Appendix 1

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An examination of the linear no-threshold hypothesis of cancer risk assessment: Introduction to a series of reviews documenting the lack of biological plausibility of LNT

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The linear no-threshold (LNT) single-hit dose response model for mutagenicity and carcinogenicity has dominated the field of regulatory risk assessment of carcinogenic agents since 1956 for radiation [8] and 1977 for chemicals [11]. The fundamental biological assumptions upon which the LNT model relied at its early adoption at best reflected a primitive understanding of key biological processes controlling mutation and development of cancer. However, breakthrough advancements contributed by modern molecular biology over the last several decades have provided experimental tools and evidence challenging the LNT model for use in risk assessment of radiation or chemicals. Those science advancements have revealed that DNA is not simply an inert chemical target such that even a single "hit" potentially results in cancer, or that multiple hits additively cumulate over time. Modern biology has now unequivocally demonstrated that biological systems mount a plethora of highly integrated defenses to a continuous chorus of endogenous and exogenous attacks (e.g., ROS) on core genetic material and function. These defenses (expressed at subcellular, cellular, organ and whole body levels) are essential to sustaining cell and organism homeostasis. This massive explosion in fundamental understanding of cell and organism function now clearly points to the need to examine the impact of this vast body of knowledge on the scientific legitimacy of maintaining the LNT model as a continuing and scientifically defensible driver of radiation and chemical carcinogen risk assessment.

The concept of LNT responses to exogenous agent exposures had its origins well before its application in carcinogen risk assessment when it was first proposed as the mechanistic explanation of biological evolution. Inspired by the research of Hermann J. Muller demonstrating that very high doses of radiation induced transgenerational phenotypic changes claimed as caused by heritable point mutations [18], two physical chemists from the University of California at Berkeley proposed that cosmic and terrestrial ionizing radiation provided the mechanistic driving force for the evolution of life on earth [22]. Despite this perceived need to identify a plausible mechanistic explanation for evolution and the prominence of co-author Gilbert Lewis, who would be nominated for the Nobel Prize some 42 times, this idea generated much heat but little light. This hypothesis was soon found to be unable to account for spontaneous mutation rates, underestimating such events by a factor of greater than 1000-fold [19].

Despite this rather inauspicious start for the LNT model, Muller would rescue it from obscurity, giving it vast public health and medical implications, even proclaiming it a scientific principle by calling it the Proportionality Rule [20]. While initially conceived as a driving force for evolution, Muller gave the LNT concept a second chance at scientific life and tirelessly promoted it as a plausible basis for radiation safety assessment for the remainder of his scientific career. Muller soon would link a mechanism to his model via the collaboration of leading physicists who saw creative advances occurring at disciplinary interfaces, such as genetics and nuclear physics. Soon this interdisciplinary grouping would devise a mechanism via target theory for Muller's data and the LNT-single hit model was born [6,24].

Technology in the form of X-and gamma ray generation and their associated medical applications and the ensuing development of nuclear weapons would provide the impetus for propelling scientific recognition and application of LNT to societal risk concerns. This early knowledge of mutation and its proposed mechanism and associated LNT-driven dose response features would become transformed into a massive public policy issue following the dropping of the two atomic bombs in 1945. Society became terrified of the thought of generations of deformed children and predictions of a plethora of inescapable cancers arising from the rapid proliferation of these new ionizing radiation based technologies [3,4,7].

As for Muller, his now decades old research discovery took center stage and he was soon awarded the Nobel Prize in December of 1946 for his discovery of radiation-induced gene mutations in fruit flies. Notably, while unequivocally stating in his Nobel lecture that "*there is no escape from the conclusion that there is no threshold for radiation-induced mutation*", as a paid consultant, Muller had seen the results of a classified

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study from the Manhattan Project by Ernst Caspari which demonstrated a clear mutation threshold when a lower radiation dose rate was used [5,12]. As a relevant aside, Caspari's threshold findings have been replicated in more recent fruit fly studies with additional observations of low-dose hormesis [16,21]. Following his Nobel Prize, Muller now had the public and scientific platform he had so long sought and he capitalized on this, leading radiation geneticists on a quest to exchange longstanding regulatory beliefs in a threshold dose response model for mutagens and carcinogens for the LNT-single hit model. This objective required some critical orchestrated political and financial assistance at a key strategic time from the Rockefeller Foundation and the US NAS (both organizations ironically headed by Detlev Bronk), but Muller and his radiation geneticist colleagues ultimately prevailed in bringing about a profound change in risk assessment policy in 1956, convincing members of the Genetics Panel at the US NAS to recommend the adoption of LNT for low-dose radiation cancer risk assessment [7,9].

The fear of radiation-caused cancer was soon extended to chemicals by the US congressional passage of the “Delaney clause” in the Food Additives Amendment of 1958, which pronounced that any new substance found to cause cancer in humans or animals, regardless of dose, could not be used as a food additive. Soon thereafter in 1959 the Secretary of the US Department of Health, Education and Welfare created the first wave of chemical cancer panic by declaring low-level residues of the herbicide aminotriazole in cranberries to be a cancer threat. This pesticide had recently been reported to induce cancer in experimental animals at very much higher test doses (equivalent to humans eating 15,000 pounds of cranberries every day for many years). That warning, which occurred during the week of Thanksgiving, caused an immediate plummet in the sales of this holiday staple and was a key catalyst for decades of ensuing public cancer chemophobia [11,17].

These early profound shifts in the basic approach to cancer risk assessment, all of which were based on mostly poorly-understood toxicological phenomena of the time, had major ripple effects. Perhaps most substantively, they provided key inspiration to Rachael Carson in her seminal [13] book, *Silent Spring*. Her book awakened and scared both the world and President John F. Kennedy with concerns of widespread chemical contamination, especially in the form of chlorinated pesticides such as DDT. It was indeed her influence that would further fuse and synergize with Muller's LNT revolution, leading the US Congress to create the National Environmental Protection Act (NEPA) in December of 1969 and Environmental Protection Agency (EPA) only months later. While Muller would die in 1967, his transformational advocacy of LNT would find their target and inspire the actions of the soon-be-created US regulatory agencies.

In 1972, a new NAS Committee i.e., Biological Effects of Ionizing Radiation (BEIR I), would “revalidate” the LNT using a mammalian rather than fruit fly model based on the massive research by William Russell at Oakridge involving more than two million mice. The LNT concept was preserved despite the recognition that the BEAR I had made a crucial error in claiming that risk was based on total dose rather than dose rate. The re-affirmation of LNT by BEIR I, based on the Russell work, was adopted by EPA as the “gold” standard in 1975 for radiation and chemical carcinogens. It provides the key fundamental grounding and rationalization for the LNT. This was crucial since epidemiological methods were incapable of quantitatively assessing the shape of the dose response in the low dose zone due to background variation and other methodological limitations. The Russell studies and the BEIR recommendation would become a toxicological/risk assessment “homing” approach that would help to ensure the adoption and continued use of LNT. This scientific foundation for LNT has been challenged since, as it has been learned [10], the Russell control group mutation estimates were substantially flawed. After appropriate corrections, the massive data revealed not only a clear mutation threshold in male mice, but an hormetic response in female mice. These unequivocal findings, which have never played a meaningful role in EPA's subsequent regulatory policy, further erode trust in use of LNT in science-supported cancer risk assessment actions.

By the mid-1970s the Carson-inspired environmental revolution was fully engaged, with the emerging belief that 80% of human cancers were due to environmental factors, mostly manmade, and therefore giving hope of potentially eliminating this dreaded disease with strong regulations. Driven by such beliefs as well as the obvious visual recognition at the time that air and water pollution needed to be comprehensively addressed, Congress was inspired to create a plethora of environmental legislation and an associated aggressive and well-funded research framework “to end cancer in our lifetime”.

The emerging regulatory efforts were more successful than Muller and Carson could ever have imagined. By the mid-1970s the LNT model would be adopted by EPA for risk assessment of both ionizing radiation and chemical carcinogens, with no dose, even a single ionization or molecule, absent of potential harm. Before society really knew what happened, there was an environmental transformation, actually an intellectual and emotional revolution, that could be seen in many ways, such as trying to manage industrial waste practices, toxicity screening of new chemicals prior to entering commerce and those already in wide use, drastically reducing automobile emissions, profoundly reducing lead in the environment, and many other such actions. These and other actions also created profound fears of “toxic” environmental and occupational agents even at extremely low doses. These activities were soon seen and felt in the cost of new environmental and occupational regulations, massive clean-up costs for all types of contamination, leaving many in the regulated community to wonder “how clean is clean? What is clear is that LNT-based cancer risk assessments resulted in estimates of acceptable environmental exposures and implementation of regulatory actions that were increasingly challenging, if not impossible, to meet, and with every additional increment of exposure reduction often resulting in disproportionate clean-up and/or control standards. For example, with respect to remediation of radiation-contaminated sites, Pete Lyons, then with the Department of Energy (DOE), noted that removing soil down to 25 mrem instead of 15 mrem would save billions of dollars in unnecessary remediation costs (personal communications with Robert Golden).

Nonetheless, the revolution had occurred on multiple levels and it happened so fast that it essentially outstripped needed scientific foundations upon which to base regulatory decisions. In 1976 the EPA created the Cancer Assessment Group (CAG) that was quickly followed by an interagency report lead by David Hoel (NIEHS) and David Gaylor (FDA), two leading biostatisticians, which recommended the LNT-single hit model. The CAG, under political pressure to establish functional cancer risk assessment practices, reached into the NAS BEAR and BEIR radiation risk guidance documents to quickly adopt the LNT-single hit model and apply it for chemicals and radiation based on the assumption that both types of carcinogens acted via a mutational mechanism that was assumed as linear at low dose. At the same time Kenny Crump, David Hoel and others co-authored a paper proposing that chemically induced tumors acted in an additive to background manner, explicitly following identical no-threshold mechanisms [14]. The additive to background assumption was subtle but significant. Its acceptance would essentially assure that linearity would occur under virtually all modeled situations, and surprisingly, even when the dose response data reflected a threshold. It took a while for this assumption to guide EPA cancer risk assessment practices, but it has done so now for more than 30 years, with no challenge and little reflection, becoming an irrefutable fixture to the risk assessment process [11].

The environmental revolution was a *fait accompli* by the early 1980s, with the OSHA Carcinogen Hearings from 1978 to 1980 assuring the final codification of LNT-based risk assessment practices. These activities were also supported by complementary efforts of the NAS Safe Drinking Water Committee (SDWC) created in 1975 in response to passage of the US Safe Drinking Water Act in 1974. The SDWC deliberations were powerfully influenced by key players such as David Hoel and the director at the National Institute of Environmental Health

Sciences (NIEHS), David Rall, in addition to two members of the original 1956 BEAR committee. Their strong advocacy for the LNT model set up a virtual storm of controversy amongst the toxicologists who argued that the threshold/safety factor approach was more than adequate to ensure protection from potentially carcinogenic chemicals. Ironically, and not generally appreciated, is that the next SDWC, under the leadership of toxicologist John Doull (to whom this Special Issue is dedicated), rescinded the NAS endorsement of LNT with the suggestion that data determine the dose-response model. However, the next SDWC reluctantly re-endorsed LNT which has remained in place to the present.

Functional consolidation of LNT was now essentially complete and in 1979 EPA would promulgate its first LNT-based drinking water standard for trihalomethanes (THM) affecting numerous public drinking water supplies using chlorinated disinfection processes. The costs to society were enormous, forcing many large cities to spend huge amounts of money on new or refined disinfection technologies. This was just the start of similar patterns affecting many aspects of society that were increasingly dependent on radiation- and chemical-based technologies to support modern and higher quality lifestyles. LNT was now the “law of the land”, and additivity to background (the validity of which has been significantly challenged [11]) would assure it could never lose. For all practical purposes the LNT had assumed the scientifically inappropriate position of the null hypothesis, which scientific convention would otherwise require starting with the assumption that very low doses to radiation and chemicals do not cause mutation or cancer [23]. Because the LNT hypothesis assumes that even a single ionization or molecule “hit” is capable of inducing mutation and ultimately cancer, the new “null” hypothesis essentially became *de facto* non-falsifiable.

Public fears that society was swimming in a sea of man-made carcinogens were further amplified by the 1973 technological advancement by Bruce Ames that used inexpensive and easily-conducted bacteria-based experimental systems to detect chemical mutagens. Relatively suddenly, large numbers of anthropogenic environmental chemicals were flagged as mutagens, and under the banner of existing LNT dogma, were considered as potential human carcinogens. These fears were further compounded by reports of approximately 50% positive cancer responses in animal bioassays using Maximum Tolerated Doses (MTD), even though such doses often were very much higher than real-world human exposures [15]. These whole animal findings, coupled to the in vitro mechanistic evidence of genotoxicity in the “Ames” assay, virtually assured defaulting to LNT dose-response risk assessments. This latter point was important for it ensured that the toxicological and risk assessment rules were “set”, and outcomes were assured in any battle over threshold versus LNT for particular chemicals. Ironically, Ames later realized that many thousands of naturally occurring chemicals, including many present in clearly “healthy” diets of fruits and vegetables, also were mutagens in genotoxicity testing systems. And even more importantly, he recognized that human exposures to these natural substances *in toto* and in some cases individually were far greater than exposures to anthropogenic agents. This fundamental observation led Ames to vehemently challenge the assumption that exposures to man-made chemicals were, with few exceptions, primary contributors to human mutagenicity and cancer [1].

In the aftermath of this now nearly half-century environmental revolution, what has been accomplished and learned? Great progress has been made in the cleaning and greening of the environment. The air and water in developed countries are indisputably far cleaner today compared to the 1960s. All of this is good news, and was a highly worthwhile focus of attention. However, after many trillions of dollars spent worldwide in efforts to clean up the environment to avoid promised cancer epidemics, with the exception of smoking (which directly delivers high doses of potent carcinogens to the lungs), there has been essentially little impact on the overall incidence of age-adjusted cancer in the US and in the rate of deaths from cancers per year [2]. Observed “increases” in certain cancers (e.g., breast, prostate, thyroid) can in part be attributed to improved surveillance and/or diagnosis which are not environmentally driven. Early post-WWII generations, despite indisputably experiencing the highest levels of environmental pollution during the 1950's to 70's, are not exhibiting the cancer epidemic otherwise expected from LNT assumptions. The cancer statistics are strikingly, surprisingly and depressingly essentially static. This suggests that despite commitment of enormous societal resources to comply with LNT-based risk assessments, LNT-based cancer regulatory practices have failed to fulfill the promise of making meaningful differences in overall cancer incidence and mortality.

Would society have been better served by the threshold model? Certainly, advances in modern biology as described in some of the papers of this Special Issue exposed the lack of biological plausibility of the LNT. And as observed by Ames, it should be deeply troubling to all health professionals that the LNT paradigm counterintuitively infers that the greatest risks for environmentally-induced cancer are due to consumption of foods, i.e., fruits and vegetables, that otherwise are viewed as the baseline for the healthiest of human exposures.

The problem then is whether rapid and profound advancements in biological sciences are sufficient and appropriate to displace a default LNT-based risk paradigm that was undeniably structured on simplistic and erroneous interpretations of 1940's era biological experimentation. It is clear that in no other scientific endeavors, other than LNT-based cancer risk assessments, has the practical progression and application of advancements in science knowledge been so irrevocably held hostage to what not only is a functionally untestable hypothesis but also what is now robustly counter to modern biological science.

It was in the above-noted spirit of thinking and concern that this project convened a group of highly qualified experts in toxicology, radiation biology, epidemiology and related areas to undertake a systematic review of the scientific validity of the LNT model from both historical as well as modern molecular biology perspectives. This approach entailed a fresh look at Muller and his historical contributions and brings it up to the present with modern molecular biology-driven considerations of mutation and cancer mechanisms and how they inform the dose-response of environmental radiation- and chemically-induced human cancer. These perspectives have been integrated into a comprehensive evaluation of the LNT model, examining whether this risk assessment strategy that has dominated the past half century is scientifically sustainable.

Given the enormous and increasingly precious societal resources required to address the broad functional implications of the LNT risk assessment paradigm, the stakes are too high for the scientific and regulatory communities to continue to ignore the ever-growing mountain of evidence directly challenging the biological underpinnings of the LNT model. The primary objective of the perspectives presented in the series of papers comprising this special issue of Chemico-Biological Interactions is to catalyze a serious reexamination of likely the most influential and costly risk assessment practice that should have been recognized as substantially flawed from its inception. Such a need is further emphasized by the fact that, book-ending Muller's 1946 Nobel, the 2015 Nobel Prize for chemistry was awarded to three scientists from the Francis Crick Institute, Duke University and the University of North Carolina for their detailed mechanistic studies of three different kinds of DNA repair (i.e., base excision repair, mismatch repair and nucleotide excision). It is these and countless other complementary cellular mechanisms which describe the evolutionarily-conserved and multilayered processes that function for the important purpose of protecting DNA integrity, and unquestionably are modulators of mutational and cancer thresholds associated with low dose radiation or chemical exposures.

It was not an objective of this project, however, to propose alternative approaches to cancer risk assessment beyond LNT, other than to build the case that both history and present-day biological knowledge justify moving from LNT to threshold-based risk assessments. It is clear

that continuing advances in toxicological and biological sciences are revealing a multiplicity of threshold-based options that will support substantially improved science-versus policy-based risk assessments.

It is the hope of the contributing authors of this Special Issue that this volume (Calabrese, E., 2019; A comprehensive assessment of its historical and scientific foundations; Costantini, D. and Borremans, B. The linear no-threshold model is less realistic than threshold or hormesis-based models: An evolutionary perspective; Scott, B. The LNT model for cancer induction is not supported by radiobiological data; Tharmalingam et al.; Re-evaluation of the linear no-threshold (LNT) model using new paradigms and modern molecular studies; Brooks, A. The impact of dose rate on the linear no threshold hypothesis; Andrew, M. Zarnke et al. BEIR VI radon: The rest of the story; Kobets, T. and Williams, G. Review of the evidence for thresholds for DNA-reactive and epigenetic experimental chemical carcinogens; Clewell et al. Mechanistic aspects of chemical carcinogens demonstrating thresholds; Ricci, P. and Tharmalingam, S. Ionizing radiation epidemiology does not support the LNT model; Williams, R.A. Economic benefit-cost implications of the LNT model.) will stimulate a broad scientific and policy reevaluation of cancer risk assessment policy and practices. The flawed history of LNT and its lack of biological plausibility as revealed by robust advancements of modern-day biological sciences should compel regulatory agencies to immediately remove it as the default model in cancer risk assessment. Indeed, the time is ripe, if not long overdue, to seize the opportunity to place cancer risk assessment on sounder, more biologically based and fully transparent foundations.

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Appendix A. Supplementary data

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Transparency document

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The linear No-Threshold (LNT) dose response model: A comprehensive assessment of its historical and scientific foundations

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ABSTRACT

The linear no-threshold (LNT) single-hit (SH) dose response model for cancer risk assessment is comprehensively assessed with respect to its historical foundations. This paper also examines how mistakes, ideological biases, and scientific misconduct by key scientists affected the acceptance, validity, and applications of the LNT model for cancer risk assessment. In addition, the analysis demonstrates that the LNT single-hit model was inappropriately adopted for governmental risk assessment, regulatory policy, practices, and for risk communication.

1. Introduction

This paper provides a detailed historical assessment of the origin and progressive development of the linear no-threshold dose response (LNT single-hit model). The time period of this historical assessment starts in 1927 after Muller reported inducing transgenerational phenotypic changes (i.e., heritable mutations) in *Drosophila* via the use of very high doses of X-rays[1] to the present time, with the recent discovery of critical errors made by the U.S. NAS (National Academy of Sciences) BEIR (Biological Effects of Ionizing Radiation) I Genetics Subcommittee (1972) [105] leading to the acceptance of LNT and perpetuating these errors via the subsequent BEIR committees now through BEIR VII. The paper not only details the peer-reviewed literature but also makes extensive use of the personal papers of numerous leading individuals that helped to determine the acceptance of LNT by the scientific and regulatory communities as well as the general public. Despite its standard toxicological analysis framework, this paper also has elements of a scientific detective story with its many unexpected historical twists and turns. This analysis is also different than the traditional scientific review as it documents a disturbing effort by some leaders of the radiation genetics community of the 1940s-1960s to force the acceptance of the LNT model, at almost any cost. Also discussed is the well-documented evidence of deceptions, obfuscations, and deliberate scientific misconduct, all of which significantly affected the broader scientific and medical communities, and regulatory agencies of the U.S., such as EPA, and worldwide. This, in turn, affected cancer risk assessment policies, practices, and recommendations, and had a major impact on environmental regulation, the public health and medical practices throughout the world.

2. LNT and biological evolution

The linear no-threshold dose response (LNT) in biology was first proposed in 1928, making it now 90 years old [118]. This idea emerged from a stellar duo of physical chemists from the University of California at Berkeley. The leader was Professor Gilbert N. Lewis, a world famous scientist, who would be nominated for the Nobel Prize some 42 times. However, on this occasion, Lewis would step out of his field and enter the more uncertain, murky and speculative domain of biology, postulating a possible mechanism for biological evolution.[2] Finding the principal mechanism for evolution was perhaps the most fundamental question challenging the biological community in the aftermath of Darwin's Origin of the Species and Mendel's discoveries concerning heredity. The situation created a profound intellectual challenge and great competition within the biological sciences. The search centered on the belief that the answer to the evolution question would be directly

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[1] In December of 1927 at the AAAS meeting in Nashville Muller [85] discussed the possibility that he may not have induced gene mutation but massive large scale heritable chromosomal deletions and aberrations.

[2] This 1928 paper [118] on the mechanism of evolution was preceded by Lewis's 1925 Silliman Lecture at Yale University in which he addressed the broad question of evolution, exploring possible mechanisms. Lewis was therefore predisposed to applying his knowledge of the chemical and physical sciences to the study of evolution.

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Abbreviations

AAAS	American Association for the Advancement of Science
AEC	Atomic Energy Commission
APS	American Philosophical Society
BEAR	Biological Effects of Atomic Radiation
BEIR	Biological Effects of Ionizing Radiation
CAG	Carcinogen Assessment Group
EPA	Environmental Protection Agency
ERDA	Energy Research and Development Administration
FDA	Food and Drug Administration
ICRP	International Commission on Radiological Protection
JCAE	Joint Commission on Atomic Energy
LNT	Linear No-Threshold
NAS	National Academy of Sciences
NCRPM	National Council of Radiological Protection and Measurement
NEPA	National Environmental Protection Act
NRC	National Regulatory Commission
PNAS	Proceedings of the National Academy of Sciences
RF	Rockefeller Foundation
SH	Single-Hit

linked to how gene mutations were induced and passed on to subsequent generations.[3]

As early as 1910, Thomas H. Morgan had oriented his *Drosophila* laboratory at Columbia University to the study of genes via the pursuit of heritable mutations. However, despite conducting a vast number of experiments designed to 'artificially' induce genomic mutations by a host of noxious chemicals and physical agents, all attempts seemed to fail. A century later this seems hard to believe, since Morgan's group threw nearly everything it could at the fruit fly genome including high doses of ionizing radiation, all without apparent success. This cascading series of experimental failures, while not discouraging future experimental attempts to induce mutation, led to the belief that the genome must be very stable, nearly immutable. Nonetheless, the answer to the "mechanism of evolution" question required finding a means to mutate the genetic material. What was needed was a novel approach. A solution to this challenge was eventually developed by Hermann J. Muller, at the University of Texas at Austin, and former graduate student of Morgan. Muller modified the experimental fruit fly model, making mutations more readily and unequivocally detected.[4] Muller discovered that very high doses of X-rays administered to the male parental generation induced numerous phenotypic changes in subsequent generations, entitling his paper the 'artificial transmutation of the gene'. Muller was very strategic in the framing of the title since mutation of the gene was believed to be the mechanism for evolutionary change. Moreover, while others had previously reported success with the induction of chromosomal aberrations ([53,77–79] [5]), Muller would clearly emphasize that it was the transmutation of the gene that was the fundamental feature of evolutionary change.

The actual data to support Muller's assertions were reserved for a presentation at the 5th International Genetics Congress in Berlin during September (11th-18th) of 1927 [87]. Muller, who inexplicably failed to cite the prior publication of Gager and Blakeslee [53], quickly became an international figure with much public attention. However, the paper containing the data for the major findings was relegated to an obscure conference proceedings [87]. A review of the Muller proceedings paper reveals it lacked an adequate presentation of research methods, cited no references, was sloppy in the presentation of data, and with three experiments, each with study design limitations. An assessment of this publication strongly suggests that this Nobel Prize research was not peer-reviewed [27].[6] Nonetheless, the findings were reproducible, broadly accepted as novel and highly important, principally because of the enhanced capacity to induce what were believed to be gene mutations [168]. In this rush for discovery primacy, Muller [86] acted by publishing his key paper in *Science* (July 22, 1927) three months prior to the Congress, without showing any data. How this occurred was never explained by Muller or the journal nor did the Congress Proceedings [87] paper receive criticism, possibly because others confirmed its basic findings and/or never read it, being content to read the more highly cited, but data-deficient *Science* publication [86]. On April 24, 1928, Muller would make a follow up presentation of his mutation findings, publishing a substantial discussion of the data in the Proceedings of the U.S. National Academy of Sciences (PNAS) on September 15,1928[7] [88].

Inspired by the findings of Muller, Gilbert Lewis and his colleague Alex Olson soon published a follow-up paper in Nature [118], proposing that the mechanism of evolution was genomic mutation induced by cosmic and terrestrial radiation following a linear dose response (they used the term proportional rather than linear). The linear relationship was significant since it would explain observed changes in all species, ranging from least to most susceptible to mutation. The Olson and Gilbert [118] explanation was based on the findings of Goodspeed and Olson [55] [8] on radiation-induced mutation in the primrose plant at a dose some 500,000 fold greater than background.

The LNT model (using the term 'proportional' for linear) was thus first applied to the concept of biological evolution, not cancer/genetic risk assessment. Initially the hypothesis of Olson and Lewis [118] was supported by research of Hanson and Heys [61] from Muller's laboratory and Washington University, who stated that "natural radiation may be responsible for the mutations which are the grist of the natural selection mill with the resulting evolution of new forms." This remarkable conclusion was derived from an investigation of fruit fly mutations in an abandoned uranium mine. Other support for the Olson and Lewis hypothesis was provided by Refs. [46,47,72].

Due to the prominence of the theory of evolution and the reputations of Lewis and Muller, this hypothesis of Olson and Lewis quickly drew considerable attention. However, in the end it failed to gain traction within the genetics and evolutionary biology communities

[3] As early as 1916, Muller would state in a lecture that "the central problem of biological evolution is the nature of mutation …" [29].

[4] Muller's flies had a mutant X chromosome with a crossover suppresser. This was a large inverted segment that blocked the crossing over phenomenon. These flies also had a recessive lethal mutation along with a dominant bar-eye mutation. This permitted the heterozygous females to be visually identified, thereby essentially eliminating an error in phenotype identification.

[5] Gager and Blakeslee [53] would report the occurrence of radium-induce mutation in the Jipson Weed in January, 1927, this being the first report of an exogenous agent inducing gene mutation. This discovery of Gager and Blakeslee [53] has been sustained over nearly a century. However, the findings of Muller [86] simply overwhelmed the field with his far greater capacity to produce mutations. Gager and Blakeslee would occasionally remind the field of their priority while giving credit to Muller for his findings. See Ref. [28] for a discussion of the Gager/Blakeslee and Muller interactions.

[6] A July 8, 1946 letter of Muller to Edgar Altenburg [92] revealed that the paper he read at the 1927 Genetics Congress was published in the proceedings without any change from the presentation text, thus strongly supporting the belief that the key Nobel Prize winning paper was not peer-reviewed [181,182].

[7] Muller [88] cited his Congress Proceedings paper in his PNAS September 15, 1928 paper with the correct page numbers, but with the incorrect year of 1927 rather than 1928. In fact, Muller's PNAS citation was not listed in the basic Web of Science search. This citation was detected in a "cited reference search" of the Web of Science. The 1927 publication date was again used by Muller in a paper by Muller and Mott-Smith [100]. It appears that Muller may have used the 1927 date rather than the actual publication date (1928) to enhance his claim to primacy for the discovery of gene mutation.

[8] The Goodspeed and Olson [55] paper was used since it provided data rather than the "discussional" paper in *Science* by Muller [86].

since cosmic and terrestrial radiation were only able to account for about 1/1300 of the background mutation rate in the control group fruit flies to what would become the Muller Nobel Prize research using linear modeling [100]. The hypothesis of Olson and Lewis [118] would not be revived, with attention eventually being redirected toward endogenous metabolism with its vast generation of reactive oxygen species (ROS) as a likely mechanistic engine of evolution [43,73].

The reason for the striking failure of the normally highly astute Lewis to discern the problem may have been related to the fact that Muller failed to show any data in his epoch-making *Science* paper, while the proceedings of the 5th International Genetics Congress was published too late and not generally available. As noted above, Olson and Lewis [118] had to rely on the limited findings of Goodspeed and Olson [55]. Thus, it is likely that Lewis was forced to be too speculative in his quest to be the first to offer a plausible mechanism for evolution. A chronology of the history of the LNT is provided in Appendix 1.

3. LNT and the proportionality rule

Even though Muller rejected the hypothesis that background radiation-induced mutation acting via a LNT dose response was the mechanistic engine of evolution, he nevertheless would soon accept the validity of the LNT theory and its later linkage with the single-hit mechanism for ionizing radiation-induced mutation and eventually for cancer as well. While it is not yet possible to pinpoint the timing of the adoption of his belief in LNT, it was probably directly linked to the results of two investigations under his direction. It appears that Muller accepted the validity of the LNT for mutation from findings reported in several published studies [57,59–61,115] [89]. Muller had made the assumption that the dose response was linear down to a single ionization event though the **lowest** cumulative dose from these investigations was extraordinarily high, i.e., approximately 285 rads (r), administered at a high dose rate. In contemporary terms, this was roughly the equivalent of receiving 1000 modern chest X-rays in 3.5 min or about five chest X-rays/second. In fact, the lowest dose using the C1B *Drosophila* model in Muller's Nobel Prize research was nearly 6 fold greater than this dose.

While the above papers were commonly cited by Muller as supporting the LNT perspective during this period of concept consolidation, he failed to properly balance and integrate other contemporaneous publications from similar credible studies that did not support the LNT perspective [125,155,175]. Table 1 provides a listing of contemporary radiation-induced mutation studies that supported the LNT/proportionality relationship and those that contradicted it. All of the studies supporting the LNT were conducted at very high doses/dose-rates, hundreds of thousands times greater than background. While Muller remained silent with respect to the challenging studies, he would eventually need to address the issue of cumulative dose, dose-rate, and the nature of the dose response in the low dose zone via improved and more insightful experimental protocols.

The period from about 1927 to 1932 represents the first stage in the historical assessment of the LNT model. While Muller provided the experimental vehicle, Olson and Gilbert [118] created the conceptual framework (i.e., linear dose response) and application (i.e., evolution mechanism), even if those were ultimately rejected. Oliver [115] and Hanson and Heys [59–61] provided evidence to support the occurrence of LNT, even though at extremely high doses/dose-rates. In fact, as this period would come to a close, Muller would transform these developments into no less than a quasi-biological law called the Proportionality Rule, the term Muller used for the LNT concept. The 'proportionality' term was apparently borrowed by Muller from the Olson and Lewis [118] paper, transformed into a 'Rule', which quickly gained standing within the radiation genetics community, but not much further. Table 2 provides a series of citations and a quote within each, showing how the radiation genetics community used the concept of proportional dose response to describe the linear dose response for ionizing radiation and mutation. As described with the quote from Hanson [58] the mutation incidence was directly proportional to dosage and that 'Muller named this the proportionality rule'.

The seminal work of Muller [86] reported for the first time that an external agent, ionizing radiation, could induce gene mutations (i.e., 'artificial transformation of the gene') in the fruit fly genome as inferred from phenotypic changes observed in the next generation. While this was the principal focus for Muller and about which most observers focused, he also directed attention to the concept of dose response, since his Nobel Prize study designs [87] were inadequate to assess the dose-response relationship issue.

Muller's Nobel Prize research initially involved experimentation with a homogenous strain of *Drosophila* females with heterozygous males. In this first experiment he exposed the flies to four 'doses' which were Dose x Duration of X-ray exposures [i.e., 12 (i.e., 810 r total dose over the 12 min), 24, 36, and 48 min]. The two highest doses/durations were quite toxic, inducing sterility in 70–80% of the males. At the lowest dose/duration tested Muller induced a single apparent mutant offspring with a phenotypic change. The phenotypic change rate would increase notably for the 24 min treatment (i.e., 1620 r) over the response of the 12 min exposed group. Choosing not to replicate this four dose/duration treatment study, which suggested the possibility of a threshold at the 12 min duration, Muller switched to his new C1B fruit fly strain, which was a model that gave unambiguous sex-linked mortality results. However, instead of testing over the original four doses, Muller opted to use only the 24 and 48 min duration periods, thereby seemingly attempting to prevent a possible no effect dose at the low end while still maintaining a dose that retained a high risk of toxicity/sterility. This follow up two dose experiment yielded a limited dose response that also was not linear with Muller reporting the increase as a square root function (√2) rather than a doubling (2-fold increase) for a linear response.

Follow up research by Oliver [115] using the C1B strain model would be critical in establishing Muller's belief in LNT. In this four dose study the lowest dose tested was sufficiently effective in that it increased mutant lethals by nearly six fold over control values, making a linear dose response. However, when a legitimate challenge to an LNT mutation interpretation was published, as in the case of Stadler [155], it was ignored. For example, Stadler [155] assessed mutagenicity involving 13 radiation doses in barley with the three lowest doses showing no enhanced mutation over the control, reflecting the possibility of a threshold dose response and a challenge to the LNT concept. Despite its enhanced power and greater dose response relevance, such findings were apparently ignored even though Stadler [155], raised the possibility of there being a threshold in his discussion by stating that 'the absence of mutation in the cultures given the three lowest doses might suggest the possibility of a threshold intensity below which mutations do not occur … '.

Stadler would more seriously challenge Muller for the rest of his life (dying of cancer in 1954) over the key assumption that Muller had actually established what he claimed: induced the artificial transmutation of the gene (i.e., mutation) [96,101,127,158,159].[9] While Muller continued to assert that the X-rays induced precise 'point' mutations in single genes (e.g., what today would be called base pair mutations) Stadler [156–158] hypothesized that Muller's mutations were not precise but often, and perhaps totally, manifestations of massive deletions and various genic rearrangements that could involve multiple genes [26]. In contrast to Stadler's description, Patterson and Muller [127] referred to these transgenerational phenotypic alterations as due to 'progressive' mutations/changes, which they argued were the essential foundations of evolutionary change. If Stadler's views were to be persuasive then the

[9] Even after his death, Stadler would challenge the Muller interpretation as his last Ph.D. student Gerry Nuffer [113], who acknowledged the help of Stadler, would publish detailed findings in maize showing no evidence that X-ray-induced transgenerational phenotypic changes were due to gene mutation. He identified a variety of other chromosomal/gene interactions (e.g., position effects) that might account for the findings, thereby challenging the generality of the Muller findings to plant genetics. The challenge of the Stadler/Nuffer findings were broadly applied by others although the authors were astutely careful in their wording.

significance of Muller's findings would be profoundly diminished, with the results representing a more modest extension of earlier X-ray induced chromosomal (i.e., non-gene) aberration research. While these two titans (i.e., Muller and Stadler) of radiation genetics were unrelenting in their debates (since the stakes were so high) Muller [90] would temporarily prevail (as 'validated' by his Nobel Prize in 1946), possibly due to the power of his personality, and that he outlived Stadler[10] who struggled with cancer over the last eight years of his life. However, once molecular techniques had advanced following the deaths of Muller and Stadler, the data would clearly reveal that Stadler's views were largely vindicated [50,52,111,112,114,123,139,165–167,170]. In contrast, at the high doses used by Muller [86,87] the damage to the mature fruit fly spermatozoa genome would be dominated by massive deletions and other large genetic lesions [117], making the progressive point mutation hypothesis untenable. Reflecting the view that Stadler's interpretations were not only correct but also vindicated can be seen in the judgments of two of Muller's closest radiation geneticist colleagues, Crow and Abrahamson [41]. Nonetheless, the early and widespread acceptance of Muller's far more poorly supported interpretation of the nature of the X-ray-induced genetic damage at high doses would lead directly to the creation of the clearly flawed LNT Single-Hit model.

4. Linking LNT with single-hit

By the end of 1932, Muller had developed what seemed to be a firm belief in LNT for X-ray induced gene mutation. However, this belief was without an underlying mechanism or an experimental study in which the protocol would be able to test the legitimacy of the LNT model. These ostensible weaknesses (e.g., very high doses, lack of mechanisms, weak cytogenetic analysis) of the data supporting LNT would be partially rectified by the end of the decade, even if the studies themselves providing the 'rectification' had important limitations. In the case of mechanisms, Muller received a huge boost when he linked up with Timofeeff-Ressovsky, the outstanding Russian radiation geneticist, working in Berlin from 1932 to 1934 and several other international leaders in the physics community such as Neils Bohr, Max Delbruck and Kevin Zimmer. Muller and Timofeeff-Ressovsky would provide some of the key mutational data while the physicists contributed the mechanism, based on X-ray exposure and target theory. While target theory was first developed for use in predicting how chlorine disinfection might kill bacteria (see Refs. [22,36]), it was soon adopted by physicists ([42,130,178]) to explain X-ray-induced mutagenicity. The physicists demonstrated that the more hits needed to produce a gene mutational effect, the more threshold-like the dose responses would appear ([10,180]). In contrast, as the number of hits approaches one, the more linear the dose response would appear (Fig. 1). Thus, the conclusion was judged to be clear. The X-ray induced linear dose response for genomic mutations in the male fruit fly mature spermatozoa was best accounted for with a single hit model using target theory. As a result of this radiation biologist-physicist collaboration, the LNT-Single Hit (SH) model was created.[11] The result of this collaboration was published in 1935 by Timofeeff-Ressovsky and colleagues. Unfortunately, this potentially groundbreaking paper was published in a new journal that was cancelled after only one year, profoundly reducing its potential impact on the scientific community. The obscurity of the Journal and the fact that it only lasted one year, prevented the paper from citation in leading

Table 1
Dose response mutagenicity data at the time of linearity concept consolidation (Circa 1928–1934) (Source: [13]).

Reference		#Doses		
Supportive of Linearity				
Oliver [115]	*Drosophila*	5 doses	X-ray	Lowest dose 275 r
Hanson and Heys [62]	*Drosophila*	2 doses	Radium	Lowest dose 6315 r
Hanson and Heys [63]	*Drosophila*	13 doses	X-ray	Lowest dose 445 r
Timofeeff-Ressovsky [169]	*Drosophila*	5 doses	X-ray	Lowest dose 1400 r
Not Supportive of Linearity				
Muller [86,87] (Exp 1)	*Drosophila*	4 doses	X-ray	
Muller [86,87] (Exp 2)	*Drosophila*	2 doses	X-ray	
Weinstein [175]	*Drosophila*	2 doses	X-ray	
Hanson [57]	*Drosophila*	2 doses	X-ray	
Hanson and Heys [60]	*Drosophila*	2 doses	X-ray	
Stadler [155]	Barley	15 doses	X-ray	
Serebrovsky and Dubinin [146]	*Drosophila*	3 doses	X-ray	

[10] In order to preserve the uniqueness and significance of the artificial transmutation of the gene concept/findings, Muller would publish an 82 page paper in 1930 with his University of Texas colleague J.T Patterson. The focus of the paper centered on whether the X-ray-induced transgenerational phenotypic changes were due to losses (deletions) and rearrangements of portions of chromosomes or rather the so-called "progressive" point-like, genetic changes that he believed drove evolution. This article, in many ways, reflected the pattern of Muller's professional life. He marshaled as much evidence as possible, presented it in excruciating detail and never compromised on an essential point knowing in advance that he had to defend the artificial transmutation of the gene concept [26]. What then was the basis of his belief that he had induced intra-genic (i.e., "real") mutations. The cited reasons for this belief/conclusion included: (1) the general randomness and specificity of induced phenotypic changes (called mutations); (2) identical phenotypes were independently affected; (3) that phenotypic changes were dose dependent; (4) that numerous toxic chemicals were not effective in producing such changes and (5) (similar to 4) "most important of all, probably, is the fact that a direct and simple proportionality has been shown to exist between the frequency of the induced mutations and the amount (energy) of the radiation absorbed." Patterson and Muller [127] cited Hanson and Heys [60] and Oliver [115] to support this conclusion. Patterson and Muller [127] then stated that "there is no indication in the results of any lower critical intensity, or threshold value, beneath which there is no (or a relatively lesser) effect." In the body of the paper Patterson and Muller [127] would also emphasize that X-rays could on occasion induce reversible changes such as white eyes to red and the reverse, supporting a view that relatively modest phenotypic changes could be induced that reflected normal "spontaneous mutations". The problem with the Muller argument was that it was based on logic, inference and parsed arguments. Missing from his views was actual proof concerning the nature of the radiation-induced genetic lesions over the tested dose range. Thus, from 1930 to the mid-1950s the Stadler and Muller perspectives would collide, awaiting advances in methods that would permit determination of the nature and size of genetic lesions. Of importance was that Painter [120–122] provided novel cytogenetic staining techniques for *Drosophila* chromosomes based on the earlier work of McClintock [80–82] for corn. These techniques would clearly show that the X-ray treatments used by Muller produced a very high level of chromosomal aberrations, weakening his point mutation argument. The reverse mutation explanation offered by Muller was also refuted in multiple studies (e.g., [70,71,174]. Additional methodological advances would emerge with the development of the Southern blot [153] and PCR a decade later [102]) which further supported the Stadler position.

[11] The mutational effect was viewed as being caused by one or a few discrete, basic biophysical effects, which were conceived to be "hits" on a "target". The genetic mutation was assumed to be a "pure physical event" with no physiological or biological involvement [173]. From a range of ideas as to what constituted a hit, it was possible to then derive statistical models of dose-responses. If only a single hit on a single target was needed to induce the effect, with the percentage rate of the effect graphed on a logarithmic scale, the dose response would appear then as a straight line. The various mathematical model predictions were then compared to actual data in the dose-response studies. The visual confirmation of the emerging theory with actual mutagenic dose response data made the LNT single-hit model believable and readily accepted. This process led Timofeeff-Ressovsky et al. [169] to assert that gene mutation was a "one-hit" process, caused by a single ionization from a quantum of radiation on a sensitive region of the genome. They even went so far as to estimate the physical features of a sensitive region, it being about the size of a large organic molecule [10].

Table 2
Documentation of the introduction of the proportionality rule concept into the mutation literature, 1929–1960 (Source: [17]).

References	Quotes
Hanson and Heys [60]	"It is only to be expected that the number of mutations be directly *proportional* to the number of rays to which the organisms are exposed." Page 207
Muller [89]	"Since then Hanson, using radium, and Oliver in our laboratories using X-rays, have both found that the frequency of mutations produced is exactly *proportional* to the energy of the dosage absorbed … There is, then, no trace of a critical or threshold dosage beneath which the treatment is too dilute to work." Page 236
Oliver [115]	"That is there is a direct *proportionality* between the percent of lethals and the length of time of treatment may be seen more readily by a comparison of the t1 values calculated from the results for each of the given doses." Page 45
Stadler [155]	"Mutation frequency increased approximately in direct *proportion* to dosage." Page 13
Hanson and Heys [63]	"Taking the amount of ionization in air as a measure, the mutation rate seems to vary approximately in direct *proportion* to the intensity." Page 142
Oliver [116]	"By inference it can be added that the cosmic and the terrestrial radiations of higher energy content also are capable of producing mutations in *proportion* to their power of ionization." Page 480
Oliver [116]	"The relation of *proportionality* to the dosage applies not merely to the lethals in general, but, more specifically, to the lethal gene mutations." Page 485
Oliver [116]	"… [gene mutations and gene rearrangements] … all probably occur in direct *proportion* to the dosage, no matter how small a dose is used." Page 486
Patterson [126]	"In general their results [i.e. [59,115]] justify the conclusion that the rate is directly *proportional* to the dosage employed." Page 133
Hanson and Heys [62]	"Further evidence of the *proportionality rule* from a study of the effects of equivalent doses differently applied." Page 335
Hanson and Heys [62]	"Experiments planned with a view to determining within what limits the *proportionality rule* holds show again a strict correspondence existing between the amount of radium administered and the consequent biological effect, the induced mutation frequency obtained varying directly with the dosage." Page 343
Hanson [58]	"The rate seems to be directly *proportional* to the dosage. Muller has named this the '*proportionality rule.*' For example, when all other factors are kept constant, doubling the time of exposure also doubles the number of lethal mutations." Page 486
Oliver [117]	"The frequency of induced mutations is directly *proportional* to the intensity of the treatment." Page 391
Delbruck [44]	"The *proportionality rule* gave the basis for the single-hit interpretation …" Page 359
Stern [161]	"The *proportionality rule* has been proven to hold over a wide range. Figure 155 shows that, for Drosophila, the relation is essentially linear over the range from 25 r to several thousand r. It has further been shown that the frequency of induced mutations is independent of the time over which the radiation is applied." Page 433
Stern [162]	"It has been established for a variety of experimental organisms that the number of mutations induced by radiation is proportional to the dose. This *proportionality* has been proven to hold over a wide range of dosages." Page 491

indexes, creating what would have been a virtual academic death sentence had these authors not been so prestigious and professionally connected. Nonetheless, Muller's Proportionality Rule now had a potential mechanism that could account for its findings and a new name: Linear-No-Threshold (LNT) single hit model.

5. Dose-rate and LNT

While the professional disputes between Muller and Stadler over transgenerational phenotypic changes and mutations were significant, acceptance of Muller's gene mutation view was essential for the development of the LNT single-hit dose-response model. It is now known that the lowest doses employed by Muller in his Nobel Prize research induced massive deletions throughout the genome with many probably approaching and exceeding 100 kb (kilobase) size along with other major genetic alterations [50].[12] The physical deletion of large chunks of DNA, damaging dozens to multiple hundreds of thousands of nucleotides, affecting numerous genes in large numbers of cells, as well as inducing substantial inflammatory responses within and between cells, is not compatible with the basic features of the LNT-SH model as described by Timofeef-Ressovsky et al. [169]. Despite the fact that modern advances [69,139] refuted the single-hit interpretation by Timofeef-Ressovsky et al. [169], these critical insights have only recently been used to reassess the validity and historical foundations of the LNT single-hit model [24–26].

The scientific basis for the LNT single-hit theory as developed by Refs. [86,169] and others was improperly framed, based upon incorrect assumptions, lacking essential understanding of induced genetic damage and its biological significance. Thus, from a theoretical basis, the LNT single-hit theory-model represented a type of biological reach that was excessively ambitious, lacking credible genotoxic information required for the development of a mechanistic model for risk assessment/regulatory purposes. Yet, despite such serious limitations, the LNT findings would be integrated with the one-hit mechanism; it was easily understood from a conceptual perspective and would be later readily (if uncritically) adopted by governmental regulatory agencies.

While the LNT single-hit model was a key step forward, its credibility remained limited, having only a descriptive high dose experimental basis requiring substantial extrapolation from high to low dose and a mechanism that was expressed with mathematical simplicity through experimental validation at low doses. Despite such multiple challenges coupled with the lingering and documented doubts of Stadler and others, Muller would eventually develop a way to attack the model-validation question experimentally.

This new experimental approach was based on the assumption that X-ray induced mutations were cumulative and irreversible. Under such a set of conditions, it was predicted that the total/cumulative mutation damage would be the same regardless of whether the dose of radiation was given acutely or spread over a prolonged period of time. It is not clear where Muller first got this idea but the concept was similar to the Bunsen-Roscoe Law (1862) and Haber's Law [12,84,176,177] both of which described a type of Concentration X Time = Constant relationship. Several references relating to application of the Bunsen-Roscoe Law were available to Muller and his graduate student Ray-Chaudhuri in the years leading up to the research and may have influenced them [68,143,160]. The Bunsen-Roscoe Law has been referred to as the 'Reciprocity Law', discovered by the famous chemist Robert Bunsen and his colleague H. Roscoe in 1862. This 'law' indicates that the amount of product of a photochemical reaction is the result of the total amount of radiation energy hitting the photochemical system, in effect, an intensity x time formulation. The strengths and limitations of Haber's Law had been broadly assessed but usually within the framework of inhalation toxicity, not radiation genetics, or dose-genomic mutation incidence. When Muller became the advisor of Ray-Chaudhuri at the University of Edinburgh in the late 1930s, this new experimental dose-time framework became his dissertation area, working with mature

[12] If Painter's cytogenetic staining of *Drosophila* had been available a decade earlier so that the extensive X-ray induced damage to chromosomes of Muller's fruit flies in 1927 were better appreciated, it may have averted the development of the LNT Single-Hit theory. However, by the time Painter had published his findings, the Timofeeff-Ressovsky et al. LNT Single-Hit model concept was well on the way to being finalized.

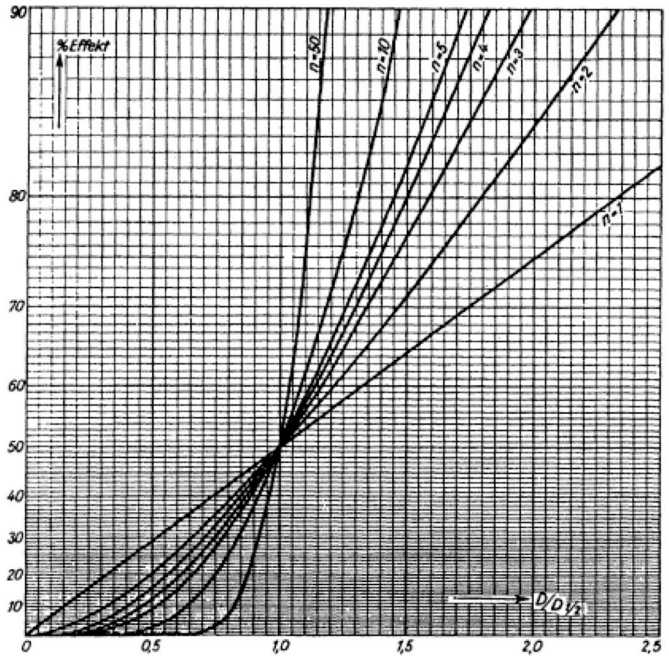

Fig. 1. Model dose-response curves, calculated theoretically for various number of "hits," n on a single "target" assumed necessary to produce the effects (From [180]).

male fruit fly spermatozoa. Ray-Chaudhuri would cite the Bunsen-Roscoe Law as the theoretical framework for his research on the dose-intensity x time relationship [131,132]. The most likely reason for citing the Bunsen-Roscoe Law rather than Haber's Law was that several papers had been reported on the effects of X-rays on biological end-points based on the Bunsen-Roscoe Law, whereas Haber's Law had yet to be applied in such a manner.

Using the Muller fruit fly model [132], Ray-Chaudhuri obtained data that provided support for the LNT hypothesis for radiation-induced mutagenicity based on the mature male spermatozoa, all with a quantitative model that appeared theoretically sound. An important problem remained however, since the research of Ray-Chaudhuri had critical limitations. In general, these problems included inadequate control groups, acknowledged major statistical errors by this Ph.D. Committee [56], and issues with experimental quality control features, some of which were recognized by Caspari in correspondence with Stern (American Philosophical Society [3,4]). In specific terms, the Ray-Chaudhuri study was of modest size and lacked the reporting and documentation of multiple essential methodological parameters. The paper also did not include data on lethal clusters, the occurrence of female sterility and fertility, sex ratios, as well as the age of the males, and other factors. He also made a decision to change to a different fruit fly strain midway through the set of experiments, a decision without any explanation. Of significance was that the new strain displayed a control group mutation incidence of approximately one third of the strain it replaced. Yet, Ray-Chaudhuri simply combined the data of the different strains claiming there were no strain differences. None of these limitations were noted by Professors Haldane or Muller in their written assessment of Ray-Chaudhuri's dissertation (Ray-Chaudhuri material – Muller File, Lilly Library, Indiana University). Furthermore, in letter exchanges between Ray-Chaudhuri and Muller, it was clear that Muller was not present for most, if any, of his experiments due to travel, failing to offer crucial, timely and hands on guidance (Muller to Ray-Chaudhuri, Muller File, Lilly Library). Nonetheless, all these weaknesses were carefully submerged, as Muller was seemingly intent on using the Ray-Chaudhuri study to promote the LNT in the face of much skepticism. In fact, Muller [90] would use the Ray-Chaudhuri data to promote the LNT model in his Nobel Prize Lecture.

Muller would frame the dose-rate study of Ray-Chaudhuri as a possible game changer. However, this would not be the case if the experimental limitations could not be overcome. In fact, the soon to be initiated Manhattan Project mutation studies would provide such an opportunity. Muller once again found himself involved, this time as a paid consultant with Professor Curt Stern at the University of Rochester. The research was to evaluate the effects of X-rays/gamma rays on fruit flies, with the intent of assessing the nature of the dose response and dose-time response for transgenerational genomic mutations. That is, Stern was going to assess whether linearity at low doses occurred, making use of Muller's dose-rate methodology, and the Muller-5 strain of fruit fly. This study would be a far stronger one than Ray-Chaudhuri's with respect to technical capacity, study design, sample size, statistical power, quality control, professional supervision, and at a dose rate approximately 1/6 of the Ray-Chaudhuri studies.

6. The Manhattan Project: testing the LNT hypothesis

One component of the Manhattan Project assessed whether the dose-response for mutagenicity induced by ionizing radiation was linear via a total dose/dose-rate experimental protocol using the fruit fly. Those associated with the study expected the X-ray-induced mutations to be independent of dose-rate, and explained by the total dose received based on the Ray-Chaudhuri [131,132] findings. However, a significant problem occurred when Ernst Caspari, who was conducting the chronic exposure part of the research, reported to Stern in August 1946. His findings not only did not support the total dose hypothesis, but also displayed a threshold, showing a tolerance dose [14]. These findings were potentially important since they challenged those of Ray-Chaudhuri [131,132], which in turn had been used as key foundational support for the LNT. As noted above, the Caspari study had much going for it. In fact, the integration of the Spencer/Caspari experiments combined to yield a dose-rate study that was designed to 'settle' the dose-rate question that the Ray-Chaudhuri report initiated.

Upon learning of the Caspari chronic experimental data, which supported a threshold and/or tolerance dose, Stern refused to accept the findings as valid, claiming that the problem was not with the dose-rate and linearity hypotheses but with Caspari's control group. This involved Stern's claim that because mutations in this control group were aberrantly high it would lead to the absence of a treatment effect, and show a false threshold dose response [5].[13]

What eventually evolved in response to the Caspari/Stern dispute would be both surprising and historically significant. Caspari refuted the claims of Stern by showing that his control group was consistent with the published literature, forcing Stern to withdraw the criticism [5]. However, Stern (and perhaps with Caspari's consent) wrote in their discussion of the manuscript that the strikingly new threshold supportive findings should not be accepted until it could be determined why these results seemed to conflict with the acute data of the Spencer experiment [154]. A problem with this position was that the Spencer and Caspari studies used significantly different methods and it was not practically possible to resolve their divergent results (see Table 3 for differences). Stern seemed, however, very comfortable accepting the methodologically inferior Spencer report [154] rather than the threshold-supporting findings of Caspari.

Stern asked Muller to review the now drafted Caspari manuscript just prior to his Nobel Prize trip/lecture. Muller acknowledged its receipt and commented on it in a letter to Stern (November 12, 1946 - [91]) stating that these findings (1) challenged the current LNT dose-response paradigm, (2) needed to be replicated, and (3) that Caspari was a competent researcher and he would not dispute his research findings. Despite such a written statement just prior to his Nobel Prize,

[13] See Calabrese [14] for a detailed, point-by-point discussion of this dispute.

Muller would go on to claim in his Nobel Prize lecture that there was no scientific evidence to support even the possibility of a threshold dose response. The only option he insisted that was possible was the linear dose response. He believed there is 'no escape from the conclusion that there is no threshold dose' (Nobel Prize lecture, HJ Muller, December 12, 1946). The above described sequence of events suggests that scientific certainty about this key issue had devolved into a belief system.

The Caspari and Stern draft manuscript sent to Muller on November 6, 1946 contained the following sentence in the conclusion: 'From the practical viewpoint, the results presented open up the possibility that a tolerance dose for radiation may be found, as far as the production of mutation is concerned'. This statement of Caspari and Stern ([31]- page 15) which was sent to the AEC archives and to Muller, made it clear that Caspari and Stern believed that a threshold for ionizing radiation induced mutation was 'possible'. In fact, the only changes in the entire paper after Muller's review was the elimination of this sentence in the summary and the addition of Muller's name in the acknowledgement section. These insights into Muller's dynamic intervention with the Caspari and Stern manuscript reflects that both Stern and Caspari believed that a threshold interpretation best applied to their mutation data. Yet, Muller would inexplicably override this possibility, while recommending replication of the study in private communications with Stern [Muller Letters to Stern - November 12, 1946 [91] and Jan 14, 1947 [93]]. The conflict that Muller had with the Caspari data became obvious. How he dealt with it became problematic.

We see that Muller not only had a strong belief in the LNT but a profound bias as reflected in his misleading and deceptive comments at his Nobel Prize lecture. Muller would go on to repeat the original criticisms of the Caspari work [94,95],[14] relating to the control group while Muller's own research produced copious data indicating that the Caspari control group data was fully consistent with his own data and the published literature [19].

The situation became more complicated when Stern tried to replicate the Caspari findings from the fall of 1946–1948, now working with a new graduate student, Delta Uphoff. The first major experiment by Uphoff was problematic, as it appeared that the control was aberrantly low, about 40% below normal. Having just gone through the Caspari control group dispute, Stern needed to get the control group issue settled. He then entered into a series of letter exchanges with Muller on the topic, focusing on the Caspari and Uphoff data. Since Muller was extensively researching control group spontaneous mutation variability with the same model (in his continuing dispute with Stadler over gene mutation), he was in an ideal situation to inform Stern. In these letter exchanges Muller was highly supportive of the Caspari data.[15]

As a result of this evaluation, the Uphoff data were viewed as 'uninterpretable' as Uphoff and Stern [171] wrote in a then classified full-length manuscript for the Atomic Energy Commission (AEC). Further complicating the matter, these authors (Stern and Uphoff) blamed the problem on 'investigator bias' (i.e., 'may reflect a personal bias of the experimenter') in the discussion of the paper without explaining what this meant and who was to blame for the bias, as well as whether such bias affected other ongoing experiments and staff, and how Caspari's study would be interpreted. The second of the replication experiments of Uphoff would also yield a similarly aberrant low control group, thereby making two major experiments uninterpretable. The final Uphoff experiment was also problematic, not because of the control group, but because the low dose radiation treatment induced mutations that exceeded predictions of the LNT model by several fold [14].

With World War II over, the Atomic Energy Commission (AEC) needed to have reliable data to guide it on the assessment of the health effects of low doses of radiation. The AEC had invested in mammalian and insect (i.e., fruit fly) studies at the University of Rochester. In the case of the mammalian studies as lead by Professor Donald Charles, no effective guidance was forthcoming. This was believed to be due, at least in part, to the well-known and highly frustrating reluctance of Charles to release/publish his findings unless fully confident with the experimental results. Even after the use of over 400,000 mice, little of value was shared with the scientific community by that group. This included a brief, three-page summary in 1950 [34], some five years after the war ended. By 1954 with no follow-up publication, Charles had resigned from the University and a year later he committed suicide [6]. A summary paper was eventually published in 1961 that was far too late [35], as these efforts had been surpassed by the striking research findings on dose-rate using the mouse model by William L. Russell as discussed below.

For differing reasons, Stern was also being challenged to produce results. In his case, the problem was that each large experiment had a significant concern or flaw as discussed above. Refusing to accept the idea that his research would not achieve the stated goals, Stern decided to rehabilitate the uninterpretable (and experimenter biased) data of Uphoff and to re-marginalize the findings of Caspari, without sharing the detailed 'inside' story validating the Caspari control group as described earlier. This resulted in Uphoff and Stern [172] publishing a one page technical note in *Science* summarizing all five major experiments (i.e., Spencer, Caspari and three by Uphoff), integrating them to claim support for the LNT. They promised the *Science* readership a subsequent highly detailed paper with all the necessary methods, materials, and data. However, they failed to fulfill this pledge. Nevertheless, lacking supporting data the *Science* paper became highly influential, propelling the acceptance of LNT, even though there is no evidence that Stern's radiation geneticist colleagues ever requested the detailed follow-up paper.

6.1. Facilitating the acceptance of LNT: role of Rockefeller Foundation and the National Academy of sciences – the BEAR I Genetics Panel

During the early 1950's aboveground testing of nuclear weapons led to increased worldwide exposure to various radionuclides prompting public health concerns. This would lead to the Rockefeller Foundation (RF) funding the U.S. NAS to undertake a detailed assessment of multiple areas of concern (i.e., oceanography and fisheries, meteorology, waste disposal and dispersal, agriculture, pathology, and genetics). The President of the Rockefeller Institute for Medical Research (later renamed Rockefeller University) as well as the President of the National Academy of Sciences (NAS) at that time was Detlev Bronk. In reality, therefore, Bronk was responsible for funding some of his activities as President of the NAS. In this dual role, Bronk selected Warren Weaver, a mathematician, and the long-term scientific director of the RF, to chair the NAS Biological Effects of Atomic Radiation (BEAR) Genetics Panel. Furthermore, Weaver knew most, if not all, of the BEAR I Genetics Panel members, and had long funded some of them such as Sonneborn and Muller, contemporaneously, during the time of the Genetics Panel proceedings. As reported by Wynchank [179], prior to the creation of the Genetics Panel, the RF had funded close to four million dollars to the University of Indiana for research in the area of radiation genetics alone.

At the start of the BEAR I Genetics Panel Weaver showed his power

[14] In footnote 1 on page 10 of [94] Muller stated that "Uphoff and Stern have published a report of further work, with doses as low as 50 r, given an intensity as low as 0.00165 r per minute. The results obtained are entirely in conformity with the one-hit principle. A consideration of these results, together with the early work, leads to the conclusion that the deviation first referred to (the Caspari and Stern [32] findings) was caused by a value for spontaneous mutation rate that happened to be unusually high." Muller [95] would continue his discredited criticism of the Caspari and Stern [32] paper, repeating the "unusually high control frequency" (page 476) conclusion as a basis to reject its challenge to linearity. These statements of Muller complemented the deceptions of Stern, thereby further enhancing the acceptance of LNT while also preventing his deceptive remarks at the Nobel Prize lecture from being discovered.

[15] See Appendix A, Calabrese [16] for a set of the Muller-Stern exchanges.

over funding support by stating that he would 'try to get a very substantial amount of free support for genetics if at the end of this thing we have a case for it. I am not talking about a few thousand dollars, gentlemen. I am talking about a substantial amount of flexible and free support to geneticists' ([104]; BEAR I Genetics Panel Transcripts February 5, page 35). This is a significant statement as it had the intention of encouraging Panel members to align their recommendations with what the RF believed was important if they wanted to continue to receive Foundation funding. Weaver would further contextualize his funding remarks with the statement: 'There may be some very practical results – and here is the dangerous remark – don't misunderstand me, we are all just conspirators here together'. Weaver's comments are unambiguous, linking project outcomes to RF funding interests and the needs of the radiation geneticists of the Panel.[16]

At the time of the convening of the BEAR I Genetics Panel, the threshold model guided U.S. government policy for assessing risks for both non-carcinogens and carcinogens. Members of the radiation geneticist community, as lead by Muller, had long challenged this view hoping to change it to a proportionality/LNT model. However, on multiple occasions on national and international advisory committees, radiation geneticists had failed to be sufficiently persuasive, never having the votes to replace the threshold with the LNT model. This was principally due to the fact that these Advisory Committees were dominated by persons trained in medicine rather than radiation genetics. This situation would change by design of the RF, via Bronk and Weaver. They made sure that there would be a distinct Genetics Panel that would be separate from a Medical (i.e., pathology) Panel and the RF would uniquely highlight and distribute its findings via multiple, highly visible and influential venues. This is really all it would take to affect a change in national policy for the next six decades.

I was interested in reading the meeting transcripts of the BEAR I Genetics Panel, seeing a substantial debate between proponents of the threshold and LNT models. While I knew that LNT had won the dispute, I wanted to see how these experts debated, which arguments dominated, and which geneticists were most persuasive. My expectations were far too high. As it turned out there was no debate. What happened early on in Panel activities was that Tracey Sonneborn, Muller's colleague at the University of Indiana, read into the record the equivalent of the Radiation Geneticist Mantra, indicating a belief that all radiation-induced mutational damage was cumulative, irreversible, and lacking repair. This combination of factors led to their belief that the dose response for radiation induced mutations was linear down to a single ionization. Radiation genetic risk assessment was best explained based upon the total dose; dose-rate, regardless of how low, would only result in cumulative damage. Sonneborn was not challenged on any point. This was a curious situation since most of the members of the Panel were often opinionated, at times had disputes with each other, and sometimes these interpersonal disputes were rather inflammatory. For example, Muller resigned from the editorial board of Advances in Genetics in a dispute with Demerec, the Editor-in-Chief, over publication of a manuscript by Ref. [33]. Demerec accused Muller of attempting to impose his version of scientific censorship [45]. Yet, despite their apparent personal rift, they were in full agreement on LNT.

Since the BEAR I Genetics Panel was in agreement that the dose-response was linear for radiation-induced mutagenicity it soon found itself with little to do. To fill this void, Weaver challenged the geneticists on the Panel to independently provide their detailed written estimates (including methodology) concerning the number of genetic defects the American public would experience assuming the gonads received a specific dose of ionizing radiation. This genetic damage was to be estimated for the next ten generations. Since the Panel was comprised of a broad range of geneticists (e.g., bacterial, paramecium, fruit fly, mammalian, clinical, population-based, etc.) each was encouraged to use their own education, training, and research methods to derive their independent answers. It was felt that if the estimates of harm from such divergent, but complementary perspectives closely converged, it would enhance confidence in policy recommendations.

Following their meeting on February 6, 1956, the Panel members were expected to complete their analyses over the next month. Of the 12 geneticists on the Panel (i.e., there were 13 to start but one resigned due to academic obligations), nine took up the challenge and provided detailed separate reports within the next month. The other three Panelists declined to submit comments, principally because they believed there was too much uncertainty such that any estimates would not be reliable. In the case of the human geneticist, James Neel, at the University of Michigan and expert on the effects of the atomic bomb on Japanese survivors, he was particularly animated in asserting his position based on written correspondence with Weaver. Neel believed that the uncertainty would be so substantial that it would be unethical to even provide them. More specifically, Neel wrote to Weaver the following on April 17, 1956:

> "The geneticist has social responsibilities, but he also has the responsibilities as a scientist. One is that in an area as critical as this one is, he must beware of letting his conjectures get too far in advance of his facts. It is to me an exceedingly tenable position, having stated the general genetic argument, to say flatly that we know so little about the quantitative aspects (see Ref. [20])."

The letter once again reflected the opinion of Neel that providing population-based estimates of genetic damage was an indefensible exercise and that he would oppose doing such that 'he would go down with flags flying and guns booming to the last' [108].

When the nine separate assessments were received, they were given to Jim Crow to collate, organize, and summarize so that the Panel could more easily assess the submitted reports (his specific function was 'to go through all the damage estimates, compare them, and display assumptions, methods, input, and results in some sort of chart or graphic form'. [20]; page 4). Upon his initial review of the received estimates, Crow sent a letter to Weaver on March 7, 1956 [37] stating the following:

> "Upon looking at the estimates I realized two things. One is that nobody seems to have very much confidence in them. The second point is that those who arrived at comparable estimates usually did it by comparable procedure so that they are not very independent."

Less than one week later Crow again wrote to Weaver [March 12, 1956 [38]]. 'The groups differ widely in their confidence in the best estimate, as indicated by their grossly discrepant minimum and maximum estimates.' In a follow up March 29, 1956 letter to Weaver [39], Crow wrote, 'The limits presented on our estimates of genetic damage are so wide that the reader will, I believe, not have any confidence in them at all.' That is, even though the expert radiation geneticists were told to assume that the dose response would be linear (thus already restricting group variability), the estimates nonetheless profoundly varied. The degree of disagreement was so substantial that Crow asserted that if these values were shared with the scientific community and the general public that it was likely that the Panel's scientific and policy recommendations would have little credibility. This was the key

[16] Obtaining grants to support research was important to some NAS BEAR Genetics Panel members. Personal Correspondence of some of the BEAR Genetics Panel members reveals they were motivated, at least in part, by self-interest, to overstate public health risks to promote their scientific and personal/professional agenda [18]. The fact that distinguished radiation geneticists of the BEAR Genetics Panel may have been willing to exaggerate risks (i.e., be dishonest - in their words: "stretch a point") to enhance their chances to obtain funding, is a critical finding as this type of self-interest has been usually only applied to scientists funded by private interests. What the comments reveal is that academic researchers who are dependent on government/foundation grants may be similarly susceptible. In the present case, these findings may be profoundly significant as the switch to the LNT model had major public policy implications.

Table 3
Differences between Spencer/Stern and Caspari/Stern [32,154] (Source: [14]).

	Spencer/Stern	Caspari/Stern
Exposure	X-rays	Gamma rays (radium needle)
Animal Model	Males exposed prior to mating	Females exposed after mating
Exposure Duration	Acute exposure (minutes)	Chronic exposure (21 days)
Dose Rate	~15,000-fold greater than Caspari	~1/15,000 of Spencer
Vials	Plastic vials to hold flies	Glass vials to hold flies
Temperature	24 °C	18 °C
Diet	Cornmeal molasses	Honey yeast agar
Age (males)	≤7 days, most 2–4 days old	≥5 days
Controls	Controls poorly matched with treatment exposure period	Controls closely matched with treatment exposure period
Temperature Control	Poor, highly variable based on external conditions	Good
50-r Treatment Group	2 groups with different dose rates and exposure period all combined	A single 50-r treatment group all treated similarly
Mold Control	Used Moldex throughout study	Possibly less Moldex used in the 21 day radiation exposure period due to the lower temperature (18 °C vs. 25 °C)
Lethal Clusters	Not corrected for lethal clusters. If so, the treatment group (50 r) used would have had its mutation rate decrease by −8% versus 4% for controls.	Corrected for lethal clusters. No differences between control and treatment
Control/Background Radiation	Background exposure not given	Background exposure reported as 0.6 r
Sample Size Comparison	50-r treatment group had 20,400 less flies than the Caspari experiment	
Study Design	The study was not designed to affect the occurrence of lethal clusters	The study was designed to minimize the possibility of lethal clusters
F_0 Breeding Protocol Differed	40 females/40 males; females – 2 days old	50 females/100 males; females ≤16-h old
Radiation Exposure Condition Differed	20 males/capsule; no food in capsule	50 females/capsule; food in capsule
Lethal Designation Protocol Differed	Used 6 heterozygote females in F_2 generation to identify lethality	Used 2 female heterozygotes in F_2 to identify lethality
Viability criteria	A single wild-type male offspring lead to a designation of viable culture.	A single wild-type male offspring lead to a designation of a semi-lethal.

factor for Crow and the entire Panel. The assessment by Crow of the profound disagreement in the estimates amongst the participating nine geneticists would now be seen against the backdrop and context of the Sonneborn statement:

> … "the thing of most value in all this calculation would be to show how one can use different methods to make estimates, and see to what extent methods, if possible, variations in approach, lead to different answers. So that if they converge, or tend to converge, then we might have more willingness to put them forth." (BEAR I Genetics Panel Transcript, page 257)."

The problem of a lack of genetic damage estimate convergence by the panelists came to a head. There was really no way to proceed with confident recommendations. Further, these 'discrepant' estimates would have been even more discrepant had the views of the three non-participating geneticists, such as Neel, somehow been integrated into overall analysis. So what was the next step forward?

Without having the authority or having been so instructed to do so, Crow decided to save the 'single-hit theory', like Muller and Stern did a decade earlier. Crow excluded several of the independently provided expert assessments. This action was taken despite the fact that each of the Panel members was considered a legitimate world-renowned geneticist with a unique area of specialization.[17] Crow made the decision to eliminate the contribution of Demerec based on bacterial estimates. This action was taken for the stated reason that Demerec's values differed the most from the other estimates due to the use of different methods and approaches. More specifically, Crow wrote to Weaver on March 12, 1956 [38] stating, 'I haven't included Dr. Demerec's estimate on the graph for it, too, is based on quite different assumptions that lead to a greatly different value than the others obtained.' The bottom line is that Demerec's estimate of genetic damage was far below the other eight expert estimates and added significantly to the lack of desired convergence that Sonneborn emphasized was necessary.

The diversity and complementarity of approaches for the genetic damage risk estimates had been deemed to be a key strength of this exercise. However, upon seeing the results, Crow now thought otherwise and eliminated the human population based estimates of Wright and Kaufmann, without justification. The Wright and Kaufmann estimates were the next to lowest estimates. Given that Crow repeatedly expressed concern about the substantial variability amongst damage estimates of the panelists it was not surprising that the three estimates that he eliminated were the lowest. By eliminating these three from the total, it markedly reduced the range of the 'discrepant' estimates.[18]

The actions of Crow to eliminate three expert estimates and the willingness of the Panel to follow his lead is striking. In many ways, this situation became perversely humorous, especially after reading the basis of Crow's personal 'expert' estimates. For example, consider the methodology of Crow, which, of course he accepted. Crow combined three methods to provide a 'best' estimate of genetic damage, including his version of upper and lower bounds. He first decided to use data from the fruit fly for a lower bound. He then decided that human risks from the Japanese bomb survivor data would comprise the upper bound. The 'best' estimate was the mouse data of Russell since it rested conveniently between the fruit fly and human data. While each of these biological models may be used to construct their own best estimate and upper and lower bounds of uncertainty, the integration of each of the models as described by Crow is strikingly inappropriate. The bizarre manner in which he did these was amateurish and revealed that Crow had little understanding of how to proceed. Yet, there is no record that this approach was criticized by any member of the Panel. Further, Crow had stated that his actions to eliminate the estimates of Demerec,

[17] There is a strong general impression/belief that the BEAR I Genetics Panel members were top experts on radiation genetics, based on experience/publication record. In fact, the majority of the geneticist panel members had never published an article on radiation induced mutations prior to their selection on the Panel. Several others had a limited (i.e., several papers) publication record in this area. The bottom line is that the "expert" Panel image was a myth created by the Rockefeller Foundation and U.S. NAS to enhance the acceptance of the policy recommendations of the final reports.

[18] The best estimates of genetic damage for these three eliminated geneticists was: Kaufmann – 195,000; Wright – 50,000; and Demerec – 5220. These collective estimates are approximately 70% lower than those of the remaining five geneticists (i.e., 275,000). George Beadle did not provide an estimate for generation #1 [19].

Wright and Kauffman meant that the Genetics Panel estimates would only be based on the data from fruit fly and mice [109,110]. Yet, Crow ignored his own imposed 'rules' as he used human data for the upper bound, again without receiving any criticism. This simple vignette of Crow's methodology, its inappropriateness and his violation of his own exclusionary rules and with his actions never being challenged, may explain why the Panel voted not to share their methods and findings with the public and scientific community. Not only would these estimates have been rejected, but their highly acclaimed expert status would soon be challenged and perhaps ridiculed.

The actions of Crow were probably not criticized by other Panel members because significant uncertainties reigned, even by those submitting 'detailed' estimates. For example, in his letter to Weaver on February 20, 1956 [163]; Sturtevant stated

> "After going through these calculations I come out with a feeling that they are rather futile. At almost every step it has been necessary to make a guess, often with little to go on and with no real basis for setting limits within which the true value probably lies."

In effect, Sturtevant was agreeing with the above sentiment of Neel.

These insights of Sturtevant clearly contradict the subsequent more politically correct statement in the *Science* article of the Genetics Panel which asserted that – Each (i.e., expert geneticist on the Panel) thus said, in effect: 'I feel reasonably confident that the true value is greater than my minimum estimate and less than my maximum.' Based on the Sturtevant letter, the statement in *Science* is not true. Even if this statement in *Science* were accurate, it is extremely weak, given the very large range between upper and lower bounds for most estimates.

To make matters even more suspect, consider a further and insightful criticism from Jim Neel, Panel member. He states that the reason for converging of estimates following the elimination of the Demerec, Wright and Kauffman estimates was due to the strategy of Crow to select estimates that were not independent but that used essentially the same assumptions for gene number, mutation rates and other parameters [109,110]. In fact, Neel exposed the bias of Crow's decision to restrict estimates to *Drosophila* and mice, as this would yield the false impression of scientific agreement where there was little or none. Thus, according to Neel[107,108], Crow knowingly biased the assessment in order to create the impression of a high level of Panel expert convergence. This plan fell apart when the Panel had to construct uncertainty estimates (i.e. upper and lower bounds), and an effort to seek a group consensus failed. A similar consideration of Crow's own approach for upper and lower bounds illustrates the unreliability of their estimates.

The continuing duplicity of Crow is displayed in his March 29, 1956 letter to Weaver [39] in which he tells Weaver that 'I suggest one of two things: (a) omit the estimates entirely, or (b) give a single best estimate of the number of mutations, or a narrow range of estimates, based on direct extrapolation from mouse and Drosophila'. Crow then writes 'We then state that these are based on mouse data and let the reader add his own uncertainty factor.'

This letter of Crow illustrates two significant points. The first is that he wanted to opt for showing either no estimates or only a 'narrow range' assuming the convergence of estimates based on mouse and fruit fly data. This suggests that he was trying to ensure that the report would be censored to reflect only the conclusion that he favored.

Secondly, after informing Weaver that he recommended using only the mouse and fruit fly, he states that the reader should be told that the estimates are based only on the mouse. This amounts to flagrant dishonesty. Perhaps he did not want the public to know that predictions for people were based on a fruit fly. He also inexplicably suggested that the reader should construct their own uncertainty factor, using highly censored (i.e., inappropriate) data, lacking upper and lower bounds. This last suggestion reveals that Crow recognized that he and the panel were not able to provide competent expert guidance and that each 'guess' was as good as the next.

Having revealed the internal communications of Crow, Weaver and other Panel members on how they derived genetic risks, it becomes clear that the process employed was scientifically chaotic, inherently flawed, had significant elements of deception and dishonesty, as well as signs of widespread professional incompetence. Yet, while this was being hidden from the public, the process was fully enveloped by an appeal to authority (i.e., U.S. NAS and members who were world leaders and promoted as experts on the topic of radiation induced mutations).

The 100-fold range of uncertainty for the first generation U.S. population mutational responses reported in the *Science* article for the six remaining experts misrepresents data that had already been highly censored. The statement that the uncertainty range for the first generation was based on the six selected expert estimates, as reported in *Science* is not correct, as George Beadle, one of the remaining six, only provided an estimate over 10 generations, not for generation #1. Thus, the article could have stated the uncertainty range of 10–2000 fold for the tenth generation effect (745 mean value) (based on six estimates) and the mean of 756 (100–2857) for the first generation (based on five estimates). Since only five or six estimates were used, an entire listing was very feasible, thereby being fully transparent. However, Crow did not want to show the actual figures or how they were derived as he repeatedly emphasized in letters to Weaver.

The recommendation of the BEAR I Genetics Panel to switch to the LNT for genetic risk assessment was a major event, affecting policy, politics, public perceptions of risk, risk communication strategies, as well as providing scientific foundational support for the efforts of Rachel Carson, in her groundbreaking and highly influential book *Silent Spring* published in 1962.[19] As a result of the publicity generated by the Genetics Panel report the US Congress would hold Congressional Hearings in 1957 on the topic of radiation health risk assessment, with multiple Panel members testifying before Congress in support of the switch to the LNT [66]. The process of getting their message out would achieve a significant and very practical milestone in December 1958 when the US National Committee for Radiation Protection and Measurement (NCRPM) [106] generalized the recommendations of the BEAR I Genetics Panel to include somatic cells, and so by doing, applied the LNT model to the process of cancer risk assessment. Since members of the NCRPM also were members of other high-level advisory committees such as the International Commission on Radiological Protection (ICRP), the adoption of LNT by other advisory groups and regulatory agencies in other countries would follow. The inclusion of the same geneticists on multiple 'independent' Advisory Committees represented a strategy to advance specific policies, giving the LNT supporting committee members more opportunities to promote LNT.

7. The BEAR I Genetics Panel in perspective

While LNT became the accepted dose response model for cancer risk assessment as a result of the recommendations of the BEAR I Genetics Panel in 1956, it was readily susceptible to challenges, especially on the grounds that the data were based on fruit flies, not mammals. Making this situation even more potentially contentious was that the BEAR I Genetics Panel voted (i.e., written ballot) not to provide written documentation of the scientific foundations for their decision to recommend the LNT. The reliance upon the fruit fly was rooted in the failed efforts of Donald Charles at the University of Rochester with funding from the AEC during the Manhattan Project to provide findings within a reasonable time period with rodent models concerning ionizing radiation and mutation. Regardless of this striking failure, it was

[19] The historian Ralph [75] states: "not only did she [Carson] tap into this anxiety [about fallout] and direct it toward pesticides, she also used the public's understanding about the hazards of fallout to teach about the similar hazards of chemical poisons."

generally recognized that the fruit fly model would be an interim one for risk assessment purposes. In fact, this was a principal motivation behind the massive investment on the extensive mouse specific locus test program at the Oak Ridge National Laboratory. Alexander Hollaender, who created and oversaw this initiative, understood that the public health debate would require data from mammalian models just as was provided in other areas of hazard and risk assessment, especially as seen with chemical and pharmaceutical products. With respect to the Genetics Panel not providing a scientific basis for the LNT recommendation, this decision [8,21,54] was passed up the administrative ladder, eventually to the President of the NAS, Dr. Bronk, who did not challenge their decision [9] thereby establishing an inexplicable precedent. The actual underlying explanation was, at least in part, about money, that is, grant money for geneticists. The Panel saw the situation as a type of zero-sum game. That is, if the Panel spent their limited time researching, writing, refining and attempting to obtain agreement on the scientific foundations of their recommendations, then there would be insufficient time to identify funding research priorities for the RF via the actions and leadership of Warren Weaver [9].

Lost in this debate over whether to provide a written scientific justification for the various policy recommendations, including LNT, were the written comments of Muller and his dispute with Demerec and how he could not accept Demerec's bacterial model and his critical judgements of Wright's human genetic damage assessment. Muller would note in a letter about this to the new BEAR Genetics Panel Chairman, George Beadle (see below), 'why should the Panel share its dirty laundry for the world to see?' He had already accepted this fact, stating that 'quarreling geneticists' could not resolve their scientific differences. Based on a copious record of personal correspondence over decades Muller and Crow had a close personal relationship. Consequently, previously undisclosed information as revealed in letters from Muller to Beadle might provide an historical insight as to why Crow excluded the data of Demerec and Wright when assembling the genetic damage estimates of the BEAR I Genetics Panel members as previously noted. The comments of Muller to Beadle permit one to speculate whether Crow's decision to drop Demerec and Wright were initiated by a communication between Muller to Crow. The coincidence seems too great to dismiss the possibility.

The attitude that Muller brought to the issue of providing a technical report to the scientific community that would provide the basis for the Genetics Panel's recommendation is enlightening. He stated to Beadle [97] (now the Chair of the Panel – having just replaced Weaver):

> "As for the preparation of a technical report …, it seems to me that it would involve us in a lot of thankless work and disputatious rehash of points we have already considered, as well as airing our dirty linen before the public unnecessarily."

Muller then goes on to state:

> "After all, only geneticists would be competent to judge the validity of our technical report and geneticists do not need it…."

Of particular importance were his follow up comments:

> "So far as I can see, it would be a matter of quarrelling over what would be the most important points to put in and to what extent they were valid, things on which I thought we had agreed to disagree. Why, for instance, should I enter into a public dispute with Demerec on whether a bacterial generation[20] should be taken as corresponding just as closely to a human generation as a Drosophila generation does? This is only one little example of many … … Similarly, I think I would have to disagree with Wright concerning the frequency and importance of small detrimental mutations as contrasted with the conspicuous ones known as lethals and visibles."

This correspondence of Beadle and Muller may therefore provide pivotal insight into the dynamics of the Panel, their need for grant funding, why they failed in their responsibility to the country, and how Muller sought to blunt the influence of Demerec and Wright in the internal Panel disputes. It also revealed how Weaver and Crow were willing to disrespect one person's area of expertise, even after it was stated that it was their goal to integrate and assess the estimates of each expert from the diverse fields within genetics.

8. Dose-rate: Russell's challenge to LNT

While December 1958 would prove to be significant for the adoption of LNT for cancer risk assessment based on the actions of the NCRPM, it would also be ironically important for a potentially significant challenge to the scientific foundations of LNT. This challenge would become evident on December 19, 1958 when the journal *Science* published a significant paper by William L. Russell and colleagues [141] from the Oakridge National Laboratories demonstrating the effect of dose-rate for ionizing radiation-induced mutation in spermatogonia and oocytes in the mouse model. The findings of Russell were broadly significant enough to become a front-page story in the *Buffalo Evening News* as written by the Pulitzer Prize Winner Nate Finney [49], who had a long and serious interest in the societal and public health implications of atomic energy and nuclear weapons. The first public sensing of Russell's work was revealed four months earlier in an August 16, 1958 story in the *New York Times* [142]. However, at the time of the *Science* publication in December 1958 the *New York Times* was on strike, leaving the reporting field wide open for the *Buffalo Evening News* reporter [138].

The Russell findings were significant because over time they would unequivocally refute the LNT mantra of the radiation genetics community. These mammalian findings with spermatogonia and oocytes would indicate that radiation-induced mutation damage was not cumulative and could be reversible and the dose response therefore should not be assumed to be linear. The findings also suggested to Russell that DNA-repair must occur even though it had not yet been discovered.[21] In fact, Russell's (and Altenburg's) inferences were correct. The Russell data were seen as a possible game changer and would quickly affect research directions for the field. It was indeed ironic that within a week or two of the accepting of LNT by the NCRPM, its possible demise was being featured in the most prestigious scientific journal in the world.

Analyses of the Russell scientific writings and correspondence reveals that he tried hard not to explicitly and directly challenge the radiation geneticist community and the seemingly exquisite sensitivities of Muller. Russell was performing a type of balancing act, that is, he was trying to promote his findings while adhering to the radiation geneticist mantra and still supporting the LNT. As can be seen from the published literature and correspondence (Russell letters/memos to Muller [133–135], Muller letter to Russel [98], Russell would maintain this (and perhaps torturous) position until Muller's death in April 1967 when he would finally and unashamedly confront the radiation geneticist mantra on each of its fundamental tenets with the mammalian data he had accumulated over more than two decades on dose-rate

[20] It should be noted that Demerec had an extensive publication record with *Drosophila*, spanning two decades and more than 50 papers in the peer-reviewed literature. He also had a strong publication record with bacterial mutations. Thus, Demerec was uniquely qualified to see the relationship of bacterial susceptibility with that of *Drosophila*. In fact, he was far more experienced in this than Muller. Furthermore, Demerec was originally trained as a maize geneticist with Emerson at Cornell for his Ph.D. in the most prestigious group in the U.S.

(*footnote continued*)
Demerec was perhaps the most broadly experienced geneticist in the country.

[21] Edgar Altenburg would write Muller about the novel Russell findings, likewise suggesting the existence of DNA repair (Altenburg to Muller, December 27 [2]).

[137]. More immediate, however, was the fact that within a few months after the publication of the Russell findings, Muller had shifted over his lab to now incorporate dose-rate studies with *Drosophila* based on the research methodology of Russell [24,25]. This represented a significant shift as Muller's earlier research on dose-rate with Ray-Chaudhuri [131,132] involved only mature fruit fly spermatozoa. With the switch to the use of earlier stages of reproductive cells, Muller was reporting that he, too, now had observed the dose-rate phenomenon [119].

The findings of the Russell and Muller dose-rate research found their convergence in the report of the next BEAR Genetics Panel (i.e., BEAR II) chaired by George Beadle, Nobel Prize recipient (1958) in its 1960 publication ([103] – BEAR II). The incorporation of this information came late in the Panel process and probably would not have happened without a last minute intercession by Russell and his director at Oak Ridge, Alexander Hollaender, who requested/challenged George Beadle to ensure that the dose-rate information be included. Beadle agreed and instructed Russell and Hollaender to write that section of the report [64]. The re-written report was then sent to all members of the Panel, including Muller, with a summary of the preliminary fruit fly dose response data of Muller. However, unlike the BEAR I Genetics report, the BEAR II Genetics Panel Report (1960) was not widely distributed, had little to no acclaim and no ostensible impact on the field or public policy based upon citation, follow up debate, and other possible spin-off activities. Nonetheless, the BEAR II Genetics Panel acknowledged the existence of dose-rate in their 1960 report in both mice and fruit flies. However, while the Genetics Panel finally recognized the biological reality of dose-rate, they failed to confront the issue of the generalization of the 1958 NCRPM LNT recommendation to somatic cells. The new dose-rate findings were a potentially significant scientific problem that could discredit the major dose response policy recommendation to support LNT.

Within a few years, it would become clear that a possible explanation for why the dose-rate phenomenon might not have been observed in the earlier Ray-Chaudhuri study (1939, 1944) was because the mature spermatozoa lacked the capacity for DNA repair while this capacity was present in somatic cells and spermatogonia and oocytes. Thus, reliance on mature spermatozoa, which lack the capability of DNA repair, as the basis for cancer risk assessment using the LNT model was/is a fundamentally flawed approach. Yet, it was within this framework that LNT was created and 'matured' into broad acceptance within the scientific and regulatory worlds of the 1950s and 1960s as guided by Muller and the radiation genetics community.

The 1960s revealed that Russell's research would be extended so that it enabled a clear threshold response to be observed for mouse oocytes at a 'relatively' low dose-rate. The oocytes displayed a threshold for genetic damage at an exposure rate that was 27,000 times above normal exposure to radiation in the U.S. from background and other exposures [136].

The data of Russell created an important rift within the radiation genetics community. This was highlighted by an article of Harold Plough [129], a professor of biology at Amherst College, and former genetics graduate student with Muller at Columbia. Plough was also the person who helped to facilitate a position (i.e., Amherst College, 1940–1945) for Muller in the U.S. upon his return after an eight-year hiatus and with no other available offers. This rift was significant as Muller and another (future) Nobel Prize recipient Salvador Luria, excoriated Plough as seen in letter exchanges and in articles/letters-to-the-editors to the *Boston Globe* (Menzies, June 19, [83]); Luria, July 2, [74]; page 18) and *Washington Post* (Simons, June 19, [147]). During this dispute, Jim Crow wrote to Muller, telling him that Plough was totally out of step with the rest of the radiation genetics community and that no one believed that thresholds for radiation-induced mutation exist [40]. The letter of Crow was curious since it was written after the BEAR II Genetics Panel (1960) (of which he and Muller were members) acknowledged the findings on dose-rate for Russell and Muller and after reports of Russell which clearly showed that a threshold exists for the mouse oocyte for ionizing radiation induced mutation. The letter of Crow to Muller was never challenged or corrected by Muller, despite its obvious factual flaws.

During the same time interval, Muller would become engaged in a substantial debate over the role of dose-rate in human risk assessment especially within the context of his role on expert committees of the ICRP [23–25]. In these debates, he claimed that the dose-rate data were inadequate to apply to human risk assessment. Part of his rationale was that differences in dose-rate responses between insects and mammals had not been resolved and therefore the mammalian data of Russell should not be used in human risk assessment. Yet, he argued that there was an evolutionary basis for this apparent interspecies difference in which dose-rate would have been more strongly selected for in mammals than in insects [99]. The point here is that at every possible turn Muller would attempt to preserve LNT, even if it meant being deceptive and dishonest (e.g., his comments about Caspari's control group) or inconsistent, as in this case, or imposing of censorship as in the case of his dispute with Demerec [45] and in his attempt to prevent Neel from speaking at an international symposium on his Japanese atom bomb survivor data ([25]; footnote 1).

9. Russell challenges radiation genetics mantra

While Russell finally broke ranks with the radiation geneticist community, it was not until the 1969–1970 time period as revealed in several publications and conference presentations (Table 4) [136]. In his 1970 presentation at the 14th International Congress of Radiation Research at Evian, France, Russell [137] stated that the original estimates of genetic risk (which were made by the BEAR I Genetics Panel) [7] for radiation (and, as noted by Ref. [17]) and later for chemical carcinogens were based on the two major assumptions that: (1) radiation-induced gene mutation frequencies in the fruit fly have extrapolative relevance to humans and (2) results from radiation experiments on fruit fly spermatozoa illustrate general principles of radiation genetics and thus can be applied to humans (i.e., the mantra of the radiation geneticist). What followed from these two overreaching assumptions was a series of six specific and fundamental risk assessment tenets (i.e., "general principles") upon which genetic and, as noted by Calabrese [17], cancer risk assessments were based. According to Russell [137], his radiation geneticist colleagues believed that,

> 1) Gene mutation rate is directly proportional to radiation dose; 2) Gene mutation rate is independent of radiation dose rate; 3) Gene mutation rate is independent of dose fractionation; 4) There is no repair of gene mutational damage; 5) There is no threshold below which no genetic damage occurs; and 6) There is no recovery from mutation with time after irradiation.

Following two decades of conducting genetics research on mice at Oak Ridge National Laboratory, Russell had evaluated the effects of ionizing radiation on over a million mice (radiation-exposed and control groups combined) in the largest progressive/cumulative mammalian study ever conducted. From this extensive experience, Russell [137] concluded that, ' … the first assumption is probably not valid, that the second is definitely incorrect, and that none of the six 'general' principles applied to mouse spermatogonia and/or oocytes.' During his presentation, Russell offered scientific evidence supporting these conclusions. This presentation had the potential to be a major galvanizing event that led to substantial debate while offering the opportunity for a significant mid-course correction concerning the nature of the dose response in the low dose/dose-rate zone. However, it failed to do so.

During this period (i.e., 1970) Russell would accept membership on the first NAS BEIR Genetics Subcommittee (BEIR I) which was to be chaired by Jim Crow. The central issue of this Genetics Subcommittee would be how it would address the nature of the dose response in the low dose zone. In effect, this was to be the next battle in the threshold versus LNT confrontation. It was then about 15 years since the

precedent-setting BEAR I Genetics Panel report of 1956. During that time, the environmental revolution had started in earnest following Carson's [30] book, *Silent Spring*, the passage of the National Environmental Protection Act (NEPA) following the massive Santa Barbara oil spill in January/February 1969, signed into law by the U.S. Congress in December 1969, and the creation of EPA in 1970. Likewise, the role of quantitative risk assessment using low dose modeling received a strong boost by the seminal publication of Mantel and Bryan [76] that introduced the concept of low dose modeling for cancer risk assessment. This publication originated from the herbicide (i.e., aminotriazole) Cranberry scare during Thanksgiving of 1959 in the U.S. during the Presidential campaign between John F. Kennedy and Richard M. Nixon [65].

Mantel and Bryan [76] proposed that an arbitrary acceptable risk for carcinogens be set at 1/100,000,000 (1×10^{-8}) over a lifetime using the probit model. Regardless of the model, the concept of acceptable risk rather than reliance on a true biological threshold had taken hold at the National Cancer Institute (NCI) for chemical carcinogens and at the NAS for ionizing radiation. The creation of the BEIR I Genetics Subcommittee in 1970 occurred at a strategic moment as it was at the time of EPA creation, yet, before the Agency had constructed guiding principles for carcinogen regulation in the mid-1970s. Thus, even though the NAS BEIR I Committee was created to offer guidance to the country on the health concerns associated with the expansion of the domestic use of ionizing radiation, its recommendations would be more broadly influential, serving as an ideal source of highly respected scientific/public health guidance for environmental cancer risk assessment.

10. BEIR I

Following the death of Muller on April 5, 1967, the BEIR I Genetics Subcommittee (1970–1972) addressed the question of cancer risk assessment anew. They did this by reviewing what the BEAR I Genetics Panel wrote some 15 years before and reflecting upon what had been learned in the interim years. While much was discussed, several key concepts and findings emerged. The most important conclusion of the BEIR I Genetics Subcommittee was that the BEAR I Genetics Panel of 1956 made a mistake on the key concept of dose-rate. This conclusion was based on the data of Russell from the mouse specific locus test, which subsequently had matured and expanded, now having more than a decade of widespread exposure and scrutiny within the scientific community. Being wrong on dose rate was not a simple or singular point. It meant that genetic damage was not cumulative, could be reversed, and was repairable. These findings exposed multiple flaws in radiation geneticists' central beliefs. In the period between the discoveries of Russell in 1958, to the creation of the BEIR I Committee in 1970 DNA repair had been discovered, as predicted by Russell. The basis for the recommendation of LNT had, therefore, been convincingly challenged on scientific grounds.

The BEIR I Genetics Subcommittee also raised another fundamental point that challenged the BEAR I Genetics Panel report. This concerned the fact that the LNT, as derived from fruit fly data via the research of Muller and Ray-Chaudhuri and the Stern-Manhattan Project studies, used mature spermatozoa that were now known to lack DNA repair. The use of a biological model lacking DNA repair to estimate risks in somatic cells possessing DNA repair is fundamentally inappropriate. Yet, that is precisely what the LNT-based cancer risk assessment paradigm had long been based on. BEIR I also knew that it had to transition to the so-called modern era-that is, adopting a rodent model with cells that possessed DNA repair. The real challenge was whether they could do this and still retain LNT. This was an especially significant challenge since the dominant intellectual and ideological paradigm amongst the geneticists was LNT, a perspective that had become rooted not only in the science, but also within their culture.

In contrast to the BEAR I Genetics Panel, the BEIR I Genetics Subcommittee provided a written basis for their recommendation of the adoption of the LNT. This recommendation was very much like a reaffirmation of the status quo, lacking the fairness of an independent competition between two ideas (i.e., LNT vs threshold). In the case of the threshold vs LNT debate the Genetics Subcommittee would not only play a significant role but so to would the findings from animal studies and epidemiology.

These two disciplines represent important components in the overall risk assessment process. However, neither of these complementary methodologies is capable of adequately addressing the LNT question. This can be best appreciated by the fact that the mega-mouse study of the U.S. FDA, which used over 24,000 mice could only confidently estimate risk down to the 10^{-2} (1/100) area and is therefore referred as the ED01 study [11]. The limitations of epidemiology are also widely known within the legal system in the U.S. only accepted as a causal judgement when the odds ratio is ≥ 2, that is, when the risk at least doubles [164]. This is far greater than values of $1/10^{6}$ (or even 1/10) that are implicit in present risk assessment practice.

The BEIR I Genetics Subcommittee based their judgement in large part upon a belief in the mechanisms of radiation induced cancer, and this was due to an initial event that involved mutagenicity, a view now widely seen as insufficient, requiring multiple steps/stages [48].[22]

If it could be shown that the dose response for mutagenicity was linear at low doses, it was widely believed that the dose response for radiation-induced cancer would also be linear. This was precisely why the mantra of the radiation genetics community of cumulative, irreversible and linear was central to the risk assessment process and regulatory Agency policy. The challenge facing the BEIR I Genetics Subcommittee was that now the paradigm-changing data of Russell had taken center stage. Russell's data was not trivial but based on the findings of more than a million mice in the largest cumulative mammalian genetic toxicity program ever undertaken. It was an example of Big Science and was funded and located within the AEC, which later became the ERDA and later still the Department of Energy. As such, it was a program that involved a large number of professional staff over several decades. The government had made a massive investment in this area for the explicit purpose of having a solid scientific foundation for the risk assessment process for ionizing radiation.

Over the decades since the 1950s Russell and his team published numerous papers on their progressive studies, with accumulating sample size. The initial striking findings of the 1958 *Science* journal paper, which demonstrated the existence of dose rate effects in spermatogonia and oocytes were confirmed and strengthened with its massive cumulative size. The findings for the male indicated that by lowering the dose-rate the mutation damage incidence could be significantly reduced as compared to the same total dose given acutely. The research demonstrated that the mutation incidence could be reduced by about 70% in males. In a series of parallel experiments with females, they demonstrated that at 'low' dose-rates that the amount of genetic damage could be reduced by 100%, that is, the low dose-rate females became indistinguishable from the controls. The findings of Russell were of striking significance, especially for the females since they demonstrated the unequivocal existence of a threshold for genomic mutation as induced by ionizing radiation. The mechanisms by which these decreases in mutation rate occurred was explained by the presence of DNA repair. Why the male did not return to the control group value as did the female was not known at that time. While it would seem that answering the question of why the females achieved a

[22] The actions of the BEIR I Genetics Subcommittee were deeply rooted in the Somatic mutation theory (SMT), a view that has directed cancer risk assessment to the present. While not the focus of the present paper, the SMT has been challenged from multiple perspectives [124,151,152] over the past decade. How such developments may affect the federal cancer risk assessment criteria remains to be seen.

threshold and the male did not was extremely important, it was never resolved by the BEIR Genetics Subcommittee. A possible technical reason why it could not be easily addressed was because the number of exposure days was limited by the duration of spermatogonial development. This placed a constraint on what dose/dose-rate could be delivered to a particular stage of cell development, essentially limiting research to resolve the male threshold issue.

When the BEIR I Genetics Subcommittee [105] evaluated the Russell data it acknowledged the existence of dose-rate and the threshold response of the female. It also noted that the male spermatozoa showed a decrease by 70% in mutation rate as compared to the acute exposure, but still not a threshold. Based on their report, there was no discussion of why, from an evolutionary perspective, the oocytes would display a threshold while the spermatogonia did not. For example, perhaps the spermatogonia were simply progressively lessening their DNA repair capacity that would eventually result in the DNA repair deficient mature spermatozoa. Alternatively, perhaps a threshold may have been detected had lower dose-rates been evaluated. Is there an evolutionary reason why such a gender-difference would exist? Would such a difference exist in somatic cells? Of course, these questions were all premised on the assumption that the Russell findings were correct, accurately presented and interpreted. The judgement of the BEIR I Genetics Subcommittee was that the LNT should be retained/adopted based upon the spermatogonia of the Russell data. They decided to construct a linear dose response from the lowest dose tested (i.e., dose associated with the ~70% decrease in mutations in males) to the origin. They also made the assumption that the spermatogonial cells would be a better representation of somatic cells than mature spermatozoa. Thus, the BEIR I Genetics Subcommittee transitioned from dependence on the mature spermatozoa of the fruit fly for the LNT recommendation to the Russell findings with mouse spermatogonia while still retaining the LNT.

The BEIR I Genetics Subcommittee report [105] proved to be highly influential as it would serve as the basis for how U.S. regulatory agencies would estimate risk for both ionizing radiation and chemical carcinogens. This was first reported in 1975 (and reaffirmed two years later in 1977) by the US EPA. The agency explicitly cited the Genetics Subcommittee report and the dose-rate findings of Russell as described in the following quote from Calabrese, 2017b [25]-see quote, page 456):

> "EPA uses primarily the recommendations of the National Academy of Sciences Committee on the Biological Effects of Ionizing Radiation (BEIR) as expressed in the November 1972 report to arrive at dose to health conversion factors. Besides the concept of linearity expressed in the policy statement (i.e., EPA, 1975– EPA Policy Statement on Relationship between radiation dose and effect. 41 Federal Register, 28409), it is further assumed that health effects that have been observed at dose rates much greater than those represented in this report are indicative of radiation effects at lower dose rates. Any difference in biological recovery from pre-carcinogenic radiation damage due to low dose rates is neglected in the BEIR health estimates."

The U.S. EPA Carcinogen Assessment Group (CAG) under the direction of Roy Albert [1] would also explicitly cite the recommendations and rationale of the BEIR I Genetics Subcommittee (1972) [105] as providing the basis for the use of LNT for the assessment of risk for chemical carcinogens. The EPA accepted the LNT model of the AEC/BEAR I Genetics Panel. This model was adopted by EPA since it was easy to apply. From a toxicological perspective, the agency simply had to identify the lowest dose of carcinogen that induced a statistically significant response and then draw a straight line to the origin of the graph in order to estimate cancer incidence at any exposure level. The biological plausibility of the LNT model was based on the assumed linearity of mutation dose response as recommended by BEAR I and BEIR I, within the framework of target theory. Albert [1] indicated that ' … any difference between chemical carcinogens and ionizing radiation could be waived aside as they both cause genetic damage … '. Thus, in retrospect, the long term investment in the research by Russell on the mouse specific locus test, which started in 1949 at Oak Ridge National Laboratory, proved to be a highly successful endeavor as it now provided the scientific rationale for carcinogen risk assessment for all U.S. regulatory and public health agencies.

The Russell findings were so massive and credible that they served as the fundamental basis for understanding how ionizing radiation and genotoxic carcinogens would act at low doses/dose-rates. This became a type of toxicological 'homing device' that complemented the necessary (and significant) but insufficient whole animal and epidemiological data which lacked the power to confidently assess low dose/ambient exposure effects. In effect, the Russell findings became the gold standard, providing the intellectual rationale for linearizing carcinogen dose responses. Despite this reaffirmation of the LNT, it was insufficiently appreciated that this foundation, based on the Russell data, had its own significant inconsistencies. For example, the oocyte showed convincing evidence of a threshold even at doses about 27,000 fold greater than background. There was also no data to indicate that the male, even though not showing a threshold at doses comparably greater than background, might not show one at lower doses/dose-rates. Nonetheless, this was the basis of the LNT over the next half century. Over this period of time many thousands of new research papers were used by proponents and opponents of the LNT but the rationale for the LNT would remain the same. It would revert back to BEIR I, the Genetics Subcommittee and the Russell findings. Even multiple studies showing that cosmic/terrestrial ionizing radiation appears necessary for improving a wide range of health indices in multiple species [51,67,128] was not sufficient to make a change from LNT.

11. BEIR I error discovered and corrected

Nearly 25 years (in 1995) after the convening of the BEIR I Genetics Subcommittee, Paul B. Selby, a senior geneticist at Oak Ridge National Labs, and a former Ph.D. student of William L. Russell, uncovered significant irregularities in the construction of the historical control group used in all the major mouse specific locus test studies and risk assessment applications. The irregularities were of such potential magnitude as to warrant an external assessment by a committee of four leaders in the field. The external expert committee, plus the Russells' and Selby agreed that the control group required correction, with an adjustment upwards for mutational incidence. The Committee requested the Russells and Selby publish their adjustments in the scientific literature. The Russells adjusted the mutational rate upwards of 120% [140] while Selby argued that the control values were wrong by 5–7 fold [144]. While this dispute was contentious, the tone of these published articles was non-inflammatory making it difficult for the field to appreciate the seriousness of the debate and its widespread implications. Over time, publications accumulated which addressed many of the issues debated by the Russells and Selby [140,144,145]. The net result was that the arguments of Selby had grown in statue with broad acceptance by leading radiation geneticists [24,25].

Despite this ongoing process, it was only recently that the question was raised concerning how would the Russell and Selby adjustments have affected the judgements/conclusions of the BEIR I Genetics Subcommittee [23–25]. This was a relevant question, for if the Russells had provided accurate control group information, it would have been available for the BEIR I Genetics Subcommittee through their 1970–1972 meeting period. In a recent paper, it was shown that if the Russells' upward correction had been made at the time of the BEIR I, the data would have revealed that the male mutation incidence at the low dose-rate would have displayed a threshold (i.e. the 70% decrease in mutation would be 100% with the error correction) [24,25]. If this had been the case, then the argument used by the BEIR I Genetics Subcommittee for the adoption of the LNT would have been invalid.

Furthermore, if the analysis of Selby had been available and used, it would have supported a possible hormetic dose response interpretation.

These new findings are significant, since they argue that the basis of the modern LNT as originated with recommendations of BEIR I, was based upon a mistake and are therefore invalid. While science is supposed to be self-correcting, it is clear that it has taken nearly half a century for this error to be recognized and a correction proposed. The reasons for such a prolonged failure to detect the control group error are likely many, but require speculation. Perhaps the most reasonable is that the mouse SLT was a unique bioassay, requiring massive resources. It could only be conducted in large governmental laboratories. There was only one such location in the U.S. This would become an issue because many technical questions and methodologies were unique to the specific locus test, limiting the number of people with adequate expertise to review and correct possible errors. It also exposed flaws in the peer-review process. Journal editors may have been at a loss as to whom to send the Russell manuscripts to. This leads to an appeal to authority and an unwillingness to challenge authorities such as Russell. In fact, the only challenge would originate internally, which is not a surprise, as very few would have known as much as Selby and to have been in a position to offer highly technical criticisms.

Such corrections, when applied to the risk assessment actions of BEIR I, indicate that those actions would also need to be adjusted. This adjustment would confront the issue of whether this central and dominating recommendation of BEIR I that lead to the reaffirmation of LNT should be changed. In retrospect, the data indicate that the NAS BEIR I Genetics Subcommittee used the Russell data to re-affirm the LNT model and did so not knowing that the historical control data used in the Russell publications was incorrect by from 2 to 7 fold. Given the prestige of the NAS, the complexity of the mouse SLT, and the high esteem of the Russells, the data and the recommendation were assumed to be accurate. This unprecedented situation created the perfect scientific storm: the entire carcinogen risk assessment process of the US and essentially all other countries with appropriate regulatory governmental structures was based on a significant undetected mistake that is still guiding cancer risk assessment today.

12. Discussion

The history of the LNT is shown to have originated as an attempt to discover a biological mechanism that could explain evolution. While this proposal of Olson and Lewis [118] failed to be convincing, their idea that the dose response for radiation-induced mutation should follow a dose-related direct proportional relationship (i.e., a linear dose-response) was persuasive, at least to the radiation geneticist community. This view was quickly adopted by Muller and supported by laboratory findings under his direction. Muller would soon become the dominant influence in formulating the proportional response concept, its generality and scientific implications (i.e., Proportionality Rule). Soon after these descriptive developments of the Proportionality Rule model, the next step was the development of a proposed mechanism. This was achieved in 1935 by Timofeeff-Ressovsky et al. [169] in their classic paper that has been rediscovered, translated, and given modern prominence [150]. This action added the concept of target theory by leading physicists to provide the mechanism. Complementing the mechanism, Zimmer [180], one of three authors of the key 1935 [169] paper, provided the mathematical formulation, which functionally showed that the LNT model was due to a single hit (Fig. 1). It was this sequence of actions, which were the fundamental scientific building blocks of the modern LNT-single-hit model. Muller would then secure the biological credibility of the LNT in subsequent studies with Ray-Chaudhuri [131,132]; which indicated no support for the dose-rate concept. Total dose was all that counted, regardless of whether ionizing radiation was given acutely or chronically. This perspective would translate into a linear dose response model with adverse effects being predicted down to a single ionization. While Muller strongly supported the LNT, it is important to note that highly credible data challenging the LNT judgement were generally ignored or marginalized, even though having scientific credibility (Table 1).

The radiation genetics community was intellectually led by Muller, even though there were many strong personalities within the group. Muller was unique amongst the other talented radiation geneticists, showing a very strong commitment, extremely attentive to detail, with a highly critical and combative demeanor. As a result of his leadership, the field adopted his view that radiation induces mutation in a linear fashion. This group of radiation geneticists wanted this view to guide medical treatments and health/exposure standards for the general public and workers.

The entire scenario just described was based on the incorrect interpretation by Muller of X-ray induced gene mutation in *Drosophila* at very high doses and how this error mesmerized the scientific community and government leaders even in the presence of credible and devastating criticism by Stadler and others. Thus, the LNT-SH model was based on a mistake and consequently led to the flawed cancer risk assessment recommendations of the NAS BEAR I (1956) [7] and BEIR I (1972) [105] expert panels. Muller was therefore able to mislead the field, regulatory agencies and even the Nobel Prize committee. In fact, despite having been shown to be incorrect on his interpretation that he induced gene mutation, his views still control the textbooks and governmental risk assessment policies worldwide, even in 2019, despite overwhelming modern data to the contrary.

From my perspective, the initial two decades of LNT development occurred in a manner that was typical of novel concept challenges and acceptance within science and society. This process became problematic and controversial only after Ernst Caspari, in August 1946, presented his data to Stern. These data did not support the Muller-Ray Chaudhuri lack of dose-rate findings. When seen in the perspective established above, one can better appreciate why Stern rejected the contrary findings of Caspari and why Demerec [3] was so concerned that he implored Caspari with the statement, 'What can we do to save the hit theory.' Stern and Muller, key leaders of the radiation genetics community, were strikingly challenged by the new data. The Manhattan Project was far more advanced than the research of Ray-Chaudhuri with Caspari's study having improved quality control and study design features. Earlier papers (e.g. [15]), revealed a series of

Table 4
Summary of the effects of dose-rate on the induction of mutations by radiation in the mouse (Source: [136]; page 623)*.

Russell Quote:
"Using the genetic techniques available today and the data discussed above, it is of interest to estimate the frequency of mutations that would be expected if a population of mice received the maximum gonadal dose of radiation (5 rem over a 30 year period) allowable for the general population in addition to background radiation. This radiation dose of 5 rem would be received at a dose rate of approximately 3.3×10^{-7} r/minute (0.00000033r/min). This is a dose rate that is over 27,000 times smaller than the lowest dose-rate used in studies with female mice in which no induced genetic effect was observed even when a dose of 400r was used. Therefore, no significant effect would be expected from this low dose (5 rem) even if it were delivered at a considerable higher dose-rate. This dose-rate is also 3000 times smaller than the lowest rate used in experiments with male mice. The lack of a threshold dose-rate, however, when males are considered means that one would expect mutations to be induced at the seven specific loci, and these could be detected, but an extremely large and costly experiment would be necessary. For example, if one uses the mutation rate obtained in the low dose-rate experiments, 8×10^{-8} mutations/locus/gamete/r, one would expect 280 mutations/100 million gametes or progeny tested (8×10^{-8} mutations/locus/gamete/r) (7 loci/gamete) (5 rem). This, obviously, is an experiment which is not feasible to carry out from any standpoint."

*Even this assessment by Russell is now recognized to have significantly overstated the mutation risk due to an error in the historical control group. Correction of this error using the Russell adjustment reveals a threshold response. Correction of this error using the Selby adjustment suggests an hormetic response [24,25].

irregularities in judgements and behavior by Stern and Muller, first occurring after Caspari presented his data to Stern. These include:

1. Stern directing the writing of the manuscript discussion that challenged the acceptance of the Caspari data.
2. Writing a discussion that placed greater credibility on the acute exposure Spencer experiment that had numerous limitations. The support for the Spencer data was due to its apparent demonstration of a linear dose response (i.e., supported the geneticist mantra) – not to its scientific quality.
3. Muller's disavowing the possibility of a threshold at his Nobel Prize lecture, even after he had seen the Caspari data supporting a threshold and had strongly recommended that funds be obtained to replicate it.
4. Both Stern and Muller promoting the validity of the Delta Uphoff experiments which had aberrantly low control group values, which Uphoff and Stern stated in writing made these data uninterpretable.
5. Uphoff and Stern publishing a note in *Science* that included the uninterpretable findings and not sharing with the readership why data, unacceptable less than a year before in the formal report to the AEC, were now acceptable.
6. The failure of Uphoff and Stern to fulfill their pledge to the *Science* readership that they would publish a follow up paper with detailed methods, materials and supportive data.
7. The false reporting by Muller [94,95] that Caspari had an aberrantly high control group value, while his own data and memos explicitly confirmed the findings of Caspari and discredited the control data of Uphoff.

These obfuscations and deceptions by Stern and Muller would not only enhance the acceptance of the Uphoff and Stern [172] paper but would also lead to marginalization of the Caspari findings [148,149]. The goal of the Stern and Muller actions was no less than that of Demerec, which was to save the LNT SH model and to promote its acceptance. The perspectives of Stern, Muller, and others in the radiation genetics community were also shared by the leadership of the Rockefeller Foundation who selected geneticists who were LNT advocates for the NAS BEAR Genetics Panel. This bias was also seen in the selection of Weaver to Chair the Panel and his inappropriate remarks that (1) raised the possibility of sizable and highly flexible grant money for geneticists if their report was 'appropriate', (2) the inappropriate actions of Crow to exclude three technical estimates of genetic damage by the contributing geneticists, (3) the false reporting in *Science* by the BEAR I Genetics Panel concerning the number of panel members who provided radiation risk estimations, (4) the misrepresentation of variability of the six (i.e., five) estimates of the Panel and (5) the actions of the President of the NAS to support a decision of the BEAR I Genetics Panel not to provide a written report explaining the scientific basis of their recommendations.

Led principally by Muller, the BEAR I Genetics Panel was successful in convincing essentially all major advisory groups and countries to adopt their LNT recommendation. This scientific saga would be renewed with the dose-rate findings of Russell in the mouse model. Even though the findings of Russell would essentially disprove the radiation genetics core concepts of cumulative, irreversible and linear responses, the BEIR I Genetics Subcommittee, some 15 years after BEAR I, could not break free from the hold on the field that Muller had imposed and passed on to his scientist protégées, such as Jim Crow, who chaired the BEIR I Genetics Subcommittee. Finally, due to the vigilance and courage of Paul B. Selby [144,145,], key mistakes by Russell were revealed, forcing a revision of the Russell dose-response findings in 2017 [24,25], leading to a highly credible challenge to the LNT model.

Not to be forgotten in the LNT story is Muller's Nobel Prize. The international prestige of the Nobel Prize, received by Muller in 1946, provided enormous and enduring support for Muller's career, the field of radiation genetics, and the LNT. The awarding of the Nobel Prize for the production of x-ray-induced gene mutations provided the necessary credibility for the transformation of a clearly flawed hypothesis into a major environmental and public health belief system and cancer risk assessment policy. The Nobel Prize Committee's decision transformed a progressively discredited hypothesis (in light of the research of Stadler, McClintock and others) into a biological 'truth' following health concerns generated by the dropping of the atomic bomb in 1945 in Japan. The widespread adoption of the LNT may be directly tied to a flawed decision by the Committee to award Muller the Nobel Prize. It is likely that without the 'boost' provided by the Nobel Prize for Muller the history and acceptance of LNT would have been significantly affected.

The history of the LNT is complex, strikingly revealing the intersections of science, personalities, politics, power, financial temptations, and most importantly, beliefs. While a substantial part of this story was pieced together from the peer-review literature, other findings and insights were revealed via the NAS meeting transcripts and numerous letters, memos, and preserved papers of members of the NAS Genetics Panels and others. In fact, unless strenuous efforts were made to obtain and explore these additional sources of information, the story of LNT would still remain obscured and a false representation would persist regarding what the historical record now reveals.

13. Conclusions

The LNT single-hit dose-response model for cancer risk assessment was conceived, formulated, and applied in a manner which is now known to have been scientifically invalid. Contributing to the embrace of the LNT model were a series of scientific errors and the unfounded assumption that one could accurately extrapolate potential risk from very high to very low doses of ionizing radiation. This occurred despite findings indicating that (1) the type of genetic damage/mutation spectra is highly dose dependent (i.e., mostly gene deletions at the high doses used by Muller and not gene mutations), precluding accurate and valid low dose extrapolation, (2) the use of mature *Drosophila* spermatozoa which are haploid and lacking of DNA repair to extrapolate to mammalian somatic cells which are diploid and possess efficient DNA repair, and (3) the rejection of dose-rate in risk assessment which is now an important concept in ionizing radiation risk assessment. Thus, the concept of LNT single-hit for cancer risk assessment is shown to have multiple flaws that reveal its lack of scientific validity. However, despite these flaws the radiation genetics community of the 1940s-1960s promoted and strongly advocated the adoption of the LNT single-hit model to replace the threshold model. As documented in this review, on numerous occasions leading members of the radiation genetics community abandoned their scientific role and instead became ideological advocates for the LNT single-hit model, displaying questionable judgements and behaviors that reflected efforts to obfuscate, deceive, and even misrepresent the scientific record. These actions clearly played a significant role in the successful adoption of the LNT by the scientific and regulatory communities, as well as in widespread public health policy. By the early 1970s numerous limitations of the BEAR I Genetics Panel cancer risk assessment approach were recognized by the BEIR I Genetics Subcommittee, by replacing the fruit fly with a mammalian model and using diploid cells with DNA repair showing clear dose-rate effects. Nevertheless, LNT was still retained since a threshold for mutagenicity was only found in oocytes, and not in spermatogonia. However, more recent re-evaluations of the scientific basis of the BEIR I Genetics Subcommittee show that the data upon which its judgement was based were in error, requiring a significant historical control group adjustment. These adjustments now unequivocally reveal that the responses of both male and females displayed threshold dose responses, indicating that the basis of cancer risk assessment as recommended by the NAS BEIR I Subcommittee and accepted by virtually all regulatory agencies, is demonstrateably incorrect. These new findings have profound implications for regulatory agency cancer risk assessment, cost-benefit analyses, numerous public health practices, technological

developments, use of nuclear power, and risk communication messages to the general public for both radiation and chemicals.

Declaration of interest

Author declares no potential conflict of interest.

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Appendix 1. A 90-year LNT Chronology: From mutation to cancer risk assessment

Statement	Year
First report of induced mutation; Gager and Blakeslee	January 1927
Muller report on X-ray induced mutation in Science	July 1927
Muller (and Gager and Blakeslee) presented data on mutations at Genetics Congress	September 1927
Stadler-presentation of X-ray induced mutation in plants-AAAS Conference	December 1927
Muller and Gager and Blakeslee - 5th Genetics Congress proceedings	undetermined date but published before Sept. 15, 1928
Muller - presentation to National Academy of Sciences on X-ray induced mutations	April 24, 1928-pub. Sept. 15, 1928
Stadler - publication of mutation data in Science	August 24, 1928
Muller - publication of mutation data in PNAS; in this publication he cited the proceedings of the 5th international genetics congress with correct page numbers but with a 1927 publication date which was incorrect.	September 15, 1928
Alex Olson and Gilbert Lewis - proposed linear dose response for mutation to be mechanism of evolution; published in Nature	1928
Oliver (Muller student) dissertation showing linear dose response for radiation induced mutations	1930
Muller proposes Proportionality Rule	1930
Stadler challenges Muller on gene mutation interpretation for reported transgenerational phenotypic changes induced by ionizing radiation. Challenge based on novel cytogenetic advances of McClintock.	1931 and then at 1932 6th international Genetics Congress
Timofeeff-Ressovsky et al. propose single hit model and link to Muller's linear dose response mutational data	1935
McClintock demonstrates new mechanism for radiation-induced mutation	1935
Ray-Chaudhuri (Muller's student) dissertation supports total dose/linear theory	1939
Manhattan Project-genetic mutation study starts at U. Rochester with Curt Stern directing project	1943
McClintock develops the transposition gene theory – new mutation mechanism	1944
Ernst Caspari's data support threshold rather than linear dose response in Manhattan Project research with Curt Stern	fall 1946-Muller sent data (November 1946)
Muller receives Nobel Prize for 1927 findings – misleads Nobel audience in lecture on dose response	December 1946
Stern fails to adequately replicate Caspari study with Delta Uphoff	1946–1948
Stern published Warren Spencer and Caspari papers in Genetics	January 1948
Salvador Lauria (future Nobel prize recipient) tries to convince Muller to incorporate McClintock's transposon findings into mutation theory	1948
Stern and Uphoff publish mini-meta analysis of Manhattan Project mutation research in Science	1949
Robley Evans, MIT, supports threshold model, based, in part, on Caspari threshold evidence in a Science publication	1949
Muller tries to get Stern to challenge Robely Evans; fails on this and then writes articles misrepresenting the Caspari control group data	1950 and repeats this argument again in 1954
Edgar Altenburg tries to convince Muller to incorporate McClintock's tranposon model into gene mutation theory	1952
Stadler criticizes Muller gene mutation explanation and single hit model in Science	1954
National Academy of Sciences BEAR I Genetics Panel, 1955–1956 recommend switch to LNT, misrepresent findings in Science paper and later refuse to provide scientific justification for their recommendation	Summer 1956
NCRPM applies LNT model for cancer risk assessment	December 1958
William L. Russell (Oak Ridge National Labs) published first evidence of dose rate for mutations with ionizing radiation, suggesting the existence of DNA repair	December 1958
NAS BEAR II Genetics Panel, report acknowledges dose rate in mouse and Drosophila	1960
Russell and Muller have debates in international advisory committees over the role of dose rate in human risk assessment	1963–1965
Muller dies	April 1967
Russell publicly renounces radiation genetics dose response mantra	1969 and 1970 based on dose rate findings
NAS creates BEIR I (1970) which retains LNT while rejecting total dose; it switches to use of Russell mouse data from fruit fly reliance. Committee is unaware of significant error in Russell control group data	report in 1972
EPA adopts LNT based on the use of the Russell data (which is still in error)	1975 and reaffirms it in 1977
EPA adopts single-hit LNT model for radiation and chemical carcinogen risk assessment, incorporating an independence of background modeling feature	1979 – notice in Federal Register
EPA switches from single-hit to multi-stage model for cancer risk assessment	November 1980
EPA adopts additive to background assumption for cancer risk assessment, drops independent to background	1986 – EPA cancer guidelines
Paul B Selby reports error in Russell control group in 1995; error confirmed by the Russells and corrected in the scientific literature separately by Russells [140] and Selby [144,145]	1996 and 1998
Calabrese applies Russells' and Selby corrections to BEIR 1972 risk assessment and reports that a threshold or hormesis response would have been reported if the control group error had been detected and corrected at the time of BEIR I	2017

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.cbi.2018.11.020.

Appendix A. Supplementary data

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The linear no-threshold model is less realistic than threshold or hormesis-based models: An evolutionary perspective

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ABSTRACT

The linear no-threshold (LNT) risk model is the current human health risk assessment paradigm. This model states that adverse stochastic biological responses to high levels of a stressor can be used to estimate the response to low or moderate levels of that stressor. In recent years the validity of the LNT risk model has increasingly been questioned because of the recurring observation that an organism's response to high stressor doses differs from that to low doses. This raises important questions about the biological and evolutionary validity of the LNT model. In this review we reiterate that the LNT model as applied to stochastic biological effects of low and moderate stressor levels has less biological validity than threshold or, particularly, hormetic models. In so doing, we rely heavily on literature from disciplines like ecophysiology or evolutionary ecology showing how exposure to moderate amounts of stress can have severe impacts on phenotype and organism reproductive fitness. We present a mathematical model that illustrates and explores the hypothetical conditions that make a particular kind of hormesis (conditioning hormesis) ecologically and evolutionarily plausible.

1. Introduction

The origin of aerobic life is one of the most fascinating and elusive topics in biology. Certainly, one of the great leaps in the history of life on earth was the evolution of the capacity to use oxygen to generate energy [1]. As far as we know, oxygen expanded the metabolic and biochemical capacities of organisms, possibly contributing to the diversification of life [2]. Harnessing oxygen in aerobic metabolism to generate energy is not without hazards though, the most important being the generation of reactive oxygen species (ROS). If uncontrolled, these highly reactive by-products can wreak havoc, attacking all of the main building blocks from which bodies are made, including DNA, thus resulting in oxidative stress [3]. As a consequence, the need to evolve adequate defenses against the generation or accumulation of such damage arose in parallel with the use of oxygen by cells to generate energy [4]. Aside from oxygen metabolism, there have been numerous other potential sources of stress, such as changes in abiotic conditions.

Diversification of life and the spread of species into new and different environments meant that organisms faced new challenges, such as adaptation to new thermal regimes, fluctuations in water availability or salinity, variation in natural background ionizing radiation and in ultraviolet light. The nature of, and the interplay between, the costs and benefits involved in balancing offspring or energy generation against damage mitigation is a major area of research that cuts across many biological disciplines and levels of enquiry. What this research has taught us so far is that, while substantial molecular and higher-level damage can be detrimental for the organism, exposure to tiny amounts of such damage or to mild doses of environmental stressors (e.g., heat stress, ROS, radiation) may be essential for the organism [5–18].

In this review, we discuss current evolutionary thinking on the costs and benefits of stress exposure with a special reference to ionizing radiation, but also relying on examples of other environmental stressors. Although these examples include endpoints spanning the molecular to the organism level, the main focus is on reproductive fitness (or output) because to be successful in evolutionary terms an organism must pass its genes to the next generations, without mutations detrimental for fitness. To further explore these concepts, we present a novel mathematical model that explores and tests the hypothetical conditions that make a particular kind of hormesis (conditioning hormesis) ecologically and evolutionarily plausible.

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2. Dose–response models in toxicology and stress physiology

Axioms such as 'forewarned is forearmed' and 'that which harms often teaches' are cemented into our society, but not necessarily into our science. It is often assumed for an individual that the health or reproductive fitness declines (or e.g., cancer risk increases) as an LNT function of the dose of the stressor (e.g., chemical toxicant, abiotic stressor). This LNT decline which differs for different individuals is assumed to impact the risk of a given stochastic health effect (e.g., cancer), which is also characterized by an LNT model and can be justified on the basis of a Poisson distribution of cancer cases when there is a linear decline in health fitness.

The LNT model for characterizing the risk (or related endpoint such as relative risk) of a specific health effect has traditionally been a leading concept in several core disciplines (e.g., toxicology, radiation biology), but in recent years its validity has increasingly been questioned because it failed to accurately predict organism responses and outcomes (stochastic) related to low-dose stressor exposure [19–23]. The main reason for this is that knowledge about an individual's response to high doses has proven insufficient to predict its response to low doses, which raises important questions about the validity in evolutionary biology of the LNT risk model as it is currently used [e.g. [14,24,25]].

This research along with other research in this Special Issue and prior published work [e.g. [14]] shows that the LNT model is in most cases considered not biologically realistic. According to the threshold model, there is no significant effect (e.g., cancer induction) until the dose reaches a given threshold value (termed the 'No Observed Adverse Effect Level' in the toxicological literature), above which reproductive or other fitness traits decline (or physiological stress level increases) linearly or non-linearly with dose. Often, the dose-response relationship is biphasic, with low doses eliciting a stimulatory or beneficial organism response and high doses causing inhibition or toxicity, respectively. This form of biphasic dose-response is characteristic of hormesis [e.g. [5,19]].

In the following paragraphs, we review the findings of a number of ecological and evolutionary studies that show how the LNT model is less accurate than threshold and hormetic models, and present a hypothetical mathematical model that explores the conditions that make a particular kind of hormesis [termed "preconditioning hormesis" by Ref. [26]] evolutionary plausible.

3. Is the LNT model evolutionarily realistic and compatible with the need of DNA integrity maintenance?

DNA is the repository of genetic information in each organism. Its integrity and stability are both essential to life. DNA is also not inert because it is open to damage when an organism is being exposed to an environmental stressor. The premise of the LNT model is that the impact of some forms of environmental stressors, be it ionizing radiation or heat stress, on a biological endpoint, like DNA damage and consequent mutation frequency and cancer incidence, is directly proportional to the dose. Implicit in the assumptions of the LNT model is that an observable detrimental biological effect becomes evident when the magnitude of a given environmental stressor an organism is exposed to increases relative to a control situation (e.g., no radiation, no heat stress) and that the frequency of such a biological effect increases linearly with the dose. The assumptions justifying the LNT model are irreconcilable with current evolutionary theory for several reasons.

First, life on earth appeared and evolved in highly stressful environments where, for example, levels of ionizing radiation were much higher than background radiation levels today [27]. Thus, selection favoured evolution of numerous adaptive molecular mechanisms to deal with constant exposure to natural background radiation and other stressful conditions that organisms retain at present, such as those repairing damage to DNA. Consequently, any detrimental effects on reproductive or other fitness traits would be expected to be evident only above certain stress levels.

Second, all life on earth is exposed to background radiation with highly variable absorbed doses ranging from 0.01 to 260 mGy y^{-1} in humans [28,29]. Epidemiological and physiological studies did not find consistent differences in endpoints like DNA damage, cancer markers or chromosome aberrations between people living in areas with high naturally occurring background radiation and those living in areas with low background radiation [e.g. [28,30–32]].

Third, if organisms have adapted to thrive in the presence of radiation, how would they react to a significant decrease in environmental radiation dose? Work on protozoans, bacteria and fruitflies (*Drosophila melanogaster*) has shown that exposure to lower-than-background radiation levels can result in negative effects on fitness-related traits, compared to individuals exposed to background radiation [e.g. [33–35]]. Experiments on the protozoan *Paramecium tetraurelia* and the cyanobacterium *Synechococcus lividus* showed that shielding against background radiation was detrimental and that radiation hormesis only occurred in a limited range of doses above background level and disappeared for doses higher than 50 mGy y^{-1} [35]. Follow-up experiments on other organisms have found similar deleterious effects for exposure to below-background radiation, including (1) decreased protection to mutational damage in *Saccharomyces cerevisiae* [36], (2) higher sensitivity to apoptosis and intracellular oxidative stress in *Cricetulus griseus* [37], (3) reduction of growth rate in *Mus musculus* L5178Y cells [38], and (4) changes in the concentration of antioxidant enzymes [39] and in the expression of genes regulating DNA repair and response to oxidative stress in *Shewanella oneidensis* and *Deinococcus radiodurans* [40,41]. Recent work has actually shown that organisms exposed to lower-than-background radiation experience this unusual environment as stressful, leading to upregulation of many genes involved in protection against oxidative stress and down-regulation of those regulating protection of DNA [42].

Fourth, the LNT model is not accurate or biologically meaningful in predicting the organism response to low doses of a given stressor because it ignores the mechanisms that govern the organism's physiological adaptive stress responses. Implicit in the LNT model is the assumption that organism responses are elicited passively by environmental stimuli. Contrary to this outdated view, the organism more often actively decodes the information content of a given environmental stimulus and orchestrates a response to it. These potential responses are wide-ranging, including genetic and epigenetic mechanisms and phenotypic plasticity that translate into the activation of protective mechanisms of molecular integrity or of systems of damage repair [6–8,11,14–18].

Exposure to chemical toxicants or other kinds of stressors can also result in selection of resistant phenotypes or genotypes [43–47]. Translocation of these resistant individuals in areas free of that particular toxicant can cause reduced survival and reproduction [e.g. [43,44,46]]. This indicates that organisms adapted to a specific stressor might have developed a need of it, as otherwise the costs of maintaining protective mechanisms against a particular stressor would be too high to sustain. This is further supported by studies on phenotypic plasticity, which is the ability of an organism to change its phenotype [e.g., via preconditioning and postconditioning hormesis, 26] in response to changes in the environment.

In rapidly changing environmental conditions, the contribution of plasticity has critical implications for individuals and the evolution of populations by allowing adaptive traits to be rapidly introduced within a single generation [48,49]. This is particularly important when exposure to stress occurs early in life when the conditioning of the physiological system (preconditional hormesis) prepares the organism to withstand stress later in life [e.g. [16–18,50]]. Conditioning of stress responses in early life may carry fitness benefits providing the stressor is then encountered in the adult environment, while there might be a cost of phenotypic adjustment if there is no subsequent exposure to that

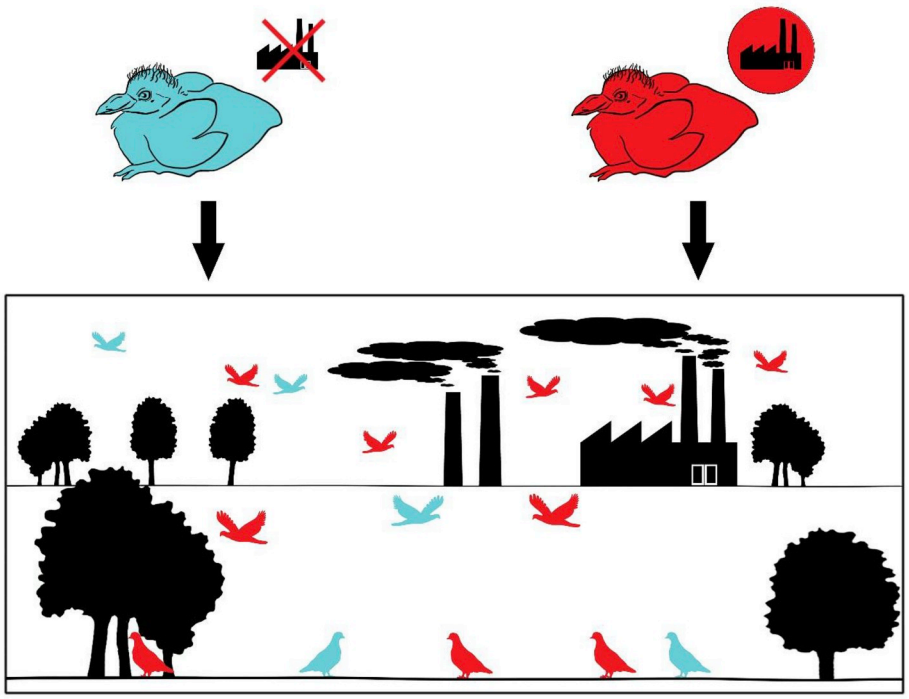

Fig. 1. Environmental conditions experienced while developing may have long-lasting consequences for the individual chances of surviving and reproducing. This illustration shows one hypothetical scenario about the consequences of developing in either a toxicant (or stress) free environment (blue nestling) or a mild polluted (or stressful) environment (red nestling). In adulthood, the population size of blue and red birds will be similar in low to mild stressful environments as long as there is not a cost due to mismatching between young and adult environmental conditions [e.g., 10]. In contrast, in environments where there is high pollution or stress red birds will outperform blue birds because of the early life conditioning hormesis of mechanisms to deal with stress. This hypothetical scenario would apply to species with low vagility, such as those that cannot rapidly disperse or migrate. Serena Costantini ©.

stressor in adulthood [10]. This may occur when the early life environment does not match the conditions experienced in adulthood. In other words, early life exposure to a mild dose of a chemical toxicant or of another environmental stressor (e.g., radiation, heat stress) might trigger phenotypic adjustments that would then translate in that individual being able to tolerate them better than when exposure to a high amount of the same stressor occurs in later life and, consequently, to flourish in challenging environments (Fig. 1). In the following paragraphs, we present further examples of experimental work supporting the idea that the key assumptions of the LNT model are generally wrong.

4. Evidence against the LNT model

4.1. Genetic evolution

Observational and experimental studies have shown that organisms can exhibit higher evolutionary rates when living under stressful conditions [e.g. [51,52]]. Earlier experiments on *Drosophila melanogaster* showed that genetic recombination (generation of a novel combination of genetic information that can be passed from the parents to the offspring) increases at development temperatures above or below normal culture temperatures, resulting in a U-shaped curve [53,54]. At near lethal temperature extremes, however, there is some evidence for a fall in recombination. Later experiments on *Drosophila melanogaster* [55,56] and other species [*Neurospora crassa* in Refs. [57,58]; *Coprinus lagopus* in Ref. [59]; *Caenorhabditis elegans* in Ref. [60]] found qualitatively similar results, implying that in mildly stressful environments, variability generated by recombination may increase. These results suggest potential for threshold or hormetic responses to facilitate evolutionary change.

A recent study on *Escherichia coli* showed that genetic innovations involving pre-existing DNA repair functions can play a predominant role in the acquisition of a phenotype resistant to increasing ionizing radiation [61]. Examples of evolutionary change in a short time can also be found in wild vertebrates. A recent study of Darwin's finches in the Galápagos Islands tells us that a complex trait, such as beak size, can evolve significantly in less than 1 year when the environment is stressful [62].

4.2. Stress resistance and survival

Some organism responses to radiation described in numerous species echo those resulting from some other types of stressors, which implies that mechanisms underlying threshold or hormetic responses are highly conserved across a wide range of species and stress agents (see below). For example, recent work on a bird species (zebra finch, *Taeniopygia guttata*) showed that exposure to relatively mild stress may have long-lasting positive consequences.

In two studies by Refs. [9,10], zebra finches encountering warmer-than-normal environments in adulthood showed increased resistance to molecular oxidative damage and long-term survival and resilience. However, this was observed only when they had been exposed to episodes of mild thermal stress before reaching sexual maturity. This work suggests that early-life exposure to mild stress may be beneficial when it matches (to some degree) the environmental conditions experienced later in life. When no heat stress was encountered in adulthood, however, survival was poorer in birds that experienced mild heat stress in early life than in those that did not, demonstrating a cost of preconditioning hormesis in the absence of a challenge in adulthood.

In another study on the zebra finch [63], showed that repeated exposure to the stressful conditions caused by unpredictable food availability (which induced no changes in body mass) was associated with an increase in lifespan. The birds responded to the unpredictable food supply by increasing baseline glucocorticoid stress hormones without any signs of habituation of this hormonal response to the treatment across time [63]. The increase of plasma concentration of glucocorticoids induced by the treatment was significant, but relatively mild, since the baseline glucocorticoid concentrations in the treated birds were substantially lower than the peak levels that occur during an acute stress response in this species [63]. These results led the authors to hypothesize that in a range of nutritional or other mild and unpredictable environmental stressors, hormetic responses via moderate stimulation of the Hypothalamic-Pituitary-Adrenal axis (which is responsible for the secretion of glucocorticoids) may represent an evolutionary conserved mechanism that promotes survival and reduces the

rate of ageing [63].

4.3. Variation among co-specific populations and species

Exposure to environmental stressors can contribute to shaping life diversity, and stress is something all living organisms experience. However, the intensity of environmental stressors varies in time and space, as does the coping capacity of populations and species. For example, while adverse health effects (e.g., cancer) do not show any appreciable increase in individuals living in areas with high natural background radiation, some aspects of cell resistance to stress appear to improve [28,30,64,65].

A recent meta-analysis of studies testing the effect of chronic low dose radiation on metrics of oxidative status (markers of oxidative damage, enzymatic and non-enzymatic antioxidants) found significant heterogeneity in effect size across species and tissues [66]. This conclusion suggests that there may be selection that acts on the capacity of organisms to cope with ionizing radiation (e.g., upregulation of DNA repair mechanisms, antioxidants). For example, while controlling for a number of potentially confounding variables, [67] showed that glutathione (an important intracellular antioxidant) levels increased, and lipid peroxidation and DNA damage decreased with increasing background radiation in some species of birds. These results might be due to genetic selection. For example, through directed evolution in the laboratory [61], generated populations of *Escherichia coli* exhibited a new phenotype characterized by extreme resistance to ionizing radiation due to increased DNA repair functions. Similarly, [68] suggested that hormetic mechanisms induced by environmental stressors might drive the evolution of genes regulating mechanisms that extend longevity. Alternatively, these results might be due to hormetic preconditioning [26] of the physiological system in order to tolerate higher levels of radiation. For example, experiments on genetically similar laboratory rodents found that individuals chronically exposed to doses of radiation slightly higher than background level lived longer than those exposed to background radiation [e.g. [69–71]].

Inter-species variation in tolerance of ionizing radiation might also be inferred from estimates of local abundance (i.e., number of individuals of a species living in a given area). The prediction is as follows: if external and internal exposure from radionuclides has no discernible impact on the health status of a species, abundance of that species in a highly radionuclide contaminated site would be expected to be similar to that in control sites. Although early work has shown negative effects on local abundance, number of eggs produced, immunity or body colourations in some bird species living in the highly contaminated Chernobyl Exclusion Zone [72], recent evidence suggests that populations of several mid-to large-sized carnivores, and of Eurasian boars, increased within the Chernobyl Exclusion Zone during the decades after the accident, and that mammal distributions across sites are uncorrelated with the severity of local radiation contamination [73,74]. These results suggest that the response of populations to radiation may vary across time, raising a difficulty in predicting responses in the long-term.

It is clear that it is not straightforward to predict whether in the long-term a species is going to flourish or perish in areas where there has been a small increase in background level of radiation. This conclusion does not seem surprising given that over the incipient stages of evolution of life the intensity of natural background radiation was much higher than it is now [27]. The conservative nature of DNA damage repair mechanisms in modern organisms suggests that these mechanisms evolved in the distant past and that living organisms retain the capability of efficiently repairing DNA damage from present radiation levels [27].

It is also important to consider that the biological effects of a given environmental stressor also depend on the co-exposure of the organism to additional stressors. For example, when experimentally exposed to high doses of gamma radiation (200–400 Gy), Caribbean fruit flies (*Anastrepha suspense*) suffered reduced survival, but when exposed to a combination of radiation and anoxic stress, survival of females was unaffected, while that of males was lower than controls yet significantly higher than males exposed only to irradiation. Exposure to a combination of irradiation and anoxic stress also improved resistance to oxidative stress and mating success of males. The strong tolerance of the marine tardigrade *Echiniscoides sigismundi* to radiation is due to molecular mechanisms that have evolved to allow survival of this organism to extreme dry environments [75]. These results further support the conclusion that evolution would not have been successful if the LNT model for stochastic biological effects were valid.

5. Modelling the conditions that make hormesis evolutionarily possible

Having presented substantial evidence that the LNT model is unlikely to be a good description of biological reality, we propose mathematical simulations to establish a better understanding of the evolutionary implications of hormesis. Relying on a number of realistic assumptions based on data available in the literature, mathematical simulations allow us to test relevant concepts without the need to perform experiments. Simulation models also allow us to formalize ideas, and have the added benefit of forcing us to define the most essential aspects of stress-response mechanisms. Although it would be possible to build a detailed model based upon empirical data on a well-studied model organism, we deliberately chose a general approach because the aim is to improve understanding at a conceptual level. Such models are a highly useful first step towards understanding the potential effects of environmental stressors on reproductive fitness [76].

Here, we present a simple hypothetical model that investigates the conditions under which mechanisms that allow preconditional hormesis [26] experienced during development can be expected to persist in a population. We focus on preconditional hormesis because much empirical research on stress physiology or longevity has provided convincing support for the biological relevance of this kind of hormesis and its applicability to many kinds of environmental stressors [e.g., 9–10].

More specifically, we investigate (i) how the degree of stress predictability during early and late life stages (stress (mis)match) is expected to affect the reproductive fitness of individuals in a population that have hormesis potential (HP), and (ii) how this reproductive fitness is affected by trade-offs between the benefits and costs of having HP. With use of the term HP we allow for genetic and epigenetic mechanisms driving hormetic responses to environmental stressors [77]. For example, [78] investigated the genetic variation of hormetic effects on lifespan induced by heat stress and the associated quantitative trait loci in various strains of *Caenorhabditis elegans*. Wild type CB4856 worms exposed to heat stress survived 18% longer than controls of the same strain. Using recombinant inbred lines (RILs) derived from a cross between wild types N2 and CB4856, [78] also found natural variation in stress-response hormesis in lifespan. More than one quarter (28%) of the RILs displayed a hormetic effect in lifespan induced by heat stress. Importantly, the ability to recover from heat-shock mapped to a significant quantitative trait locus (QTL) on chromosome II. The QTL was confirmed by infiltrating relatively small CB4856 regions into chromosome II of N2.

The model builds on realistic numbers of offspring that many bird and mammal species can generate at each reproductive event. It simulates a population of a generic species in which all individuals go through a young and an adult stage, after which they produce a certain number of offspring and die (i.e., no overlapping generations). We allow via our modelling for young stage individuals of a given species to experience three levels of stress (none, mild, severe), while during the adult stage they can experience two (none, severe).

While it would be possible to implement a range of stress levels experienced during adulthood, stress levels were intentionally limited to two in order not to make the final number of potential outcomes too

Table 1
Model conditions based on a set of simple rules. Each row describes a rule, the stage (young or adult) and stress condition (none, mild and severe for the young stage, and none or severe for the adult) at which it is applied, which individual class it applies to (Hormesis potential – HP and/or Hormesis negative – HN individuals), and the effect size by which it changes the number of offspring. The final number of offspring for each individual class at the end of a generation (young + adult stage) is calculated by summing each rule that was applicable for the conditions experienced during the young and adult stages.

Rule	Stage	Stress Level	Affects HP	Affects HN	Effect
1. Cost for being HP [Assumes that there is a cost for having hormesis potential, regardless of the experienced stress conditions]	Young	Any	X	–	−1
2. Cost of stress	Young	Mild	X	x	−1
3. Cost of stress	Young	Severe	X	x	−3
4. Cost of hormetic conditioning activation [Assumes that there is a cost for activating hormetic conditioning mechanisms]	Young	Mild	X	–	−0.5
5. Cost of stress	Adult	Severe	x	x	−2.5
6. Benefit of standard plasticity in case of mild stress during youth and severe stress during adulthood [Assumes that all individuals, regardless of whether they have hormetic conditioning potential, still develop a low level of resistance against future stress if they were exposed to mild stress in early life]	Adult	Severe	x	x	1.5
7. Benefit of hormetic conditioning in case of mild stress during youth	Adult	Severe	x	–	3.5
8. Benefit of standard plasticity in case of severe stress during youth and adulthood [Assumes that all individuals, regardless of whether they have hormetic conditioning potential, still develop a low level of resistance against future stress if they were exposed to severe stress in early life]	Adult	Severe	x	x	1

Table 2
Resulting total number of offspring for the different combinations of stress conditions, based on the standard set of rules described in Table 1.

Condition while young	Condition while adult	Final offspring number for HP individuals	Final offspring number for HN individuals
No stress	No stress	5	6
No stress	Severe stress	2.5	3.5
Mild stress	No stress	3.5	5
Mild stress	Severe stress	6	4
Severe stress	No stress	2	3
Severe stress	Severe stress	0.5	1.5

large, as this would make result interpretation unnecessarily difficult. For instance, the addition of a mild stress level during adulthood would generate a number of intermediate results between those generated by no or severe stress, but our interest lies in developing a conceptual understanding of the most extreme scenarios.

Exposure to a given stressor affects the number of offspring generated, but within the same species, individuals with HP are affected differently from those that are hormesis negative (HN, i.e., unable to generate an hormetic response): HP individuals can develop resistance against stress experienced during adulthood, but only if they were exposed to mild stress levels early in life. All model scenarios (which include a number of simulations) include a cost for being HP, which reflects the expected trade-off between investing in hormesis potential (self-maintenance) and in other traits (e.g., growth, reproduction). The model is based on a simple set of rules, described in Table 1, that determine how HP and HN offspring numbers are affected each generation, using a reference offspring number of 6 individuals (but note that the exact value is irrelevant and can be any number). For example, if individuals experience mild stress when young, and severe stress when adults, the number of offspring for HP individuals will be as follows: 6 (reference offspring number) −1 (rule 1: cost for being HP) −1 (cost of mild stress when young) −0.5 (cost for activating hormetic conditioning) −2.5 (cost of severe stress when adult) +1.5 (benefit of standard plasticity) +3.5 (benefit of hormetic conditioning), which adds up to a final offspring number of 6. Under the same conditions, the number of offspring for HN individuals will be: 6 (reference) −1 (cost of mild stress when young) −2.5 (cost of severe stress when adult) +1.5 (benefit of standard plasticity), adding up to a final offspring number of 4. In this situation, hormetic conditioning potential therefore yields a higher reproductive fitness. Table 2 shows the final offspring numbers for all possible stress combinations.

Each model run consists of 30 generations, where all individuals (starting with 50% HP and 50% HN) in each generation go through a young stage and an adult stage. At the start of a model run, one of three stress levels (none, mild, severe) is randomly chosen to be experienced by young individuals. Next, a stress level is generated for the following adult stage, according to a certain 'stress match probability' (SMP)

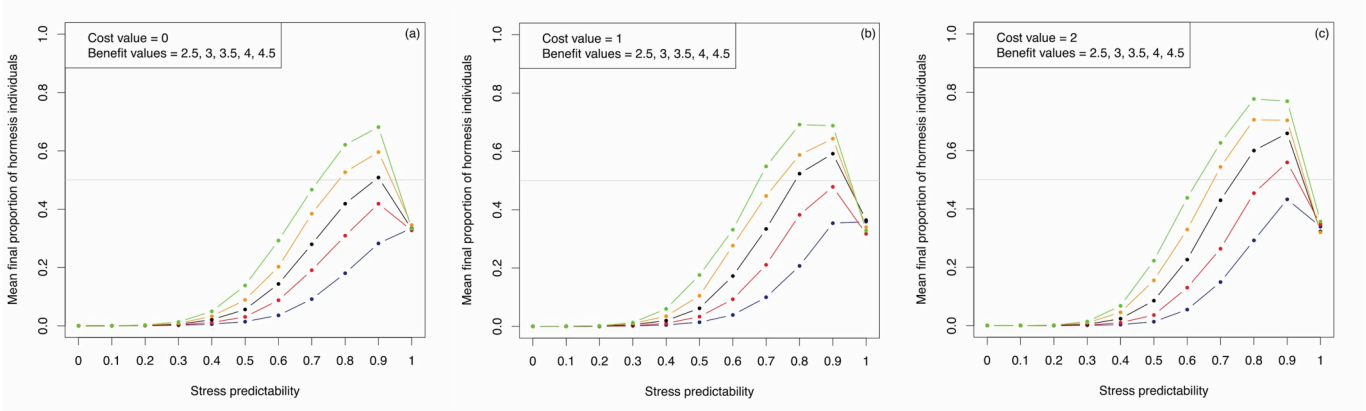

Fig. 2. Mean final proportion of HP individuals in the population for a range of stress match probabilities, under different combinations of mild stress cost values during youth and HP benefit values in the case of mild youth stress followed by adult stress. Benefit values shown in decreasing order from top (green line) to bottom (blue line). All other model values are the same as those shown in Table 1. The horizontal line indicates the transition where the reproductive fitness of HP is higher than that of HN.

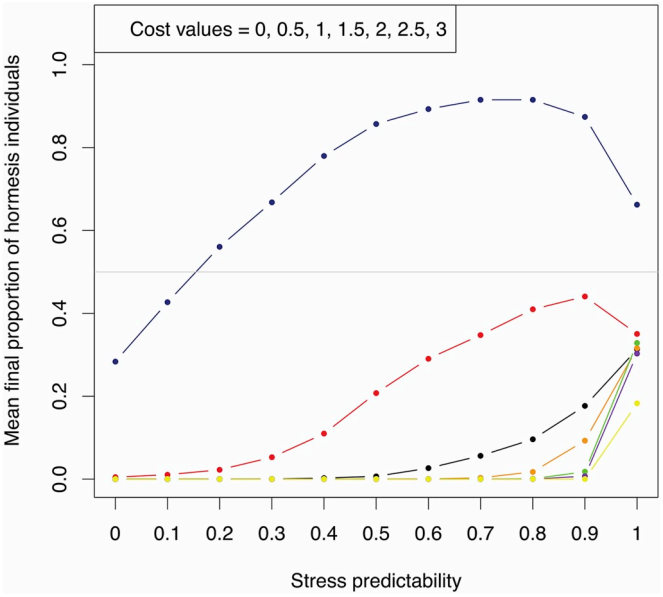

Fig. 3. Mean final proportion of HP individuals in the population for a range of stress match probabilities, and a range of cost values for hormesis potential, shown in increasing order from top (blue line) to bottom (yellow line). All other model values are the same as those shown in Table 1. The horizontal line indicates the transition where the reproductive fitness of HP is higher than that of HN.

value that is selected for that given model run. This value between 0 and 1 gives the probability of experiencing the same environmental conditions as those that were present during the young stage. For example, if the SMP is 1, the subsequent stress situation will always be the same as that during the previous stage. If the SMP is 0.7, there is a 70% chance that the subsequent situation will be the same, and a 30% chance that it changes. If the SMP is 0, the stress situation will always change. This means that for SMP values above 0.5 the stress level is more likely to be the same as the previous one, while for values below 0.5 the stress level is more likely to be different. The model goes through 30 generations, and the proportion of HP individuals in the population at the end of the 30^{th} generation is used as a proxy for HP reproductive fitness (i.e., evolutionary success). Thirty generations were chosen because this is sufficiently long to lose the influence of initial model stochasticity, and choosing a higher number of generations would not have affected the results (not shown).

A next step is to investigate which environmental stress conditions enable hormesis to persist in the population. We approach this question by analysing a number of model situations: (i) the effect of a range of stress match probabilities on HP reproductive fitness, (ii) the effect of the magnitude of the benefit offered by hormetic conditioning relative to the cost of mild stress during youth, (iii) the effect of the magnitude of the cost of being a HP individual. For each model situation, a set of cost and benefit values was chosen (based on the values shown in Table 1), and for each SMP value (all values from 0 to 1, in steps of 0.1) 1000 model runs were performed. This procedure was necessary because each model run is a random and stochastic outcome of the model. For each model run, the final proportion of HP individuals was retained, and the mean was calculated so that each model scenario had one single value of reproductive fitness.

The most important pattern that emerged from the simulations is that in most situations HP individuals can only survive in the population if stress conditions did not change too often. For all tested combinations of mild stress cost and hormetic conditioning benefit (Fig. 2), we observe a threshold behaviour, where HP individuals can only survive in the population at SMP values of 0.3 or higher, regardless of the magnitude of stress cost (Fig. 2a vs 2b vs 2c). In other words, if there is a low probability of stress conditions remaining the same, the benefit of conditioning never exceeds the cost of stress, even for high benefit values. It is also interesting to note that when stress occurrence is completely random (SMP = 0.5), there is always a proportion of HP individuals that can survive in the population (Fig. 2), except when the cost of being HP is high (Fig. 3). The cost of being HP has strong effects on HP reproductive fitness (Fig. 3): when there is no cost, HP individuals can always survive in the population and reproductively outperform HN individuals in most cases except when stress conditions are very likely to change (SMP < 0.2). As soon as there is a cost, however, HP reproductive fitness decreases rapidly, although there always seem to be some conditions of high stress predictability that allow a proportion of HP individuals to survive together with HN individuals.

6. Conclusions

Our review provides both empirical and theoretical evidence to conclude that the LNT model is not only invalid but also biologically unrealistic as compared to either threshold or hormetic models. It is beyond dispute that evolution has taken place in a number of extraordinarily stressful environments with simultaneous exposure to radiation, chemicals and abiotic factors (e.g., heat stress). This complex process has resulted in the appearance and positive selection of a number of stress response mechanisms (including mechanisms of damage repair), most of which are now highly conserved across species and underlie many of the threshold or hormetic responses to environmental stressors (including ionizing radiation) characterized to date. Our simulation models allowed us to define the most essential aspects of stress-response mechanisms underlying hormesis. It will be important to validate or refine our models using empirical data collected from well-defined experiments.

In conclusion, based on a large body of empirical data, in addition to theoretical assumptions, it is logical to conclude that if LNT were a biologically valid dose-response model, the evolution of life on Earth would not have been possible.

Conflicts of interest

Authors declare no conflict of interest.

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The LNT model for cancer induction is not supported by radiobiological data

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ABSTRACT

The hallmarks of cancer have been the focus of much research and have influenced the development of risk models for radiation-induced cancer. However, natural defenses against cancer, which constitute the hallmarks of cancer prevention, have largely been neglected in developing cancer risk models. These natural defenses are enhanced by low doses and dose rates of ionizing radiation, which has aided in the continuation of human life over many generations. Our natural defenses operate at the molecular, cellular, tissue, and whole-body levels and include epigenetically regulated (epiregulated) DNA damage repair and antioxidant production, selective p53-independent apoptosis of aberrant cells (e.g. neoplastically transformed and tumor cells), suppression of cancer-promoting inflammation, and anticancer immunity (both innate and adaptive components). This publication reviews the scientific bases for the indicated cancer-preventing natural defenses and evaluates their implication for assessing cancer risk after exposure to low radiation doses and dose rates. Based on the extensive radiobiological evidence reviewed, it is concluded that the linear-no-threshold (LNT) model (which ignores natural defenses against cancer), as it relates to cancer risk from ionizing radiation, is highly implausible. Plausible models include dose-threshold and hormetic models. More research is needed to establish when a given model (threshold, hormetic, or other) applies to a given low-dose-radiation exposure scenario.

1. Introduction

"It is true that life exists in a sea of radiation, radioactivity, and chemicals for most populations. Moreover, all living things consist of chemicals constantly undergoing complex interactions microsecond by microsecond in an elegant and well controlled manner consistent with population having healthy lives that extend on average over 75 years in most industrialized countries" [1].

"The species, which have been selected by evolution during 3.5 billion years for unicellular organisms and 600 million years for multi-cellular organisms, are those which benefit from protective mechanisms against mutagenic and carcinogenic agents. Life has developed in a bath of ultraviolet and ionizing radiation. It should therefore be expected that living organisms have particularly efficient systems within the dose range which has been delivered during evolution (2–20 mSv/year)" [2].

"The number of lives in the world that can be saved and prolonged by low dose ionizing radiation in one year is considerably greater than all the American combat losses in our entire history" [3].

The quotations above highlight the importance of understanding radiobiological mechanisms, including those that relate to disease prevention and increased longevity after low radiation doses versus harm after high doses. Ionizing radiation is a ubiquitous feature of the cosmos [4] and the stimulatory effects of low doses of ionizing radiation were observed shortly after the discovery of X rays by Wilhelm Röntgen in 1895 and during intervening years [5–8].

Natural background ionizing radiation has exerted a stress to organisms since life first appeared on Earth and microorganisms have been demonstrated to be sensitive to the loss of natural background radiation [9–11]. While high radiation doses are clearly harmful, reducing natural background ionizing radiation has been demonstrated to also be harmful [12]. Thus, reducing radiation dose is not always beneficial, depending on the dose range. When exposed to less than natural background radiation levels, achieved through shielding, single cell organisms could not proliferate [13]. *In vitro* and *in vivo* exposures to low doses of sparsely ionizing radiations such as gamma or X rays were found to invoke adaptive changes in DNA that are protective [14–18]. The nature of the response depends on the complexity of the damage [19].

Complex and efficacious defense mechanisms against cancer which are enhanced by low-dose radiation have evolved since life forms first originated on our planet [20–23]. At the subcellular and cellular levels, DNA repair and apoptosis (programmed cell death) are key defenses. At

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Abbreviations

ADCC antibody-dependent cellular cytotoxicity
ANP activated natural protection
ATM Ataxia-Telangiesctasia mutated
ATP adenosine triphosphate
CD8+ cytotoxic T cells of type $CD8^+$
CD4+ T cells of type $CD4^+$
CDKN1A cyclin dependent kinase inhibitor 1A
CTLA-4 cytoxic T-lymphocyte-associated protein 4
DNP 2,4-dinitrophenyated ascaris extract
eNOS endothelial nitric oxide synthase
epiregulated epigenetically regulated
epicellcom epigenetically regulated, cell-community-wide
ERK1/2 extracellular signal-regulated kinases 1 and 2
GADD45A growth arrest and DNA-damage-inducible protein GADD45 alpha
γ-GCS γ-glutamylcysteine synthetase
γ-H2AX phosphorylated histone H2AX
GPx glutathione peroxidase
GR glutathione reductase
GSH glutathione
HBEC human bronchial epithelial cells
HDG high-dose-rate group;
HDR high-dose radiation
HFL1 fibroblast cell line;
HGF/MET hepatocyte growth factor/mesenchymal-epithelial transition
HLNRA high-level, natural radiation area
HOCl hypochlorous acid
HO-1 heme oxygenase 1
HRR hormetic relative risk
HSP heat shock protein
H2O2 hydrogen peroxide;
INF-γ: interferon gamma
iNOS+/M1 inducible nitric oxide synthase/M1 macrophage polarization
IL-6R interleukin-6 receptor
LDG low-dose-rate group;
LDR low-dose radiation
MC methylcholanthrene
NADPH nicotinamide adenine dinucleotide phosphate
NBS1 protein Nibrin
NK natural killer
•NO nitric oxide radical
Nrf2 nuclear factor erythroid 2-related factor 2
•OH hydroxyl radical
O2-• Superoxide radical
ONOO- peroxynitrite;
PAM protective apoptosis mediated
p38MAPK p38 mitogen-activated protein kinase
SASP senescence-associated secretory phenotype
STAT3 signal transducer and activator of transcription 3
Trx-1 thioredoxin-1
TSCE two-stage clonal expansion
UFD-2 U-box-containing ubiquitylation enzyme
uPAR Urokinase receptor

the tissue level, intercellular signaling can remove precancerous cells. At the whole-body level, the immune system can eliminate (e.g. via abscopal effects) both precancerous and cancer cells. Thus, there is a hierarchy of natural defense mechanisms (cancer barriers) that must be overcome in order for cancer to occur [16,20–25]. This hierarchy of natural defenses against cancer and their enhancement by low-dose radiation is a focus of this review. Another focus is on implications of these factors for the linear-no-threshold (LNT) model, which is essentially devoid of biological mechanisms and whose support now comes mainly from some poorly-designed and unreliable epidemiologic studies [26].

2. The hallmarks of cancer and implications for LNT

Carcinogenesis is a complex phenomenon that cannot be solely reduced to a series of mutations caused by independent stochastic lesions occurring in the same cell [2,27–29]. Rather, the carcinogenesis process impacts all aspects of genome function [30,31]. Further, the influences of genetic and epigenetic mechanisms are now well-established [29]. During the carcinogenesis process, modifications of the genome via several stages then confer a selective advantage to the impacted cell and its progeny [32].

A number of changes are therefore needed before aberrant cells are formed and start to grow uncontrollably to form cancer. A seminal paper [33] outlined how cells acquire a cancer-like phenotype by detailing key changes or features called the “hallmarks of cancer.”

To understand the implausibility of low radiation doses causing cancer, it is important to be aware of the multiple hallmarks of cancer and what natural defenses (barriers) need to be overcome for cancer to occur and how unlikely it is that a single radiation ionization (radiation hit) can lead to cancer. With the LNT model, all of the different cancer hallmarks can arise from a single radiation hit. The indicated hallmarks are briefly discussed below.

2.1. Self-sufficiency in growth signals and insensitivity to anti-growth signals

Cells must acquire the ability to continually grow in order to lead to cancer [33]. To become self-sufficient in providing their own growth signals, cancer cells constitutively activate signaling pathways making them no longer dependent on external signals to prompt progression through the cell cycle. Anti-growth signaling (a component of the hierarchy of natural defense mechanisms) from the host which occurs as a barrier to cancer must therefore be overcome. However, cancers can become resistant to anti-growth signals from the host, which facilitate abnormal cell division.

The age of the host can influence host-to-tumor signaling as revealed by a recent mouse study [34–36]. Changes in the spleen (an immune system interconnection in mice) with increasing age were examined for potential influences on anticancer immunity. A tumor implant strategy with monitoring of immune system responses was employed. The animal model used was C57BL/6 male mice (adolescent, young adult, middle-aged, and old; 68, 143, 551 and 736 days old, respectively) with and without a syngeneic (genetically similar or identical) murine tumor implant. By using global transcriptome analysis, immune-system-related functions were found to be key regulators in the spleen associated with tumor growth as a function of age, with T-cell associated CD2 (cluster of differentiation 2), CD3ε (cluster of differentiation 3 related), chemokine (C-C motif) ligand 19 (CCL19), and chemokine (C-C motif) ligand 5 (CCL5) being the key molecules involved.

Recent findings of Tape et al. [37] indicate that oncogenic mutations regulate tumor-growth-related signaling and involve both tumor cells and adjacent stromal cells. They showed that tumor cell oncogenic KRAS (which is called $KRAS^{G12D}$) can indirectly regulate tumor cell signaling via stromal cells. The researchers analyzed heterocellular (i.e. composed of cells of different kinds) $KRAS^{G12D}$ signaling in pancreatic ductal adenocarcinoma cells and observed that tumor cell $KRAS^{G12D}$ signals to fibroblasts which then signal back to the tumor cells (i.e.

reciprocal signaling). This reciprocal signaling from fibroblast to tumor cells can cause amplification of the number of regulated signaling nodes from tumor-cell-related $KRAS^{G12D}$, thereby facilitating tumor growth. However, the reciprocal signaling from fibroblasts also regulates apoptosis of the tumor cells. The LNT model [38,39] relies on the premise that even a single radiation ionizing event can cause tumor formation and/or be responsible for reciprocal signaling that promotes growth of an existing tumor. This is quite implausible. Otherwise, life as we know it could not exist since everyone is radioactive with many ionizing events taking place in our bodies every second during life.

2.2. Evading apoptosis

When encountering aberrant and potentially cancerous growth signaling, normal cells can activate programmed cell death (apoptosis) signaling. However, cancer cells can acquire the ability to evade the induction of apoptosis, which is crucial for both maintaining tumor growth and allowing cancer cells to form in the first stage of disease development. Interestingly, low-dose radiation stimulation of selective apoptosis of neoplastically transformed cells has been demonstrated [40,41], which does not support the LNT model for cancer induction. The dose-response relationship for neoplastic transformation has been found to be hormetic (Fig. 1 [20]; in agreement with observations of [40,41]. The figure shows a hormetic response for neoplastic transformation relative risk (RR) after gamma-ray exposure of cells *in vitro* based on data of [42]. Redpath's group also studied the importance of dose rate in connection with low-dose, gamma-ray protection against neoplastic transformation [43]. A dose-rate threshold (approximately 1 mGy/day) was revealed. The research group also showed that the relative risk for *in vitro* neoplastic transformation (which was hormetic) after gamma-ray exposure was consistent with the possibility of hormetic responses for cancer induction in humans. Quite similar relative risk dose-response relationships were found for *in vitro* neoplastic transformation and for cancer induction in humans for moderate and higher doses [42]; however, the data for low doses where hormetic responses (relative risk < 1) occurred were based on neoplastic transformation. Moderate and high but not low doses were involved in the cancer risk studies. Because similar responses for neoplastic transformation relative risk and cancer induction relative risk were observed for moderate and higher doses, similar responses might also be expected for low doses where hormetic responses were observed for neoplastic transformation.

2.3. Enabling replicative immortality

Most cancers are considered to arise from a single cell [33]. To become a visible and palpable mass, this cell must divide many times. Most normal cells cannot divide indefinitely because they are limited in the number of times they can reliably and effectively make copies of the entire genome. This is because small amounts of DNA (telomeres) on the ends of the cell's chromosomes are lost during every replication cycle eventually stopping more cell division. The cells then enter a non-dividing state called senescence (covered in sections 3.1.5 and 3.2). Interestingly, low-dose radiation-induced senescent stromal fibroblasts have been demonstrated *in vitro* to make nearby breast cancer cells more radioresistant [44].

Cancer cells must overcome the senescence barrier in order to divide indefinitely to form tumors. Some tumors have been found to contain mutations that lead to reactivation of telomerase, facilitating continuous replication. Another method of maintaining telomeres is ALT (alternative lengthening of telomeres), which doesn't require telomerase, and instead resembles a mechanism of DNA damage repair. Some cancers have also been found to involve ALT.

2.4. Sustained angiogenesis

A tumor mass requires a blood supply in order to grow [33]. Angiogenesis (blood vessel formation) provides this need. Angiogenesis is facilitated by interactions between the tumor mass and its environment (the normal host tissue). Low oxygen levels and secretion of pro-angiogenic factors promote angiogenesis.

2.5. Invasion and metastasis

The invasion of the normal host tissue by the tumor and the spreading of cancer to other sites in the body (metastasis) increase the risk of death. Changes that promote invasion take place at the cellular level, including changes in the expression of cell surface markers which facilitates attaching to surrounding tissues [33].

Metastasis usually occurs by cancer cells first invading blood vessels and then being transported via the circulatory system to other sites of the body. These processes are known to involve a large number of secreted factors that break down tissue which allows invasion into blood vessels and then establishment of a new tumor at the site of deposition.

Results from molecular oncology studies suggest that the progression of a solid tumor to a metastatic phenotype is not simply the result of dysregulated signal transduction pathways, but is achieved through a stepwise selection process that is driven by the lack of oxygen [45,46]. The adaptation of populations of neoplastic cells to a hypoxic environment facilitates cancer cell dissemination through the up- or down-regulation of critical metastasis-associated genes. Such genes include E−/N-cadherin for epithelial-mesenchymal transition [47,48], urokinase receptor (uPAR) for degradation of the basement membrane and extracellular matrix [49], hepatocyte growth factor/mesenchymal-epithelial transition (HGF/MET) for cell motility [50,51] and vascular endothelial growth factor (VEGF) for stromal interactions, intra/extravasation and angiogenesis [52]. The systematic alteration of these phenotype regulators allows cells to escape the hostile microenvironment of the primary tumor and to colonize at a different location within the body [45]. Importantly, low-dose radiation has been demonstrated to suppress cancer metastases in animal models [53–58], possibly via abscopal effects related to anticancer immunity.

2.6. Additional emerging hallmarks since year 2000

After the seminal paper of Hanahan and Weinberg, the six hallmarks of cancer discussed above were revised to include four additional

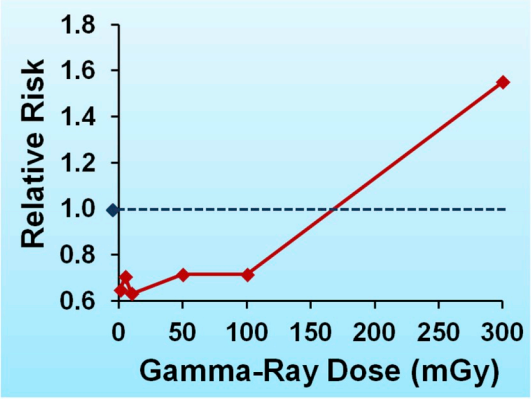

Fig. 1. Redrawn hormetic relative risk dose-response relationship for gamma-ray induced neoplastic transformation of HeLa x skin fibroblast human hybrid cells, as evaluated by Scott [20] based on *in vitro* data from Redpath et al. [42]. The reduction in transformation relative risk (RR) at low doses is related to the systems-biology-associated protective processes [DNA damage repair and possibly selective apoptosis of transformed cells [40]] that operate at the molecular, cellular, and tissue levels.

malignant traits that are referred to as emerging hallmarks [59]: evading immune destruction (another barrier), altered cellular energetics, cancer-enabling inflammation, and cancer-enabling genetic instability. These traits also promote the development, survival and evolution of the tumor mass and its constituent cells.

Achieving all the hallmarks of cancer requires overwhelming a hierarchy of natural defenses (barriers). Those natural defenses are enhanced by low radiation doses, an observation which is not in support of the LNT model for cancer induction [23].

3. Biological, biochemical, and other principals inconsistent with LNT

> *"At the early stages of evolution, increasingly complex organisms developed powerful defense mechanisms against such adverse radiation effects as mutation and malignant change. These effects originate in the cell nucleus, where the DNA is their primary target. That evolution has apparently proceeded for so long is proof, in part, of the effectiveness of living things' defenses against radiation"* [60].

> *"The notion of radiation hormesis, that exposure to low levels of ionizing radiation could produce beneficial effects, developed seriously in the late 1950's, and was, to most radiation scientists, incredible…More recent understanding of the mechanisms of radiation damage and repair, and discoveries of induction of gene expression by radiation and other genotoxic agents make it seem inevitable that under suitable conditions, irradiation will produce beneficial effects"* [61].

It has been estimated that life on Earth originated about 3.9 billion years ago in a more hostile natural radiation environment [62–64]. The radiation exposures comprised low linear-energy-transfer (LET) (e.g. beta and gamma radiations) and high-LET (e.g. alpha radiation) sources. The level of natural background radiation exposure during that era is estimated to have been about five-fold larger than for recent times [64]. Mammals later emerged, and survived via adapting to the harsher radiation and also oxygen environments. The evolutionary adaptations led to the present-day hierarchical system of mild-stress activated natural protection (ANP). The molecular, cellular, tissue and whole-body level ANP-related defenses against carcinogenic processes must be successively overcome for cancer as a disease to occur [15,23–25,65].

3.1. Molecular-level defenses

3.1.1. Low-dose radiation stimulates protection from oxidative damage

High-radiation-dose toxicity can arise from reactive oxygen species (ROS; e.g. O_2^- and H_2O_2) generated by the radiolysis of the water in living cells [66–68]. ROS are also generated in cells through metabolic processes that include respiration, ischemia/reperfusion, and oxidation of fatty acids. High concentrations of ROS that overwhelm cellular defenses can in addition to damaging DNA, lipids and enzymes, ultimately lead to the onset and progression of diseases such as cancer [69]. Evolution has however provided cells with sophisticated defense systems (i.e. systems biology) which protect them from ROS attack, including enzymatic mechanisms such as superoxide dismutase, catalase, and glutathione peroxidase, as well as non-enzymatic mechanisms involving the reduced forms of molecules such as glutathione (GSH), thioredoxin-1 (Trx-1), vitamin C, and vitamin E. Trx-1 is a multifunctional, low molecular-weight (12 kDa) antioxidant protein that contains an active thiol/disulfide site with oxidoreductase activity. Trx-1 enhances the catalytic activity of peroxiredoxin and glutathione peroxidase, which decompose hydroperoxides and hydrogen peroxide, respectively [70]. It also reduces levels of glutathione disulfide and hydroxyl radicals, serving a key role in controlling the cellular reductive/oxidative (redox) balance [69,71]. Through the antioxidant and other defense systems, intracellular ROS levels are controlled and prevented from becoming overabundant [69,72–76].

Kataoka [72] demonstrated that a whole-body X-ray dose of 200 mGy increased superoxide dismutase (SOD), glutathione peroxidase (GPx), and GPx mRNA in spleens of C57BL/6NJcl and BALB/c mice. This was not the case for a large dose of 4Gy [72]. The author did not report the dose rate used. Another study suggested that the levels of reduced glutathione (GSH), glutathione reductase (GR), γ-glutamylcysteine synthetase (γ-GCS), and Trx increased in liver shortly after whole-body irradiation with 500 mGy of gamma rays delivered at the very high rate 1.16 Gy/min [74]. In addition, the levels of GSH, GR, γ-GCS, and Trx increased in the brain shortly after 500 mGy of gamma rays [75]. The activation of antioxidant functions is mediated via transcriptional regulation of the γ-GCS gene, predominantly through the activator protein-1 binding site in its promoter region [77]. These findings support the view that exposure to low and moderate radiation doses (mild stresses) increases natural protective antioxidants. Stochastic low-dose-radiation thresholds are likely involved as well as intercellular signaling, which may be epiregulated [78].

Kataoka [79] reviewed information on inhibition of ROS-related diseases via use of low-dose X rays or radon inhalation to stimulate antioxidant production and key findings are as follows: Total-body X-ray exposure (500 mGy) before or after carbon tetrachloride (CCl_4) treatment inhibited hepatopathy (liver disease) in mice. X-ray exposure (500 mGy) before ischemia-reperfusion injury or cold-induced brain injury inhibited edema. These findings suggest that low-dose X rays have potent antioxidative effects related to blocking damage induced by free radicals or ROS. In addition, radon inhalation by mice increased superoxide dismutase activity in different organs and inhibited CCl_4-induced hepatic and renal damage and streptozotocin-induced type I diabetes. These findings implicate radon inhalation as likely having potent antioxidative effects. In addition, radon inhalation inhibits carrageenan-induced inflammatory paw edema, suggesting that radon inhalation has both anti-inflammatory and anti-pain effects. Indeed, radon therapy has provided relief to humans from suffering from a

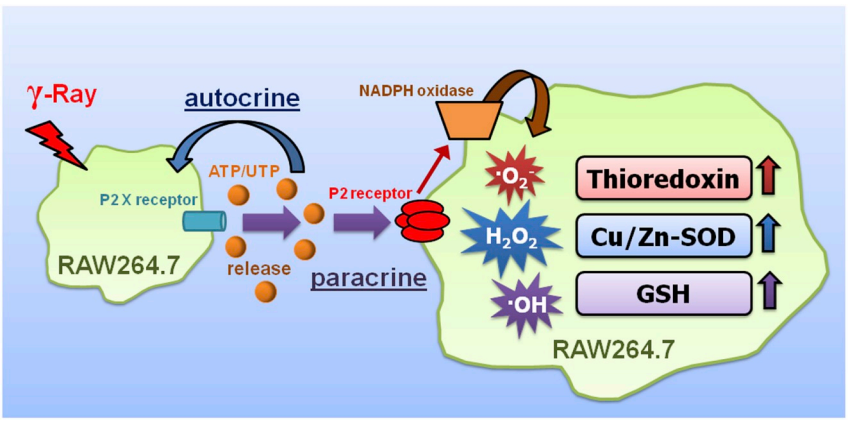

Fig. 2. Redrawn conceptual model of [69,81] for radiation-induced reactive oxygen species (ROS) production in RAW264.7 cells associated with adenosine triphosphate (ATP) signaling. ATP is released from the irradiated cells leading to production of reactive oxygen species (ROS). This is brought about via activation of cell membrane nicotiamide adenine dinucleotide (NADPH) oxidase through purinergic signaling. Antioxidants such as thioredoxin (Trx-1), Cu/Zn superoxide dismutase (Cu/Zn-SOD), and glutathione (GSH) are thought to be induced as an adaptive response to newly released intracellular ROS. This includes the ROS arising from the interaction of ionizing radiation with water. Both autocrine and paracrine pathways are involved. With autocrine signaling the cell secretes a messenger (autocrine agent) that binds to the autocrine receptors on the same cell causing the cell to change. Paracrine signaling affects nearby cells.

variety of inflammatory diseases [80].

Growing evidence points to adenosine triphosphate (ATP) signaling as being important in radiation adaptive responses including DNA damage repair, stimulating the production of endogenous antioxidants, cell-mediated immune responses, and differentiation of regulatory T (Treg) cells [81].

Detailed review of new studies by Ref. [81] revealed that transient receptor potential melastatin 2, a calcium-permeable non-selective cation channel, is activated in a P2X7-receptor-dependent manner, which results in release of nucleotides such as ATP through the connexin 43 hemichannel. The P2Y6/P2Y12 receptor is then activated, which leads to a range of low-dose-radiation-induced molecular events that include the activation of epidermal growth factor receptor signaling to extracellular signal-regulated kinases (ERK1/2), repair of DNA damage, ROS production, and induction of endogenous antioxidants.

[81] also pointed out that it has been suggested that ATP signaling is involved in the "bystander effect" (i.e., impacting nearby cells). If so and if low radiation doses are involved, then ATP signaling may be epiregulated. Both ATP and connexin 43 were found to participate in the bystander effect in mouse-model experiments. Related to this [81], reported wave-like releases of ATP from single cells irradiated with an X-ray microbeam.

[81] also pointed out the relation between gamma-radiation-induced ATP release and the induction of cellular Trx-1 (thioredoxin-1) via purinergic signaling. Exposure to gamma rays or exogenously adding ATP led to an increase in Trx-1 expression. It was found that ATP-generated intracellular ROS increased Trx-1 expression (as an adaptive response to ROS). ATP released from the irradiated cells may stimulate ROS production by the activation of cell membrane NADPH oxidase via purinergic signaling. Fig. 2 shows the related conceptual model of [81]. The details of the signaling pathways by which ATP activates NADPH oxidase through purinergic receptors are not known. In addition, the mechanism by which radiation induces ATP release is also not known. Ongoing research is addressing these unknowns [81,82].

Einor et al. [83] surveyed the scientific literature on the effects of chronic low-dose ionizing radiation on oxidative damage and the antioxidant responses. Their findings indicated resistance to oxidative stress via antioxidants as one possible mechanism associated with variation in species responses to low-dose ionizing radiation. If so, then genetic background would be expected to be important for mounting antioxidant defenses in humans.

Based on an extensive literature review, Feinendegen [84] summarized experimental data on the biological effects of different concentrations of ROS in mammalian cells and on their potential role in modifying the response of mammalian cells to agents such as ionizing radiation and genotoxic chemicals. He also attempted to contrast the role of a steady production of metabolic ROS at various concentrations in mammalian cells to that of environmental-exposure-related sudden and infrequent ROS bursts from background ionizing radiation. Both the steady production and infrequent bursts of ROS can cause biological damage and alter intra- and inter-cellular signaling, depending on their ROS concentration. At low concentrations as are associated with low-level, low-LET radiation exposure, signaling effects of ROS appear to aid cellular survival and protection dominates over damage occurrence. The reverse occurs at high ROS concentrations such as are associated with high radiation doses and dose rates. Background radiation encountered on Earth generates suprabasal ROS bursts along charged particle tracks several times a year in each nanogram (ng) of tissue [84]. The average mass of a mammalian cell is about 1 ng.

A burst of about 200 ROS occurs within less than a microsecond from low-LET irradiation (such as with X-rays) along the track of a Compton electron (about 6 keV energy, ranging about 1 μm in tissue) [84]. One such electron track in 1 ng of tissue deposits a microdose (dose to microscopic target) of about 1 mGy. The number of instantaneous ROS per burst along the track of a 4-meV high-LET alpha particle in 1 ng tissue reaches about 70 000 [84]. Knowledge of the magnitudes, types and sites of these bursts in and around cells and the variable time intervals between them helps to understand low-dose and low dose-rate radiobiological effects. At background and low-dose (above background) radiation exposure, a major role of ROS bursts along particle tracks relates to ROS-induced apoptosis of damage-carrying cells [e.g. neoplastically transformed cells [40,41]], and also on prevention and removal of DNA damage from endogenous sources by way of transient protective, adaptive, cellular responses [84,85]. Based on his extensive literature review, Feinendegen [84] concluded that low-dose radiation exposure of humans and other mammals aids their systems-biology-related physiological mechanisms for tissue homoeostasis. The conclusion argues against the validity of the LNT model for cancer induction.

3.1.2. Low-dose radiation stimulates protective epigenetic changes

With the advent of high-throughput technologies such as DNA microarrays in the late 1990s, changes in gene expression have been found to be prevalent after high radiation doses. However, only a very limited number of genes have been shown to be consistently up-regulated by low radiation doses. Research on the biological effects of exposure of human cells to low radiation doses demonstrated that the molecular and cellular processes observed are often related to adaptive responses manifested via ANP [25]. The adaptation appears to be regulated by changes in gene expression that involve mRNA and miRNA (i.e. epiregulated). Such epiregulated changes are much more likely than are gene mutations after exposure to low radiation doses [86,87]. Indeed, epigenetic changes appear to have been quite important in evolutionary adaptation to environmental and other stresses [88–91]. At present and at the cellular level, both radiation and chemical low-level stresses elicit a limited repertoire of evolutionarily derived adaptive responses [92].

Epigenetic alterations are heritable changes that govern gene expression. The changes are important for regulating the structure and function of the genome without changes in the DNA sequence. The alterations include different molecular changes such as DNA methylation, histone modifications, remodeling of chromatin, genetic imprinting, random chromosome (X) inactivation and noncoding-RNA (microRNA, long non-coding RNA, short interfering RNA, etc.)-regulated gene expression [93]. The principal mechanisms of epigenetic change occurrences are via modifications in DNA methylation and changes in how DNA is packaged around the core histones. Both mechanisms can result in gene activation or silencing [87].

Signaling proteins respond to both radiation-induced DNA damage and chromatin modifications. Their activity is modulated by the number of DNA lesions (which depend on dose, dose-rate, and the type of radiation) and by intercellular signaling. These proteins activate phosphokinase transmitters, in particular the protein encoded by the ataxia-telangiectasia gene [29]. Those transmitters, along with other signals (e.g. ROS), regulate radiation adaptive responses (e.g. cell cycle control, DNA repair, and triggering apoptosis of precancerous cells) [94–96], likely with the aid of epigenetic changes which are much more prevalent than are radiation-induced mutations. At low radiation doses, miRNA changes are involved in stimulating DNA repair, suppressing cell lethality, and suppressing cancer progression [97].

Epigenetic changes are the main mechanism for medium-to long-term adaptation to accumulated (intense, long-term, or repeated) stress [92]. The indicated authors proposed the 'adaptive deregulation of the epigenome in response to stress' hypothesis which assumes that the general adaptive response to stress grows stronger with the increasing stress level, epigenetically activating response-gene clusters while progressively deregulating other cellular processes. With mild stresses, the epiregulated adaptation could be beneficial in maintaining or improving homeostasis capability.

Furusawa and Kaneko [98] used a simple theoretical cell model (consisting of a gene regulatory network with epigenetic feedback regulation) to evaluate the effect of epigenetic dynamics on adaptation

and evolution. They found that the type of epigenetic dynamics considered enables a cell to adapt to unfamiliar environmental changes (e.g. low-dose-radiation or chemical exposure) for which no regulatory program has been prepared, through selection of a cellular state with a high growth rate. They also demonstrated that the addition of epigenetic regulation promotes evolutionary development of a regulatory network that can respond to environmental changes in a rapid and precise manner. Their results strongly suggest that epigenetic feedback regulation in gene expression dynamics (an adaptive response) provides a significant increase in fitness by engendering an increase in cellular plasticity during adaptation and evolution. These theoretical findings are consistent with the view that rapid adaptation (e.g. within seconds or minutes) of cells to mild environmental stress likely involves epigenetic rather than very-low-probability mutational changes.

Bernal et al. [99] utilized the viable yellow agouti (A^{vy}) mouse model [100] to determine if deleterious or protective epigenetic changes occur when exposed to low-dose radiation during the proper stage of gestation. This mouse strain is sensitive to environmental stresses (e.g. low-dose radiation) that alter the fetal epigenome. Variable expression of the A^{vy} metastable epiallele is regulated by epigenetic modifications such as cytosine phosphate-guanine site methylation and histone marks that are established early during development in and around the cryptic promoter in a transgene upstream of the Agouti gene [99]. Transgenes are exogenous genes that are introduced into an organism so that it will have a new characteristic that can be transmitted to offspring. Metastable (i.e. stable if not disturbed) epialleles are expressed differently in genetically identical individuals because of epigenetic modifications of genes that occur during early development. Hypomethylation of the alternative promoter results in inappropriate Agouti gene expression in all tissues in A^{vy} mice [99]. This leads to a yellow coat color (morbidity-promoting phenotype) and also antagonizes the melanocortin 4 receptor in the hypothalamus, which leads to high prevalence of obesity, cancer, and diabetes.

Imposing mild radiation stresses (14–30 mGy) during the proper stage of gestation led to protective epigenetic changes (coat color shifted from yellow towards brown [$p < 0.01$]) in offspring in a sex-specific manner, with males benefiting (reduced risks for obesity, cancer, and diabetes) more than females. The protective changes were inhibited by antioxidants, thereby implicating ROS as having an important signaling role in the mild stress adaptive response observed [99].

3.1.3. Low-dose radiation activates DNA damage repair and related molecular changes

Eukaryotic cells are subjected daily to a significant amount of spontaneous DNA damage related to normal metabolic activities within cells and normal microenvironmental changes [16,101]. Reported counts of damaging apurination/apyrimidination events are as high as 1000 to 10,000 hits per mammalian cell each day and the overall damage rate may reach about 1 million DNA damaging events per genome each day on average [102,103]. Even with this high DNA damage rate, the mutation rate of eukaryotic DNA is held in the range 0.1–100 deleterious mutations per genome per sexual generation [104]. Thus, the DNA repair system is quite efficient (with the relatively rare exceptions of inherited DNA repair deficiencies) in preventing deleterious alterations of the genetic content which is to be passed to cell progeny [101]. This remarkable achievement relates to the system of DNA damage repair. As might be expected, DNA damage response is influenced by genetic factors [105].

DNA double strand breaks are the most serious type of genomic damage and are induced as an LNT function of radiation dose [16]. Sophisticated homeostatic mechanisms (components of the system of DNA damage repair) evolved to mitigate such damage [106].

There are three known mechanisms of repair of double-stand breaks: non-homologous end joining, microhomology-mediated end joining, and homologous recombination. Low-dose-radiation stochastic thresholds are likely involved in DNA double-strand-break repair activation by radiation and genotoxic chemicals and may involve intercellular communications arising as an epiregulated cell-community-wide (epicellcom) process [78]. With an epicellcom process, damage to a small number of cells leads to intercellular signaling (stress response) that involves a large number of bystander cells, thus bringing about a cell-community response rather than an individual cell response. Epicellcom processes may be responsible for some hormesis phenotypes [107].

Studies of genetic diseases that are characterized by genome instability have provided novel insights into the underlying mechanisms of DNA damage response [108]. NBS1 (the protein Nibrin), which is responsible for the radiation-sensitive autosomal recessive disorder, Nijmegen breakage syndrome, is one of the first factors to accumulate at sites of DNA double-strand breaks. NBS1 is involved in regulating chromatin remodeling, cell cycle checkpoint control and the repair of DNA double strand breaks.

Some information related to DNA damage repair activation by low radiation doses has been derived from studies of radiation-induced mutations. A sex-linked recessive lethal mutation assay was performed by Koana et al. [109] in *Drosophila melanogaster* using immature spermatocytes and spermatogonia irradiation with 150-kVp X rays at a high (500 mGy/min) or low (50 mGy/min) rate. The mutation frequency in the sperm irradiated with a low dose at a low rate was significantly lower than that for controls, whereas irradiation with a high dose and rate resulted in a significant increase in the mutation frequency (i.e. hormetic response: low-dose-enhanced natural protection and high-dose/high-rate suppression of protection leading to harm). When cells deficient in DNA excision repair were used instead of using wild-type cells, low-dose irradiation at a low rate did not reduce the mutation frequency (i.e. no evidence for radiation ANP). These findings are consistent with the possibility that error-free DNA repair functions were activated as an epicellcom process by low-dose/low-dose-rate irradiation and that this led to repair of spontaneous DNA damage throughout the target cell population as well as radiation-related damage, thus producing a practical threshold for induced mutation-related harm (e.g. mutation-facilitated cancer). The findings contradict the LNT hypothesis as it relates to mutation and cancer induction.

In a more recent study by Koana et al. [110], the third instar larvae of Drosophila were irradiated with X rays, and the somatic mutation frequency in their wings was measured after their eclosion (i.e. emergence). In the flies with normal DNA repair and apoptosis functions, 200-mGy irradiation at 50 mGy/min reduced the frequency of the small spot (mutant cell clone with reduced reproductive activity) compared with that in the control flies. Suppression of apoptosis using the baculovirus p35 gene caused the small spot frequency to increase four fold in the un-irradiated control group; however a reduction by the 200-mGy irradiation was still evident, suggesting that apoptosis (protective barrier against mutation propagation) inhibition was reversed by the mild radiation stress. The small spot frequency was also reduced by 200-mGy irradiation of non-homologous end joining-deficient mutants. No reduction in the small spot frequency by 200-mGy X rays was observed in a mutant that was deficient in single-strand break repair, and the small spot frequency increased as radiation dose increased. Large spot (mutant cell clone with normal reproductive activity) frequency was not affected by suppression of apoptosis and increased in wild-type larvae and in mutants for single- or double-strand break repair as radiation dose increased. The authors hypothesized that some of the small spots resulted from DNA single-strand damage and, in wild-type larvae, 200-mGy irradiation activated the normal single-strand break repair gene, which reduced the background somatic mutation frequency.

Unlike the robust activation of DNA damage repair after high radiation doses, the efficiency of activation of DNA damage repair and related signaling pathways after low doses and dose rates vary greatly between different individuals. Genomic and functional assays measuring low-dose and dose-rate ionizing radiation responses repeatedly

show increased inter-individual variability when cells and tissues experience DNA damage levels that are similar to those that arise endogenously (due to aerobic metabolism, diet, lifestyle, etc.) [111].

The level of natural background gamma radiation in Kerala, India varies from <1 mGy/year to about 45 mGy/year. Residents of the area have been studied for possible DNA damaging effects of natural background gamma rays. A recent study by Jain et al. [112] quantified spontaneous levels of DNA double strand breaks (DSBs) in peripheral blood mononuclear cells of 91 randomly selected individuals from a high-level, natural radiation area (HLNRA) and reference lower level natural radiation area (N = 30) using γ-H2AX as a biological marker. Average annual whole-body gamma-ray doses received by the HLNRA and reference groups were 8.28 ± 4.96 mGy/year and 1.28 ± 0.086 mGy/year, respectively. The average spontaneous frequency of DSBs (based on γ-H2AX foci) among reference and HLNRA groups were 0.095 ± 0.009 and 0.084 ± 0.004 per cell, which is not significantly different (p = 0.22). The individuals from HLNRA were further classified by Jain et al. [112] as low dose-rate group (LDG, 1.51–5.0 mGy/year), moderate dose rate group (2.63 ± 0.76 mGy/year), and high dose rate group (HDG, >5.0 mGy/year, group average dose rate 11.04 ± 3.57 mGy/year). The spontaneous frequencies of γ-H2AX foci per cell in reference, LDG and HDG groups were found to be 0.095 ± 0.009, 0.096 ± 0.008, and 0.078 ± 0.004, respectively. Individuals belonging to HDG showed marginally lower frequency of DSBs as compared to the reference and LDG groups. These findings suggest that residual DNA damage under conditions of continuous irradiation from natural environmental sources is not an LNT function of average dose rate.

Jain et al. [112] interpreted their findings as suggesting that either a lower induction of DNA damage by background radiation or enhanced repair of DSBs for individuals from the high dose-rate group (HDG) of the HLNRA (high-level natural radiation area). Their data are consistent with the view that natural background radiation exposure may help (via simulating homeostatic mechanisms) to prevent the accumulation of DNA DSBs caused by exposure to other carcinogens or endogenous processes. Also, the observation (hidden in their data) that the variance of the measured DSBs was less for the HLNRA group than for the reference group, while averages were not significantly different, suggest that normal homeostasis (related to controlling cellular DNA damage burden) is more efficiently maintained in high natural background gamma-ray areas than for low natural background gamma-ray areas. In addition, for the HLNRA group, the DSB frequency was not correlated (R^2 = 0.04) with age (a surrogate for cumulative exposure to all carcinogens), which is supportive of the view that natural background radiation may be acting to prevent DSB accumulation over time.

Spontaneous intrinsic modification of cellular DNA occurs throughout nature [113]. Researchers [114,115] summarizing their findings indicated that approximately 10,000 measurable DNA-altering events per hour occur in each mammalian cell due to intrinsic natural processes. Billen [113] interpreted the radiation research literature as showing that only about 10 (or fewer) measurable DNA alterations occur per mGy of low-LET radiation, per mammalian cell. Thus, each hour we humans and other mammals undergo at least 1000 times as many spontaneous or natural DNA damaging events per cell as would be expected from exposure of each cell in the body to 1 mGy of ionizing radiation. Since background radiation exposure in the United States is on the order of 1–2 mSv/y (whole body effective dose), Billen [113] concluded that spontaneous DNA damage in mammalian cells is mainly caused by factors other than natural background radiation.

The LNT hypothesis was initially justified on the basis of the dose-response function for mutation induction in germ cells of *Drosophila melanogaster* interpreted to be of the LNT type, based on the very high X-ray doses used by Muller [116]. However, a more recent, better designed, and more reliable study [117] using gamma rays that included orders of magnitude lower radiation doses (delivered at 22.4 mGy/h) revealed that a strong adaptive response occurs at doses less than about 100 mGy with a significant reduction ($p < 0.01$) in the mutation frequency to well below the spontaneous level at a dose of 0.5 mGy. Because there is on average less than 1 electron track (from ionizations) per cell at the indicated absorbed dose, this is likely a protective bystander effect that relates to *epigenetic activation (epiactivation)* of adaptive-response genes [78]. Thus, the initial mutational basis for use of the LNT risk model for cancer induction has been invalidated [118]. Interestingly, the 0.5 mGy dose up-regulated genes for protective heat-shock proteins and apoptosis as well as for other mild-stress responses; however, DNA-repair-related genes were not up-regulated [117]. Somewhat higher doses appear to be required for up-regulation of DNA repair genes [119]. Rather than relying only on DNA repair for mutation and cancer avoidance, damaged cells may be removed via selective apoptosis as a mild-stress response when signaled to divide [119]. These adaptive responses are probably regulated epigenetically and involve intercellular signaling. Apoptosis [a powerful natural barrier against mutations and cancer [23,107]] and other modes of cell death are discussed in the next section.

Interestingly, while the 1927 publication by Muller had an important role in the acceptance of the LNT model for ionizing-radiation-induced stochastic effects, his 1954 publication (i.e. 27 years later) with other researchers [120] demonstrated that the LNT model was not supported by data for UV-induced mutations in Drosophila. It appears that the 1954 publication was not widely known.

Some radiation-adaptive-response-related molecular changes that are induced by low radiation doses are linear for a range of doses [121]. In some cases they also depend on dose rate. In the ML-1 human myeloid leukemia cell line used by the researchers, reducing the dose rate by over three orders of magnitude led to a linear induction of the p53-regulated, stress-response genes: cyclin dependent kinase inhibitor 1A (CDKN1A), growth arrest and DNA-damage-inducible protein GADD45 alpha (GADD45A), and mouse double minute 2 homolog (MDM2), for radiation doses between 20 and 500 mGy. However, this resulted in some protection against apoptosis. Reducing the dose rate reduced the magnitude of induction of CDKN1A and GADD45A, but not the duration of cell-cycle delay. In contrast, MDM2 induction did not depend on dose rate for the rates studied by Amundson et al. [121]. Microarray analysis revealed additional low-dose-rate inducible genes and indicated the existence of two general classes (groups) of low-dose-rate responding ML-1 cell genes. One group of genes was induced in a dose-rate-dependent fashion, like was the case for GADD45A and CDKN1A. Functional annotation of the gene clusters indicated a majority of these genes were involved in apoptosis regulation. Another group of genes with dose-rate-independent induction (as the case for MDM2) was also identified. The majority of genes in this group are involved in cell cycle regulation. These observations are consistent with low-dose-radiation stimulated adaptive protection and inconsistent with the LNT risk model for cancer induction.

3.1.4. Coordinating DNA repair and apoptosis

Multiple protein ubiquitination events that occur at DSBs regulate the detection of threatening damage, the damage-response signaling, and the resultant repair of damage [a barrier to cancer [23]]. Ackermann et al. [122] investigated how DSB repair is coordinated with the apoptotic response. They identified a central role of the E4 ubiquitin ligase UFD-2 in the coordination between the DNA-repair process and the apoptotic response.

3.1.5. DNA damage response and immune defense

Research findings have linked DNA damage response (DDR) and immune defenses [123]. In a recent review, Nakad and Schumacher [124] describe advances on the understanding of the role of the DDR in activating immune signaling. They point out that in response to genotoxic insults such as from low-dose ionizing radiation, the DDR can arouse the immune system, e.g. by inducing the expression of antimicrobial peptides as well as ligands for receptors found on immune

cells. The activation of immune signaling is triggered by different components of the DDR that include DNA damage sensors, transducer kinases, and effectors. Nakad and Schumacher [124] also stated the following on how DNA damage leads to the activation of innate immunity and how innate immunity can then cause additional DNA damage: *The DNA damage response leads to apoptosis, transient cell cycle arrest or cellular senescence. Cellular senescence can cause senescent cells to modify their tissue environment through the senescence-associated secretory phenotype (SASP). This in turn can result in cytokine secretion that activates the innate immune system which can suppress tumourigenesis by clearing senescent cells with oncogene activation or chronic DNA damage. However, SASP can also lead to tumourigenesis through cytokine signaling which promotes proliferation of tumor cells. The activation of innate immunity involves the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) which could promote chronic inflammation. The generation of ROS/RNS by innate immunity and chronic inflammation can promote tumorigenesis through causing mutations in bystander cells, or by impairing DDR.*

Pateras et al. [125], after an extensive literature review, pointed out that compelling evidence indicates that the DNA damage response and repair (DDR/R) and immune response signaling networks work together for the benefit of the organism. DNA and RNA viruses can directly and indirectly activate the DDR/R machinery in the host cells. Activation of DDR/R then increases the likelihood for the immunogenicity of the recipient cell. Further, stimulation of DDR/R by exogenous or endogenous factors (e.g. radiation) can trigger both innate and adaptive immune responses. The immune system stimulating properties of ionizing radiation (a DDR/R inducer) provides a way to study how DDR/R stimulation can alert host immunity. As reported in the review by Pateras et al. [125], it has been found that critical cellular danger signals stimulate defense at the systemic level and vice versa. They also point out that disruption of DDR/R–immune-system cross talk can compromise tissue integrity and lead to immune defects.

3.2. Cellular-level defenses

Cells in the human body are continuously exposed to various external and internal stresses that, in addition to ionizing radiation, include hypoxia, chemical and other toxins, oxidative stress, and others. The ability of cells that make up our tissue and organs to adapt to these stresses (an evolutionary gift) is crucial for survival of our species. Complex cellular adaptation strategies have evolved to combat environmental, physiological and other threats [126,127].

As already indicated, cellular senescence (a metabolically active form of irreversible growth arrest) can protect against cancer occurrence. The characterization of senescent cells is mainly based on the following morphological and molecular features which distinguish them from quiescent or terminal differentiated cells [128]: (1) flattened, elongated and enlarged shape; (2) high lysosomal β-D-galactosidase activity at pH 6 due to increased numbers of lysosomes; (3) irreversible RB-dependent heterochromatin structures, called Senescence-Associated Heterochromatin Foci; (4) SASP that includes proinflammatory cytokines, chemokines and extracellular matrix metalloproteinases; and (5) cell cycle arrest in the early G1 phase which is mediated by p53, p21 and p16.

The senescence occurs in response to a variety of intrinsic and extrinsic genotoxic stimuli such as ionizing radiation [129–132] and is mediated through tumor suppressor pathways [133,134]. The initiation of senescence leads to the inhibition of cancer-facilitating, cyclin-dependent kinases [135,136]. Importantly, senescence can stop proliferation of cells with genomic instability, thereby preventing the transmission of cancer-facilitating genomic damage to daughter cells. Cellular senescence as an adaptive response to mild genomic stress has been considered a natural tumor-suppressor mechanism [137].

The functioning of cellular senescence as a tumor suppressor was demonstrated by cell fusion experiments [138]. The fusion of proliferating cells with senescent cells inhibited DNA replication in the fused cells, even in the presence of mitogens. These cell fusion experiments implicated senescent cells as containing control entities capable of exerting a dominant effect over proliferating, pre-senescent cells. Further, the tumor suppressive capacity of cellular senescence has been implicated in both mice and humans [139].

Senescence also evokes some concerns. It has recently been recognized that pro-inflammatory factors such as those encompassing the SASP are linked to cellular proliferation, a persistent low-grade inflammation, elevated DNA damage foci, and transformation of preneoplastic cells [137]. Thus, there is a concern that via the SASP, mild stress-invoked-premature senescence could increase the chance of cancer development [137]. However, unlike high-dose radiation which enhances inflammation, low-dose radiation can suppress inflammation [107,140]. Further, in mice that had a high spontaneous incidence of lung cancers, exposure to single low doses (mild stress) of gamma rays significantly reduced the lung cancer incidence rather than increasing it [107]. Similar observations were made for human exposures (chronic) to residential radon and may relate to suppression of smoking-related cancer [26,107].

Death of aberrant cells is also an important barrier to cancer [40,41,141–147] and in some cases is p53-independent [for neoplastically transformed cells [143]]. Cell death manifested as apoptosis, autophagy, or necrosis is a fundamental cellular response to stress. Apoptosis (which can selectively eliminate aberrant cells) is a regulated cell death process that reflects the cellular decision to die in response to cues from the cellular environment and is executed by intrinsic cellular machinery [40,148]; Elmore S 2007; [149]. In contrast, necrosis is uncontrolled cell death brought on by massive stress (e.g. from high dose radiation and toxic chemicals). Autophagy involves self destruction starting with engulfment of cytoplasmic material by the phagophore and sequestration of material to the autophagic vacuoles, where they are eventually destroyed [150]. The type and intensity of stimuli, type of tissue, developmental stage of the tissue, and the physiologic cellular microenvironment determines the cell death process that occurs [151].

The apoptotic effect of low-dose ionizing radiation on male germ cells has been of interest to radiation researchers for the last two decades. Apoptosis of male germ cells is essential for normal spermatogenesis and often occurs through highly conserved events that include the transfer of vital cellular materials to the growing gametes following the loss neighboring cells. Apoptosis of germ cells also functions in diverse processes that include the removal of abnormal or superfluous cells at specific cell cycle checkpoints, establishment of caste differentiation, and individualization of gametes [152].

Because of their high radiation sensitivity, induction of germ-cell apoptosis has been observed in the testis of animals exposed not only to high-dose radiation (HDR) but also to low-dose radiation (LDR). Exposure of male germ cells to LDR induces a protective (stimulating) effect, while exposure to HDR causes an inhibitory effect on the metabolism, antioxidant capacity, and proliferation and maturation of cells [152]. Pre-exposure to low dose radiation protects germ cells from subsequently high-radiation-dose-induced genomic and cytological effects (an adaptive response). Fig. 3, which is based on the conceptual model of Liu et al. [152], summarizes what is currently known about radiation adaptive responses of male germ cells.

3.3. Tissue-level defenses

3.3.1. Tissue interactions suppress and control tumors

Tissue level interactions (contact inhibition of cell proliferation, signaling and exchange of regulatory molecules via intercellular junctions, protective bystander interactions, secretion of regulatory factors by neighboring cells and stroma) are important in tumor suppression and control [27,153]. There are multiple interactions between a cell in which a potentially oncogenic event has occurred and the neighboring

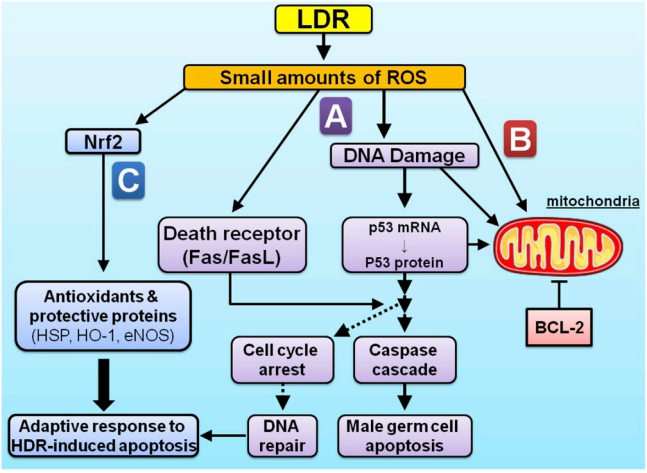

Fig. 3. Redrawn conceptual model of Liu et al. [152] for the possible biological effects of low-dose ionizing radiation (LRD) in male germ cells. LDR stimulates one or more sequences of events, designated by Liu et al. as pathways A, B, or C. *Pathway A*: germ cell apoptotic death via p53 and Fas/FasL signaling. *Pathway B*: germ cell apoptotic death via mitochondrial damage. *Pathway C*: LDR-induced adaptive survival response of germ cells which protects against high-dose ionizing radiation (HDR), via inducing the antioxidant system. For this pathway, exposure of cells to LDR triggers in addition to antioxidants, protective molecules that include HSP, HO-1, and eNOS via activation of Nrf2 transcription factor (nuclear factor erythroid 2-related factor 2) activity. For all of these pathways, LDR triggers intracellular ROS production that then stimulates one or more of the pathways, depending on radiation dose.

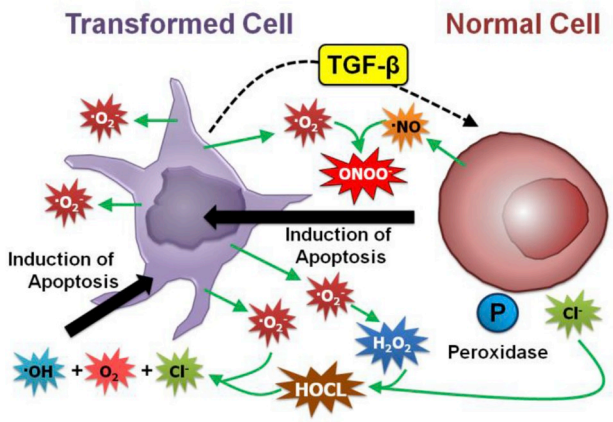

Fig. 4. Simplified version of the systems-biology-related, signaling pathways for the protective apoptosis mediated (PAM) process in fibroblast based on [141,156,157]; as drawn by Ref. [22]; redrawn for this publication. A key early event is the release of transforming growth factor beta (TGF-β1) by transformed cells. Nontransformed cells, when activated, release peroxidase (P) and nitric oxide (•NO). Superoxide anions (O_2•$^-$) generated and released by the transformed cells participate in the intercellular signaling and make transformed cells the selective target for intercellular induction of apoptosis (i.e. transformed cells are selectively eliminated via p53-independent apoptosis). Chloride ions (Cl^-) and hydrogen peroxide (H_2O_2) also participate in the intercellular signaling. The interactions of the indicated molecules result in two major signaling pathways that bring about protective apoptosis. These pathways are based on hypochlorous acid (HOCl)/hydroxyl radicals (•OH) and •NO/peroxynitrite ($ONOO^-$). H_2O_2 plays a key role by fostering the HOCl/•OH pathway and inhibiting the •NO/$ONOO^-$ pathway.

cells of the same type, the extra-cellular matrix, and the stroma. These interactions (via signaling) can impact the carcinogenic process. Indeed, signaling between the cell undergoing malignant changes and its microenvironment can slow the carcinogenic process [29]; however, the signaling can in some circumstances also augment the carcinogenesis process [37].

3.3.2. *Low-dose radiation stimulates selective removal of precancerous cells*

The ability of a precancerous cell to escape natural anticancer signals imposed on them by neighboring cells and the microenvironment is an important stage in tumorigenesis; Portess et al. [154] used a cell co-culture approach to characterize a system of intercellular induction of apoptosis whereby nontransformed cells stimulate selective removal of neoplastically transformed cells via cytokine, ROS and RNS signaling. This p53-independent phenomena has been called a protective apoptosis mediated (PAM) process [20,22]. Portess et al. [154] demonstrated that irradiation of nontransformed cells with low doses of either high-LET alpha particles or low-LET gamma rays led to stimulation of intercellular induction of apoptosis (i.e. the PAM process). By using scavengers and inhibitors they demonstrated the involvement of ROS/RNS signaling and the importance of transformed cell secreted NADPH oxidase in the selectivity of the system against transformed cells. Absorbed radiation doses as low as 2 mGy of gamma rays and 0.29 mGy of alpha radiation produced an observable increase in selective-apoptotic removal of transformed cells. However, this adaptive response process appears to saturate at somewhat higher doses (50 mGy for gamma rays and 25 mGy for alpha radiation, implying a relative biological effectiveness of 50 mGy/25 mGy = 2 for alpha radiation for this effect) under the exposure scenarios employed. By applying a neutralizing antibody assay, the researchers confirmed an important role for transforming growth factor β (TGF-β) in the radiation-induced intercellular signaling. The indicated protective signaling appears to represent natural anticancer mechanisms which may have evolved many years ago when background radiation levels on earth were much higher than today.

Temme and Bauer [155] also studied signaling between irradiated transformed (or tumor) and unirradiated, nontransformed cells using a co-culture system involving both cells types, with a focus on the PAM process (although not called that by the researchers). They found that low-dose gamma rays substantially increased superoxide anion production in oncogenically transformed cells and tumor cells but not in nontransformed cells. The enhancement was independent of radiation dose over the range 20–200 mGy. This finding is consistent with the notion of an epicellcom response to mild stress. The transfer of a few irradiated transformed cells to nonirradiated control cultures (bystander study) was sufficient for transmission of a signal leading to the induction of superoxide anion production in the nonirradiated cells. SiRNA-related knockdown and reconstitution experiments revealed that TGF-β1 was involved in the protective bystander effect triggered by low-dose gamma rays in their experimental system. Fig. 4 is a simplified version of the conceptual model of [141,156,157] for intercellular signaling-related triggering of apoptosis of transformed (or tumor) cells as modified from Refs. [21,141,156,157]. and others in his research group used the terminology “intercellular induction of apoptosis” rather than PAM process (terminology used by Ref. [25] and by Ref. [21]. The protective process involves a sophisticated system of interdependencies and interactions of ROS and RNS. Different pathways leading to selective apoptosis are likely associated with the auxiliary PAM process (based on [40], with the selected path possibly depending on the cell type to be eliminated via apoptosis (mutants, neoplastically transformed cells, micronucleated cells, etc.), the local environment, the type of DNA damage, and the stimulating agent [25].

Other researchers demonstrated that low doses of low-LET photon radiation can lead to a reduction in the neoplastic transformation frequency to below the spontaneous level [42,158–160] while high doses lead to elevated transformation frequencies that increase as the dose increases further (i.e. hormetic responses) as presented in Fig. 1. The reduction in the spontaneous frequency may relate to intercellular signaling between transformed and non-transformed cells, leading to selective removal of the transformed cells as proposed by Bauer [40].

3.3.3. Low-dose radiation suppresses inflammation

Inflammation is a homeostatic mechanism which in some circumstances can lead to diseases including cancer. The underlying immunological mechanisms and the interrelationship between ionizing radiation and inflammation are complex. Acute radiation doses to the total body exceeding 1 Gy when delivered at a high rate may initiate inflammatory reactions possibly facilitating cancer development [140]; however, low radiation doses and dose rates can attenuate an ongoing inflammatory process and this strategy has been used in treating inflammatory and degenerative diseases [140]. Unfortunately, wide application and progress in this form of radiation therapy has been greatly hampered by LNT-related radiation phobia.

A large body of experimental evidence has accumulated which demonstrates that small radiation doses modulate several inflammatory processes [72,161]. The modulations include hindered leukocyte adhesion to endothelial cells, reduced activity of inducible nitric oxide synthase, and reduced oxidative burst in macrophages [161].

Cigarette smoke contains the chemical benzo[a]pyrene (BaP) that when metabolized in the body produces the inflammation-promoting carcinogen BaP diol epoxide (BPDE). The metabolite induces lung tumors (often multiple) in animal models when given at high immunosuppressive levels [162]. Further, cigarette smoke constituents are known to cause inflammation and related lung cancer in humans. Importantly, lung cancer in humans has been found to be suppressed by low-level exposure of radon in the home [26,163,164]. Low-level radon has also been demonstrated to suppress inflammation in mice [72].

Because BPDE modifies the microenvironment (e.g. stromal cells) of potential-cancer-causing lung epithelial cells (if neoplastically transformed), Chen et al. [165] investigated whether low-dose-gamma rays could alter the *in vitro* response of stromal cells to BPDE exposure. The strategy employed was based on neoplastic transformation of human bronchial epithelial cells (HBEC) being an essential step in the lung cancer development. The researchers employed a cell-culture/media-transfer approach. Results obtained indicated that BPDE induces secretion of the pro-inflammatory cytokines (e.g. IL-6) from human lung fibroblast. More importantly, a single low dose (90 mGy) of gamma rays inhibited IL-6 secretion.

Chen et al. [165] also investigated the mechanism by which IL-6 secretion by fibroblasts promotes transformation of HBEC. Condition media from fibroblast (cell line HFL1) treated with cigarette-smoke carcinogen (BPDE) strongly induced the phosphorylation of STAT3 in HBEC in an IL-6-dependent manner. Direct application of IL-6 markedly potentiated BPDE-induced HBEC neoplastic transformation. This observation supports the finding that IL-6 secretion from fibroblasts aids HBEC transformation. The finding that low-dose gamma rays suppress fibroblast-derived, IL-6-mediated transformation is supportive of complementary findings of Vicent et al. [166] that are discussed below.

Vicent et al. [166] carried out gene expression analysis comparing normal mouse lung fibroblast and cancer-associated fibroblasts (CAF) from mice. The researchers identified a gene set (or gene signature) related to the CAF phenotype. The gene signature for the CAFs is an independent marker of poor survival for patients with non-small-cell lung cancer. Genes comprising the desired gene signature were up-regulated in normal lung fibroblast after they were exposed to tumor cells for an extended period. This suggested that lung fibroblast can be influenced by bystander tumor cells and take on a CAF-like phenotype. Functional studies demonstrated important roles for IL-6 to interlukin-6 receptor (IL-6R) signaling and cytokine-like factor 1 to ciliary neutrophilic factor receptor signaling, in promoting non-small-cell lung cancer. Based on the work of Chen et al. [165], low-dose gamma rays would be expected to suppress IL-6 to IL-6R signaling providing protection against lung cancer.

3.4. Whole-body-level defenses

At the whole-body level, anticancer immunity can eliminate cancer cells. A highly complex and coordinated cellular and humoral biological system (including abscopal effects) mediates tumor destruction. Unfortunately, cancer also suppresses anticancer immunity, facilitating further cancer development. However, low-dose (but not high-dose) radiation can activate components of anticancer immunity as discussed below.

As indicated in a review by Farooque et al. [167] and information already provided, it is now recognized that while high doses of radiation suppress the immune system, low doses and dose rates can stimulate anticancer immunity which can aid in cancer prevention [56,152,153,168,169] and can be used in cancer therapy [170]. Epidemiologic data which supports this view have shown that inhabitants of elevated but relatively low natural-background radiation in India (Kerala), Brazil, China, the USA, the Misasa radon spa area of Japan and elsewhere, have lower cancer mortality than those living in areas with significantly lower background radiation levels [171–173]. In addition, a significantly lower rate of cancer mortality among the population residing in the Guangdong area of China with elevated background radiation has been found to be correlated with immune system enhancement [167,174]. Similar results have been reported in occupational radiation workers, patients exposed to low-dose radiation used for diagnostic purposes, and in experimental studies with laboratory animals [167,175–177].

The activation of several immune-system-related cells such as natural killer (NK) cells, dendritic cells, macrophages and T cells, as well as increase in mast cell activity, was observed after use of low-dose radiation in treating tumors [168,178]. A decrease in T-regulatory cells, altered cytokine responses (e.g. an increase in IL-2) and IFN-γ secretion, and a decrease in TGF-β levels [169,179,180] and antibody production have also been observed [152].

Experimental studies using low-dose X-rays and gamma rays in different strains of mice have demonstrated a decrease in the growth rate of tumors as well as inhibition of metastasis and the indicated findings correlate with anticancer immunity enhancement [57,169,181]. Low-dose-radiation-induced immune enhancement is reported to occur at least in part via the induction of both the antigen-presenting cells (APCs) and T lymphocytes, facilitating intercellular reactions within the immunological synapse [182]. Expression of molecules that are involved in negative regulation of the immune system (i.e. immunosuppression) such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), cytokines such as interleukin-10 (IL-10), interleukin-4 (IL-4) and cyclic adenosine monophosphate (c-AMP), as well as protein kinase A, decreases after low-dose irradiation, leading to immune system enhancement [182]. Low-dose irradiation also

Table 1
Effects of low-dose ionizing radiation on *innate immunity*.[a]

Cellular component	Modification	Immune system role in low-dose response	Reference
Natural killer	Increased functionality	Lysis of tumor cells	[53,54]
Antibody-dependent cell-mediated cytotoxicity	Increases	Lysis of tumor cells	[183]
Macrophage	Increased functionality	Phagocytosis and antigenic presentation	[184]
Dendritic cell	Activated	Increase in T-cell proliferation and antigenic presentation	[147]

[a] Reference: Farooque et al. [167].

upregulates several other anticancer factors such as the natural killer (NK) and antibody-dependent cellular cytotoxicity (ADCC) activity of splenocytes, surface molecules such as CD25 (IL-2 receptor), CD71, CD28, CD2 and CD48, DNA repair, and immune system stimulating signaling molecules (e.g. calcium, c-GMP and p38MAPK) [182]. However, the immune system response to low-dose irradiation varies with cell type, dose range, and dose rate pattern [167].

Tables 1–3 summarize key findings by Farooque et al. [167] on the effects of low doses of ionizing radiation on the immune system, with emphasis on cancer prevention. Fig. 5 shows the conceptual model of Farooque et al. [167] for immune system components (innate, cytokine, adaptive) modulated during low-dose-radiation-induced tumor regression.

Sakai et al. [24] have used a mouse model for skin-tumor induction by an injected chemical carcinogen (0.5 mg of 20-methylcholanthrene [MC] in olive oil) to examine the efficiency of preventing MC-induced skin tumors using chronic low-rate exposure to Cs-137 gamma rays (which stimulate the body's natural anticancer defenses). Dose rates used were 0.3, 0.95, or 2.6 mGy/h. Thirty-five days after the start of irradiation the mice were injected via the groin with MC and radiation exposure was then continued at the same rate as before the injection. The cumulative tumor incidences after 216 days following MC injection were 94% in mice irradiated at 0.3 mGy/h, 76% for 0.95 mGy/h, 89% for 2.6 mGy/h, and 94% in non-irradiated control mice. The result (76% incident) for the 0.95 mGy/h group was significantly below ($p < 0.05$) the reference group (MC only) level. The implied protection afforded by the chronic, low-rate gamma-ray exposure was attributed to a hierarchy of adaptive response mechanisms that include increased antioxidant capacity, stimulated repair of DNA damage, stimulated removal of neoplastically transformed cells via apoptosis, and stimulated removal of proliferating cancer cells by the immune system. Sakai and his colleagues were one of the first groups to propose a hierarchical nature (multiple cancer barriers) of radiation adaptation in mammals. This was based not only on their research but also on research findings by other groups. Now a hierarchy of natural defenses (barriers) against cancer that are enhanced by low-dose irradiation is well established [23,65].

Internal exposure to high-level BaP causes inflammation and in mouse models has been demonstrated to cause multiple lung tumors in each exposed animal. Using chemopreventative agents, researcher have successfully protected from BaP-exposure related lung cancers by using specific agents that reduce the dose of ultimate carcinogen (e.g. BPDE) that arises in the body via metabolism of BaP. Such studies however do not relate to boosting the body's natural defenses against cancer. Given that low-dose radiation suppresses cancer-facilitating inflammation, it might be expected that low-dose radiation may reduce the number of lung tumors in mice exposed to high-level BaP, provided anti-inflammatory genes are not irreversibly epigenetically silenced via the high level BaP exposure. Bruce et al. [162] examined the effects of injected BaP alone or in combination with fractionated low-dose gamma radiation (60–600 mGy total doses) on the induction of lung adenomas in A/J mice [162]. The results obtained demonstrated that 600 mGy to the total body delivered in six biweekly fractions of 100 mGy starting one month after BaP injection significantly reduced the number of lung tumors (adenomas but not carcinomas) induced per animal. The 60 mGy group (10 mGy fractions) did not reveal any radiation protection against BaP-induced lung adenomas. This finding suggests that DNA double-strand-break repair (which should be induced by both 10 mGy and 100 mGy fractions [119]) may not explain the protection observed for the 600 mGy group. Suppression of inflammation and/or stimulation of anticancer immunity appear to be more plausible explanations. The data of Bruce et al. [162] also indicated that the six biweekly doses of 100 mGy suppressed the occurrence of spontaneous hyperplastic foci in the lung; however, this suppression failed to reach statistical significance when analyzed based on average foci per lung, possibly related to the small sample sizes used for the control and test groups.

Kojima et al. [183] examined whether the increase of glutathione levels induced by low-dose gamma rays is involved in the appearance of enhanced natural killer (NK) cell activity and ADCC, leading to a suppression of tumor growth in Ehrlich solid tumor-bearing mice. NK cell activity in ICR mouse splenocytes increased from 4 to 6 h after whole-body exposure to 500 mGy of gamma rays and thereafter decreased to near the baseline level by 24 h after exposure. The pattern for ADCC over time was similar. Adding reduced glutathione exogenously to

Table 3
Effects of low-dose ionizing radiation on *secretory components* of the immune system.[a]

Cytokine	Modification	Immune system role in low-dose response	References
IL-2	Increase	T-cell proliferation	[169,179]
IL-12	Increase	Proinflammatory response	[187]
IFN-γ	Increase	Phagocytosis and antigen presentation	[188]
TGF-β	Decrease	Maturation and proliferation of T and B cells	[180]
IL-10	Decrease	Immune activation	[180]
TNF-α	Increase	Proinflammatory response	[189]

[a] Reference: Farooque et al. [167].

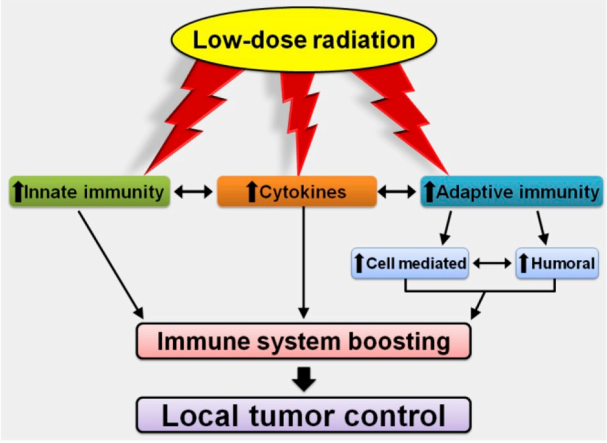

Fig. 5. Redrawn conceptual model of Farooque et al. [167] for immune system components modulation during low-dose-radiation-induced tumor regression. The indicated modulations lead to immune system boosting and local tumor control.

Table 2
Effects on low-dose ionizing radiation on *adaptive immunity*.[a]

Cellular component	Modification	Immune system role in low-dose response	Reference
$CD8^+$ (CTL)	Increase in cytolysis	Lysis of tumor cells	[184]
$CD4^+$	Enhanced responsiveness	Helping other immune cells	[185]
Th1	Increase	Anti-tumor activity	[186]
Th2	No change	Pro-inflammatory response	[55]
T-regulatory	Decrease	Breaking of tumor tolerance during carcinogenesis and induction of anti-tumor immunity	[180]

[a] Reference: Farooque et al. [167].

splenocytes in culture (obtained from normal mice) enhanced both NK activity and ADCC in a dose-related manner. Tumor growth was also examined in tumor-bearing mice and the growth rate after inoculation was significantly reduced by low-dose gamma rays. The results suggested that low-dose gamma rays activate immune functions in the body via an induction of glutathione, which led to a reduction in the tumor growth rate.

The influence of repeated (fractionated) 500 mGy gamma-ray doses on the Th1/Th2 immunity balance in mice with Ehrlich-Solid-Tumors was investigated by Hayase et al. [186]. Fractionating the dose helps prevent severe damage to normal tissue. The repeated doses significantly delayed the growth of the tumors. In addition, the cytotoxic activities of natural killer cells and cytotoxic T lymphocytes were enhanced by repeated low doses. The irradiation also increased the production of IFN-γ by splenocytes of tumor-bearing mice but interleukin 4 (IL-4) was not altered, resulting in an increased IFN-γ/IL-4 ratio, a hallmark of a shift to a Th1 phenotype. The repeated gamma-ray exposure also increased IL-12 production and levels of reduced-glutathione in macrophages.

Klug et al. [190] demonstrated that low-dose irradiation programs macrophage differentiation to an iNOS + /M1 phenotype that leads to effective T cell immunity against cancer. They showed that local low-dose-gamma irradiation causes efficient recruitment of tumor-specific T cells in human pancreatic carcinomas as well as T-cell-mediated tumor rejection. Survival was also prolonged in otherwise immune refractory tumor-bearing mice.

Using an “artificial tumor metastasis” model where tumor cells were injected into mice, Cheda et al. [53,54] conducted studies of tumor growth suppression by low-dose radiation (which stimulates anticancer immunity). They demonstrated that single, total-body exposure of mice to 100 or 200 mGy of X rays inhibited the development of artificial tumor metastases in the lungs and that the effect was related in part to radiation-exposure enhanced activity of natural killer cells. They also demonstrated in another study [191] that inhibition of the growth of the injected tumor cells by single exposure of mice to 100 or 200 mGy of X rays results mainly from stimulation of the cytocidal (i.e., killing) activity of macrophages that secrete increased amounts of nitric oxide.

Zhou et al. [192] employed an *in vivo* (mouse) “artificial-lung-cancer” suppression model to investigate immune system enhancement with low-dose X-rays (75 mGy at 12.5 mGy/min). Suppression by radiation of tumor (artificial) growth in the lung after subcutaneously injecting lung tumor cells (to form lung tumors) in C57BL/6 mice serves as a marker of immune system enhancement. Increase tumor growth (from injected C57BL/6 mouse-derived Lewis lung cancer cells) serves as a marker for immune system suppression (which is caused by high radiation doses). The researchers demonstrated the pivotal role of immune system enhancement by low-dose/low-dose-rate exposure in contrast to its suppression by a high-dose/high-dose-rate exposure (1 Gy at 1 Gy/min). They found that low-dose/low-dose-rate radiation activated T cells and natural killer cells and increased the cytotoxicity of splenocytes and the infiltration of T cells into tumorous tissues. In contrast, when immune function was suppressed by high-dose/high-dose-rate radiation pretreatment, low-dose/low-dose-rate radiation did not inhibit artificial tumor growth. However, when low-dose/low-dose-rate radiation was administered before the high dose (at a high rate), immunity was protected from suppression by the high-dose/high-dose-rate exposure and artificial tumor growth was inhibited somewhat. This was interpreted to indicate the induction of immune system adaptation by low-dose/low-dose-rate exposure.

Growing research findings support the view that low- and moderate-level radon suppresses inflammation and stimulates the immune system. Suppressing inflammation can indirectly be inferred via suppressing inflammation-related diseases. Stimulation of anticancer immunity can be inferred from a reduction of metastatic cancer. Takahashi and Kojima [58] examined the effect of radon (^{222}Rn, $t_{1/2}$ = 3.82 days, alpha particle energy = 5.49 MeV) in ingested water in

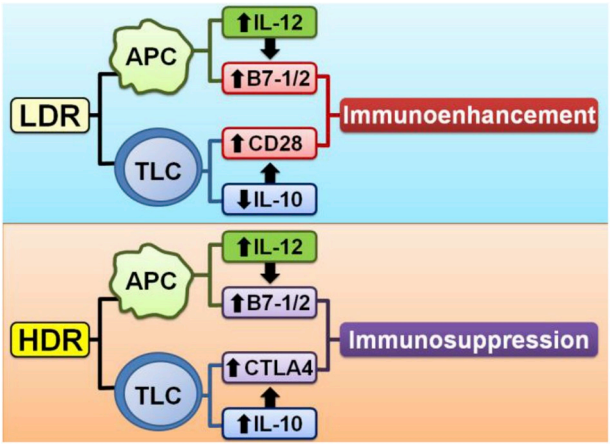

Fig. 6. Redrawn conceptual model of Liu [182] for immunoehancement or immunosuppression interactions between antigen presenting cells (APC) and T lymphocytes (TLC) via surface molecules and cytokines in response to low- or high-dose radiation. LDR = low dose radiation; HDR = high dose radiation; up and down arrows on the left side of the symbols indicate stimulated up- and down-regulation, respectively; arrows between symbols indicate facilitation. LDR leads to immunoenhancement which serves as a barrier to cancer. HDR leads to immunosuppression thereby facilitating cancer occurrence.

suppressing inflammation-related diseases and metastatic cancer using two experimental mouse models (radon concentrations varied over a wide range). Model 1 (suppression of inflammation): ingestion exposure of five-week-old SPF NC/Nga mice to radon significantly delayed the progression of atopic dermatitis-like skin lesions induced by the explosive picryl chloride (2,4,6-trinitrochlorobenzene). Model 2 (stimulation of anticancer immunity): the number of pulmonary metastatic foci in six-week-old male C57BL/6 mice inoculated with B16 melanoma cells two weeks after the start of radon ingestion was reduced significantly by the radon intake. In addition, the IFN-γ/IL-4 ratio in splenocytes from BALB/c mice immunized with DNP-Ascaris was significantly increased by ingested water containing elevated radon. These results were interpreted to indicate beneficial suppression of inflammation and beneficial modulation of the immune system (anticancer immunity) by the ingested radon.

Fig. 6 provides the systems-biology-related conceptual model of Liu [182] for immunoenhancement and immunosuppression interactions between antigen presenting cells and T lymphocytes via surface molecules and cytokines in response to low- or high-dose radiation. Low doses stimulate immunity (immuneenhancement) while high doses are immunosuppressive.

With the LNT hypothesis, adding radiation on top of a known carcinogenic dose always increases cancer risk. This should be the case whether the added radiation comes after or before the known carcinogenic dose. Interestingly, when a low dose or a low or moderate dose precedes [193] or follows [194] a known carcinogenic or mutational high dose, risk from the known high dose can decrease, which essentially invalidates the LNT model. The observations discussed next are from adaptive-response studies which essentially invalidate the LNT model as it relates to cancer induction by ionizing radiation and are based on Nenoi et al. [193].

Bhattacharjee [195] found that the yield of thymic lymphoma among Swiss mice induced by a high dose of 2 Gy of gamma rays was substantially decreased when the mice were pre-irradiated with a priming low rate of 10 mGy per day for 5 or 10 consecutive days. According to the LNT model, the low rate exposure added to the high carcinogenic dose of 2 Gy should have increased the cancer risk, while the added radiation actually decreased the risk.

Ina et al. [196] found that the induction of thymic lymphomas by four separated doses of 1.8 Gy each (7.2 Gy in total) in C57BL/6 mice

was consistently reduced by pre-irradiation with 75 mGy of X-rays given 6 h before each 1.8 Gy irradiation. They also showed that induction of thymic lymphomas was more effectively reduced by continuous whole-body irradiation with gamma rays at 1.2 mGy per hour for 450 days starting 35 days before the large known carcinogenic radiation dose. According to the LNT model, these observations of reduced cancer risk should not have occurred, pointing to LNT as being invalid in this instance.

It was reported by Mitchel et al. [197] that the latent period for development of acute myeloid leukemia induced by a challenge carcinogenic dose of 1 Gy in CBA/Harwell mice was significantly extended when the mice were first irradiated with a 100-mGy dose 24 h before a large known carcinogenic dose. Such an observation would not be expected were the LNT model valid. Mitchel et al. [198] later reported that a single exposure of either 10 or 100 mGy alone reduced (to below the spontaneous level) rather than increased cancer development in p53 heterozygous mice. These and other finding are also inconsistent with the LNT model which predicts an increase risk above the baseline level [199].

Kakinuma et al. [200] reported that four deliveries (1 per week) of a dose of 200 mGy (800 mGy in total) suppressed N-ethyl-N-nitrosourea-induced thymic lymphoma in B6C3F1 mice. This result is consistent with the view that small radiation doses can activate the body's natural defenses against cancer and thereby prevent cancer induction by environmental, dietary, and other chemical carcinogens. The indicated finding does not support a combined exposure (radiation + carcinogenic chemical) carcinogenesis model where cancer risk increases linearly as radiation dose increases and linearly as chemical dose increases, which is now being considered by regulatory agencies.

Findings reported here are consistent with those in other papers [[201–205]] in this Special Issue.

3.5. Mild-stress-induced epigenetic changes and increased longevity

The dysregulations in epigenetic control by high level of stressors (e.g. large chemical and radiation doses) appear to have a major promotional impact on aging and age-related diseases that include cancer [87]. According to Vaiserman [87], mild stress (e.g. from low-dose radiation and chemicals) may slow the aging process. The mechanisms that underlie such benefits may be associated with an increased ability to adapt to the mild stresses [87,206].

An “epigenetic regulation” explanation was proposed by Arking and Giroux [207] for the late-life mortality-rate plateau (paradoxical reduced mortality rate from cancer and other diseases at older ages). The authors suggested that this could be attributed to epigenetic changes in response to a wide variety of environmental stressors, with subsequent transient increases in the basal level of expression of the antioxidant and heat shock protein genes in the long-lived subset of the population. As a result, a hormetically-responding subpopulation would exhibit a reduced late-life mortality rate and increased longevity.

3.6. Old-age-related cancer suppression and related mechanisms

Progression of existing cancers is now recognized to involve elaborate tumor-host interactions including immune editing and angiogenesis which strongly depends on host age [34–36,170]. From adolescence through middle age, cancer incidence rate increases with age, while during middle-age the rate of new occurrences begins to decrease and the rate decrease continues to advanced ages [34–36,208–210]. Understanding how the cancer risk modifying effects of organs change with age and understanding low-dose radiation and age interactions will aid in improving radiation risk assessment.

3.7. Hallmarks of cancer suppression

Fig. 7 presents some currently known hallmarks of cancer suppression and this terminology is based on a recent publication [23]. All of the protective mechanisms indicated are stimulated by low-dose radiation and may also be stimulated by other forms of mild stress, including some chemical stresses. The existence of the indicated multiple protective mechanisms against cancer and their stimulation by low-dose radiation make the LNT model for radiogenic cancer highly implausible.

3.8. Threshold effects of in utero radiation exposure

There is heightened sensitivity related to harm from *in utero* exposure. Effects of *in utero* radiation exposure were reviewed by Shaw et al. [211]. They point out in their review that radiation risks from *in utero* exposure depend on the stage of pregnancy and the radiation absorbed dose. The type of radiation and dose rate are likely also important. Potential radiation health effects vary, depending on the fetal stage of development. According to Shaw et al. [211], the radiation risks are higher during organogenesis and in the early fetal period, lower during the second trimester, and least during the third trimester. The estimated threshold doses for malformations were reported to range from 100 to 200 mGy or higher and are mainly associated with central nervous system abnormalities. These threshold dose values relate to low-LET photon radiation.

The prenatal period is the most sensitive for radiation exposure. This is also the time when about 50%–75% of all human pregnancies abort [212] and has been attributed to abnormal development. Because there is a high incidence of spontaneous abortion (a stochastic effect) for this period, finding evidence of significant harm from small radiation doses is challenging. Based on findings from animal studies, it has been suggested that radiation-induced prenatal death might occur at doses of 50–100 mGy and above, if delivered before implantation [211]. The suggestions implicate possible dose thresholds for these effects. Radiation-induced prenatal death and other non-cancer effects in humans occur at other stages of gestation but at doses of about 250 mGy and higher [211,213]. The other effects include growth retardation, malignancies, and neurologic effects such as small head size, severe mental retardation, intellectual deficit, and seizures. The risk of cancer for offspring exposed to high radiation doses (e.g., >500 mGy of X rays) is clearly elevated [214,215]. Whether doses <100 mGy elevate

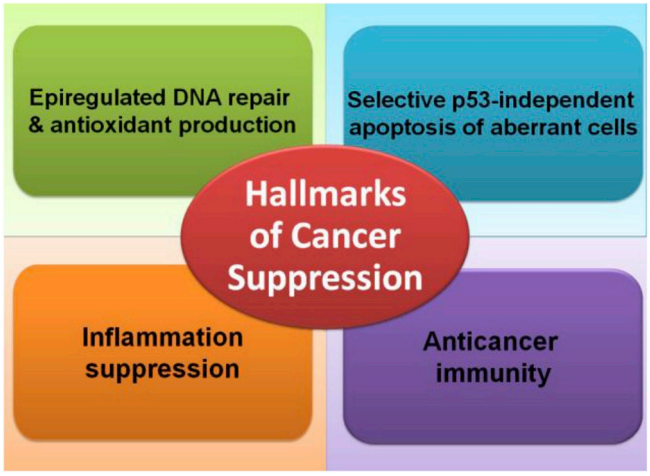

Fig. 7. Hallmarks of cancer suppression (based on [23]: epigenetically-regulated DNA repair and antioxidant production (protects from oxidative damage), selective apoptosis (p53-independent) of aberrant cells (e.g. transformed cells), inflammation suppression (reduces cancer risk), and anticancer immunity (destroys cancer cells). All of these hallmarks are stimulated by low radiation doses and dose rates with an efficiency that depends on the type of radiation, the radiation dose, the dose-rate history (how dose rate varied over time), and the endpoint considered.

cancer risk is however controversial [214,215].

Cancer risk from low radiation doses (< 100 mGy) *in utero* is mainly based on use of the LNT model and extrapolating from high to low doses, although there are exceptions [211]. Based on the literature review we conducted, there is extensive evidence against the validity of the LNT model as applied to radiation-induced cancer.

3.9. Evidence for dose threshold for cardiovascular disease

Like for low-dose-radiation-induced cancer (risk) being an LNT function of radiation dose, the possibility for low-dose-radiation-induced cardiovascular disease (risk) being an LNT function of dose is also controversial. Using the loss of angiogenic capacity in a human aorta endothelial cells assay (*in vitro*), a dose-rate-dependent cobalt-60 gamma-ray threshold was found to be between 500 mGy and 1 Gy [216]. This *in vitro* finding does not support use of the LNT model for cardiovascular disease induction; however, *in vivo* studies are also needed before firm conclusions can be made.

4. Systems–biology–related models that do not support LNT

Systems biology is directed at a holistic approach to understanding the complexity of regulating biological systems [218]. The definition of systems biology used here relates to mammals and is as follows: It is the study of systems of biological and physiological components of the body, which include molecules, cells, tissue, and organs and related interactions. Living systems such as humans are dynamic and quite complex. As with all complex, multivariate, multi-parameter processes, accurately predicting outcomes (e.g. cancer occurrence in a given organ or tissue at a given age or follow-up time) as well as related variability and uncertainty, is quite challenging. Both conceptual (not related to mathematics) and quantitative (mathematical description) systems-biology-related models have been developed that relate to cancer suppression by low radiation doses.

Fig. 8 summarizes a conceptual model for low-dose radiation suppression of carcinogenesis as formulated by Ulsh [217] from a systems biology perspective. Natural barriers to cancer [which are enhanced by low radiation doses [23,107]] include DNA repair, apoptosis of cells with genomic instability, terminal differentiation of aberrant cells, and immune system elimination (via immune surveillance) of both transformed and proliferating cells.

Fig. 9 summarizes the conceptual model of Janiak et al. [170] for low-dose radiation stimulation of immunoediting that enhances anticancer immunity. Based on an extensive literature review [170], the researchers stated that many, if not all, of the tumor promoting immune mechanisms are likely to be blocked and/or reversed by low dose radiation exposure; however, many of the underlying mechanisms are unknown. They postulated that in addition to the direct activation of NK lymphocytes (and possibly other antitumor cytotoxic cells), low radiation doses enhance the “visibility” and/or “susceptibility” of cancer cells to immune-surveillance-related assaults via stimulating the expression by neoplastic and immune cells of molecules and ligands (e.g., CD2, B7, CD28, NKG2D) needed for triggering cytotoxic reactions and/or turning on “danger signals” in the neoplastic tissue. They also state that low-level radiation exposures are likely to alleviate or reverse tumor-associated-immune degeneracy through elimination or inhibition of the multiple cells, cytokines, and other factors associated with immunosuppressive loops induced by a tumor. This could lead to the following [170]: (a) shifting of the immune response to favor the anti-neoplastic phenotypes (e.g. Th1 in the case of $CD4^+$T cells, M1 for macrophages, and N1 for neutrophils); (b) targeting of Treg-Th17 and Th17-DC interactions (aids tumor regression); (c) activation of Toll-like receptor-mediated signaling in phagocytes and antigen-presenting cells; (d) attenuating cancer-initiation-promotion-progression-facilitating chronic inflammation; (e) and/or down-regulation of immune checkpoint molecules (e.g., CTLA-4, PD-1, and/or PD-L1 on T cells).

Janiak et al. [170] pointed out based on their review as well as their own research that there are numerous non-immune mechanisms that are stimulated by low radiation doses that benefit normal but not malignant cells. These include more efficient DNA repair, stimulation of anti-oxidant reactions (which helps to reduce tissue injury), increased cell proliferation, and a metabolic shift away from oxidative phosphorylation to aerobic glycolysis (which results in increased radioresistance of healthy tissues).

At this time, nobody has reliably applied the whole-body-level, systems-biology-related-dynamics approach for quantitatively characterizing cancer risk from radiation exposure for reasons pointed out below. Systems-biology-based cancer risk models for radiation exposure need to address all of the following and possibly more: (a) Molecular changes, their epigenetic regulation, the related variability between different individuals, and related stochastic radiation thresholds for gene activation and silencing. (b) Cellular changes, intercellular interaction effects including bystander effects, and related variability for different tissue, organs, and individuals. (c) Tissue changes (for different tissues and individuals) and influences of abscopal effects. (d) Age and radiation interactions as well as age and tumor interactions. (e) Radiation dose and dose-rate influences. (f) Radiation quality (i.e. type of radiation) influences. (g) Radiation dose distribution (over the body) influences.

Because of the above major challenges with the complex-dynamics approach, only less complicated, outcome-focused and systems-biology-related, non-dynamic risk models have so for been developed. Some non-dynamic outcome quantitative models are discussed below. A more extensive review of modeling (e.g. microdosimetric, track-structure and other models) is beyond the scope of this paper.

4.1. Hierarchical defenses model with deterministic thresholds

Feinendegen [65] systems-biology-based model for low-dose-radiation related cancer risks treats the human body as a hierarchy of

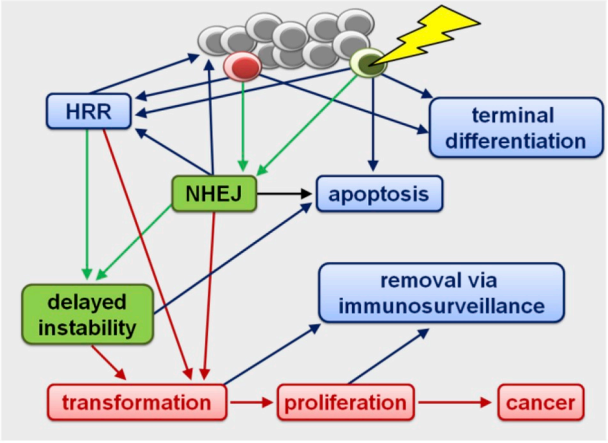

Fig. 8. Redrawn conceptual systems-biology-based model of Ulsh [217] for the web of biological responses to low-dose-radiation damage. Low-dose-radiation-induced damage to a target cell (green shaded central circle surrounded by white circle) is depicted as a lightning bolt. The population of cells includes normal cells (gray-shaded central circle surrounded by white circle) and cells carrying potentially carcinogenic damage (dark-red-shaded central circle surrounded by light-red circle) from endogenous and other exogenous processes and agents. Processes (HRR, removal via immunosurveillance, terminal differentiation) that are most likely associated with low or decreasing cancer risk due to radiation adaptation are shown in blue and serve as barriers to cancer [23]. Processes (neoplastic transformation, proliferation) that are most likely associated with increasing cancer risk (unlikely low-dose-radiation-related) are shown in red. Processes (NHEJ, delayed instability) with uncertain consequences for cancer risk are shown in green. HRR, homologous recombinational DNA repair; NHEJ, nonhomologous end joining DNA repair.

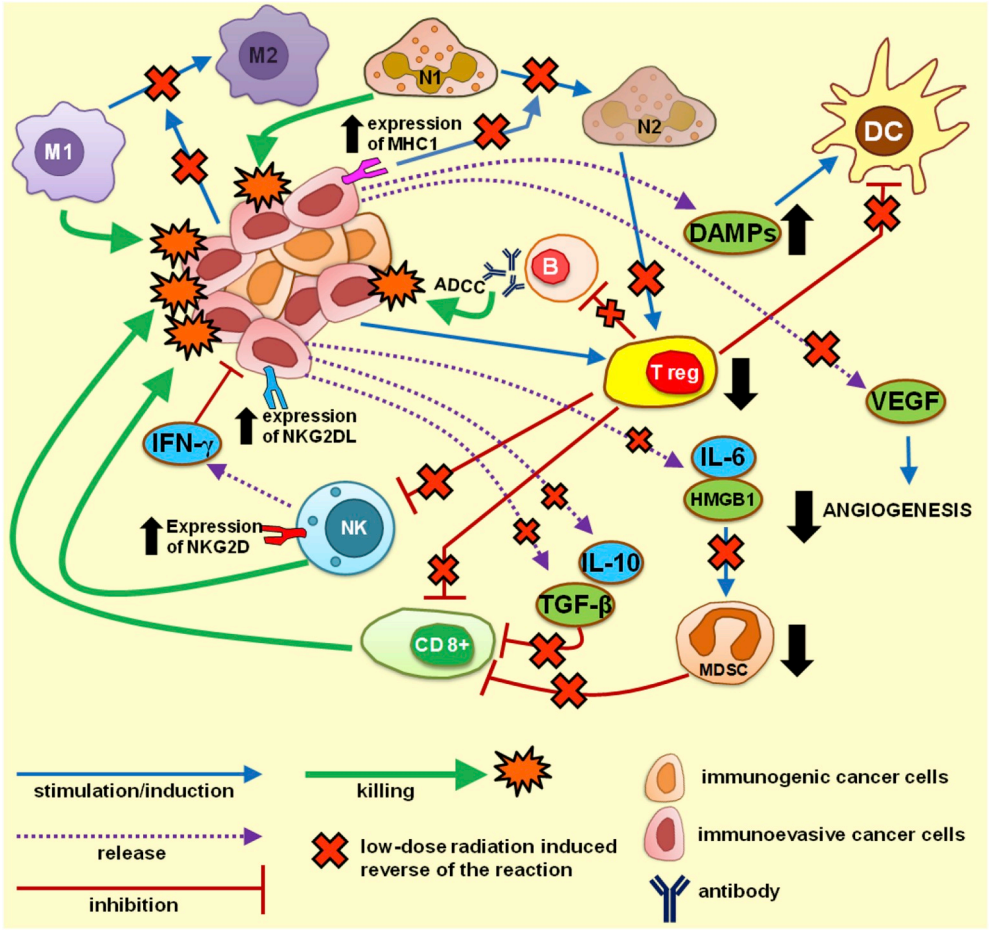

Fig. 9. Redrawn systems-biology-based conceptual model of Janiak et al. [170] for low-level radiation blocking and/or reversing different tumor-promoting immune mechanisms: *ADCC*, antibody-dependent cellular cytotoxicity; *B*, B lymphocytes; *CD8*$^+$, CD8$^+$ T lymphocytes; *DAMPs*, damage-associated molecular pattern molecules; *HMGB1*, high-mobility group box 1 protein; *M1*, phenotype 1 macrophages; *M2*, phenotype 2 macrophages; *N1*, phenotype 1 neutrophils; *N2*, phenotype 2 neutrophils; *Treg*, regulatory T lymphocytes; *NKG2DL*, ligand for the natural killer group 2D receptor; *NKG2D*, natural killer group 2D receptor; *VEGF*, vascular endothelial growth factor. See main text for more details.

different levels of organization (Fig. 10). In order for radiation or other perturbations to damage a given system level, there is a threshold (deterministic) for harm at each level. The model distinguishes between three principal signaling loops: (1) between molecules and cells; (2) between cells and tissue; and (3) between cells and the entire body. With ascending levels the biological organization comes more complexity. Signaling-related kinetics is not quantitatively modeled. Natural biological defenses (cancer barriers) that must be overcome in order for cancer (spontaneous or other) risk to increase include scavaging of internal toxins, DNA damage repair, protective apoptosis (which removes aberrant cells), cell senescence, and anticancer immunity which suppresses cancer occurrence. These protective processes are differentially stimulated by low radiation doses, making LNT implausible.

Equations employed for the Feinendegen model allow for evaluating cancer risk as a function of radiation dose to a given part of the body. The model yields nonlinear threshold or hormetic responses (for risk vs. dose) after low radiation doses.

4.2. Hierarchical defenses model with stochastic thresholds

Like with the Feinedengen [65] model, Scott et al. [78] also used a non-dynamic, system-biology-based, hierarchical-defenses model which focused on the body's natural defenses against cancer that are differentially stimulated by low radiation doses and inhibited by high doses. The natural protection (assumed epigenetically regulated) considered includes DNA damage repair, selective apoptosis of pre-cancer cells, and anticancer immunity. Unlike with the Feinendegen model, stochastic thresholds (stimulatory and inhibitory) were assumed with stimulatory thresholds for natural defenses being involved at low doses and inhibitory thresholds (related to inhibition of natural defenses) being involved at high doses. Because of the low-dose stimulation and high-dose inhibition of the natural defenses and the related stochastic thresholds, non-linear, hormetic dose-response relationships for cancer relative risk can arise. The model has therefore been called the hormetic relative risk (HRR) model and allows evaluation of the proportion (indicated by a protection factor (PROFAC)) of cancers that are prevented as a result of radiation exposure. The HRR model has been applied to lung cancer [78,219] and to total cancers [219] for different irradiated groups of humans. PROFAC values for lung cancer for the

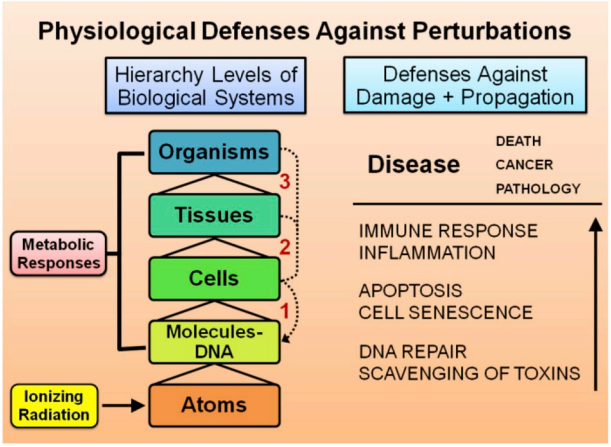

Fig. 10. Redrawn systems-biology-based conceptual model of Feinendegen [65] for hierarchy of natural protective processes that are stimulated by low-dose radiation. See main text for additional information.

different irradiated groups ranged from 0.07 to 1.0 (i.e. 7–100% of cancers prevented). For all cancers combined, PROFAC values ranged from 0.06 to 0.49 (6–49% of cancers prevented). Here, prevented cancers relate to those that make up the baseline cancer risk. Interestingly, even residential radon at low and moderate levels appears to prevent rather than cause lung cancer [26,163].

Stochastic stimulatory (for adaptation) and inhibitory (adaptation prevention) thresholds have also been applied to characterizing nonlinear mutation dose-response relationships [194,220,221] and nonlinear neoplastic transformation relationships [221].

4.3. Other models

Some other modelers have focused on characterizing multistage carcinogenesis within the framework of stochastic multistage clonal expansion models which are extensions of the two-stage clonal expansion (TSCE) model of carcinogenesis usually attributed to Moolgavkar and Venzon [222] and Moolgavkar and Knudson [223]. The systems-biology-related TSCE model is based on the assumption that initiated cells, have a slight growth advantage over normal neighboring cells, and arise from stem cells according to a non-homogeneous Poisson process. Once induced, initiated cells are then considered to undergo a stochastic birth–death–mutation process with the birth and death process leading to clones of initiated cells and the mutation process then leads to the conversion of an initiated cell into a fully malignant cell. Unfortunately, the TSCE model does not address the hierarchy of natural protective processes that prevent cancer and are differentially stimulated by low radiation doses. Multistage extensions of the TSCE model have also been proposed by various investigators but most are similarly deficient in addressing natural protection against cancer and its enhancement by low radiation doses.

A novel nonparametric statistical modeling approach, based on a special algorithm for artificial neural networks, was developed by Sasaki et al. [224] and employed in analyzing cancer databases established by the Radiation Effects Research Foundation for A-bomb survivors. Interestingly, the novel analysis demonstrated unique features at low doses that could not be accounted for by the LNT model. These features included the presence of a threshold radiation dose for increased cancer risk that varied with organ, gender and age at exposure, and a small but significant “bumping increase” in cancer risk at low doses for Nagasaki that may reflect dose misclassifications [224] and missing dose from fallout radionuclides [225]. The threshold was implicated by the derived negative excess relative risk. The thresholds may have been underestimated because doses from internal radionuclides (from fallout radioactivity) were missing and may be rather large as has been recently implicated for Hiroshima [225].

A multistage State-Vector Model for *in vitro* neoplastic transformation was introduced [226,227]. The model was influenced by the work of Fleishman et al. [228] and was successfully applied to published hormetic dose-response data from *in vitro* studies (data sources: [42,158,159]. Interest in the neoplastic transformation dose-response data can be justified based on the fact that cancer relative risk and neoplastic transformation relative risk dose-response relationships were found to be quite similar [42]. With the State-Vector Model, initiation is assumed to arise from DNA double strand breaks induced by radiation and also by endogenous processes. Promotion is assumed to arise from a disruption of intercellular communication and a compensatory proliferation of initiated cells. Cell death is modeled as being related in part to radiation-induced necrosis and also to low dose related bystander-cells-induced apoptosis. The apoptosis mode of cell death has been hypothesized to be responsible for the observed decrease of the *in vitro* neoplastic transformation frequency to below the spontaneous level as reported by the cited studies [42,158,159].

5. Application of Hill's criteria to demonstrate LNT implausibility

Hill [229] proposed 9 criteria by which disease (e.g. cancer) causation could be distinguished from simple associations related to a risk-factor (e.g. ionizing radiation) exposure. Ulsh [230] used the Hill's criteria to evaluate the plausibility of the LNT, threshold, and hormetic risk models for characterizing low-dose and low-dose-rate radiation effects (cancer focus). With the focus on low radiation doses and dose rates and cancer, the situation is different than when high radiation doses and dose rates are involved and one extrapolates to low doses and dose rates (approach that has been used for LNT). The criteria evaluated were as follows: (1) strength of the association, (2) consistency (repeatability or generality), (3) specificity, (4) temporality (risk factor exposure precedes outcome), (5) biological gradient (demonstrated dose-response relationship), (6) plausibility (consistency with biological mechanisms), (7) coherence (outcome should not conflict with known disease history), (8) experimental support (suspected causation should be supported by experimental data), and (9) analogy (similar causation by other known agents). In using the indicated criteria and extensively reviewing radiation biology data including some of the data discussed in this publication, Ulsh [230] concluded that the case for low-dose and low dose-rate radiation causation of cancer as predicted by the LNT advocates fail to satisfy the indicated objective criteria. Instead, hormetic and threshold models were found to have more compelling weights of evidence.

As pointed out in Section 4.3, the once popular multistage carcinogenesis models (which need to be modernized) do not account for the hierarchy of natural defenses (barriers) against cancer occurrence that are enhanced by low radiation doses. Thus, it can be stated with confidence that the multistage carcinogenesis models (e.g. as the one used by Little et al. [231]), so far as they apply to low-dose-radiation exposure, fail the Hill's criterion of plausibility and are biologically deficient (with respect to radiation adaptive responses).

Based on the research findings discussed, there is no basis (other than ease of use) for relying on the LNT model for low-dose and low-dose-rate radiation risk assessment for cancer. It is highly implausible that the multiple hallmarks of cancer could result from a single ionizing event (radiation hit), as required by the LNT model; further, the natural barriers against cancer occurring are enhanced by low-dose radiation. Thus, rather than an LNT response, a threshold or hormetic response for cancer is more plausible, depending on the circumstance considered [232]. As stated by Katz and Waligórski [79]: *“Existing data obtained with beams of electrons, protons, X ray photons, incorporated tritium, and ^{125}I demonstrate that hundreds of electrons may traverse a cell for inactivation and millions may be required for cancer induction. If linear extrapolation were valid these numbers would be reduced to one.”* As stated by Tubiana et al. [232]: “*Preconceived concepts impede progress; in the case of the LNT model, they have resulted in substantial medical, economic, and other societal harm.*” When the harm is evaluated today, it includes thousands of radiation-phobia-related deaths (Chernobyl-related abortions and Fukushima-evacuation-stress-related deaths [233].

6. Conclusions

The following conclusions are made based on an extensive review of publications related to the molecular-, cellular-, tissue-, and whole-body-level changes after exposure to low doses of ionizing radiation:

- A hierarchy of natural barriers (defenses) to cancer occurrence exist and must be overcome for cancer to occur. The cancer barriers include epiregulated DNA damage repair and antioxidant production, selective p53-independent apoptosis of aberrant cells, suppression of cancer-promoting inflammation, and anticancer immunity.
- The natural barriers make up the hallmarks of cancer suppression and each is enhanced by low radiation doses and dose rates, thereby making cancer less likely for beneficiaries.

- The dose range over which the cancer barriers are enhanced likely depends on dose rate (increasing as dose rate decreases) and probably depends on the type of radiation, radiation energy, and type of cancer.
- The LNT model, as it relates to radiation-induced cancer, is highly implausible because it does not account for the natural cancer barriers and their elevation by low radiation doses (and dose rates) and their reduction by high doses and dose rates.
- Threshold and hormetic dose-response models are more consistent with the existence of a hierarchy of low-dose-enhanced cancer barriers.
- New research is needed to determine which model [threshold, hormetic, or other (excluding LNT)] applies for a given endpoint and radiation exposure scenario.

Declaration of interest

The authors were part of the research team that produced this Special Issue publication and interacted on a regular basis with other team members. The authors had the sole responsibility for the writing and content of the paper. Dr. Scott is a retired Scientist from Lovelace Respiratory Research Institute, Albuquerque, NM, U.S.

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Re-evaluation of the linear no-threshold (LNT) model using new paradigms and modern molecular studies

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ABSTRACT

The linear no-threshold (LNT) model is currently used to estimate low dose radiation (LDR) induced health risks. This model lacks safety thresholds and postulates that health risks caused by ionizing radiation is directly proportional to dose. Therefore even the smallest radiation dose has the potential to cause an increase in cancer risk. Advances in LDR biology and cell molecular techniques demonstrate that the LNT model does not appropriately reflect the biology or the health effects at the low dose range. The main pitfall of the LNT model is due to the extrapolation of mutation and DNA damage studies that were conducted at high radiation doses delivered at a high dose-rate. These studies formed the basis of several outdated paradigms that are either incorrect or do not hold for LDR doses. Thus, the goal of this review is to summarize the modern cellular and molecular literature in LDR biology and provide new paradigms that better represent the biological effects in the low dose range. We demonstrate that LDR activates a variety of cellular defense mechanisms including DNA repair systems, programmed cell death (apoptosis), cell cycle arrest, senescence, adaptive memory, bystander effects, epigenetics, immune stimulation, and tumor suppression. The evidence presented in this review reveals that there are minimal health risks (cancer) with LDR exposure, and that a dose higher than some threshold value is necessary to achieve the harmful effects classically observed with high doses of radiation. Knowledge gained from this review can help the radiation protection community in making informed decisions regarding radiation policy and limits.

1. Introduction

There is widespread consensus regarding the harmful effects of high doses of radiation (HDR), however the biological risks induced by low dose radiation (LDR) is controversial [1,2]. Since the 1970s, the radiation protection agencies have used the linear no-threshold (LNT) model to estimate the risk of LDR by extrapolating risks determined using high dose studies [3,4]. In this model, radiation is always considered harmful with no safety threshold, and even the smallest radiation dose has the potential to cause an increase in health risk [5]. However, advances in radiation biology and cell molecular techniques demonstrate that the LNT model does not appropriately reflect the health effects in the low-dose range and is inconsistent with current molecular findings in LDR biology [6–11].

The main pitfall of the LNT model is due to the inappropriate extrapolation of mutation and DNA damage studies that were conducted at high radiation doses delivered at a high dose-rate [4,12–14]. These studies formed the basis of several outdated paradigms [1] that are either incorrect or do not hold for LDR doses. First, it was assumed that the primary radiation-related cause of cancer was due to DNA damage, and since DNA mutation increases as a linear function of radiation dose it was postulated that cancer frequency would also increase as a linear function of dose [15]. The second paradigm was based on the idea that ionizing radiation always produced adverse effects and that each ionization event increased the health risk proportionately. Finally, the radiation dose-response curves were interpreted using the “hit theory”, which determined biological risks as the number of cells traversed by discrete ionizing radiation energy packets [16]. Here, only the cells that were “hit” with the ionizing energy packet responded to the radiation exposure [17].

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This review of cellular mechanisms will demonstrate that studies conducted in LDR biology in the last 20 years largely invalidates the outdated paradigms discussed above [18]. First, cell systems employ a variety of mechanisms (DNA repair, apoptosis, autophagy, cell-cycle arrest) for effective removal/repair of LDR mediated DNA damage [19–22]. Therefore, LDR does not result in DNA mutation accumulation and has no detrimental effect on cell transformation which may reflect in no change in cancer risks [23–25]. Furthermore, large scale molecular expression profile analyses demonstrate that cells express different gene/protein profiles at low doses compared to HDR [26–29]. This shows that the molecular effects of HDR and LDR do not fit a linear biological response. Another well established cell mechanism due to LDR includes radiation-induced bystander effect. Here, un-irradiated cells display molecular gene-expression profiles similar to neighboring irradiated cells [30–32]. The recent bystander studies emphasize cell-to-cell communication as an underlying mechanism that allows tissues to respond as a whole and not as single cells, thereby dismissing the traditionally believed "hit theory" [12]. Moreover, numerous studies report that LDR is harmless, and in many systems show a decrease in cancer frequency or an adaptive phenotype, including LDR mediated enhancement of immune response to pathogens and tumour suppression [33–35]. This notion that low levels of radiation can produce beneficial effects is known as radiation hormesis [36–39]. Taken together, the evidence presented in this review demonstrates that there are minimal health risks with LDR exposure, and that a dose above a threshold level is necessary to achieve the harmful effects classically observed with HDR.

The advent of newer techniques in cell and molecular biology combined with advances in radiation techniques have made it possible to measure radiation-induced changes at low doses and dose-rates which were not possible in the past. However, the LDR field is diverse and often conflicting which contributes to the controversy surrounding the biological effects at low doses [40]. The literature in radiation biology demonstrates that the effects of LDR are dependent on numerous factors including radiation dose, dose-rate, tissue-type, cell-type, gender and species [41–45]. This illustrates that LDR effects are context dependent, and is one of the main reasons why LDR studies are often conflicting. For example, the choice of a cell to undergo DNA repair, apoptosis or cell-cycle arrest when exposed to LDR is cell-type, species and gender dependent [46–50]. Therefore careful review of the literature is essential to tease out the underlying mechanisms in LDR biology. Thus, the goal of this review is to summarize the modern cellular and molecular literature in LDR biology and provide new paradigms that better represent the biological effects in the low dose range. The following sections will be discussed in detail below:

1. Repair/removal of radiation induced DNA damage
2. Comparison of LDR and HDR high-throughput expression profile studies
3. LDR and epigenetics
4. LDR mediated bystander effects
5. LDR mediated tumor suppression

Knowledge gained from this review can help the radiation protection community in making informed decisions regarding radiation policy and limits. This review will also emphasize the need for more studies using modern systems-level analyses to fully understand the health effects of LDR biology.

2. Repair/removal of radiation induced DNA damage

One of the most well-established molecular phenotypes of LDR is the positive correlation between radiation dose and DNA damage. Radiation mediated DNA damage is seen across all doses, demonstrating linearity from 20 mGy to 100 Gy [51–58]. The linearity is based on numerous studies which measured DNA damage using markers of DNA double strand breaks (DSB) including γH2AX, 53BP1, Ser1981-pATM, Rad51, Ku70, and micronuclei formation. There are some studies which also demonstrate DNA damage between 1 and 20 mGy, however this region seems to be cell type and strain specific [10,51,59]. For example, micronuclei formation is seen under 20 mGy in lymphocytes of Balb/c mice but not evident in C57Bl/6 mice [52]. Interestingly, when similar doses are given at a very low dose rate, DNA damage is unnoticeable compared to controls. For instance, analysis of blood samples of mice exposed to 5 weeks of chronic LDR at 0.002 cGy/min (approximately 400x background levels) demonstrated no change in DNA damage analyzed via micronuclei formation, DNA fragmentation, and markers of DNA damage repair [60].

Despite evidence for linear dose-dependent increase in radiation mediated DNA damage, cancer risks remain unchanged at the low dose or low dose rates [3,25,61]. Similarly, numerous studies report lack of genome instability below 1 Gy demonstrating that LDR does not contribute to genomic rearrangements [45,62–64]. Likewise mitochondrial DNA is also resistant to DNA damage below 0.5 Gy [65,66]. Taken together, these studies suggest that although LDR results in DNA damage, the cell is able to either repair the damage or remove itself from the cell population thereby protecting the organism from accumulating DNA mutations. The sections below will review three molecular mechanisms (Fig. 1) utilized by LDR exposed cells to prevent cancer formation: (1) repair of damaged DNA, (2) removal of damaged cell via apoptosis/autophagy, or (3) cell cycle arrest thereby rendering the cell unavailable for propagating the damaged DNA. These sections collectively demonstrate that ionization events under LDR exposures do not alter health risks in terms of carcinogenesis.

2.1. DNA damage repair mechanisms

An average cell endures up to one million DNA damages per day from natural endogenous oxidative metabolism and environmental exposures [67]. However, this rate of DNA damage is counteracted by DNA repair mechanisms which ensure that DNA damage is efficiently repaired (Fig. 2) [68]. Indeed, lack of DNA repair mechanisms may lead to cancer formation with LDR exposures [69].

Radiation induces DNA damage via direct and indirect mechanisms. Direct radiation damage occurs when energy from radiation creates fast-moving electrons which physically breaks the sugar phosphate backbone or alters the base pairs of the DNA. DNA damage via indirect

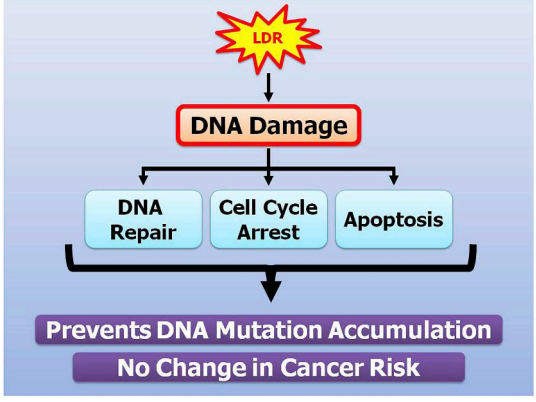

Fig. 1. Molecular mechanisms utilized by LDR exposed cells from preventing DNA mutation accumulation. Irradiated cells employ numerous repair mechanisms to protect from DNA damaging insults: (1) repair of damaged DNA, (2) removal of damaged cell via apoptosis, or (3) cell cycle arrest thereby rendering the cell unavailable for propagating the damaged DNA. These repair mechanisms not only protects cells from the immediate DNA damaging events, but often results in long-term adaptive memory that is capable of protecting the cell from future oxidative exposures. The lack of DNA mutation accumulation demonstrates that there is no change in cancer risk with LDR exposures.

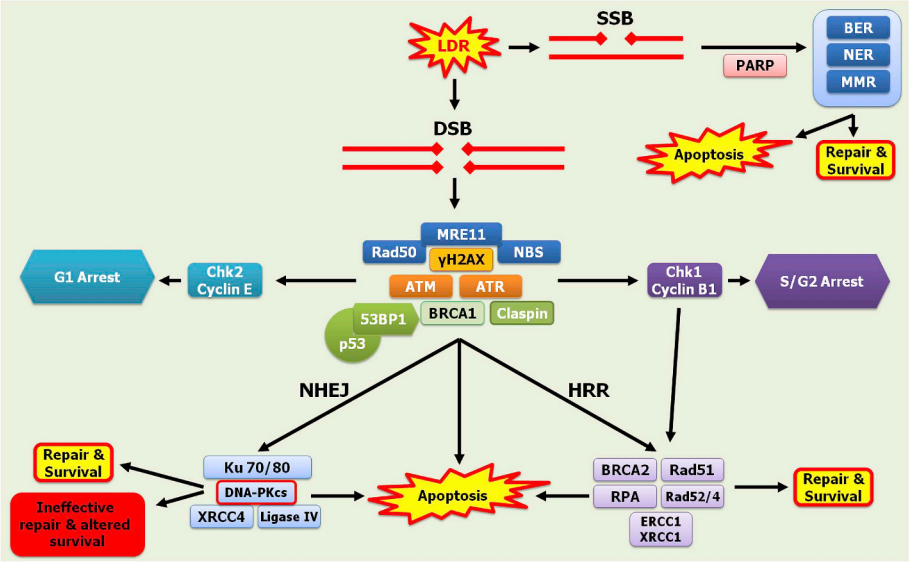

Fig. 2. LDR mediated DNA damage response. Low LET LDR indirectly damages DNA by radiolysis of water molecules resulting in the production of reactive oxygen species (ROS). These free radicals damage DNA via single stranded breaks (SSBs), double-strand breaks (DSBs) and locally multiple damaged sites. Radiation mediated SSBs are initially detected by PARP1 enzymes and repaired by mechanisms including base excision repair (BER), nucleotide excision repair (NER), and mismatch repair (MMR). DSBs are detected by a cluster of proteins including γH2AX, ATM, ATR, BRCA1, 53BP1, Rad50, MRE11, NBS, and claspin. The cells utilize two mechanisms for repair of DSB: nonhomologous end joining (NHEJ) and homologous recombination repair (HRR). NHEJ is often error-prone and dominates during the G1 cell cycle whereas HRR predominantly functions at G2 cell cycle and is considered less error-prone than NHEJ.

mechanism occurs whereby radiation causes radiolysis of water molecules resulting in the production of reactive oxygen species (ROS). These ROS free radicals oxidize (8-oxo-7,8-dihydroguanine), alkylate (7-methylguanine), or hydrolyze (deamination, depurination and depyrimidination) the DNA bases [70].

LDR with low linear energy transfer (LET) is predominantly associated with indirect DNA damage capable of producing single stranded breaks (SSBs), double-strand breaks (DSBs) and locally multiple damaged sites [71]. Radiation mediated SSBs are efficiently repaired using the complementary undamaged strand as a guide template (Fig. 2). SSBs are initially detected by PARP1 enzymes and repaired by mechanisms including base excision repair (BER), nucleotide excision repair (NER), and mismatch repair (MMR) [72]. On the contrary, DSBs and multiple damages in a local region are more challenging to repair and are highly deleterious if left unrepaired. DSBs are detected by a cluster of proteins including γH2AX, ATM, ATR, BRCA1, 53BP1, Rad50, MRE11, NBS, and claspin [73,74]. The cells then utilize two mechanisms for repair of DSB, nonhomologous end joining (NHEJ) and homologous recombination repair (HRR). NHEJ is often error-prone and dominates during the G1 cell cycle [75]. Members of the NHEJ repair system includes Ku70/80, DNA-PKcs and an enzyme called DNA ligase IV which uses overhanging pieces of DNA adjacent to the break site to reattach the broken ends. NHEJ activity is upregulated during genomic instability due to HDR exposures above 1 Gy [76,77]. In contrast, HRR predominantly functions at G2 cell cycle and is considered less error-prone than NHEJ since it utilizes the homologous chromosome or sister chromatids as the template for repair. Members of the HRR pathway include BRCA2, Rad51, Rad52 and XRCC1. Taken together, these mechanisms demonstrate that the cell employs numerous repair mechanisms at its disposal in order to mitigate DNA damaging insults (Fig. 2). Interestingly, this basic DNA repair process is highly conserved among both prokaryotes and eukaryotes suggesting its evolutionary importance in maintaining the integrity of the genetic code from multiple insults including exposures to oxidative stress, chemicals and background ionizing events [78].

LDR results in the upregulation and increased activity of numerous DNA repair proteins described above. For instance, fibroblasts demonstrated enhanced NHEJ repair following a single 100 mGy exposure as evident by the robust nuclear enrichment of ATM, γH2AX, 53BP1, Ku70/80 and translationally controlled tumor protein (TCTP) [22]. Conversely, 10 mGy fractionated exposure for 7 consecutive days promoted HRR pathway [20]. Here, elevated DNA damage was sensed by ATM which downregulated protein phosphatase 2A (PP2A) activity. PP2A activity was further reduced by ROS mediated activation of AKT signalling [79]. PP2A normally represses cyclin D1 activity; therefore LDR mediated inhibition of PP2A promoted cyclin D1 mediated activation of RAD51 and the HRR pathway. Furthermore, elevated cyclin D1 arrested the cells at the G2/M phase of the cell cycle (more on this below). Similarly, another study with chronic 10 mGy exposures in epidermal stem cells revealed a reduction in NHEJ activity coupled with enhanced shunting of DSB repair via the HRR pathway [80]. This may be an adaptive mechanism used by cells to help undergo HRR mediated DNA damage response which is prevalent during G2/M cycles while avoiding the error-prone NHEJ system.

LDR mediated activation of DNA repair mechanisms not only protects DNA from the immediate DNA damaging events, but often results in long-term adaptive responses that are capable of protecting the cell from future oxidative insult (such as HDR) [81–86]. There are numerous studies which demonstrate that either single or fractionated conditioning dose of 100 mGy or less is sufficient to enhance production of DNA repair systems capable of protecting from DNA damage induced from challenge doses ranging from 1 to 4 Gy [57,83,87,88]; [89]. These studies also demonstrate that fractionated or chronic LDR is more effective than a single acute LDR exposure for the development and maintenance of the prolonged DNA repair adaptive responses [90–93]. Indeed, numerous studies report increased expression of DNA repair genes including H2AX, ATM, Lig4 and RRM2 under chronic LDR radiation exposure [22,52]. Here, the adapted cells appear to be error-free since these cells are less susceptible to radiation-induced neoplastic transformation [94].

One of the controversial pitfalls of the LNT model is that it does not take into consideration the DNA repair defence mechanisms. In fact, the LNT model is based on studies that utilized DNA repair markers such as γH2AX and 53BP1 for detecting DNA damage. Therefore, it can be argued that DNA repair is also linearly related to radiation dose since the DNA repair markers can be measured linearly from 20 mGy to 100 Gy [51,52,54,55,57,58]. This would mean that DNA damage and DNA repair mechanisms neutralize the DNA damaging effects induced by LDR.

2.2. Apoptosis

LDR mediated activation of the DNA repair defense systems often leads to programmed cell death (apoptosis) [19,95]; [92,96,97]. Apoptosis is an effective mechanism for removal of damaged genetic material thereby preventing cancer formation [98]. Certain cell types

such as stem cells have the propensity to initiate apoptosis because they have limited DNA repair capacity [96,99–104]; [105]. Therefore these progenitor cells activate apoptosis pathways rather than risk potential mistakes that can occur with DNA damage repair. For example, adult neuronal stem cells obtained from rodent subventricular zones initiate apoptosis at doses of 10–200 mGy and are thus very resistant to accumulating DNA strand breaks [99]. These stem cells demonstrate decreased expression of numerous DNA repair genes. Similarly, rodent progenitor cells of the gastrulation phase of embryonic development (around embryonic days 5.5–6.5 characterized by clonal cell expansion), have significantly reduced apoptotic-threshold characterized by upregulated expression of pro-apoptotic proteins (Bak and Bin) coupled with decreased expression of anti-apoptotic genes (bcl-2) [100]. Therefore LDR exposure during gastrulation often results in progenitor cell apoptosis. Finally, immune cell-types including white blood cells, red blood cells and platelets are also prone to LDR mediated apoptosis [106–109].

Unlike stem cells and end-stage immune cells, numerous differentiated cell-types produce a significant DNA damage repair response upon sensing DNA insults. When DNA damage becomes unrepairable, the DNA repair defense systems initiate apoptotic signaling cascades. For example, MGMT (O6-methylguanine DNA methyltransferase), the enzyme responsible for converting O6-methylguanine mutations to guanine, activates apoptosis when it is unable to repair O6-methylguanine lesions [19]. This mechanism is important for reducing genomic instability since radiation induced O6-methylguanine mutations are one of the main contributors of genomic instability. Indeed, lack of genome instability under 1 Gy is probably due to apoptotic removal of the damaged cells via MGMT signaling [76]. Similar effect is observed with DNA mismatch repair (MMR) proteins. MLH1 is an MMR protein which promotes apoptosis under chronic LDR exposures [130 mGy for 24–96 h] by reducing Rad51, a component of the HRR pathway [92]. The MMR proteins form complexes with MRE11, γH2AX and Rad51, thereby inhibiting Rad51 from participating with HRR [110]. Therefore, DNA damage repair proteins such as MGMT and MMR increases cell killing in response to LDR exposures by promoting apoptosis (Fig. 2).

In addition to DNA repair mediated initiation of apoptosis, LDR also enhances cell apoptosis via ROS and p53 mediated mechanisms [101,107,111–113]. LDR is capable of increasing ROS levels via radiolysis of water molecules. When the cellular antioxidant systems are unable to cope with the elevated ROS levels, p53 becomes activated. p53 triggers cell apoptotic pathways via mitochondrial disruption and caspase activation [114]. LDR also upregulates NADPH oxidase, iNOS and DUOX2 activity, which increases production of ROS species such as nitric oxide and hydrogen peroxide [90,111,115,116]. Nitric oxide readily combines with superoxide radicals producing highly reactive nitrogen species (RNS) that are capable of lipid peroxidation and mitochondrial disruption leading to cell death.

The choice of a cell to undergo apoptosis under LDR exposure is also dependent on the levels of NFkb and AKT [20,88,117,118]; [119]. Cells which increase NFkb expression with LDR exposure promotes AKT phosphorylation and its activation. AKT contributes to mitochondrial homeostasis, enhanced ROS inactivation, and reduced caspase activity resulting in reduced apoptosis [120,121]. However, cells which do not have NFkb stimulation under LDR exposures have reduced AKT levels, and this promotes ROS mediated mitochondrial dysfunction and enhanced cytochrome C release [111]. Therefore, NFkb and AKT are master regulators of apoptosis that demonstrate cell-type specific apoptotic signaling in response to LDR. It has been suggested that transformed cells can be differentially stimulated to undergo apoptosis by low doses of radiation [122]. Such a mechanism would decrease the risk for cancer [95].

LDR mediated apoptosis is a transient effect which demonstrates that removal of damaged cells is a direct consequence of radiation exposure, and generally cells unable to repair damage succumb to apoptosis [123,124]. The loss of cells is replenished by new cells thereby maintaining tissue integrity and function. A recent study has demonstrated the *in-vivo* benefits to this stimulation of apoptosis by LDR in Balb/c mice irradiated with a conditioning dose of 75 mGy followed by a challenge dose of 2 Gy [125]. Greater apoptosis was detected in mice that received the conditioning dose relative to both mice that received only the challenge dose and to controls. The mice that were pre-treated with the conditioning dose also had significantly delayed tumour progression and an increased survival time (median of 7 days). In contrast, HDR exposures results in uncontrolled necrotic cell death which is harmful to the tissue [126]. Overall, LDR mediated apoptosis is an important protective mechanism which prevents propagation of damaged cells.

2.3. Autophagy

Emerging evidence demonstrates that low levels of radiation can induce cell death via autophagy, a normal cellular mechanism for degradation and recycling of cellular components [103,127,128]. Autophagy is a catabolic molecular process that delivers cellular macromolecules, including damaged organelles to the lysosomes for degradation and recycling. LDR has been shown to induce accumulation of autophagic signaling molecules such as ROS, ceramide and intracellular calcium levels [127]. These molecules can enhance the removal of damaged extranuclear organelles due to radiation induced damage thereby protecting cellular function. Furthermore, prolonged autophagy or elevated autophagosome triggers non-apoptotic autophagy mediated cell killing mechanisms which ultimately helps remove damaged cells [103,123,129]. Mechanisms involved in radiation induced cell death via autophagy have been extensively reviewed previously [127,130]. Therefore, in addition to apoptosis, autophagy provides another mechanism for removal of LDR mediated damaged cells.

2.4. Cell cycle arrest and senescence

LDR arrests cells at the G2/M phase of the cell cycle [26,123,131,132]. The molecular basis of G2 arrest is a direct consequence of DNA damage induced accumulation of p53 [110,113,133,134]. Under resting conditions, p53 levels are maintained at low steady state levels and is relatively inefficient at binding to DNA. However, damaged DNA due to radiation exposure triggers a series of phosphorylation, de-phosphorylation and acetylation events on the p53 polypeptide that enhances its expression and ability to bind DNA promoters [135]. One of the main roles of p53 is activation of cyclin-dependent kinase (cdk) inhibitors p21 and p16. These biomolecules block the phosphorylation of the retinoblastoma tumor suppressor pRb, which serves as a break in the DNA replication process [136]. For cells to progress from G2 to the mitosis phase, Rb needs to be phosphorylated. Therefore, p53 plays an important role in conveying DNA damage signals to p21 ultimately resulting in cell cycle arrest.

G2/M cell cycle arrest is an important mechanism utilized by cells to prevent cancer formation by preventing unrepaired DNA alterations from undergoing mitosis while allowing time for DNA repair mechanisms to adequately restore the damaged DNA sequences (Figs. 1 and 2) [137]. For example, MSH2, an MMR gene, and ATM, a serine/threonine kinase involved in DSB repair contributes to G2 cell cycle arrest [19,22,138]. LDR also promotes G2 arrest by upregulating Wee1, a serine/threonine kinase which phosphorylates Cdk1 [131]. Another advantage of G2/M arrest is preferential repair of DSBs using the high fidelity HRR pathway while limiting the error-prone NHEJ system. For example, 100 mGy pre-exposure in fibroblasts resulted in G2/M arrest, and this was protective when challenged with 2 Gy HDR due to enhanced HRR mediated repair [20].

Prolonged cell cycle arrest due to chronic LDR exposure induces senescence in certain cell-types [139]. Senescence is a long-standing

state of cell cycle block due to unrepaired DNA damage while the cell attempts to remain metabolically active. This often results in cytoskeletal alterations that lead to cellular hypertrophy [140]. Changes in metalloproteinase (MMP) expression is a common phenotype identified in chronic LDR mediated senescent cells [26,141,142]. These cells also demonstrate altered cytoskeletal profiles and cell-cell interactions. Another common phenotype of senescent cells includes enhanced lysosomal activity measured using beta-galactosidase (SA-β-gal), an enzyme which hydrolyzes β-galactosides into monosaccharides [143]. Indeed, bone marrow stem cells demonstrated dose dependent increase in beta-galactosidase activity from 40 mGy to 2 Gy [123]. This demonstrates that stem cells may utilize a senescent phenotype to avoid radiation impacts. Here, senescence would allow the stems cells to avoid apoptosis while permitting time for DNA repair systems to repair the damaged nucleotides. Chronic LDR mediated senescence has also been demonstrated in other cell types including epithelial cells and fibroblasts [93,141,142]. Finally, senescence is a common mechanism utilized by fully differentiated somatic cells such as neurons and myocytes that are non-replicating [144]. These cells do not commonly give rise to cancer; however they do accumulate DNA damage with time which likely contributes to aging [145]. It has been suggested that cell proliferation plays a major role in the production of the high background rate of cancer [146–149]. Studies have also demonstrated that radiation at high doses interacts with cell proliferation to stimulate cancer induction in the liver [150]. Therefore cell cycle arrest and senescence prevents cancer induction by inhibiting cell proliferation.

Taken together, cell cycle arrest and senescence are adaptive mechanisms utilized by LDR exposed cells from propagating DNA mutations. These mechanisms ensure that although radiation is linearly damaging to the DNA at all doses, mutations that develop in the low dose range are removed at the cellular level. Therefore DNA repair mechanisms, apoptosis, autophagy, and cell cycle arrest are strategies that protect against cancer formation (Fig. 1).

3. Changes in molecular markers

3.1. Genomics

The advent of high-throughput microchip based array technologies has made it possible to study the full complement of biomolecules expressed in any cell under any experimental condition. Coupled with advances in ionization radiation technologies, researchers are able to study the molecular and cellular effects of LDR biology using global systems-level approaches. The breadth of available omics-based data not only includes whole-genome sequencing data, but now encompasses transcriptomics (mRNA), proteomics (protein), epigenomics (DNA methylation), miRNAomics (miRNA), metabolomics (metabolites) and lipidomics (lipids). The key goal of these approaches is integration of systems level analyses to identify unbiased biological trends. The use of this integrative approach will greatly benefit the field of radiation biology as it helps to move away from single gene-level analysis (e.g. studying a favourite gene of interest while not taking into consideration the other approximately 25,000 genes) or single phenotype analysis (e.g. looking at only DNA damage while numerous other molecular effects are ignored) to one that is driven by the biology of the cell.

The use of these large scale 'omics' based expression analyses in radiation biology demonstrate that cells express different gene/protein/miRNA profiles at low doses compared to HDR [21,26,27,151–154]. These studies provide concrete evidence that cells have unique molecular signatures depending on the dose. Therefore heath risks identified at HDR cannot be used to extrapolate the health effects at LDR since the biological responses are not linear. Examples of 'omics' based studies in radiation biology will be highlighted below to demonstrate dose specific expression profiles.

[27] performed genome-wide expression analysis on peripheral blood mononuclear cells subjected to 0.5 Gy, 1 Gy, 2.5 Gy, and 5Gy ^{60}Co gamma radiation. Gene set enrichment analysis indicated that radiation exposure promoted gene pathways involved with the immune system and cancer development, with 1 Gy being the critical threshold dosage [27]. At 1 Gy, expression levels of several genes involved with carcinogenesis and metabolic disorders (FADD, TNFRSF10B, TNFRSF8, TNFRSF10A, TNFSF10, TNFSF8, CASP1, and CASP4) showed significant alterations. Most of the genes affected by ^{60}Co gamma radiation, regardless of the dosage, appeared to be enriched on chromosomes 2, 11, 16, 17 and 19. Intriguingly, chromosome 11 contained the highest abundance of dysregulated genes from 1Gy exposure, indicating that this chromosome is particularly sensitive to ^{60}Co radiation. These results demonstrates that there is a critical threshold dose of 1 Gy that is required to elicit gene expression changes that promote immune dysregulation and cancer development. This study also illustrates that the cancer promoting gene expression changes were absent even at a relatively high dose of 0.5 Gy.

3.2. Proteomics

Transcriptomic profiling by Ref. [154] demonstrated that the brain tissue of whole-body irradiated mice showed unique gene signatures at low-dose exposure (100 mGy) compared to 2 Gy HDR [154]. Network enrichment analysis revealed that LDR significantly upregulated genes involved in MAPK/JNK signaling, estrogen receptor signaling, p38 MAPK signaling and integrin signaling. In contrast, HDR activated genes that were involved in apoptosis, inflammation, protein ubiquitination, and oxidative phosphorylation. These gene expression data demonstrate that the molecular response of the mouse brain to LDR involves protective mechanisms while HDR involves the down-regulation of neural pathways associated with cognitive dysfunction.

Proteomic analysis performed by Ref. [153] using human fibroblasts demonstrated unique patterns of differentially expressed proteins at 40, 100 and 140 mGy [153]. Here, 6, 7 and 2 proteins were significantly upregulated or downregulated at 40, 100 and 140 mGy respectively compared to controls. This study illustrated that protein expression patterns require threshold doses even in the low dose range. Similar dose dependent protein alterations were observed by Ref. [151] at 0.02, 0.1, 0.5 and 1 Gy [151].

[155] demonstrated a similar dichotomy in C57Bl/6J mice protein expression when irradiations were completed using different irradiation durations [155]. The authors reported a difference in protein expression profiles after chronic irradiation over 400 days (mice irradiated at 20 mGy per day for a total absorbed dose of 8 Gy) compared to mice that received the same dose acutely at a dose rate of 0.72 Gy per minute. Taken together, these results demonstrate the drastic difference in biological response when a lower dose rate is used, in contrast to acute high dose irradiation.

3.3. miRNAomics

[156] used miRNAomics platform to identify increased expression of several miRNAs including le7e-5p, miR-15a-5p, and miR-376b-3p due to 0.1 Gy exposure, while this response was absent at 0.5 Gy [156]. Using human B lymphoblast cells [152], also established distinct miRNA expression profiles with 0, 0.05, or 10 Gy [152]. Here, 13 miRNAs were decreased in the 0.05 Gy exposed cells (let-7f-2, miR-19a, miR-106b, miR-376a, miR-16-1, miR-23a, miR-18, miR-23b, miR-155, miR-106a, miR-17-5p, miR-21, and miR-20). In contrast, 10 Gy exposure increased 5 miRNAs (miR-324-3p, miR-328, miR-187, miR-99b, and miR-326). In addition to these, four miRNAs had similar patterns in both low and high dose irradiated cells (miR-199a-2, miR-197, miR-207, and miR-220). Of the LDR inhibited miRNAs, miR-20a is of particular interest in carcinogenesis since it is a key inhibitor that represses many cancers by regulating E2F-1 mediated cell proliferation and apoptosis. These results demonstrate that LDR could suppress the

progression of malignant cancer by controlling miRNA expression [152,156].

LDR miRNAomics studies routinely demonstrate temporal regulation. For example, miRNA profiling by Ref. [26] identified distinct miRNA responses in normal human dermal fibroblasts (NHDFs) depending on the post-irradiation time point [26]. At 6 h post-irradiation of 0.1 Gy LDR, 125 miRNAs were upregulated and 43 miRNAs were downregulated, indicating that LDR induces changes in the expression of specific miRNAs during the early adaptive response. Here, expression levels of miR-3656, miR-3125 and miR-940 were significantly increased while expression levels of miR-328, miR-885-5p and let-7d-3p were significantly downregulated, with miR-328 being the most highly downregulated miRNA in the early response group. Genes inhibited by miR-328 are involved in p53 and mammalian target of rapamycin (mTOR) signaling pathways. In contrast, 22 miRNAs were upregulated and 1 miRNA was downregulated at 24 h post-irradiation. Here miR-3937, miR-1825 and miR-369-3p were significantly upregulated while miR-634 was downregulated [21]. also revealed temporal miRNA alterations with LDR using miR-34a in breast cancer cell lines exposed to 3, 12 and 48 mGy [21]. At low doses, miR-34a was up-regulated at 4 h, but decreased 24 h post-irradiation. Here, miR-34a levels correlated with DNA damage and apoptosis. Validated miR-34a targets include several genes involved in DNA repair including Bcl-2, Notch1, Cyclin D1, Cyclin E2, CDK4, MET and SIRT1. Taken together, miRNAomic studies reveal that miRNAs are master regulators of LDR mediated cellular effects, and their expression is dose dependent and temporally regulated.

The studies presented above exemplify the benefits of 'omics' based analyses in radiation biology. One of the overall themes from these large scale experiments is that LDR cellular expression signature varies greatly from HDR. In fact, many of the network enrichment analyses demonstrated that LDR expresses cellular networks that are biologically protective or at the very least not damaging, while HDR expression profiles suggest cancer progression, immune activation and cell death [157]. These studies provide further evidence that the biological responses to ionizing radiation are not linear across all dose ranges.

4. LDR mediated epigenetic modifications

Epigenetic inheritance is the transmission of gene expression memory that occurs in the absence of changes in the DNA sequence. Cytosine methylation of DNA is the most widely studied epigenetic DNA modification (Fig. 3A). DNA methyltransferases (DNMTs) attach a methyl group at the carbon 5 residue of cytosine to form 5-methylcytosine (5meC). The addition of the methyl group to the cytosine nucleotide disrupts the major grove of the DNA leading to displacement of transcription factors that normally bind DNA. The methyl group also promotes attachment of methyl binding proteins which contribute to gene silencing and chromatin compaction. Therefore hypermethylation causes gene silencing while hypomethylation is associated with gene activation (Fig. 3A) [158].

Numerous studies report that LDR induces global hypomethylation in mature blood cells under both chronic and acute exposures [159–163]. Global hypomethylation is often associated with genome instability and thus this may be a protective method by certain cells for induction of apoptosis (Fig. 3B) [159,163,164]. Recent studies show that although LDR causes global hypomethylation, gene-specific promoter analysis demonstrated hypermethylation [43,161,165]; [162]. In fact, promoter hypermethylation rather than global hypomethylation was more stable since acute LDR caused transient genomic hypomethylation in blood 2 h post-irradiation but was not evident at 1-month [162]. Similarly DNA methyltransferases enzymes were downregulated in a tissue specific manner but these changes did not persist [43,159]. Analysis of chronic LDR revealed gene-specific hypermethylation at 811 regions which encompassed almost all important biological systems as indicated by GO and KEGG pathway analysis [162]. These included numerous hypermethylated genes such as Rad23b and Ddit3 which displayed tissue-specific methylation and downregulation that were persistent 1-month post-irradiation. In contrast to mature blood cells, lymphoblasts demonstrate the complete opposite response to LDR in terms of DNA methylation [161]. Chronic LDR in lymphoblasts induced adaptive responses that included global genomic DNA hypermethylation accompanied with increased DNMT1 and MeCP2 expression, and heterochromatin formation. This study demonstrates that the lymphoblast epigenome adapts to the initial LDR exposure by overcompensating for the decrease in DNMT1 and MeCP2 by increasing DNA hypermethylation and enhancing expression of DNMT1 and MeCP2 [161]. Taken together, LDR mediated DNA methylation alterations are tissue-, gene-, time- and dose-specific. It seems as though mature blood cells are dispensable and therefore preferentially undergoes apoptosis due to genome instability caused by LDR mediated genome hypomethylation. In contrast, lymphoblasts are master cells that do not undergo genome hypomethylation with LDR exposures and are thus resistant to apoptotic cell death.

It has been demonstrated that the phenotype of mice can be modified by radiation exposure *in utero* [165,166]. These studies utilized the viable yellow agouti (A^{vy}) mouse model which contains the A^{vy} metastable epiallele which is under the control of epigenetic modifications. Administration of agents which promote DNA hypomethylation during development promotes A^{vy} expression, leading to yellow coat color, diabetic phenotype and increased cancer frequency [166]. However, exposure of A^{vy} mice to low doses of radiation *in utero* significantly increased DNA methylation at the A^{vy} locus in a sex-specific manner resulting in a "pseudoagouti" phenotype. Here, the LDR exposure shifted to the normal A^{vy} mice phenotype which included brown coat color coupled with reduced diabetic, obese and cancer prone characteristics [165]. Such research suggests that LDR radiation produces shifts in phenotype that are protective [158,167]. More studies with LDR and epigenome profiling are necessary to properly elucidate the role of epigenetics in LDR biology [168,169].

5. LDR mediated bystander effects

The LNT model relies on the "hit theory", which assumes that only cells that are physically traversed by discrete ionizing radiation energy packets and have energy deposited in them are affected by the radiation source [16,17]. Studies looking at bystander effects have now displaced the "hit theory". The radiation-induced bystander effect demonstrates that irradiated cells are capable of transmitting signals to neighboring un-irradiated cells using either direct cell-cell communication or soluble factors [30,32,170]. The end result of this communication is that both irradiated and un-irradiated cells display phenotypes and molecular gene-expression signatures that are very similar (Fig. 4). One of the main advantages of the bystander effect is that irradiated cells are capable of transmitting stress signals to the un-irradiated cells [32,171–173]. With LDR treated cells, the irradiated cells are capable of counteracting the oxidative insult, and therefore signals sent to neighbouring un-irradiated cells often induce adaptive mechanisms such as enhanced expression of antioxidant systems and DNA repair systems [170,174,175]. With HDR treated cells, the oxidative effect is overbearing to the cells, and therefore signals sent to neighboring un-irradiated cells often induce apoptotic and DNA damage responses [176,177]. The end result of these bystander signals is that it produces cell populations that are more resistant to subsequent radiation or removal of damaged cells via apoptosis or senescence (Fig. 4).

LDR mediated bystander effect is dependent on intercellular communication methods that include gap junctional proteins, calcium signaling, cell-free DNA, and potent growth factors like TGFβ1 [30,104,178,179]. Gap junctional intercellular communication is one of the most widely studied bystander mechanism with LDR exposures and has also been shown to be important for radioadaptive responses. Mechanistically, LDR activates the connexin 43 (Cx43) promoter, a

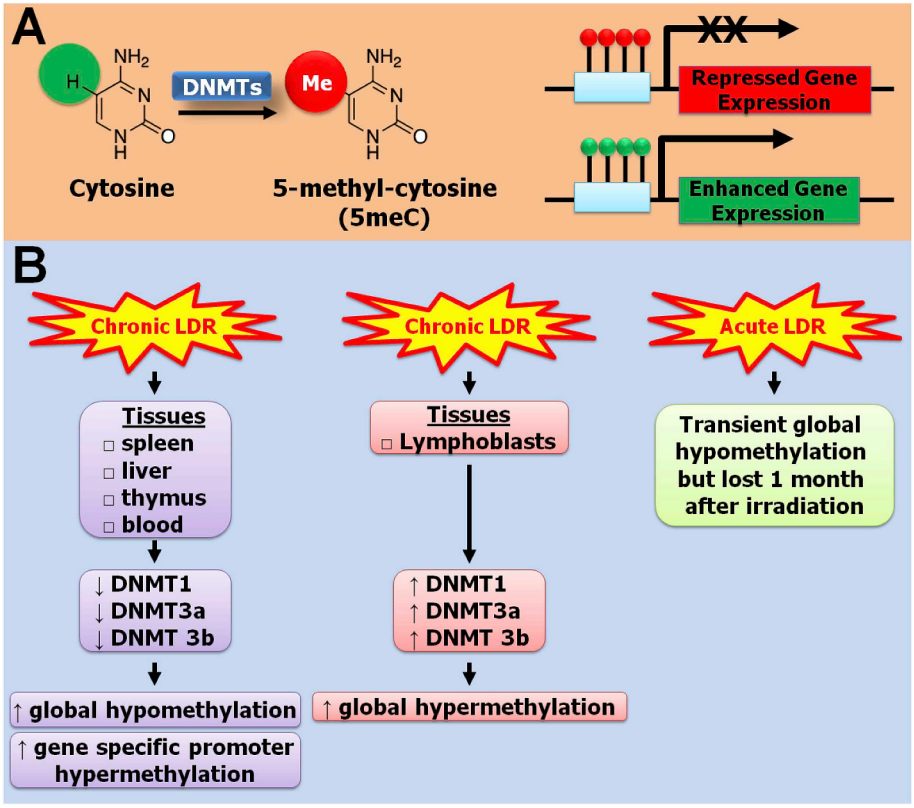

Fig. 3. LDR mediated epigenetic modification via cytosine methylation. (A) Cytosine methylation of DNA is the most widely studied epigenetic DNA modification. DNA methyltransferases (DNMTs) attach a methyl group at the carbon 5 residue of cytosine to form 5-methyl-cytosine (5meC). The addition of the methyl group to the cytosine nucleotide disrupts the major grove of the DNA leading to displacement of transcription factors that normally bind DNA. The methyl group also promotes attachment of methyl binding proteins which contribute to gene silencing and chromatin compaction. Therefore hypermethylation causes gene silencing while hypomethylation is associated with gene activation. Global hypomethylation is often associated with genome instability and is a mechanism for induction of apoptosis. (B) LDR induces global hypomethylation and gene-specific promoter hypermethylation in a cell-type specific manner. Acute LDR exposure caused transient global hypomethylation due to decreased DNMT levels, while chronic LDR exposures formed stable gene-specific promoter hypermethylation alterations. In contrast to mature blood cells, chronic LDR in lymphoblasts induced global genomic DNA hypermethylation accompanied with increased expression of DNMT levels. Taken together, LDR mediated DNA methylation alterations are tissue-, gene-, time- and dose-specific.

component of the gap junction complex in a time- and dose-dependent manner thereby promoting cell-cell communication [179]. Interestingly, Cx43 downregulation in cancer cells (a constitutive feature of G2/M phase) selectively renders tumour cells hypersensitive to LDR [132].

Rise in intracellular calcium concentration is important for transduction of bystander signals in the un-irradiated cells. For example, bystander effects from 130 mGy conditioned media were lost when calcium ions were stripped from the conditioned media [30]. Likewise, bystander screens in various glioma cell lines demonstrated that only cells capable of inducing calcium fluxes were able to demonstrate the bystander effect [178]. Interestingly, the bystander effect is related to soluble factors released by the irradiated cells that are capable of inducing calcium mobilization in the un-irradiated cells. The importance

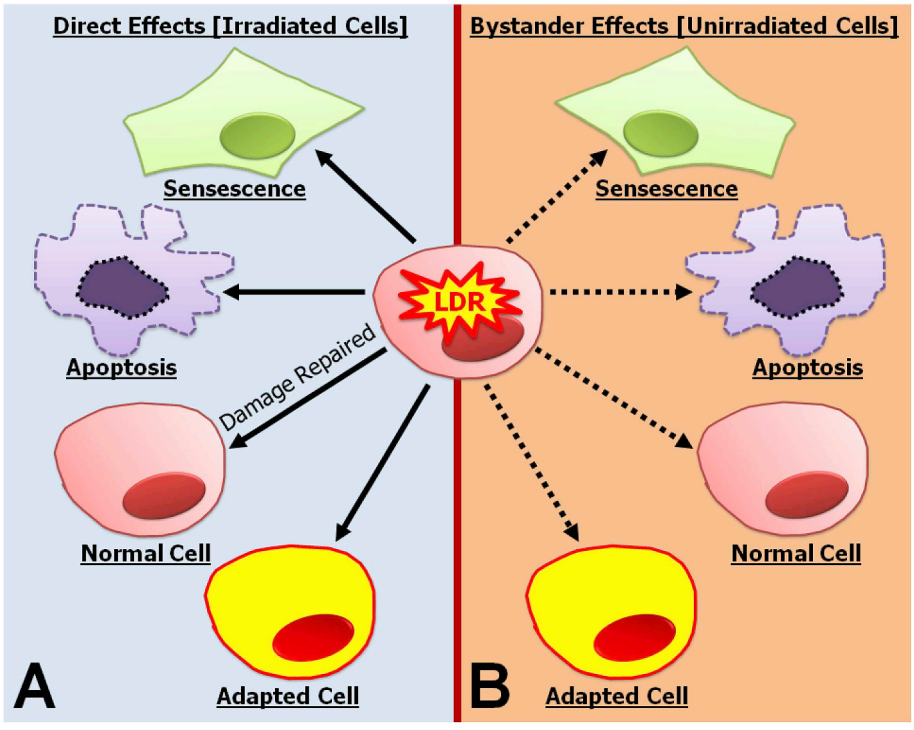

Fig. 4. LDR mediated adaptive phenotype and bystander effects. (A) Cells directly exposed to LDR undergo repair (red cell), apoptosis (purple cell) or senescence/cell cycle arrest (green cell). LDR exposed cells also develop long-term adaptive responses (yellow cell) that protect from future oxidative exposures. (B) Directly irradiated cells are capable of transmitting signals to neighboring un-irradiated cells using either direct cell-cell communication or soluble factors. This phenomenon is referred to as radiation-induced bystander effect. The end result of this communication is that both irradiated and un-irradiated cells display phenotypes and molecular gene-expression signatures that are very similar. One of the main advantages of the bystander effect is that irradiated cells are capable of transmitting stress signals to the un-irradiated cells which induce adaptive mechanisms (yellow cell). Examples of adaptive responses include enhanced expression of antioxidant systems and DNA repair systems. Therefore LDR mediated bystander effects allows tissues to respond as a whole and not as single cells.

of this is exemplified by harvesting media from LDR exposed bladder explants from C57BL6 and Balb/c mice. The media from C57BL6, but not Balb/c was able to induce clonogenic death in a keritonocyte reporter system [32]. Analysis of calcium mobilization inducing ability in both strains revealed that media from C57BL6 was able to cause rise in intracellular calcium in the keratinocyes while this was absent from the media collected from Balb/c. These results demonstrate the strain dependent difference in LDR mediated bystander effects is due to variations in intracellular calcium mobilizations. Studies with these same two mouse strains also demonstrated that mammary cancer and genomic instability was induced in Balb/c following exposure to radiation and that neither of these features were observed in C57BL6 mice [180]. Thus, genetic background is an important determinant of radiation responses.

There are numerous studies which also implicate ROS in the induction of LDR induced bystander effects. LDR mediated apoptotic cells have elevated ROS activity, which contributes to release of ATP and oxidized extracellular DNA (ecDNA) [181,182]. These soluble factors serve as candidate bystander molecules capable of affecting the un-irradiated cells. Here, ATP is detected by purigenic receptors while ecDNA is sensed by TLR9 receptors on the un-irradiated cells.

Taken together, LDR mediated bystander effects allows tissues to respond as a whole and not as single cells, thereby dismissing the traditionally believed “hit theory” [176]. Bystander effects demonstrate that the target for radiation damage is much larger than a single cell as has been proposed in the past, since “it takes a tissue to make a tumor” [183]. The studies presented above also demonstrate the importance of radiation-induced bystander effects in radioadaptive responses. Therefore, the contributions of bystander effects and adaptive responses need to be considered when developing heath risk models with LDR exposures (Fig. 4).

6. LDR mediated tumor suppression

The LNT model assumes that even the smallest radiation dose has the potential to increase cancer risks [1]. Although HDR exposure contributes to cancer formation, numerous reports demonstrate that LDR does not contribute to cancer formation, and is actually an effective anti-tumour agent which decreases cell transformation [23–25,184,185]. The section below highlights studies which demonstrate that LDR inhibits tumorigenesis by two main mechanisms (Fig. 5): (1) enhanced immune-mediated removal of tumorigenic cancer cells or (2) improved antioxidative capacity of normal cells thereby limiting tumor formation.

[186] performed an elegant study using AKR/J mice. These mice have natural leukemia incidence of 60–90% and are thus an excellent model to determine the role of radiation on leukemia incidence [187]. The AKR/J mice were exposed to chronic low dose rates of 0.7 mGy/h for 130 consecutive days until at total dose of 4.5 Gy was achieved [186]. The total dose of 4.5 Gy was also given to another cohort of animals at a high dose-rate of 0.8 Gy/min. Both the lifespan and the incidence of thymic lymphoma were changed. The life span was longer and the incidence of thymic lymphoma was lower in low-dose-rate irradiated mice compared to non-irradiated mice and high-dose-rate irradiated mice by 10 and 20% respectively. Transcriptome profiling demonstrated elevated expression of apoptosis genes (Cd5l, Fcgr3 and Pycard) in the low-dose-rate treated mice which correlated with increased cancer cell killing. Immune related genes (Pycard, Lilrb3, Igh-6, Fcgr2b and MGC60843) were also highly upregulated in the low-dose-rate irradiated mice compared to controls. In conclusion, the results demonstrate that chronic LDR promoted lymphogenesis which contributed to enhanced leukemia cell removal and subsequent reduction in leukemia incidence.

[188] exposed Kunming mice to 75 mGy whole body x-rays, and subcutaneously implanted S180 sarcoma cells 6 h post-irradiation [188]. Tumor growth and volume was monitored for 5 days post-irradiation. The authors presented remarkable reduction in tumor growth with 75 mGy pre-exposed animals compared to controls. The reduction in tumour growth was associated with increased tumor necrosis, increased infiltration of activated immune cells, improved erythrocyte function and decreased expression of VEGFR in tumour cells. This study demonstrates that LDR markedly improves the anti-tumor ability of the organism.

Molecular analyses of the effects of LDR on the immune system corroborate the LDR mediated anti-tumour findings presented above. Critical review of the effects of LDR on the immune system reveals that LDR exposures stimulate differentiation of dendritic cells and promotes activation of Natural Killer (NK) cells (Fig. 5A) [33–35,189]. Differentiated dendritic cells promote cell surface expression of proteins that help with antigen presentation (CD80, CD86, MHCI, MHCII) [189]. The

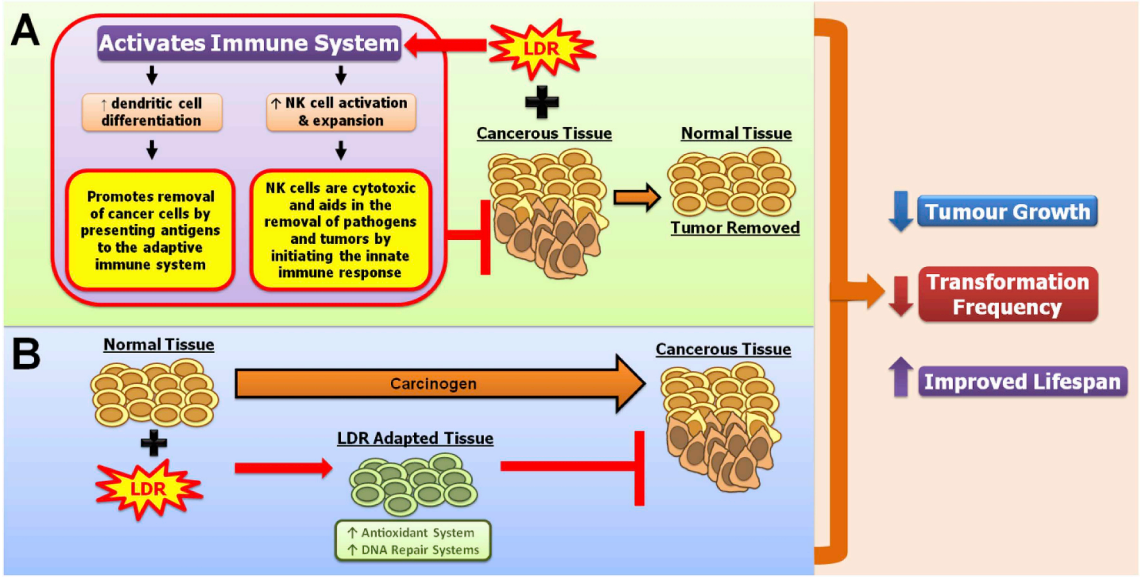

Fig. 5. LDR inhibits tumorigenesis. LDR inhibits tumorigenesis by two main mechanisms: (A) enhanced immune-mediated removal of tumorigenic cancer cells or (B) improved antioxidative capacity of normal cells thereby limiting tumor formation. LDR inhibits tumor growth, reduces transformation frequency compared to background levels and improves lifespan. Therefore LDR does not increase cancer risk.

dendritic cells also increase cytokine secretion while enhancing antigen uptake capabilities. These differentiated dendritic cells contribute to removal of tumor cells by presenting the cancer specific antigens to the adaptive immune system [190]. In contrast, activated NK cells directly contribute to tumor cell killing by taking up the cancer cells and exposing these tumours to its cytotoxic environment [191]. The NK cells also increase release of pro-inflammatory cytokines that help with tumor infiltration and removal [33–35]. In addition to activation of dendritic and NK cells, LDR also promotes lymphogenesis under very low dose rate chronic LDR exposures (0.7 mGy/h for 130 days = 2.1 Gy total dose) [192]. One of the main drawbacks of LDR exposure on the immune systems is that late-stage immune cells such as platelets, white blood cells and peripheral blood lymphocytes sensitively initiates apoptosis under very low levels of radiation [41,106,193–195]; [109,196,197]. Therefore chronic or daily fractionated LDR should be avoided to allow these cell populations to recover. Not surprisingly, continuous LDR exposure at 16 mGy/h for 30–400 days resulted in reduced lymphocyte proliferative capacity coupled with increased risk of infection and tumour formation [108]. On the contrary [198], demonstrated a reduction in high dose irradiation-induced levels of apoptosis in female *Trp53* heterozygous mice that were treated with repeated CT-scans following a high challenge dose of 4 Gy [198]. This further demonstrates the importance of fractionated dosing regimen for enhanced adaptive response.

In addition to immune stimulation, LDR also limits tumour formation by improving the antioxidative capacity of premalignant cells (Fig. 5B) [82,86,199–202]. This is exemplified by Ref. [203] which utilized radiation to protect Wistar rats from colon cancer formation [203]. Colon cancer was induced by weekly injections of dimethylhydrazine for 15 weeks with and without 0.5 Gy weekly fractionated whole body γ-rays. Remarkably, the radiation exposed animals had decreased colon anaplasia, reduced ulcerative colitis, and fewer hyperchromatic nuclei compared to un-irradiated animals. Molecular analysis revealed that the radiation treated rodents had reduced ROS production due to enhanced expression of antioxidative enzymes. The irradiated mice also had reduced expression of MDR1 and β-catenin, markers of colon cancer progression. These results illustrate that LDR promotes adaptive responses that upregulate antioxidative systems and this is an important molecular phenotype for limiting cancer risks.

[200] identified a similar role for LDR mediated enhancement of cellular antioxidant systems [200]. This study utilized KRAS over-expressing tumour cells that were exposed to a fractionated radiation regiment of 0.1 Gy for 10 consecutive days. KRAS is a plasma membrane localized small GTPase binding protein that regulates signaling pathways which control cell proliferation and survival [204]. KRAS induces ROS generation through activation of NADPH oxidase. Elevated ROS serves as a regulator for KRAS induced cellular transformation. Not surprisingly, KRAS is over-expressed in more than 35–40% malignancies including breast cancers. The study reveals that fractionated LDR treatment blocked malignant transformation formation in KRAS over-expressing breast epithelial cells [204]. Molecular analysis revealed that LDR upregulated expression of cellular antioxidative systems without altering cell death pathways which ultimately contributed to reduced ROS production.

LDR mediated modulation of miRNA targets is another mechanism whereby LDR can limit cancer progression [205]. demonstrated that miR-29c is induced with LDR exposure in hepatocellular cancers [205]. miR-29c expression correlates with its target wild-type p53-induced phosphatase 1 (WIP1), therefore LDR exposure reduces WIP1 expression. In highly proliferative cancer cells, WIP1 limits p53 function by dephosphorylating p53 at Ser-15 therefore compromising apoptosis [206]. Therefore LDR activates miR-29c, which inactivates WIP1. Limited WIP1 expression decreases p53 inactivation allowing for enhanced p53 mediated cancer cell killing, while limiting its veracious proliferation (Wang 2015) [162].

Despite numerous studies which demonstrate the benefits of LDR on tumorigenesis, the LNT model maintains that transformation frequencies are linear across all doses. A study by Ref. [24] demonstrated that there is no evidence for increased transformation frequency over the range 0.05–22 cGy using CGL-1 cells [24]. CGL-1 cells are a unique hybrid cell line (formed by combining tumorigenic HeLa and normal fibroblasts) that demonstrates normal fibroblast phenotype but undergo natural transformation to yield HeLa-type tumorigenic characteristics. Intriguingly, studies using CGL-1 cells demonstrated that transformation frequencies in the range between 0.5 and 11 mGy were below that seen spontaneously in controls [24] [25]. also demonstrated that CGL-1 cells exposed to doses of 1, 5, 10, 50 and 100 mGy had reduced transformation frequency compared to sham-irradiated cells [25]. Similarly, CGL-1 cells exposed to low doses of 60 kVp X-rays (< 10 mGy) protected CGL-1 cells from spontaneous neoplastic transformations [207]. Furthermore, chronic LDR exposures delivered at very low dose rates in the range 1–4 mGy/day was also effective in protecting against neoplastic transformation. The low dose rate chronically irradiated CGL-1 cells demonstrated reduced ROS levels, illustrating that chronic LDR enhanced the antioxidative capacity of these cells thereby limiting tumor formation [23]. Taken together, the CGL-1 studies demonstrate that LDR below 100 mGy induces adaptive mechanisms in irradiated cells that protect against spontaneous neoplastic transformation [23–25,207–209].

The studies presented above demonstrate that LDR does not increase cancer risk, but rather improves tumor removal or inhibits cancer formation. These studies are in contrast to the LNT model and further demonstrate the need for better radiation risk estimation. More importantly, many of the studies utilized doses that are typically experienced in diagnostic imaging technologies widely used in the clinical setting. Since the LNT model predicts that there is no safe threshold, individuals that may benefit from LDR therapy for cancer treatment are unnecessarily under the impression that there is increased chance of initiating new cancers from LDR exposures.

7. Conclusions

Summary of the molecular studies in LDR biology reveals that radiation alters various biomolecular processes linearly across all doses however these cellular modifications do not translate to disease progression in the low dose range due to cellular defense systems. Therefore cellular and molecular protective systems play an important role in the final biological phenotype due to LDR exposures. Thus, predications for LDR mediated health effects need to take these dynamic repair mechanisms and new low dose paradigms into consideration to appropriately determine the health risks at the low dose region. The list below highlights the LDR paradigms discussed in this review which adequately represents the biological effects in the low dose range.

New LDR paradigms

1. Radiation mediated DNA damage is linearly evident across all doses, however LDR exposures do not alter cancer risk.
2. LDR activates DNA defense mechanisms which repair damaged DNA.
3. LDR removes damaged cells that are unable to be repaired by DNA repair systems via apoptotic and autophagic mechanisms.
4. LDR initiates G2/M cell cycle arrest thereby preventing unrepaired DNA alterations from undergoing mitosis while allowing time for DNA repair mechanisms to adequately restore the damaged DNA sequences (prolonged arrest results in senescence).
5. LDR stimulates molecular gene/protein/miRNA expression profiles that are distinct from HDR exposed cells demonstrating that biological responses are not linearly related.
6. miRNAs are master regulators of LDR mediated cellular effects.
7. LDR elicits adaptive memory via epigenetic mechanisms by modifying gene-specific DNA methylation status.

8. LDR exposed cells communicate signals to the un-irradiated cells using bystander mechanisms thereby allowing tissues to respond as a whole and not as single cells.
9. LDR enhances immune-mediated removal of tumorigenic cancer cells.
10. LDR improves antioxidative capacity of normal cells thereby limiting tumor formation.
11. LDR protects against spontaneous neoplastic transformations.

This review emphasizes that LDR effects are context dependent. Dose, dose-rate, tissue-type, cell-type, gender, species and temporal regulation are all important factors that need to be considered when evaluating the health effects due to LDR exposures. Despite this complexity, the evidence presented in this review reveals that there are minimal cancer risks with LDR exposures. In fact, numerous studies demonstrate that LDR improves tumor suppression, inhibits cancer formation and protects against neoplastic transformation. Taken together, the LDR paradigms demonstrate that modern molecular biology data does not support the LNT model which assumes that even the smallest radiation dose has the potential to increase cancer risk. New paradigms need to be considered for health risk models that can help the radiation protection community in making informed decisions regarding radiation policy and limits.

An important caveat with estimating LDR mediated cancer risks is that individuals with mutations in cellular defense mechanisms (DNA repair system, apoptosis signaling, cell-cycle arrest, etc.) may be susceptible to ineffective repair resulting in mutation accumulation and increased cancer risk even at LDR exposures. Therefore personalized dosing regimen depending on the individual's genetic makeup may be an avenue that can be explored in the future. Indeed, advances in high-throughput “omics” based techniques may help make such personalized medicine become feasible. This emphasizes the need for large “omics” based data for clinical human studies in the low dose range to fully comprehend the molecular networks affected by LDR.

Declarations of interest

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