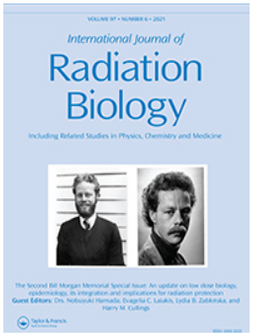

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REVIEW

Effects of low dose and low dose rate low linear energy transfer radiation on animals – review of recent studies relevant for carcinogenesis

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ABSTRACT

Purpose: Carcinogenic effects of radiation are often assumed to be universally understood, more often than, for example, carcinogenic effects of many different chemicals. This in turn leads to an assumption that any dose of radiation, delivered at any dose rate, poses a serious health challenge. This remains an issue of dispute and low dose radiation research is focused on understanding whether these exposures contribute to cancer incidence. This review is focused on the low linear energy transfer (low LET) radiation exposures for which the data is the most abundant in recent years.

Materials and methods: Review of the literature between 2008 and today, highlighting some of the most diverse studies in low dose research.

Results: Low dose and low dose rate, low LET ionizing radiation animal studies suggest that the effects of exposure very much depend on animal genotype and health status.

Conclusions: Only the integration of all of the data from different models and studies will lead to a fuller understanding of low dose radiation effects. Therefore, we hope to see an increase in international archival efforts and exchange of raw data information opening the possibilities for new types of meta analyses.

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Introduction

Current knowledge about biological effects of controlled patterns of radiation exposures is significantly dependent on information collected from animal model systems. While occupational human exposures present a good source of data, interpersonal heterogeneity in human population is substantial (e.g. Hazelton et al. 2006) and the conclusions from such studies require support from animal research work, especially when 'low dose' radiation research is done. Total doses lower or equal to 0.1 Gy are classified as low doses of radiation, while dose rates below 0.1 mGy/min are defined as low dose rates of radiation (UNSCEAR 2012), and examples of carcinogenicity of such exposures are rare. The papers included in this review fulfill at least one of these criteria, for at least one of the animal test groups; additionally a few studies including partial body radiation with moderate doses of radiation were also included. This review of the literature using animal models to study the effects of low dose ionizing radiation is focused on the period of time after the conclusion of the United States Department of Energy (DOE) Low Dose Radiation Research Program in 2008 (Brooks 2015), although several papers published earlier will also be described because their insights provide solid basis for studies of later dates described here (e.g. Ina et al. 2005; Takabatake et al. 2006; Bladen et al. 2007; Tanaka et al. 2007; Tsuruga et al. 2007). Considering the world-wide interest in environmental and biomedical low dose radiation exposures and the breath of research in this area, this review is far from comprehensive. Instead, this work attempts to display the multitude of directions that the low dose radiation research with animal models has taken during this period of time. It should also be noted that the primary thrust of this review is on radiation studies that collected animal samples and data. While many such studies concluded with publications that are used for this review, many researchers were fortunate enough to be able to continue to store the biological samples and data that were generated and preserve them for some future information and materials exchange. In other words, while several extensive databases of irradiated animal data exist for historic radiation exposures (Birschwilks et al. 2011; Haley et al. 2011; Abbott 2012; Zander et al. 2019), relatively few such concerted efforts collect the data from the animals exposed to radiation in the last decade. While such efforts would be difficult, considering the mostly de-centralized radiation biology research of the twenty-first century, potential benefits of meta analyses of these data could even outweigh the advances generated by the original research studies.

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This article will also attempt to showcase radiation biology animal research in several countries in hope that such an overview may spur on future scientific collaborations, since very few of the recently concluded studies managed to fully exploit the samples and data they generated. An encouraging example of the promise of such collaborative work is a recent study by Doi and others who have used a large volume of data generated by a different group of investigators in order to calculate the Dose-Rate Effectiveness Factor for chronic irradiation (Doi et al. 2020); see the next section.

Extended studies of low linear energy transfer (LET) irradiated wild type animals

From the earliest days of radiation biology, studies that focused on comparisons between acute external beam radiation versus different types of radiation protraction produced the most abundant data. In fact, it is primarily this type of work that has led to growth of animal radiation biology archives in the US and other countries (Birschwilks et al. 2011; Haley et al. 2011; Abbott 2012; Zander et al. 2019). In the period after 2000s on the other hand, in an effort to evaluate low dose rate radiation effects, many animal studies were focused on chronic external beam radiation. A large series of mouse studies was done in Japan at the Institute of Environmental Sciences (IES) where thousands of animals were given daily low dose rate exposures of low LET radiation. One of the recent reviews of this work (Braga-Tanaka et al. 2018) makes it clear that the differences between effects of different daily doses of radiation are very pronounced. Over the years, chronic exposures at the IES consisted of 22 h a day exposures to a ^{137}Cs source gamma irradiator at dose rates between 0.04 and 0.06 mGy/d, 0.8 and 1.1 mGy/d, and 16 and 21 mGy/d. Therefore, for total exposure periods of 400 to 485 days, total radiation doses accumulated to about 0.02 Gy, 0.4 Gy, or 8 Gy. At the conclusion of the irradiation period, most of these mice were allowed to live out their lifespans (See Table 1). Mice exposed to 0.05 mGy each day for a total dose of 0.02 Gy, show only an increase in the incidence of non-fatal liver neoplasia. No increase in any specific cause of death was noted, nor life shortening in this group of mice. Animals exposed to 1 mGy per day for a total dose of 0.4 Gy, on the other hand, had shortened, statistically significant in females, lifespans with increased incidence of many different types of neoplasia, although combined frequency of all neoplasias was not increased. In mice exposed daily to 20 mGy for a total dose of 8 Gy, increase of the combined frequency of all neoplasias was statistically significant. Life shortening was statistically significant for both genders in this group of mice (Tanaka et al. 2007). It should be noted that the total body exposure to a total dose of 8 Gy delivered acutely would be lethal for most mouse strains as well as for humans. This aspect of the IES' studies is particularly evident from a recent re-analysis of these data (Doi et al. 2020). In this study, the authors use all available data on chronically irradiated B6C3F1 mice to evaluate reduction in

Table 1. Experimental details of extended studies of low LET irradiated wild type animals.

Mouse strain	Age at first treatment	Group size	Radiation quality and delivery	Dose rate	Total dose	Period post exposure	Change in cancer incidence	Publication
B6C3F1	8 weeks	400–500	^{137}Cs gamma- ray, chronic, 22 h/day	0.05 mGy/d	20 mGy	Lifetime	Only significant increase for liver neoplasia in males	(Tanaka et al. 2007; Braga-Tanaka et al. 2018)
				1.1 mGy/d	0.4 Gy	Lifetime	Statistically significant increase of several cancers	
				21 mGy/d	8 Gy	Lifetime	Statistically significant increase of many different cancers and overall cancer incidence increase	
B6C3F1/Jcl	8 weeks	60–90	^{137}Cs gamma- ray, chronic, 22 h/day	21 mGy/d	2, 4, 6 or 8 Gy	100, 200, 300, and 400 days	Evaluation of pre-symptomatic neoplasia	(Tanaka et al. 2017)
B6C3F1/Jcl	6 weeks	60	^{137}Cs gamma- ray, chronic, 22 h/day	21 mGy/d	8Gy	Lifetime	Caloric restriction extends life; makes relative radiation detriment greater	(Yamauchi et al. 2019)
Wistar rats, females only	1 week old 5 or 15 weeks old	70	X-ray, acute, partial body (chest)	0.54 Gy/min	1, 3, or 5 Gy 1, 3, or 5 Gy	Lifetime Lifetime	Increased lung cancer with no life-shortening Increased lung and mammary cancer	(Yamada et al. 2017)
Sprague-Dawley rats	3, 7, or 13 weeks	24	^{137}Cs gamma ray, chronic	6 mGy/h 1 Gy per week (and 3–60 mGy/h)	12,468 Gy (and 4 Gy)	Lifetime	Mammary cancer frequency increases, latency decreases at 4 Gy or more	(Imaoka et al. 2019)
B6CF1	13 to 25 weeks old	200	^{60}Co gamma-ray, acute or fractionated	10 to 378 mGy/min	0.9 to 51 Gy	Lifetime	Dose fractionation decreases cancer incidence especially for total doses above 1 Gy	(Zander et al. 2020)

cancer risk due to the mode of radiation delivery and deliver the very first estimate of the Dose-Rate Effectiveness Factor based on IES data. While the Braga-Tanaka review gives an overview of the IES research in recent decades, specific research papers coming from this work warrant a closer look and will be mentioned throughout this review. The majority of the animals irradiated at IES were wild type mice, strains C3H/HeN, B6C3F1, and C57BL/6J or transgenic reporter animals. Importantly, strain to strain differences with regard to animal resilience to radiation were evident from these studies. For example, for the C57BL/6J strain of mice only males exposed to 20 mGy per day (for a total dose of 8 Gy over 400 days) demonstrated life shortening. Males exposed to the total accumulated dose of 0.02 or 0.4 Gy did not show life shortening. In comparison (Tanaka et al. 2007), in B6C3F1 strain, both sexes had shorter lifespans for the same total dose and dose rate, and females also showed life shortening with the dose rate of 1 mGy per day for a total dose of 0.4 Gy. In another study, female mice of strain B6C3F1/Jcl daily exposed to 21 mGy of ^{137}Cs gamma rays beginning with 8 weeks of age (Tanaka et al. 2017) were sacrificed at different timepoints throughout irradiation period – at 100, 200, 300, or 400 days, receiving total doses of 2, 4, 6, or 8 Gy. After conclusion of the radiation period at day 400, additional animals were also sacrificed 100, 200, 300, or 400 days later or allowed to live out their entire lifespans. Each one of these groups had 60 to 90 mice and they were matched with equal numbers of controls sacrificed at the same timepoints. In this, as well as most other IES mouse studies, malignant lymphomas were the most prevalent cause of life shortening in irradiated animals. Other neoplasias with increased frequency included hepatocellular adenomas and carcinomas, bronchioloalveolar adenomas and Harderian gland adenomas and adenocarcinomas; adrenal subcapsular cell adenomas and ovarian tubulostromal adenoma and granulosa cell tumors. In animals sacrificed immediately after the conclusion of radiation or at any time after the cessation of radiation after 4 Gy delivery, multiple coincidental neoplasias were found with increased frequency. In one of the later studies (Yamauchi et al. 2019), 6 weeks old male B6C3F1/Jcl mice were separated into two cohorts: one was put on a calorie-restricted diet and the other on a regular diet. These mice were further separated into groups of 60 and the mice on each type of diet had a non-irradiated and irradiated group. Exposure to 8 Gy was done as chronic irradiation over a period of 400 days. In all group to group comparisons, animals on a calorie restricted diet lived longer than those on a regular diet. When comparisons were done between irradiated and control mice in the same diet cohort, relative life shortening due to radiation was more pronounced in animals from the calorie restriction cohort. Animals fed a regular diet did not show statistically significant life shortening due to radiation in this study.

Partial body irradiation to high doses of x-rays was also investigated recently in 1-, 5-, or 15-week old wild type female Wistar rats. Chest exposure to 1 Gy did not cause life shortening in any of the animals; in addition, in 1-week old rats' exposures to 3 or 5 Gy also did not reduce survival. However, lung cancer incidence was increased in all groups of animals and the spectrum of lung cancers that developed in irradiated animals was different than that in controls (Yamada et al. 2017). In addition, in 5- and 15-week old rats exposed to 3 and 5 Gy mammary cancers showed increased frequency. Conversely, the frequency of pituitary tumor which was the most frequent cancer type in non-exposed rats, decreased in irradiated rats. A more recent study on breast cancer development in whole body irradiated groups of 24 female Sprague-Dawley rats exposed to ^{137}Cs irradiator gamma rays at a dose rate of 6 mGy/h, delivered for 10,000 min each week for a weekly dose of 1 Gy (Imaoka et al. 2019). Irradiations begun when animals were 3, 7, or 13 weeks of age and the total doses delivered included 1, 2, 4, 6, or 8 Gy. Seven-week old rats also received radiation with variable dose rates of a dose rate of 3, 12, 24, or 60 mGy/h until a total dose of 4 Gy accumulated. Finally, acute exposures were done as well at dose rate of 30 Gy/h. Only animals exposed to 3 or 4 Gy at high dose rate developed greater numbers of breast cancers as established at autopsy. However, age at which animals developed breast cancers was very much dependent on total dose and dose rate. In all experimental conditions except one, higher total dose or dose rate corresponded with earlier cancer onset although not always statistically significant. A single exception represented rats whose irradiation begun at 7 weeks age to dose date of 6 mGy/h: in this experimental subset, animals that received radiation for 6 weeks (for a total dose of 6 Gy) developed breast cancer earlier than animals that were exposed for 8 weeks for a total dose of 8 Gy. Causes of this 'reversal' may be many, including development of other cancers and/or other health complications that outcompeted breast cancer development.

In recent years, fractionated radiation delivery is most often studied over short periods of time and the animals are sacrificed soon after exposure. For example, apoptosis and DNA damage were studied in spleen cells of mice exposed to 0.1 to 0.5 Gy of x-rays over one to 5 days, sacrificed 6 or 24 h after final exposure (Koturbash et al. 2017). However, in the period before year 2000 numerous fractionated radiation studies were done and archival data from this work are still often used for cancer incidence analyses. Recent work by Zander et al. (2020) is one such example. This study will be mentioned here as an example of studies of archival data despite the fact that the lowest dose and dose rate used in this study do not strictly fall under the definition of low dose and low dose rate as used in this article. In this work, based on the data on gamma irradiated animals from the Northwestern University Radiation Archive for animals (NURA), formerly the Janus archive, or Argonne National Laboratory animal archive (Birschwilks et al. 2011; Haley et al. 2011; Abbott 2012; Zander et al. 2019), cancer incidence was evaluated in several pooled experiments with many hundreds of animals. Only B6CF1 mice, F1 progeny of C57BL/6J females, and BALB/cJ males from NURA were used for this work; cancer incidence was evaluated using competing risk models and cumulative

incidence function for different total doses of radiation presented. In all cases, fractionated radiation delivery decreased cancer frequency and increased life expectancy of animals that developed cancer. As expected, this effect of fractionation was more pronounced as the total dose increased.

Moderate (0.1 to 1 Gy) radiation doses delivered at low dose rate or as fractionated exposures had limited carcinogenic effect on animals with wild type genotype. Increased incidence of specific cancers in animals exposed to low doses of radiation was noted only sporadically. Total increase in number of cancers was not noted for doses less than 1 Gy, because increased incidence of few cancer types is offset by the decreased incidence of other types of cancer (Tanaka et al. 2007).

Low dose radiation of genetically modified animals with increased frequency of spontaneously developing cancers

Among the most important mouse models for study of carcinogenesis are transgenic animals with different types of mutations of tumor suppressor gene p53, protein inactivated in most cancer types (Wasylishen and Lozano 2016). Mice without p53 develop lymphoma early in life (Mao et al. 2005) and p53 heterozygotes were often used to evaluate carcinogenic potential of different DNA damaging agents including radiation. Interestingly, in low dose radiation studies a reverse effect was noted and these exposures led to increased life expectancy (Table 2). This phenomenon was entitled adaptive response and one of the earliest extensive studies focusing on it used p53 wild type and heterozygous animals on C57BL/6 strain background exposed to chronic low dose radiation (Mitchel et al. 2008). In this study, 6-week old animals were given five fractions of 0.33 mGy 5-days a week with ^{60}Co gamma rays at dose rate 0.7 mGy/h. Irradiation was done for 30, 60, or 90 days accumulating to about 48, 97 or 146 mGy. In wild type mice, exposure to 10 mGy dose did not cause a statistically significant increase in number of malignant tumors but the life expectancy of these mice was significantly shorter; these mice also had an increased proportion of T cell lymphomas. In p53+/– mice, the situation was reversed; exposure to 30 mGy was associated with a longer lifespan and a decreased proportion of T cell lymphomas compared to nonirradiated heterozygotes (Mitchel et al. 2008). In a different study, exposure to 10 mGy of x-rays increases life expectancy of p53 heterozygous animals and decreases incidence of sarcomas and carcinomas (Lemon et al. 2017b). Exposure to 4 Gy dose followed by 10 weekly 10 mGy CT scans beginning 4 weeks after challenge irradiation also decreased incidence of sarcomas and lymphomas compared to challenge dose-only irradiated animals (Lemon et al. 2017a). This is one of the rare studies where low dose radiation exposure was found to mitigate high dose exposures after they have concluded. Considering that expression of p53 gene is upregulated by radiation (Rashi-Elkeles et al. 2011) low dose effects on heterozygous mice are believed to compensate for lack of one of the gene copies. Apparently, intermittent increase of p53 in animals that have experienced a high dose exposure has a genoprotective role, for example, by reversing genomic instability triggered by the 4 Gy exposure.

A cancer prone animal model with regulated expression of oncogene Ki-ras^{G12C} was made by generating a knock-in mouse that produces oncogene in lung cells under the control of doxycycline responsive promoter (Floyd et al. 2005). This animal model was developed to enable studies of the promotion of lung cancer; in these mice exposure to doxycycline (DOX) leads to growth of multiple cancer foci in lungs by 9 to 12 months of age. When animals primed by DOX also experience 4 weekly exposures to x-ray doses between 5 and 25 mGy, they showed increased number of tumors per lung compared to DOX only controls (Munley et al. 2011). In female mice, this increase was more pronounced than in males. Animals that did not receive DOX treatment did not develop lung cancers regardless of radiation exposure.

Mice that are heterozygous for expression of gene patched (Ptch1) are another animal model frequently used for low dose radiation studies because of their propensity for development of medulloblastoma. A study on chronic versus acute ^{137}Cs gamma-ray irradiation was done with Ptch1+/– neonates and animals exposed *in utero* (Tsuruoka et al. 2016). In this study, total medulloblastoma incidence did not significantly differ between irradiated animals and controls. However, the primary focus of this work was on the types of chromosomal events that lead to functional loss of the second Ptch1 allele: in all animals exposed to 0.5 Gy acutely or over 23 h chromosomal changes were different from those found in spontaneously occurring medulloblastomas, while animals exposed to 0.1 Gy had chromosomal alterations patterns similar to that found in spontaneous medulloblastoma cases. Medulloblastoma development in Ptch1 heterozygous animals was also investigated in double mutants with knocked out DNA repair genes Rad54 or DNAPk (Tanori et al. 2019). Newborn mice were acutely exposed to x-rays at two different doses and dose rates: 0.042 Gy at 59 mGy/min or 0.25 Gy at 327 mGy/min dose rate. Development of medulloblastoma was monitored and it was noted that lack of DNAPk led to absence of this cancer in unexposed mice and decreased medulloblastoma incidence after 0.042 or 0.25 Gy exposures in comparison to Ptch+/–. At the same time, life shortening was the greatest in mice without DNAPk and heterozygous for Ptch1, 75% of which died by 30 weeks of age without radiation, or 100% after 0.25 Gy exposure. Interestingly, longevity of these double mutants was the best after 0.042 Gy exposure. Ptch1+/–Rad54–/– double mutants on the other hand had both the most medulloblastoma and the shortest lifespan after 0.042 Gy exposure.

Animal models with reduced expression of tumor suppressor genes were also used in order to evaluate transgenerational effects of low dose radiation exposures. In one such example, transgenerational effects of low dose radiation as a risk factor for development of intestinal cancer in progeny of irradiated mice. These animals had a single copy of tumor suppressor gene adenomatous polyposis coli (APC).

Table 2. Experimental details of low dose radiation experiments with genetically modified mice with increased frequency of spontaneously developing cancers.

Mouse strain	Age at first treatment	Treatment group size	Radiation quality and delivery	Dose rate	Total dose	Period post exposure	Change in cancer incidence	Publication
Wild type and p53+/–, female only	6 weeks	188–258	^{60}Co gamma ray	0.7 mGy/h; 0.33 mGy per fraction	10, 20 or 30 mGy	Lifetime	10 but not 30 mGy leads to faster cancer development and more T cell lymphoma in wt mice and opposite in heterozygotes	(Mitchel et al. 2008)
p53+/–, female only	7–8 weeks	99–188	X-ray and ^{137}Cs gamma ray	18.6 mGy/min	10 mGy	Lifetime	10 mGy x-ray exposures increases lymphoma latency	(Lemon et al. 2017b)
p53+/–, female only	7–8 weeks	~200	Weekly x-ray and ^{137}Cs gamma ray	18.6 mGy/min x-ray, 0.349 Gy/min gamma ray	4 Gy gamma-ray, 10 × 10 mGy	Lifetime	10 × 10 mGy CT scan x-ray exposure extends survival for 4 Gy exposed mice and increases latency of lymphomas	(Lemon et al. 2017a)
Ki-ras^{G12C} transgene, induced by doxycycline (DOX) at 7 weeks	8 weeks (a week after DOX exposure)	12	Weekly x-ray from a clinical CT scanner		80, 120, or 160 mGy in 4 fractions and 2 CT scans	9 months after last fraction or 3 months after last scan	Any exposure to radiation increases lung cancer burden (number of foci) in DOX exposed mice	(Munley et al. 2011)
Pthc1+/–	Neonates (days 1–5) or *in utero* (E14–18)	47–70	^{137}Cs gamma-ray	540, 0.018, or 0.09 mGy/min	100 or 500 mGy	500 days	Medulloblastoma incidence stable; 'radiation type' mutations absent only in nice exposed *in utero* to 100 mGy	(Tsuruoka et al. 2016)
Ptch1+/– single and Ptch +/– Rad 54 –/–; and Ptch +/– DNAPk –/– double mutants	Postnatal day 1	20–111	X-ray, acute	59 mGy/min	0.042 Gy	60 weeks	Doubled medulloblastoma incidence in Ptch1+/– Rad54–/–	(Tanori et al. 2019)
				327 mGy/min	0.25 Gy		Earliest death (by 30 weeks) but lowest incidence of medulloblastoma in Ptch +/– DNAPk –/–	
APC$^{1638N/+}$	6–8 weeks	20	X-ray, acute	1.56 cGy/s	250 mGy x-rays	1 day before mating	Transgenerational doubling of intestinal cancer incidence	(Suman et al. 2017)

APC is especially implicated in intestinal cancers and this study used animals heterozygous for $APC^{1638N/+}$ (Suman et al. 2017). Two days after a single exposure to 250 mGy x-rays male heterozygote animals were mated with exposed or unexposed wild type females. Heterozygous progeny from irradiated $APC^{1638N/+}$ males had increased tumor frequency regardless of the irradiation status of the wild-type female parent. Heterozygotes who were irradiated themselves also showed increased intestinal cancer frequency; it should be noted however that in all cases low dose radiation exposure increased tumor frequency twofold or less compared to non-irradiated heterozygous mice.

Heterozygous transgenic animal models that offer an opportunity to study the manner in which low dose and/or low dose rate radiation exposure contributes to the loss of the single good copy of the chromosome and consequent cancer development. While low dose exposures increased cancer incidence in most cases, one of remarkable findings of these studies was that age of animal plays a very prominent role in this process. In addition, in those studies where a single copy gene is modulated by radiation, exposure to low doses of radiation often delays loss of the second gene copy and the associated development of cancer.

Low dose radiation of stress exposed animals

Synergistic detriment from exposures to low dose radiation and other sources of stress was found in a study by Miller and others (Miller et al. 2013; Table 3). In this work, wild type A/J mice were exposed to 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK) at 8 weeks of age to simulate effects of smoking. All mice developed lung cancer due to chemical treatment, and groups of 20 male or female animals were also exposed to x-rays once a week for 4 weeks, to 10, 30, or 50 mGy fractions. Lung cancer foci were found to be more frequent and tumor volume greater in mice that were exposed to radiation. On the other hand, normal tissue damage caused by a different carcinogen, the chemotherapy drug doxorubicin, was found to be reduced when administered to animals that first received low doses of radiation. Specifically, exposure to 75 mGy of x-rays, dose rate 12.5 mGy/min, 72 h before delivery of 7.5 mg/kg of doxorubicin protects cardiomyocytes of young (4–6 weeks old) BALB/c female mice five days after chemical exposure. Decreased cardiomyocyte apoptosis in this study was explained by decreased BAX and increased Bcl2 expression (Jiang et al. 2017).

Combination of low dose radiation exposures with high dose radiation as a stress was also explored in 5- to 6-week old female C57BL/6J mice irradiated with ^{60}Co gamma rays at dose rate of 1 mGy/min and total doses of 20 or 100 mGy. Two days after whole body irradiation spleens and thymuses were isolated from these animals and exposed to 2 Gy dose ex vivo. DNA damage estimates based on numbers of gamma H2AX foci did not show any sign that the low dose pre-exposures caused either protection or detriment for irradiated splenocytes or thymocytes (Blimkie et al. 2014) with the exception of the 6 h (but not 24 h) timepoint after 2 Gy exposure where splenocytes from mice pre-exposed to 100 mGy showed doubling of gamma H2AX foci. In a different experimental setup however, low dose exposures showed long term protection against effects of high dose of radiation. Specifically, it is known that exposure to 7.2 Gy of x-rays in 1.8 Gy fractions leads to induction of thymic lymphoma in C57BL/6 strain. Nevertheless, a pre-exposure to 75 mGy x-rays 6 h before each 1.8 Gy fraction, decreased lymphoma incidence by 60%. Similarly, a chronic 450 days long exposure to gamma rays at a dose rate of 1.2 mGy/h preceding challenging irradiation by 35 days decreased lymphoma incidence to 40% (Ina et al. 2005). These types of responses are referred to as radioadaptive responses.

Low dose radiation exposure activates cellular processes that may complement cell repair events induced by other types of stress. Combined activity of distinct cell repair mechanisms has more capacity to reduce cell stress then activity of a single cell repair pathway, while low doses of radiation cause little damage to the cells. Therefore, exposures to low dose radiation can decrease odds for cancer development in those situations where low dose radiation itself is not the only stress encountered by the organism.

Research focused on DNA damage in animals exposed to low dose or low dose rate low LET radiation

Because alterations of genomic DNA are associated with cancer development, chronic exposures were investigated as a possible source of DNA mutations and chromosome aberrations. For example, several IES studies evaluated chromosomal aberrations associated with chronic irradiation (Tanaka et al. 2009, 2014). Chromosomes were evaluated in splenocytes, and the numbers of dicentric and ring chromosomes were evaluated based on pan-centromere staining. While cells carrying these aberrations undergo mitotic catastrophe which would prevent them from neoplastic progression, the frequency of ring chromosomes and dicentrics was viewed as a proxy for evaluation of all chromosomal changes. Among them are chromosomal translocations which have a high probability to develop into neoplasia (Wang et al. 2020). Chronic gamma ray ^{137}Cs irradiations were done starting with 8-week old female C3H/HeN mice. Daily doses of 1, 20, 200, or 400 mGy were delivered over 5–615 days. High-dose-rate chronic exposures between 200 and 8000 mGy were also done over 1 to 40 days. Splenocytes from animals terminated at different timepoints were cultured for 48 h, followed by Giemsa staining and scoring of ring chromosomes and fragments, or evaluation of dicentrics by Fluorescence in situ hybridization (FISH). Daily exposures to 20 mGy did not increase ring chromosome frequency until the entire dose of 8 Gy was accumulated. In animals sacrificed, the ring chromosome numbers increased linearly with the increase of total dose. On average, less than one ring chromosome in a hundred was detected in splenocytes of mice that received up to 6 Gy, while mice that received 8 Gy had four ring chromosomes per hundred. When 8-week old C3H/HeN mice were exposed to 1 mGy

Table 3. Low dose radiation of stress exposed animals.

Mouse strain	Age at first treatment	Treatment group size	Radiation quality and delivery	Other source of stress	Total dose	Period post exposure	Change in cancer or other health outcomes	Publication
AJ	8 weeks	20	X-ray	Carcinogen NNK	40–200 mGy	8 months	Lung cancer incidence per mouse increased	(Miller et al. 2013)
BALB/c, females only	4–6 weeks	10	X-ray	Doxorubicin	75 mGy	5 days	Cardio-protection	(Jiang et al. 2017)
C57BL/6J, females only	5–6 weeks old	6	^{60}Co gamma-ray, acute with pre-exposure	Ex vivo 2 Gy exposure	20 or 100 mGy	Up to 24 h	DNA damage (gamma H2AX foci), clonogenic assays show no protection from challenge dose	(Blimkie et al. 2014)
C57BL/6N JcI, females only	5 weeks	20 to 30	^{137}Cs gamma rays	7.2 Gy, 4 weekly fractions	Lifetime chronic 1.2 mGy/h	Lifetime	Simultaneous exposure to low dose decreases lymphoma incidence to 1/2	(Ina et al. 2005)

per day for total doses between 0.125 and 0.7 Gy; or to 20 mGy per day for total doses of 0.5 to 8 Gy, translocation frequencies identified by FISH showed linear increase with the total dose increase. Two translocations per hundred cells were found for total dose of 0.8 Gy and 10 for splenocytes from mice exposed to 8 Gy. In another chronic exposure IES study, chromosomal aberrations were also evaluated in lymphomas that developed in control B6C3F1 mice and their chronically exposed counterparts receiving gamma rays at the dose rate of 21 mGy per day and total dose of 8 Gy (Takabatake et al. 2006). Most of these lymphomas were of B cell origin and gain of the whole chromosome 15 was found mostly in lymphomas from the irradiated group. Other differences in chromosomal gains and losses were found as well, suggesting that radiation-induced and spontaneous lymphomagenesis differ with respect to mutation spectra.

The repertoire of the T-cell receptor (TCR) rearrangements in wild type CBA/Ca mice and Ogg1$^{-/-}$ knock out mice was recently evaluated in a longitudinal animal study conducted in Europe (Candéias et al. 2018). The premise of the study was that the normal course of TCR rearrangements may be disrupted by presence of DNA damage caused by acute radiation in wild type mice or an outcome of chronic radiation in combination with a faulty repair of DNA reactive oxygen species damage. Protein 8-oxoguanine DNA glycosylase (Ogg1) is the enzyme that removes oxidized guanine residue – 8-oxoguanine from a (Rosenquist et al. 1997) and animals deficient for Ogg1 have increased genomic instability. Four-month old wild type animals received acute x-ray irradiation to 0.1 or 1 Gy at a dose rate of 0.5 Gy/min. Three to eight months old female Ogg1$^{-/-}$ mice on a C57BL/6NTac background were exposed to gamma rays from a ^{60}Co source at a dose rate 0.43 or 2.1 mGy/h for 22 h a day for 3 weeks at the FIGARO experimental facility at the Norwegian University of Life Sciences. Total doses for chronically irradiated mice were 0.233 Gy and 1.146 Gy. Blood samples were collected 1, 3, or 6 months after radiation exposure. Frequencies of 'faulty' T lymphocyte rearrangements which would suggest genomic instability caused by radiation, were not increased in any irradiated experimental group compared to controls.

Another longitudinal study that included evaluation of chromosomal aberrations was done by Dalke and others (Dalke et al. 2018). In this work, radiation exposure was acute – total doses of 0.063, 0.125, and 0.5 Gy of gamma rays were delivered at a dose rate of 63 mGy/min from a ^{60}Co irradiator. Irradiated animals were either wild type animals or heterozygotes with a single copy of mutated Excision repair, complementing defective, in Chinese hamster, 2 (*Ercc2*) gene. While Ercc2 acts as a DNA helicase for nucleotide excision repair (Coin et al. 1998), this mutation in mice is primarily associated with increased cataract development. The wild type animals were F1 hybrids of male C3HeB/FeJ and C57BL/6J female mice (B6C3F1); the latter were F1 hybrids of male homozygous *Ercc2* mutated mice on C3HeB/FeJ background and C57BL/6J females (B6RCF1). Irradiated animals were sacrificed at 4 and 24 h or 12, 18,

and 24 months after exposure. Chromosomal aberrations were evaluated in 50 metaphase bone marrow cells from three mice of each group. A higher risk of dicentric chromosomes and other aberrations was found only in the heterozygous animals and only at 12-month timepoint after 0.5 Gy exposure. In addition, at 24 months after 0.5 Gy exposure both genotypes of animals showed increased frequency of ovarian tumors and pituitary adenomas.

A short term study example comes from work that used x-ray total doses of 8.3 to 1333 mGy given to six to 8-week-old male C57BL/6N mice (Khattab et al. 2017). Two days after exposure blood was collected and used to count the numbers of micronucleated CD71+ reticulocytes. A significant increase in micronuclei was noted for all doses. Interestingly, recent research suggests that micronuclei may be triggering the activity of the immune system (MacDonald et al. 2020). Therefore, it is possible that immune system activation caused by low dose radiation exposures could be an added factor of interest in evaluation of low dose radiation effects. Some of the short term studies also evaluated DNA damage and apoptosis in spleen cells of wild type mice (Koturbash et al. 2017). In this work, 60-day old wild type C57BL/6 male mice were sacrificed at 6 or 24 h after exposure to the last fraction of whole body x-ray irradiation at a dose rate of 50 mGy/s. Animals received 0.1 Gy per day one to five days in succession (for total doses of 0.1 to 0.5 Gy). Most numerous DNA strand breaks were found in 6 h samples, in mice that received 0.1, 0.4, and 0.5 Gy, but apoptosis was greatest at either timepoint in 0.4 and 0.5 Gy exposed animals. This work also found that phospho-ATM was increased only in 0.1 Gy exposed mice but that p53 was decreased compared to control.

A combination of chronic in vivo exposures with ex vivo exposures to a challenge dose was also done with the focus on DNA degradation as the endpoint (Osipov et al. 2013). Ten-week old male CBA/lac mice in groups of ten were chronically exposed to ^{137}Cs gamma rays at the dose rate of 0.15 mGy/h for 40 to 120 days and the accumulated radiation doses were 144, 288, and 432 mGy. Peripheral blood lymphocytes were evaluated for double stranded DNA damage by neutral Comet assay and either evaluated as they were or irradiated with a challenge dose of 4 Gy and then immediately lysed and used for Comet assay. Interestingly, only longer chronic exposures of 288 and 432 mGy protected the cells from the challenge dose. On the other hand, the shortest chronic exposure of 40 days resulting in accumulation of 144 mGy was the only dose for which DNA damage from irradiated animals exceeded the DNA double strand breaks in control cells.

Effects of low dose radiation in combination with biochemically induced DNA repair deficiency were studied in zebrafish. Ku80 is a protein involved in non-homologous end rejoining (NHEJ) repair and in this study it was suppressed because of the treatment with an antisense phosphorodiamidate morpholino oligonucleotide (Bladen et al. 2007). Fish were injected with the antisense oligo at a two--cell stage and irradiated at the 6 h old shield stage; irradiation included x-ray doses of 1, 3, 8, 20, and 50 mGy to anti-Ku80 injected fish embryos. After additional 18 h, animals were harvested for evaluation of apoptosis related DNA fragmentation by Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. All zebrafish treated with anti-Ku80 had numerous apoptotic cells, for the low total doses between 1 and 50 mGy apoptotic cells were as much as 34-fold more frequent than in controls.

Considering that low dose radiation research has found that these exposures may initiate stress response pathways, which in turn include DNA repair, we conclude that low dose exposures in the absence of active and high fidelity DNA repair lead to accumulation of unrepaired or misrepaired DNA damage. Because fixed DNA damage in somatic cells increases odds for subsequent cancer development, studies focused on accumulation of DNA damage in different model systems and associated with different doses and dose rate could form a foundation for understanding of low dose potential for carcinogenesis.

OMICS changes following radiation

Protein expression

While association between cancer development and protein expression is less direct than that between cancer and mutations, this connection is highly relevant. Post-translational protein alterations are one of the earliest cellular responses to radiation, and inevitably disturb cellular homeostasis which may result in genomic instability. Lasting changes in expression of multiple proteins, on the other hand, inform us about gene regulation changes that can also initiate or promote cancer development. Consequently, low dose radiation research should include studies focused on protein expression. Because liver neoplasia is the only cancer that is showing an increase in incidence in animals chronically exposed over 400 days to a total dose of 0.02 Gy (Braga-Tanaka et al. 2018), protein expression in this organ was evaluated in greatest detail. For example, liver protein expression was evaluated in C57BL/6J male mice after chronic daily gamma ray exposures done at IES versus acute x-ray exposures at high dose rate. Chronic, 400 days long exposures to gamma rays included dose rate of 1 mGy per day (total dose of 0.4 Gy) or 20 mGy per day (total dose of 8 Gy); while acute x-ray exposures included 4 or 8 Gy given to 10- or 24-week old animals (Nakajima et al. 2017). Chronically exposed mice were sacrificed immediately or 3 months after exposure, and the acutely exposed mice 6 days or 3 months after radiation delivery. The pattern of protein expression changes differed between acutely and chronically exposed mice. For example, laminin-2, DRAK1, BID, Bim, Bmf in chronically exposed mice was different compared to age matched controls and this change persisted over three months. In acutely exposed mice, MyD88 and Bcl-xL were changed 6 days after 8 Gy and three months after exposure to 4 Gy. Therefore, while acute irradiation affected proteins that can be considered as effectors of cell death, chronic irradiation also included proteins with pleiotropic effects on multiple cell functions. In other words, while Bcl-xL and DRAK1 both regulate apoptosis, laminin-2 affects almost

every aspect of cell life. Liver proteins were also studied by 2D gel electrophoresis after chronic exposure of C57BL/6J male mice to ^{137}Cs gamma rays (Yi et al. 2016, 2017). Exposures of 22 h per day over 90 or 180 days begun when animals were 6-week old and the dose rates used varied between less than 1 to 22 mGy per day. Twenty-two proteins, including glycine *N*-methyltransferase, catalase, and glutathione S-transferase P1 showed changes in expression. Increase of glycine N-methyltransferase, a protein responsible for regulation of the ratio of S-adenosylmethionine to S-adenosylhomocysteine and the detoxification of liver cells (Liu et al. 2007) as well as increase of antioxidant enzymes catalase and glutathione S-transferase P1 (Das and Vinayak 2015) can be considered as a self-protective activity of liver cells. Changed expression of calreticulin was the most pronounced and the authors concluded that this protein could be a marker of liver low dose radiation exposure. Considering that antioxidant enzymes decrease mutation load while chaperones such as calreticulin (Totani et al. 2020) increase functional activity of other proteins, all of these protein expression changes can be considered as adaptations to oxidative stress. Consequently, if these adaptative protein expression changes fail to meet the stress imposed on the cells, oxidative damage to genomic DNA or other cellular structures becomes inevitable.

Elementalomics

Elementalomics research focused on low doses of radiation is also in its developmental stages. For example, x-ray fluorescence microscopy was used to evaluate chemical content of peripheral blood and cardiac formalin fixed and paraffin embedded tissue in low and high dose x-ray irradiated animals (Mota et al. 2018). Low to moderate doses of 0.1 to 0.25 Gy of total body irradiation were done with male Wistar rats 24 h before sample collection. Irradiated tissues showed decreased concentrations of S, K, Ca, Fe, and Zn regardless of the dose used. Considering that elemental concentration perturbations correlate with differences in radiation sensitivity, this study may be opening a new area of investigation for radiation studies (Poropatich et al. 2019).

DNA methylation

Methylation evaluation of BALB/c mice after 10 daily exposures to 50 mGy or an acute exposure to 0.5 Gy x-rays showed global hypomethylation as well as tissue-specific and gene specific promoter hypermethylation in mice that received fractionated radiation (Wang et al. 2014). For example, DNA repair protein Rad23b (Mu et al. 2018) was hypermethylated in peripheral blood, liver, spleen, brain, and lung, while Ddit3 which is regulated by mitochondrial stress (Fessler et al. 2020) was hypermethylated in peripheral blood, liver, and lung. Evaluation of methylation caused by radiation in zebrafish was also done, in progeny of chronically exposed parents (Kamstra et al. 2018). A total dose of about 5.6 Gy ^{60}Co gamma rays was delivered over 27 days at the dose date of 8.7 mGy/h prior to mating. The progeny was harvested at 5.5 h post fertilization and DNA methylation was evaluated. As many as 5658 regions were found to be differentially methylated including many regulatory sequences modulating gene expression.

Gene expression

Gene expression modulation in response to ionizing radiation exposure has been a subject of radiation biology for a long while. Every approach to study gene expression, from Northern blots to gene arrays to next generation sequencing was applied to irradiated animal tissues. While many of these studies showed interesting findings, low dose and low dose rate radiation exposures are generally difficult to study because of the low gene expression responses to low dose exposures. In this subsection, we discuss messenger RNA (mRNA) expression as well as findings about modulators of mRNA expression – methylation of genomic DNA and expression of micro RNAs (miRs) as mRNA modulators.

RNA expression evaluation in low dose radiation research has focused on every type of molecule – from regulator molecules such as long non coding RNAs (lncRNAs) and micro RNAs (miRNAs) to messenger RNAs (mRNAs). For example, a recent miRNAs evaluation in FVB/NJ mice treated protractedly with total dose of 1.12 Gy (Liang et al. 2018) used male 12-week old animals exposed to 28 mGy of ^{137}Cs gamma rays every third day over a period of four months. Eleven miRNAs explored in this work included miR 34a, miR 375, miR 185, miR 21, miR 421, miR 193a, miR 199a, miR 146a, miR 155, miR 221, and miR 222, each known to support different aspects of cancer development. Expression of these miRNAs was evaluated of at conclusion of exposure or two months later in liver, testis, and heart. Expression of different miRNAs varied with each of these experimental conditions and organ specific miRNA expression changes were generally not statistically significant. One of the few exceptions included increased expression of oncogenic miR 21 in liver tissue at the conclusion of irradiations.

Micro RNA expression after exposures to 10 or 100 mGy of ^{60}Co gamma rays at dose rate of 1mGy/min was recently studied in wild type C57BL/6J mice (Bugden et al. 2019). Males 2 or 26 months of age were irradiated, and their blood collected one or seven days later. Micro RNAs were collected from plasma and evaluated using Qiagen microarrays and the authors reported the micro RNAs that were upregulated or downregulated compared to controls in all irradiated samples at all timepoints. Increased expression was found for mmu-miRs 329-3p, 1249-3p, 6366, 703, and 344 g-5p; mmu-miR-20a-5p and mmu-let-7j were decreased.

Gene expression was also studied in zebrafish. For example, 2.5 h post fertilization embryos were exposed to total doses of 1.62, 16.2, or 10.9 mGy delivered at dose rates of 0.54, 5.4, or 10.9 mGy/h ^{60}Co gamma rays and their RNA expression was evaluated 5.5 h post fertilization (Hurem et al. 2017). RNA sequencing analysis showed an increase in the total number of differentially expressed genes. Real time PCR confirmed differential expression of the gene 6-phosphofructo-2-kinase-fructose-2,6-biphosphatase 3 as upregulated at 1.62 mGy but downregulated at 16.2

or 10.9 mGy doses. Gene cellular retinoic acid binding protein 2 b was upregulated in all exposure groups.

Overall, OMICs changes associated with low dose radiation exposures show the greatest diversity of findings. This on one hand, suggests that it is difficult to find systematic epigenetic changes in response to low dose exposures; on the other hand, this difficulty should motivate us to develop more integrated approaches to OMICS studies. Once again, archival preservation of samples for the purpose of subsequent research such as OMICS becomes a clear low dose research imperative.

Conclusions

Historic studies of radiation effects in animal models, especially those conducted in the USA national laboratories prior to 1990s were often focused either on high doses or high dose rate exposures, resembling as much as possible whole body exposures that would be expected in the course of a nuclear catastrophe. Similarly, animals exposed to internal emitters were given through inhalation those radionuclides that word be present in a nuclear bomb fallout. Moderate and low dose radiation exposures were done as fractionated radiation, designed to simulate occupational external radiation exposures (Haley et al. 2011; Zander et al. 2019). These and analogous historic radiation studies were recently discussed in several review papers. Between 1990s and the period covered by this article, USA Department of Energy funded work focused on low doses of radiation (Brooks 2015) and similar shift in focus in radiation research could be noted world-wide. Studies of low LET low dose radiation effects have changed in the recent years, with increased focus on long term studies such as chronic low dose exposures and lifetime or transgenerational evaluation of radiation effects. At the same time, short term studies focusing on multi-omics endpoints also became more numerous. Despite these changes in low dose radiation research, the concerns about the carcinogenic potential of low doses of radiation remain. Concepts such as delayed genomic instability and role of mitochondrial disturbances in the gradual accumulation of potentially cancer-contributing events have been explored to the extent of the technical developments available to low dose researchers so far. Even though technical and methodological advances that allow exploration of new endpoints such as micro RNA expression or single cell studies continue to grow, low dose rate radiation remains a challenging field of study. New discoveries also have significant impact on understanding biological effects—for example, an awareness of the implications of epigenetics was not fully evident until recent times. In wild type animals, the effects of chronic or fractionated exposures to low doses of radiation below 100 mGy are most often negligible, while in mutant animals and those whose health was damaged by different stressors, exposures to low doses of radiation sometimes increase and sometimes decrease cancer incidence. For example, a group of 495 wild type male, but not female, B6C3F1 mice chronically exposed to 20 mGy of gamma rays show small but statistically significant increase in hepatocellular adenoma (Tanaka et al. 2007). On the other hand, a group of 62 double mutant Ptch1+/– Rad54–/– mice develop more medulloblastoma than their Ptch1+/– counterparts after 42 mGy exposure, while a group of 70 Ptch1+/– DNApK –/– double mutants develop fewer medulloblastoma than Pthc1+/– mice exposed to 42 mGy (Tanori et al. 2019). Considering that the wet bench and computational techniques in animal research keep improving and the molecular and cell biology of whole organisms are rapidly developing, we can hope that the next decades will bring us closer to understanding biological consequences of low dose radiation. In the meantime, it is important that the low dose radiation community should keep saving and sharing the data and materials allowing for new discoveries from the existing old materials and meta-analyses from the existing data. For example, the authors hope that, similarly to the recent example from Japan (Doi et al. 2020), we may see a study from Canada using old p53+/– data from studies mentioned here (Mitchel et al. 2008; Lemon et al. 2017a, 2017b). The development of a large-scale international consortium of investigators with archives and tissues would be a broad value to the community. The conclusions of such hoped for retroactive analyses of large compendiums of carefully curated data will depend on our ability to access the data and samples fully and with the complete understanding about all relevant experimental details. It is not clear where resources for such efforts could come from, but it is imperative to put more effort into preservation and organization of materials that are available to the world-wide community of radiation and cancer biologists. Future research priorities in radiation biology are as fluid as the biology research as a whole because at this moment we still do not know which direction of studies will be the most likely to allow us to grasp the effects of low doses of radiation.

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