations can be studied using bacteria, yeast, and small organism systems including C. elegans, Drosophila, and amphibian oocytes. Cell culture experiments using mammalian cells have also been adapted to study of late effects. Recent experiments demonstrate that explant of bone marrow to long-term bone marrow cultures, one year after irradiation exposure, can provide data on the late effect of irradiation function of both hematopoietic stem cells and cells of the hematopoietic microenvironment (stromal cells). Explant studies of animals irradiated one year previously allow quantitation of the effect of radiation mitigator drugs given sporadically or continuously to animals during the development of a late effect such as pulmonary fibrosis (11)..

Radiation biologists and radiation oncologists have reported recent data on the effects of volume of tissue treated, dose rate, and radiation source on the time course of appearance of irradiation late effects. Radiation fibrosis, nerve damage, vascular changes, and neurocognitive changes at the time of onset, are all directly related to radiation dose, as well as, dose rate. The availability of proton irradiation facilities has facilitated studies of radiation late effects in patients, who had proton compared to x-ray/photon irradiation. There is emerging information that the severity of late effects may be decreased by acute exposure to protons compared to photons.

Continuous low dose rate irradiation represents a separate category of induction of late effects. The National Aeronautics and Space Administration (NASA) has a great interest in continuous low dose rate irradiation, because of the expected exposure of astronauts to continuous sporadic high LET irradiation from galactic cosmic irradiation (12, 16-17). High LET particles (carbon, silicon, iron) from a galactic cosmic irradiation event (Supernovae) travel through the spacecraft (for example, on the manned MARS mission, which is estimated to be 1 ½ - 2 year duration) the astronauts will be exposed to neutrons from interaction of high LET particles with metallic and other components of the spacecraft. Continuous low dose rate neutron irradiation is a subject of intense investigation by NASA scientists. The relative biological effect (RBE) of continuous low dose rate photon compared to neutron compared to high LET particle irradiation (alpha particle-Helium nuclei and GCI high LET radiation, including iron, silicone, and carbon nuclei) is still a controversial subject. Duplication of the sporadic intermittent, but continuous irradiation by these particles is very different to simulate under laboratory conditions. The availability of neutron irradiation devices (Colorado State University, West Chester, New York – Columbia University), and LET ion irradiators (Brookhaven National Laboratories) provides facilities for such radiobiology experiments. Investigators wishing to enter this field of research must take into account the concept of low dose rate irradiation with respect to late irradiation effects. Continuous low dose rate irradiation, 600 times background levels on Earth, have been studied and reveal no detectable increase in homologous recombination using a novel FYDR mouse strain (13).

Late radiation effects also can be studied from the standpoint of physiology, pathophysiology, and metabolism. New assays are available for many aspects of metabolomics, measuring products of metabolism and changes following ionizing irradiation (14). Measurement of metabolites in saliva, urine, feces, and serum/plasma has led to new observations on the continuing expression of metabolic changes months to years after irradiation.

Conclusions

Radiation Biology presents a discipline, which includes multiple areas of basic and clinical science. Investigators entering the field of Radiation Biology must have an understanding not only of classic radiobiological methods, but also new technologies that are available for study in multiple associated disciplines. There has been a renewed appreciation for the importance of collaboration between multiple disciplines to facilitate appropriate use of modern techniques for the study of acute and late radiation effects (15).

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