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Chapter X: Late Radiation Effects

Section A: Organ Failure, CNS, Cardiac, Aging – Senescence, Cancer and Leukemia

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Understanding the mechanism of the late effects of ionizing irradiation has become one of the most important topics in biology. Research in this area encompasses topics ranging from approaches to treating radiation terrorism or radiation accidents to the effects of continuous low dose or sporadic galactic cosmic irradiation, as related to space travel (1). A common theme in all these research areas regarding radiation late effects is the lack of understanding of the mechanism. This chapter will review five topics related to late radiation effects and describe currently available research systems for both in vitro and in vivo animal model systems.

Organ Failure

The most dramatic sample of late ionizing irradiation effects is observed in the lung. Chest x-ray examination of lung cancer patients treated many years or decades previously during follow-up visits demonstrates radiation fibrosis, some confined to the irradiation field portals, but in others showing fibrosis throughout the entire ipsilateral (same side) lung, and even the contralateral (opposite side) lung (2). Pioneering work by Philip Rubin and colleagues (3) first described the role of inflammatory cytokines and in particular, circulating levels of TGF-β, as related to the induction of late pulmonary fibrosis (4). Despite research with many animal models and clinical studies, there continues to be an incomplete understanding of the mechanism by which is when late effect begins. One theory has been the concept of a slowly proliferating and late dividing cell population in the lung, which can take six months to two years to begin dividing (in the case of humans) or 100 days (in the case of C57BL/6 mice) (5).

The role of genetic factors in late radiation pulmonary fibrosis has been clearly delineated by studies with inbred mouse strains. Work by Travis (5), Franko (6), and others first demonstrated a genetic component to irradiation fibrosis. C57BL/6 mice develop fibrosis predictably at 100 days after 19 Gy thoracic irradiation, while other mouse strains including: C3H/HeJ do not (7). Genetics studies have failed to localize one or more genes responsible for the onset of fibrosis.

Another theory has been the slow, but continuous accumulation of destructive proteins in non-dividing cells within the lung endothelial cells (8). Work by Hauer-Jensen, et al. (8) first demonstrated the importance of endothelial cells in induction of radiation fibrosis. The acute irradiation event in all tissues (addressed in other chapters of this book) shows the importance of irradiation-induced DNA replication promotors for specific stress gene transcription responses, and upregulation of cytokine genes (9). The same promoters involved in radiation acute injury (SP1, AP-1, NFkb, and NRF2) are secondarily activated after the latent period, and this secondary activation induces late effects (9). The severity of late pulmonary fibrosis can be increased by co-morbid disease, in patients the conditions of emphysema, asthma, or continuous cigarette smoking (2). Animal models reveal that combined injury including: heat, traumatic wound injury, and other insults can independently elevate levels of inflammatory cytokines (9). The role of circulating monocytes and circulating progenitors of fibroblasts in the induction of the late effects has also been clearly shown (10). However, the mechanism for recruiting such cells from the bone marrow into the lung is not known.

Central Nervous System (CNS) Late Effects

A major late effect of the whole brain irradiation is cognitive decline. Pediatric Leukemia patients, who routinely received whole brain radiation, as part of prophylaxis against Leukemia cells hiding in "sanctuary sites" within the brain vasculature and blood/brain barrier, where chemotherapy drugs could not reach and destroy such cells effectively (11). Studies in the 1970s demonstrated a clear decrease in cognitive ability of children receiving total brain irradiation (TBI), and then followed for decades (12). Adult patients treated to the brain for prophylaxis of Small Cell Lung Cancer, which frequently may reappear as brain metastasis, also demonstrated cognitive decline (12).

One approach toward management of whole brain irradiation, which seeks to minimize late cognitive decline, has been the attempt to shield the hippocampus region from total brain irradiation (13). Some reports suggest that this sparing can preserve cognitive function, while not significantly increasing the chance of brain metastasis in those areas.

While the mechanism of irradiation late effects in the brain is not known. TBI (Total Body Irradiation) irradiated rodent models demonstrate cognitive decline (12). Sophisticated behavioral studies are now developed to measure cognitive decline. These are most prominently applied in the NASA research models (Readers should investigate Chapter VII on Space Irradiation.).

With respect to mouse models of cognitive behavior, there are excellent measurement techniques. The Morris Water Maze (14) is a test in which mice are challenged to swim in a water bath, where a plexiglass platform is present immediately below the water level, but not visible to the swimming animal. Mice learn where the platform is and swim toward it. Studies are done daily for usually 5 days, and those animals learn where that platform is. Animals, who have received total body irradiation or total brain irradiation, demonstrate prolongation of the period for learning and often cannot totally identify or remember the spot in the water maze, where the platform can be acquired (14).

Other assays for cognitive decline, as a late irradiation effect on the CNS include: Novel Object Recognition, and Fear Conditioning have been utilized for cognitive behavioral studies (14-15). The scientific field of neurocognitive analysis can now be applied to studies of late effects of brain irradiation. Specific neural circuitry, which is involved in memory of visually acquired information, compared to auditory (sound) acquired information is now available. Sophisticated optokinetic mouse models also facilitate the induction of nerve signaling in specific areas of the brain, which can be monitored from outside the animal using sensitive detectors of light, which is emitted from those neurons, which are firing.

The mechanism of late CNS radiation damage is not known, but research is also focused on endothelial cells (16). Neuroscience, as a discipline, has been significant advanced with the discovery of the neurolymph or endolymph pathway (17). While the blood/brain barrier has been well understood for decades, the concept of a lymphatic drainage system in the brain has only recently been described (17). During sleep, the drop in blood pressure facilitates the removal of aggregated proteins and other waste products from neurons in the brain through passive movement through the neurons and other supportive cells directly into a lymphatic system, which surrounds vessels in the brain (17). Effective radiation on endolymph studies is a

perfect opportunity for further research in this area. Radiation is known to limit circulation, slow the formation of new vasculature, and has been reported in many model systems to obstruct lymphatic flow, particularly in areas of prior trauma including surgery. Readers should explore the chapter on combined injury (brain concussion plus irradiation), which has additional information on model systems with which to study changes in blood/brain barrier and endolymph function.

How irradiation induces late changes in the blood/brain barrier is not known. One animal model system uses administration of two micron diameter fluorescent-labeled microspheres, which are injected intravenously followed by imaging of the brain. These studies demonstrate permeability of the blood/brain barrier such that these microspheres can be detected in the brain and in the spinal cord (18). Under conditions of interruption to the blood/brain barrier, the microspheres are seen throughout neural tissue. Pharmacologic manipulation (opening or closing) of blood/brain barriers has been a central research focus in understanding the mechanism of expansion of bleeding during stroke, as well as in understanding neurodegenerative diseases. In both conditions, administration of drugs that cross the blood/brain barrier is a major goal of the pharmaceutical industry. Getting therapeutic drugs, which are administered in the blood, directly into the brain and spinal cord is a challenge for many disciplines in medicine. Concentrations of chemotherapy drugs in the cerebrospinal fluid, which are required to reach adequate levels for effective chemotherapy of brain tumors or metastatic cancer, often require intrathecal (direct injection into the cerebrospinal fluid through lumbar puncture injection).

A major research area, which is recommended for scientists entering the field of radiation biology, is an understanding of radiation effects on the blood/brain barrier (BBB). How changes in the BBB relate to the major late effects of cognitive functional decline after irradiation is also a prime area for future research.

Neural stem cells have been recently discovered and their discovery has changed the entire approach for study of radiation late effects on the Central Nervous System (19). Cells lining these cerebral aquaduct system have been shown to proliferate and repopulate areas in the brain, thus, serving as brain stem cells (19). How total body or total brain irradiation effects the number of repopulating stem cells is not known. The role of bone marrow origin of progenitors of neural stem cells is also an area of great potential for research. Work by Charles Limoli, et al. (20) first demonstrated the importance of oxidative stress in the rodent model brain for inhibition of radiation repair. Application of these techniques described in recent publications, to the study of radiation late effects, appears to be a great opportunity for new research.

Cardiac Irradiation Late Effects

Radiation late effects on the heart have become a focal point for research in both clinical and animal model systems. In the 1960s and 1970s, routine use of mantle irradiation for the treatment of thoracic Hodgkin's Disease has led to the routine delivery of 35 – 40 Gy to large volumes of the heart. Important publications (21) demonstrated radiation late effects on the heart. Patients evaluated decades after radiotherapy of Hodgkin's Disease were shown to have a higher incidence of myocardial infarction, cardiac arrhythmias, and heart failure. The clinical correlation of ionizing irradiation dose to the heart, with late effects is now firmly established

(21). Radiotherapy residency programs now routinely instruct their physicians to avoid cardiac doses above 20 Gy and reduce the amount of heart in the radiation treatment field.

The mechanism of late effects on the heart is complicated. Like the lung, the heart is a high energy consuming continuously functioning organ; however, unique to the heart is the high concentration of mitochondria in cardiomyocytes (22). Myocardial function for appropriate oxidative metabolism is critical in an organ that requires considerable energy expenditure during routine function. How irradiation effects on the mitochondria are expressed years after irradiation exposure is unknown. Prevailing hypotheses for the mechanism of irradiation damage to the heart include: late radiation myocardial fibrosis, late radiation vascular changes, and damage to the electromechanical conduction system in the heart. Recent studies using continuous cardiac monitoring have demonstrated rapid induction of cardiac arrhythmias in thoracic irradiated mice. However, no such clinical correlations to thoracic irradiated patients have been shown.

Acute radiation effects on the cardiac conductive system provide a window on understanding the induction of damage, and how recovery from acute effects can lead to a latent period in the heart. As with the late effects of irradiation on other organs, the mechanism of initiation of the late cardiac effects is not known. Pharmacologic interventions to reduce cardiac late effects has been a subject of intense study, and include continuous administration of antioxidants, anti-cytokines, and reduction in blood pressure.

Several new approaches for studying cardiac late effects are now available. For clinical studies of total body irradiated or thoracic irradiated patients, and also in elegant animal models, including studies by Hauer-Jensen in irradiated rat heart (23). Several publications describe the methods used for these studies, and are encouraged.

Aging/Senescence

Ionizing irradiation accelerates aging. These studies have been well demonstrated in total body irradiated rodents, and are known for total body irradiated patients, principally, those who receive TBI for management of non-malignant diseases including Sickle Cell Anemia, auto-immune disease, and genetic replacement therapies using bone marrow as a source of unirradiated cells (24). Aging studies in total body irradiated rodents provide the background for describing the role of irradiation in accelerating aging. Hair graying, joint stiffness, bone marrow fibrosis (replacement of hematopoietic islands with fibroblast cells), neurocognitive decline, and increased incidence of age-related cancers have all been described in animal models (25).

The mechanism of acceleration of aging by irradiation is not known; however, there is a new focus for research in this, namely the role of senescence (26). Irradiated cells have multiple options for response to irradiation including cell death, return to quiescence (non-dividing state), and essentially a resting stage, carcinogenesis through accumulation of mutations, release from controls of proliferation, and senescence. The phenomenon of senescence is one of the most interesting current topics in basic biology. Senescent cells are defined as those that can no

longer divide, but do not die. These cells accumulate in aging tissues, do not move, but stay in tissues releasing a series of cytokines, some of which are thought to be deleterious to preservation of normal organ function.

The Senescence Associated Secretory Phenotype (SASP) is a subject of intense investigation. Senescent cells are quantitated by staining tissue in situ for senescence associated Beta-Galactosidase (27). Accumulation of p16 and p21 (promoters of DNA replication) (28) have also been shown in senescent cells, although the correlation of these biomarkers with each other is not clear and is different for each tissue. Rapidly proliferating tissues such as bone marrow and spleen may accumulate senescent cells more rapidly after irradiation, while slowly proliferating tissues such as the brain do not.

A critical role of accumulated senescent cells in irradiated organs leading to the acceleration of aging has been hypothesized, but not proven. The design of drugs, which remove senescent cells (senolytics) has described molecules that target p16 or p21 positive cells and remove these from tissues and organs (29). A recent topic of research is the question of whether senescent cells are good or bad for tissues. The argument that accumulation of senescent cells is beneficial follows the logic that senescent cells do not become cancer, and thus, the more senescent cells, which accumulate, the less likely are cells in that same irradiated tissue to move on for development of cancer Leukemia. In contrast, the argument that senescent cells are bad relates to the secretory phenotype, and the expression of TGF-β, IL-1, TNF-α, IL-16, and release of these and other cytokines into tissues. Senescent cells have been hypothesized to increase the likelihood of organ failure (due to cell death induced by these cytokines), and also the induction of fibrosis. Assay systems for measuring senescent cells would be greatly enhanced by having a method by which to extract senescent cells from tissues and study them separately. Unfortunately, senescent cell surface markers, which would allow sorting techniques to remove such cells from living tissues are limited, and new useful sorting techniques have not been developed. This goal is a primary area for new research (30).

Methods of cell sorting described in the chapters on bone marrow transplantation (see Chapter XXIX on this subject) should be consulted for this methodology.

Cancer and Leukemia

The most dramatic late effect of ionizing irradiation is that of carcinogenesis/leukemogenesis – the induction of cancer or Leukemia. There is a clear background genetic component in the radiation induction of cancer (31). Mice genetically engineered to express radiation response gene p53 have been shown to have an increased incidence of cancer. A priming radiation dose (low dose) followed by a higher dose has also been shown to reduce the incidence of Leukemia in animal models, suggesting that the response to irradiation can be a therapeutic prevention of the cancer induction events (32).

The first radiobiology experiments searching for increased induction of cancer first carried out in animal models first suggested that irradiation induced Leukemia and solid tumors. However, these studies were carried out in animal model systems, many of which had endogenous leukemogenic retroviruses that could be induced by irradiation (33). Mouse strains with a high

irradiation induced level of endogenous retrovirus were shown to have a higher incidence to Leukemia (AKR, C58) (34). The role of retroviruses was considered, in irradiation-induced Leukemia in animal models, but was shown to be much lower than that predicted (36-37).

The decision of a cell within an irradiated tissue to go down the path toward carcinogenesis/leukemogenesis is one of the major areas of research in Radiobiology. Do carcinogenic events only occur within true stem cells or in other cell populations within that tissue?

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