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Low-dose radiation may be a novel approach to enhance the effectiveness of cancer therapeutics

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Mini Review

It has been generally accepted that both natural and man-made sources of ionizing radiation contribute to human exposure and consequently pose a possible risk to human health. However, accumulating evidence has shown that the biological effects of low-dose radiation (LDR) are different from those of high-dose radiation. LDR can stimulate proliferation of normal cells and activate their defense systems, while these biological effects are not observed in some cancer cell types. Although there is still no concordance on this matter, the fact that LDR has the potential to enhance the effects of cancer therapeutics and reduce the toxic side effects of anti-cancer therapy has garnered significant interest. Here, we provide an overview of the current knowledge regarding the experimental data detailing the different responses of normal and cancer tissues to LDR, the underlying mechanisms, and its significance in clinical application.

Humans are consistently exposed to certain low doses of ionizing radiation including natural background radiation penetrating to the Earth's surface, medical radiation, and exposure to industrially used radioactive materials. Therefore, studying the effects of low-dose radiation (LDR) is of great interest. The radiological risk of various detrimental effects, including cancer, has been estimated by the linear no-threshold (LNT) model that assumes that even very low doses of ionizing radiation could have adverse effects on human health.[1] This has been evidenced generally by epidemiological data from Japanese atomic bomb survivors and occupationally exposed workers.[2–5] However, there was also increasing evidence indicating that radiation below certain doses could stimulate repair mechanisms to reverse the initial damage and protect the organism from subsequent radiation or other exposures that might otherwise cause cancer.[6–10] Therefore, the biological effects of LDR at certain levels are different from those of high-dose radiation (HDR), which cannot be explained by

Key words: low-dose radiation, hormesis, adaptive response, cancer

Abbreviations: APCs: antigen-presenting cells; AR: adaptive response; ATM: ataxia telangiectasia mutated kinase; CAT: catalase; DDR: DNA damage response; DSBs: double-strand breaks; ERK: extracellular signal-regulated kinases; GPX: glutathione peroxidase; GSK3β: glycogen synthase kinase 3β; HDR: high-dose radiation; HIF-1: hypoxia-inducible factor 1; HO-1: heme oxygenase-1; HR: homologous recombination; HRS: hyper-radiosensitivity; IRR: induced radioresistance; LDR: low-dose radiation; LET: linear energy transfer; LNT: linear no-threshold; MAPK: mitogen-activated protein kinases; MnSOD: manganese superoxide dismutase; miRNA: microRNA; NHEJ: non-homologous end joining; NK: nature killer; NQO-1: NAD(P)H quinone dehydrogenase 1; Nrf2: nuclear factor erythroid-2-related factor 2; PARP: poly (ADP-ribose) polymerase; ROS: reactive oxygen species; SSBs: single-strand breaks; SOD: superoxide dismutase; TGF: transforming growth factor; TNF: tumor necrosis factor

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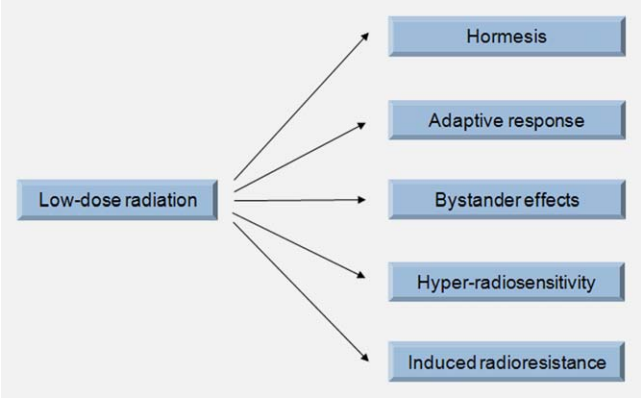

Figure 1. The biological effects of LDR. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

LNT hypothesis. For instance, the risk of radiation-induced cancer at high doses (1 Gy or higher) is statistically significant, whereas at low doses (< 0.1 Gy), the risk is uncertain.[11]

LDR is usually defined as ≤ 0.2 Gy at low linear energy transfer (LET) or ≤ 0.05 Gy at high LET.[12] In fact, since different organisms, animal species, organs, tissues or cells may respond differently to the same or different kinds of radiation under different experimental conditions, doses of LDR effectively to induce benefit vary significantly. In the past several decades, many biological effects of LDR, distinguishable from those of HDR, have been reported.[13–16] These effects include "radiation hormesis,"[17,18] which encompasses the beneficial effects of LDR in stimulating the growth and development of animals, increasing life span, enhancing fertility, and decreasing the incidence of cancer,[19,20] "adaptive response" (AR), which demonstrates that LDR-induced hormesis could also stimulate a system of protective biological processes, thereby subsequently alleviating tissue damage,[21] "bystander effects,"[22] "hyper-radiosensitivity" (HRS)[23] and "induced radioresistance" (IRR),[24] as outlined in Figure 1. Furthermore, these biological effects of LDR, particularly hormesis and AR, have been investigated for their potential applicability in the treatment of diseases such as cancer, diabetes, and tissue degeneration.[25–27] Considering that LDR increases anti-oxidant activity and DNA repairing capacity,[28–30] it has been quested whether we can use these features of LDR to protect against radiotherapy- or chemotherapy-induced damage of normal tissue, as a new modality to enhance the efficacy and reduce the toxic side-effects of radiotherapy or other chemotherapies.[31]

However, the first concern has to be addressed that whether LDR would also induce the same effects, as in normal tissue, to promote the proliferation of cancer cells or protect cancer cells from the therapeutic effects of radiation or anti-cancer drugs. Therefore, many researchers have explored the main effects of LDR, including hormesis and AR, on cancer cells, which revealed that at certain conditions there are different biological effects between some cancer cells and normal cells in response to LDR.[32–36] Based on these findings, many studies were conducted to explore the role of LDR in overcoming the obstacles of anti-cancer therapy, for example, suppression of immune function and normal tissue damage caused by radiotherapy, without alleviating the therapeutic effects. Although the health risks associated with LDR remain controversial owing to a lack of understanding of the molecular mechanisms underlying the response, it is worthwhile to further clarify and provide a prospective overview of the potential application of LDR in anti-cancer therapy. This review extensively summarizes the current knowledge on the effects of LDR in anti-cancer therapy. The multiple biological mechanisms, therapeutic modality, and the balance between the beneficial effects and the potential risk of LDR when used in clinical settings are also discussed, with an aim to provide an overview of the potential application of LDR in cancer treatment.

LDR-Induced Hormesis in Normal Cells during Anti-Cancer Therapy

LDR stimulates the proliferation of normal cells, which favors recovery of damaged tissues during anti-cancer therapy

Hormesis induced by LDR is often mirrored by its stimulation of cell proliferation. In previous studies, proliferative effects induced by LDR were documented extensively in different normal cell types including thymocytes, splenocytes, lymphocytes, lung fibroblasts and diploid cells.[37–42] Some studies showed that the activation of the Raf, AKT signaling pathway by LDR may induce the expression of genes related to cell survival by remodeling the chromatin structure and regulating the cell cycle.[39]

In addition, exposure to LDR was also found to induce hormesis in normal stem cells. Using a mouse model, Li *et al.* and Wang *et al.* demonstrated the stimulating effects of LDR on bone marrow hematopoietic progenitor cell proliferation.[43,44] Recently, the molecular mechanism underlying LDR-induced hormesis in normal stem cells was further explored by Liang *et al.*[45] These studies showed that 75 mGy X-rays can induce a significant increase in the proliferation of rat mesenchymal stem cells at 6 h post-irradiation. The increase in cell growth has been attributed to the activation of several members of the mitogen-activated protein kinases (MAPK)/extracellular signal-regulated kinases (ERK) signaling pathways since inhibition of MEK function significantly abolished LDR-induced ERK1/2 activation and LDR-stimulated cell proliferation.[45] Another recent study showed that LDR could also promote neural stem cell proliferation and enhance neurogenesis in the hippocampus of mice.[46] Stimulation of the Wnt/ß-catenin signaling pathway is assumed to be involved in the regulation of proliferation and differentiation of neural stem cells, as well as neurogenesis in the hippocampus (Fig. 2). In addition, LDR also promoted cell survival and reduced apoptosis of neuronal stem cells.[46]

Since normal stem cells are crucial to tissue repair, the direct proliferative effect of LDR on normal stem cells favors tissue repair. The effect of LDR on neural stem cell

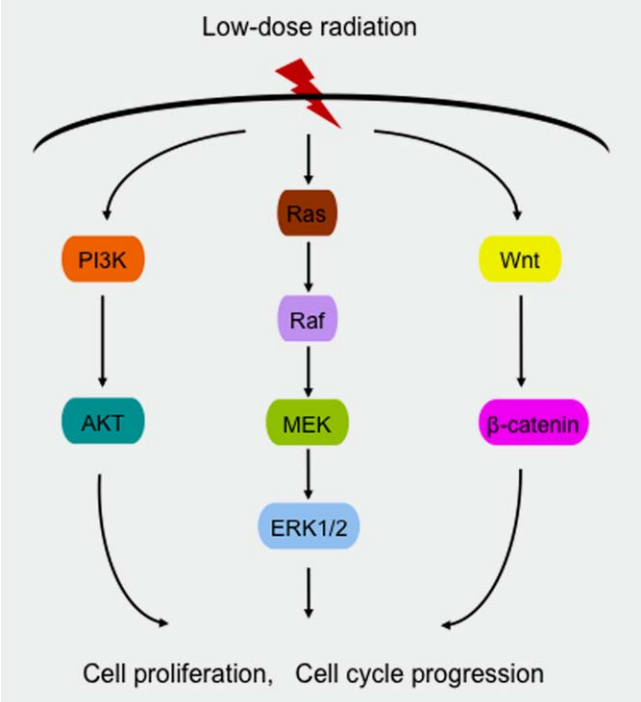

Figure 2. The multiple signaling pathways through which LDR promotes cell proliferation and cell cycle progression.

proliferation suggests its translational application in devising new therapeutic strategies for cancer treatment-related neurodegenerative disorders. In addition, the peripheral mobilizing effect of LDR on normal stem cells is also involved in the repair of damaged tissue. As an important component of the hematopoietic system, peripheral mobilization of bone marrow hematopoietic stem cells has been reported to be stimulated by LDR, which may alleviate the adverse effects of bone marrow suppression in anti-cancer therapy.[43] This effect was further confirmed in a rat model of diabetes, which showed that LDR promoted skin wound healing by stimulating the peripheral mobilization of bone marrow stem cells.[47]

Taken together, LDR affects multiple aspects of normal cells in tissue damage repair; this is a very important finding for the clinical application of LDR, which induces hormesis in normal cells and normal stem cells, favoring tissue damage repair during conventional anti-cancer therapies.

LDR induces hormesis in the immune system, which in turn enhances anti-cancer immunity

Radiotherapy with HDR induces time-restricted immune suppression by directly destroying immune cells.[48] However, in contrast to HDR, LDR offers an effective treatment for cancer through the stimulation of innate immune cells and adaptive immune response (Fig. 3).

Studies with animals subjected to whole-body irradiation have shown that LDR at a dose of either 0.1 or 0.2 Gy could significantly suppress pulmonary tumor metastases in BALB/c mice with syngeneic L1 sarcoma. This anti-cancer effect of

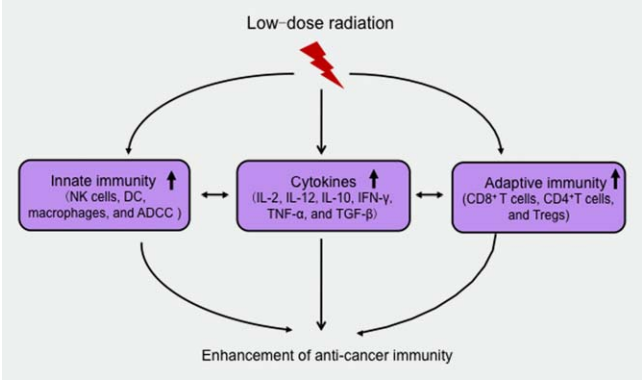

Figure 3. The mechanisms of LDR-induced anti-cancer immunity.

LDR can be abrogated by the nature killer (NK)-suppressive anti-asialo GM1 antibody.[49] Other *in vitro* studies have also confirmed this effect,[50,51] suggesting that NK cells play a role in LDR's anti-cancer effect. We found that LDR could enhance the expansion and cytotoxicity of NK cells by activating the P38-MAPK pathway.[52] Activation of macrophages in the spleen by LDR appears to contribute indirectly to the enhancement of concanavalin-A-induced proliferation of splenocytes.[53] LDR can also enhance the cytotoxic function of macrophages against P815 tumor cells in tumor-bearing mice,[54] suggesting a role of macrophages in LDR-mediated tumor response. Furthermore, LDR also programs macrophage differentiation to an $iNOS^+$/M1 phenotype that overcomes the barrier of cancer immunotherapy through efficiently recruiting tumor-specific T cells in malignant solid tumors.[55] Co-culturing of T cells with dendritic cells pre-irradiated with LDR significantly enhanced the proliferation of T cells, which was mainly caused by cytokines secreted from the dendritic cells.[56] These results suggest that LDR stimulates innate immune cells, which may further activate adaptive immune cells.

LDR was also able to enhance the adaptive immune response directly through augmentation of the proliferative response of T cells to antigenic, allogeneic, and mitogenic stimulation, with a concomitant increase in cytotoxic effects on tumor cells.[51,57,58] Moreover, LDR-induced higher expression of surface markers both on antigen-presenting cells (APCs) and on T cells leads to a reduction of self-tolerance induced by cancer cells, thereby resulting in the induction of anti-cancer immunity.[59,60] Moreover, T-regulatory cells (Tregs), a subset of $CD4^+$ T cells that comprise an important immune-evasion strategy used by cancer cells,[61] can also be affected by LDR. A single dose of LDR has been shown to reduce the Treg population, which is directly linked to a therapeutic response in the form of reduced tumor burden and prolonged survival.[62–64] Apart from the modulation of T-cell functions, LDR has been shown to increase antibody secretion and enhance the antibody-dependent cellular cytotoxicity response in tumor-bearing mice, which is well correlated with tumor regression.[65]

In addition, exposure to LDR can induce an altered cytokine profile in peripheral blood.[51,60,66,67] Treatment of tumor-

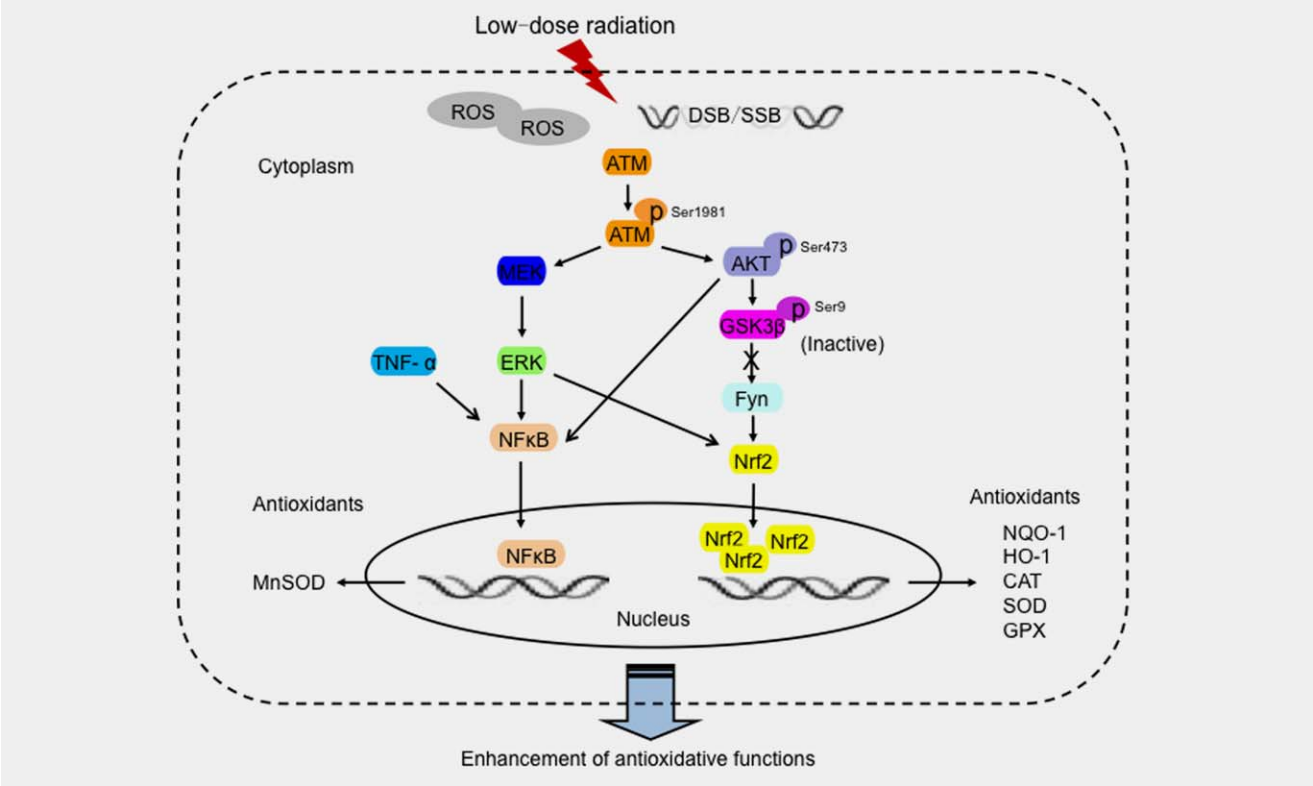

Figure 4. The multitude of mechanisms through which LDR induces the enhancement of anti-oxidative functions.

bearing mice with LDR not only reduced the secretion of immunosuppressive cytokines such as IL-10 and transforming growth factor (TGF)-β[68] but also increased the production of growth-stimulatory Th1 cytokines such as interferon (IFN)-γ, IL-2, and tumor necrosis factor (TNF)-α, which stimulate the proliferation of immune cells.[69,70]

LDR has a significant mutational effect on the IFNα-2b gene and affects the hyperimmune response in the form of lymphocytosis in the case of medical workers from radiology, nuclear medicine, and radiotherapy departments.[71] It may partially explain why people who are exposed to LDR owing to residence in high background radiation areas or through their occupation display a decreased incidence of certain cancers or have an extended life span. It also suggests that specific activation of the immune system by LDR is one of the contributory mechanisms to enhanced cancer cell killing, which enhances anti-cancer immunity and supports the use of LDR as a standard regimen.

LDR-Induced Adaptive Response (AR) in Normal Cells during Anti-Cancer Therapy

Pre-exposure to LDR can decrease chromosomal aberrations resulting from subsequent exposures to HDR, which is referred to as AR.[72–74] There appears to be three principal types of AR induced by LDR: one is stimulation of anti-oxidative functions, one is activation of DNA damage repair, and the last is the metabolic modification in normal tissues.

LDR stimulates anti-oxidant activity, thereby preventing free radical- or reactive oxygen species-induced damage to normal tissues

It is well known that radiotherapy may promote reactive oxygen species (ROS) formation in cells by water ionization, which can in turn kill tumor cells via necrosis or apoptosis. However, excess ROS can also injure normal cell structural molecules, leading to DNA fragmentation and lipid peroxidation and other effects. Therefore, it is very important to develop a strategy for activating the defense systems of normal cells to counteract these adverse effects, thus allowing for a more intensive and effective therapy. The AR of LDR has shown its potential in these aspects.

LDR has been reported to increase the levels of various kinds of anti-oxidants *in vitro* and *in vivo*. In experimental animals, a single exposure to LDR at 75 mGy or three exposures to LDR at 25 mGy have been shown to stimulate renal superoxide dismutase (SOD)−1 expression and activity.[75] Recently, the mechanism underlying LDR-stimulated anti-oxidant activity has been clarified at the molecular level (Fig. 4). It is reported that exposure to LDR resulted in increased activity of nuclear factor erythroid-2-related factor 2 (Nrf2), a major transcription factor of the anti-oxidative system, which regulates the expression of most anti-oxidants.[76] Studies on the signal pathway responsible for Nrf2-mediated anti-oxidative response showed that under conditions of mild oxidative stress, the ERK1/2-dependent signaling pathway or AKT phosphorylation may be involved in LDR-induced activation

of the anti-oxidant defense mechanism through induction of Nrf2.[77,78]

Manganese superoxide dismutase (MnSOD) is also known to play a key role in LDR-induced anti-oxidant activity by reducing the amount of toxic superoxide radicals formed following exposure to HDR. Loss or deficiency of MnSOD sensitizes cells to ionizing radiation, whereas restoring MnSOD expression in MnSOD-deficient cells reestablished the radioadaptive phenotype.[79–81] Further studies have shown that an intact TNF signaling process and NFκB activation were required for the MnSOD-mediated anti-oxidative response induced by LDR.[79–82] Studies on the molecular mechanism underlying LDR-induced anti-oxidant activity have laid a theoretical foundation for the clinical application of LDR to reduce oxidative damage of normal tissues caused by conventional anti-cancer therapies.

LDR activates DNA damage repair, thereby reducing genomic instability in normal tissues

It has been generally accepted that HDR induces a plethora of DNA lesions, including oxidative base damages, single-strand breaks (SSBs), and double-strand breaks (DSBs),[83–85] which affected the DNA integrity or alter its chemical nature. Most of SSBs are potentially reparable lesions, which can be repaired quickly and effectively mainly via poly (ADP-ribose) polymerase (PARP) activation.[86] If not repaired, SSBs can disrupt transcription and replication and can be converted into potentially chromosome aberration and/or lethal DSBs.[86] DSBs have been reported to trigger the most detrimental effects on genome stability, and have been identified as the main contributors to HDR-induced cell killing through the formation of chromosomal aberrations.[87] To ensure genome stability in irradiated cells, mammalian cells harbor cellular defense systems against radiation-induced DSBs, including activation of DNA damage response (DDR) mechanisms, cell-cycle checkpoints, and apoptosis.

It is clear that DDR is one of the mechanisms involved in LDR-induced AR.[88–90] However, how and which DNA repair pathway coordinates in the DDR in response to LDR remains unclear yet. The DDR associated with DSB repair pathways includes homologous recombination (HR) and non-homologous end joining (NHEJ) and a signal transduction process related with ataxia telangiectasia mutated kinase (ATM).[91] Recently, a study explored the genes/proteins involved in the NHEJ pathway in peripheral blood mononuclear cells at G0/G1 that have been exposed to LDR (γ-ray). The results showed that the expression of major proteins required for NHEJ was significantly increased.[92] Another study confirmed that pre-exposure of G2 cells to LDR promoted the resection step of HR when cells were exposed to subsequent challenge of radiation.[93] Moreover, increase in the use of HR due to pre-exposure to LDR was prevented by treatment with an ATM inhibitor during the incubation period between pre-exposure to LDR and challenge HDR, suggesting that ATM-dependent damage response after pre-exposure to LDR changes the cellular environment, possibly by regulating gene expression or post-transcriptional modifications in a manner that promotes resection.[93] In addition, the increased expression of ATM induced by LDR was also observed in cycling and resting human mesenchymal stromal cells by Alessio *et al.*,[94] which indicates the important role of the ATM-mediated signaling pathway in AR.

The DSB repair pathway is always controlled by the cell-cycle phase.[95,96] In G1, most irradiation-induced DSBs are repaired by NHEJ.[97,98] By contrast, HR becomes active in S/G2.[99,100] Thus, LDR may activate one pathway of DSB repair to stimulate the expression of DNA repair enzymes either in cycling or in resting normal cells, which leads to genetic stability and, eventually, radioresistance.

A unique communication between DSBs and cell cycle checkpoints is also involved in LDR-induced AR. Proliferating cells respond to DSBs by slowing down their progression through the cell cycle. It was shown that cyclin D1, which controls cell-cycle progression from G1 to S, is required for LDR-induced AR,[101,102] and that pre-exposure to LDR can effectively suppress cell apoptosis following HDR and promote survival by upregulating cyclin D1.

LDR modifies glucose metabolism, thereby increasing radioresistance in normal tissues

It has been reported that the metabolic pathways of glucose including aerobic glycolysis and oxidative phosphorylation were related with radiosensitivity and radioresistance of cells.[103] The increase of aerobic glycolysis leads to cell resistance to radiation.[104] However, oxidative phosphorylation is the main process of glucose metabolism in normal cells, which may be one of the reasons for HDR damage to normal tissues.

Lall *et al.* described a previously unrecognized cellular response in which LDR induced a metabolic shift from oxidative phosphorylation to aerobic glycolysis leading to increased radiation resistance in both cell and animal models.[105] Mechanistically, metabolic reprogramming depends on hypoxia-inducible factor 1 (HIF-1), which is induced specifically by LDR linking the metabolic pathway with cellular radiation dose response. When irradiation doses are below the threshold of causing detectable DNA damage (< 0.2 Gy) without significant activation or even inactivation of p53, HIF-1 is induced, resulting in the induction of glycolysis and an increase in radioresistance.

All together, the above experimental data offers a rationale for a new radiotherapy protocol that LDR exposure could be administered properly before radiotherapy to protect normal tissues from toxic side effects.

LDR Does Not Induce Hormesis and AR in Cancer Cells

We have discussed extensive evidence supporting the induction of hormesis and AR by LDR in different normal tissues based on the previous findings mentioned studies. However, another important issue that warrants discussion is whether

LDR could also induce the same biological effects in cancer cells. This information will be of great importance since exposure times and doses of LDR can be manipulated to favor anti-cancer therapy. We have demonstrated, for the first time, that LDR-induced proliferative effects are absent in cancer cells, including leukemia and solid tumor cells, *in vitro* and *in vivo* at the same experimental condition.[106] Although the lack of AR induced by LDR has been observed in cancer cells, including mouse skin papilloma cells (308 cells) and X-ray-sensitive lymphoma cells (L5178 Y-S and EL-4),[32,33] and Chen *et al.* also demonstrated that pre-exposure to LDR did not induce hormesis in human leukemia MOLT-4 cells, but accelerated apoptosis when exposed to a challenge radiation dose[107]; these studies did not include normal cells to be compared with this tumor cells at the same study. To extend our early study, we recently further compared human embryonic lung fibroblast 2BS and lung cancer NCI-H446 cell lines when they were irradiated with LDR at different doses (20–100 mGy). In response to 20 to 75 mGy X-rays, cell proliferation was significantly increased in 2BS but not in NCI-H446 cells. Further mechanistic study showed that LDR stimulates cell proliferation via the activation of both MAPK/ERK and PI3K/AKT signaling pathways in normal 2BS cells but not in NCI-H446 cells.[108] In addition, there is little direct evidence about the effect of LDR on glucose metabolism in tumor cells. A study by Zhang *et al.* showed that LDR improved tumor hypoxic conditions through inhibiting the expression of HIF-1,[109] which partly enhanced radiosensitivity of tumor cells. This result was supposed to be related with increase of oxidative phosphorylation.

However, there were also a few studies that have shown controversial results. For instance, Gerashchenko *et al.* showed that tumors in irradiated rats apparently grew faster than those in non-irradiated rats for up to 18 days after implantation of Guerin carcinoma cells.[110] Another earlier experimental study showed that AR was observed in two breast carcinoma cells at 2 h and persisted for up to 24 h after LDR.[111] These findings seem to suggest that it is not a common phenomenon that LDR could not induce hormesis and AR in cancer cells. In addition, it has been generally accepted that the genetic background affects biological responses to LDR, as genetic differences exist between normal and cancer cells. Significantly reduced DNA damage repair signaling and capacity have been documented *in vitro* for several cancer predisposition and chromosomal instability syndromes.[112–114] This may explain why many phenomena or LDR-induced biological effects could not be observed in some cancer cells.

Several studies have conducted with a focus on specific signaling pathways induced by LDR in normal and cancer cells. Hendrikse *et al.* examined the effects of LDR on the cell-cycle using TK6, a lymphoblast cell line with wild-type p53, and U937, a monocytic leukemia cell line with mutant and inactive p53.[115] They demonstrated that LDR exposure could induce cell-cycle arrest as an AR index in TK6 cells but not in U937 cells, suggesting the possible requirement of wild-type p53 for the AR. Our previous study also confirmed that differential expression of the p53 gene was involved in the differences between the LDR-induced biological effects observed in normal and cancer cells.[116] Moreover, LDR has been reported to activate the protective system in normal cells, by enhancing anti-oxidant activity and DNA damage repair, which were not reported in cancer cells.[35] Therefore, all these findings suggest that the difference in LDR-induced biological effects between normal and cancer cells may be related to signaling pathways involved in apoptosis, cell cycle, and cell protective system.

Some recent studies have shown that LDR induced different changes in epigenetics, including alteration of microRNA (miRNA) expression profiles and modification of DNA methylation patterns, which also affected the responses of normal and cancer cells.[117,118] miRNA microarray analysis in the study by Bae *et al.* demonstrated that LDR induced changes in the expression profiles of specific miRNAs in normal human dermal fibroblasts. Some of the deregulated miRNAs were specifically related to either the early or late radio-AR.[118] Using the same method, another study revealed that miRNAs related to cell communication and intercellular signaling transduction played important roles after normal human fibroblasts were exposed to LDR.[119]

Overall, many studies have confirmed that LDR induces different biological effects between normal and cancer cells (Table 1). However, a clear molecular mechanism responsible for the differences in LDR-induced biological effects in normal and cancer cells has yet to be found. However, it is an urgent issue to be explored since understanding the molecular mechanism underlying these differences for LDR-induced hormesis and AR between tumor and normal cells will be the theoretic basis for the clinical application of LDR in anti-cancer therapy.

Perspective Overview on the Clinical Application of LDR

Balance between the risks and benefits of LDR

Although scientists have made considerable efforts in the field of LDR-based cancer therapy, the potential health effects resulting from exposure to LDR continue to be the focus of intense debate and significant controversy. The LNT hypothesis has been generally accepted even though there were many debates for it.[120–124] The use of X-ray computed tomography scan and isotopes for diagnosis are also considered to be LDR, and are believed to be a risk of cancer. However, they are widely accepted because of their irreplaceable roles in disease diagnosis. Many anti-cancer therapies such as radiotherapy and chemotherapy are also associated with cancer risk. Compared with these methods, LDR has the potential to reduce the adverse effects of conventional anti-cancer therapies as well as cancer risk, thereby being more beneficial to cancer patients.

Table 1. Biological effects and mechanisms of LDR

	Radiation dose	Mechanisms	References
Hormesis			
Normal cells			
Mouse thymocytes	75 mGy X-ray	Enhancement of protein synthesis of RIP10	37
Mouse splenocytes	10 mGy γ-ray	Enhancement of mitogen-stimulated proliferation	38
Mouse lymphocytes	10 mGy γ-ray	Reduction in the frequency of micro-nucleated cells	42
Human lung fibroblasts	50 mGy X-ray	Activation of Raf and AKT Activation of ERK1/2 and p38	39,40
Human diploid cells	20-50 mGy X-rays	Activation of MAPK pathway	41
Normal stem cells			
Mouse hematopoietic cells, bone marrow stem cells	75 mGy X-ray	Induction of cell proliferation and peripheral mobilization	43,44,47
Rat mesenchymal stem cells	25-100mGy X-rays	Activation of MAPK/ERK signaling pathway	45
Mouse neural stem cell	300 mGy X-ray	Activation of Wnt/ß-catenin signaling pathway	46
Immune response			
NK cells	75, 100 or 200 mGy X-rays	Increase in proliferation and cytotoxicity by activating the P38-MAPK pathway	49,50,52
Macrophages	400 or 500 mGy γ-rays	Enhancement in proliferation, cytotoxic function, and differentiation	54,55,62
Dendritic cells	50 mGy γ-ray	Increase in T cell-activation capacity	56
T cells	50 or 75 mGy X-rays	Increase in cytotoxic effects and anti-tumor activity	51,59
T-regulatory cells	150 mGy X-ray	Reduction in the population and breaking of tumor tolerance during carcinogenesis	64
Cytokines	75 mGy X-ray	Reduction in immunosuppressive cytokines and increase in growth-stimulatory Th1 cytokines	68,69
Adaptive response			
Stimulation of anti-oxidant activity			
Type 1 diabetic mice	75mGyX-ray	Stimulation in renal SOD-1 expression and activity	75
Human skin fibroblast cells, type 1 diabetic mice	50 or 75 mGy X-rays	Increase in activity of nuclear factor Nrf2 via ERK1/2 or AKT phosphorylation	77,78
Mouse skin epithelial cells	5 to 100 mGy X-rays	Induction of MnSOD activity	81
Activation of DNA damage repair			
Peripheral blood mononuclear cells	100 mG γ-ray	Increase in major proteins required for NHEJ	92
Human fibroblasts	200 mGy X-ray	Increase in the use of HR	93
Human mesenchymal stromal cells	40 mGy X-ray	Increase in the expression of ATM	94
Modification of glucose metabolism			
Human normal B-celllymphocytes, human fibroblasts, BALB/c mice	100 mG X-ray	Induction of a metabolic shift from oxidative phosphorylation to aerobic glycolysis involved HIF-1	105
Anti-tumor effects			
Human leukemia and solid tumor cells	25 to 200 mGy X-rays	Absent of hormesis	106
Human leukemia cells	200 mGy X-ray	Acceleration of apoptosis	107
Mouse skin papilloma cells, X-ray-sensitive lymphoma cells	10 mGy X/γ-rays	Absent of adaptive response	32,33
Nude mice bearing ovary cancer xenografts	500 mGy X-ray	Enhancement of radiosensitivity	109

Indeed, we cannot neglect the experimental studies that have shown unfavorable evidence: for example, not supporting the application of LDR in anti-cancer therapy. For instance, a study on cultured cells suggests that long-term exposure to LDR of α-particles (0.025 Gy) could enhance the potential of malignant transformation incidence in human bronchial epithelial cells.[125] Another study using murine experimental models also demonstrated that LDR promoted tumor growth and metastasis by enhancing angiogenesis.[126] Since differences in genetic background, variability in the complexity of LDR exposure, and also since LDR may affect different intracellular signal pathways due to the alteration of other uncertain factors, differences in the performance of LDR may be observed in different individuals. With an increase in studies on LDR that explore its long-term effects and mechanisms, its complexity will be further clarified and its advantages will be applied more effectively in cancer treatment.

In addition to the experimental data, epidemiological findings also showed some factors that affected the risks and benefits of LDR. For instance, to characterize the long-term temporal trend and age-at-exposure variation in the radiation-induced risk of thyroid cancer, analysis of the thyroid cancer incidence was done for Japanese atomic-bomb survivors. Using a linear dose-response model, the excess relative risk of thyroid cancer at 1 Gy of radiation exposure was estimated as 1.28 (95% confidence interval: 0.59–2.70) at age 60 after acute exposure at age 10. The risk decreased sharply with increasing age-at-exposure and there was little evidence of increased thyroid cancer rates for those exposed after age 20.[127]

There are some uncertain factors which affected the balance evaluation between beneficial effects and potential cancer risk of LDR. Therefore, the benefit of exposure to LDR in anti-cancer treatment is still a challenging issue. Moreover, future research directed toward the identification of mechanisms associated with responses to LDR is critically needed to fully understand their beneficial effects.

The elusively multiple mechanisms underlying the effects of LDR in cancer cells and normal cells

Although considerable progression has been made in the past 50 years in understanding the molecular mechanisms underlying the effects of LDR exposure, a multitude of unresolved questions remain existing, which warrant further investigations.

Apart from the studies mentioned above, one of the most promising studies is the one evaluating HRS and IRR. HRS is characterized by an increased sensitivity to radiation doses less than 0.3 Gy, which is followed by a more radioresistant response per unit dose between 0.3 and 0.6 Gy termed IRR.[128] The HRS/IRR phenomenon has been demonstrated in normal cells as well as cancer cells *in vitro*.[129] Mechanisms underlying cell-type specific expression of HRS are still being investigated; however, they appear to be related to defective DNA repair systems and cell cycle regulation.

The HRS/IRR phenomenon has been extensively demonstrated in the past decade. Accumulating evidence suggests that it may have varied implications on radiotherapy practices.[130,131] While *in vivo* studies continue to provide insights into the potentially clinical implications of HRS/IRR in terms of exploitation of the response for a therapeutic benefit and implications for normal tissue reactions, no changes in current practices can be made until the underlying mechanism is fully understood. However, this would not be extensively discussed in here since the dose level is relatively higher than the dose levels ($\leq$ 0.1 Gy or occupationally $\leq$ 0.2 Gy low LET radiation) effectively inducing hormesis and AR.

The optimal modality of LDR: Dose, frequency and exposed area

LDR shows various effects on organisms, depending on the difference of the low LET radiation's dose, dose rate, and radiation modality. Therefore, many issues need to be clarified such as the irradiation dose, irradiation frequency, or irradiation range to be used in clinical practice. Most findings regarding these aspects have been obtained from animal studies. Cheda *et al.* found that a single X-ray irradiation of mice at a dose of either 0.1 or 0.2 Gy suppressed experimental tumor metastases.[49] Yu *et al.* found that a single dose of X-ray irradiation at 75 mGy administered 6 h before implantation significantly inhibited tumor growth in Kunming strain male mice implanted with S180 sarcoma cells.[132] Two other studies showed that pre-irradiation with X-rays at a dose of 75 mGy four times reduced the occurrence of thymic lymphoma caused by HDR.[70,133]

Since radiosensitivity varies considerably among individuals, the irradiation dose or irradiation frequency required for inducing anti-tumor effects is also different. Moreover, the biological effect induced by LDR is time-dependent. For instance, we found that LDR of X-rays at doses of 50 and 75 mGy significantly increased colony-forming unit-granulocyte/macrophage formation, starting at 48 h, reaching the maximum level at 72 h, and remaining at a high level for 96 h post-irradiation.[43] Therefore, the interval between LDR exposure and the administration of other anti-cancer therapies should be further clarified for more effective application of AR induced by LDR. Moreover, in previous studies, researchers applied the same exposure dose but for different irradiation times and at different irradiation frequencies. All these irradiation protocols induced AR. However, the protocol, that is, most beneficial to cancer patients is yet to be determined.

As for the range of irradiation, whole-body irradiation at a dose of 0.02–0.25 Gy has been reported to inhibit the growth and metastasis of tumors.[134] The study by Seiko *et al.* compared the anti-tumor effects of whole-body irradiation and local irradiation, which showed that the low-dose whole-body irradiation at a dose of 0.2 Gy significantly decreased the incidence of lung and lymph node metastasis, whereas the same dose of local irradiation had no effect on the incidence of metastasis.[135]

Different types of radiation may impact the effects of LDR. Over the past decades, some new forms of preclinical radiotherapy[136–138] have been studied, for example, microbeam radiation therapy using an extremely high dose rate and very small beam divergence, and flash therapy using sub-millisecond pulses of radiation at ultrahigh dose rate. It is shown that microbeam or short pulses with ultrahigh dose rate radiotherapy might allow complete eradication of malignant tumors and reduce the occurrence and severity of early and late complications affecting normal tissue. With the development of such new technologies, irradiation of single cells and investigation of the responses of their neighboring cells with LDR will become possible. In addition, these findings have implications for the research on dose rate or radiation pattern of LDR.

Challenges in translating the preclinical data to clinical application

Although hormesis/AR has been manifested under certain experimental conditions, it also raises challenges how to translate the mechanism from a well-controlled homogenous cell line or animal study to a heterogeneous human population. Sokolov *et al.* has reviewed the studies of different types of human cells responding to LDR exposure at the global transcriptional level.[139] They concluded that LDR responses are highly genotype, cell type, and tissue-dependent, with a remarkable degree of variability both between individuals and different cell types. Each human cell type has its own characteristic profile of gene expression alterations induced by LDR. To get an overview of the response to LDR, there has been a move to "systems biology" approaches[140] that incorporate multiple "omics" platforms in the LDR biology field.

To promote the clinical application of LDR, it is rationale to find appropriate animal models that could mimic human response to LDR. More importantly, well-designed clinical trials should be conducted, in order to study the safe and effective dose, dose rate, time interval between priming and challenging and so on. Future research is also to be focused on identifying biomarkers for detection of LDR sensitive cohorts of patients, to perform patient-specific personalized treatment.

Taken together, although there remain unresolved issues and none of the clinical trials about the application of hormesis and AR induced by LDR in anti-cancer therapy, the findings of preclinical studies provide us with a lot of evidence that could benefit the development of optimum protocols for the clinical application of LDR. This could substantially change the manner in which radiotherapy or chemotherapy is planned and performed and provide methods to treat patients more effectively.

Conclusions

The biological effects induced by LDR are different from those induced by HDR. Considerable evidence gathered over nearly half a century suggests that LDR may be used as an anti-cancer treatment strategy. In addition to its contribution to anti-cancer therapy, LDR may also play an important role in cancer prevention. Furthermore, the protective effects induced by LDR may be beneficial when used in combination with other cancer-treatment modalities. However, the other effects of LDR, for example, bystander effects, HRS, and IRR, have not yet been investigated clearly. A comprehensive understanding of the various mechanisms underlying the anti-cancer effects induced by LDR is likely to provide a fillip to the design of protocols using LDR as an adjuvant to other therapeutic modalities to enhance the effects of different cancer therapeutics. Taken together, these advances suggest that there is great potential for the application of LDR in anti-cancer therapy as well as cancer prevention. We hope that these benefits of LDR will be achieved soon and become commonplace in anti-cancer therapy.

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