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# Low-dose radiation therapy of cancer: role of immune enhancement

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The efficacy of conventional radiation therapy, one of the most widely used treatment modalities of cancer, is limited by resistance of tumors as well as normal tissue toxicity. In the last decade, several studies have shown that protocols using low-dose radiation (LDR) are more effective in providing local tumor control with negligible normal tissue toxicity. LDR stimulates antioxidant capacity, repair of DNA damage, apoptosis and induction of immune responses, which might be collectively responsible for providing effective local tumor control. This article focuses on the immunostimulatory effects of LDR in *in vivo* models and its clinical efficacy, supporting the use of LDR regimens (alone or as adjuvant) as an anticancer treatment.

**Keywords:** cancer radiotherapy • dendritic cells • immune enhancement • low-dose radiation • NK cell • T-regulatory cell

Radiotherapy is an important modality in the treatment of cancer, either employed alone in inoperable tumors or following surgery in many neoplasms [1]. Despite significant advances in medical physics, resistance of tumors to therapy in addition to systemic and normal tissue toxicity has limited the success of anticancer treatment. Several investigations during the last decade have shown that low-dose radiation (LDR) is even more effective in cancer therapy than the conventional daily doses of 1–2 Gy. While high doses of radiation are known to suppress the immune system, low doses have been shown to enhance the immune response [2–6], thereby reducing systemic toxicity, in addition to enhancing anti-tumor response. LDR has also been shown to modify several other processes such as aging, adaptive response, survival, and so on [7–9]. This interesting phenomenon exhibiting the beneficial effects of LDR is often called 'radiation hormesis' [10,11].

According to the linear no-threshold LNT theory (United Nations Scientific committee on the Effects of Atomic Radiation [UNSCEAR] 1958), the effects of ionizing radiation can be estimated by linear extrapolation from those observed at high doses [12,13]. Moreover, there appears to be no safe radiation dose, as even very low doses of ionizing radiation produce some biological effects [14]. However, epidemiological data have shown that inhabitants of high-background radiation in India (Kerala), Brazil, China, the USA, the Misasa Radon spa area of Japan and many other parts of the world have lower cancer mortality than those living in areas with a normal background radiation environment [15–17]. A significantly lower rate of cancer mortality among the population residing in the Guangdong area (China) has been found to correlate well with immune enhancement, mainly in the form of enhanced sensitivity of peripheral blood lymphocytes to mitogenic stimulation [18]. Similar results have been reported in occupational radiation workers, patients exposed to LDR used for diagnostic purposes, and in experimental studies with animals [19–21].

Low-dose radiation stimulates biological activities *in vitro* as well as *in vivo*, which include antioxidant capacity, repair of DNA damage, induction of immune responses and apoptosis in certain cancer cell types (Figure 1), as well as proliferation of normal cells [22,23]. Many other phenomena such as low-dose hypersensitivity, bystander effects, adaptive response and cell–cell communication have also been suggested to be responsible for enhanced therapeutic gain using LDR [24–27].

Enhancement of immune response with LDR treatment is one of the important factors responsible for improved therapeutic gain, since

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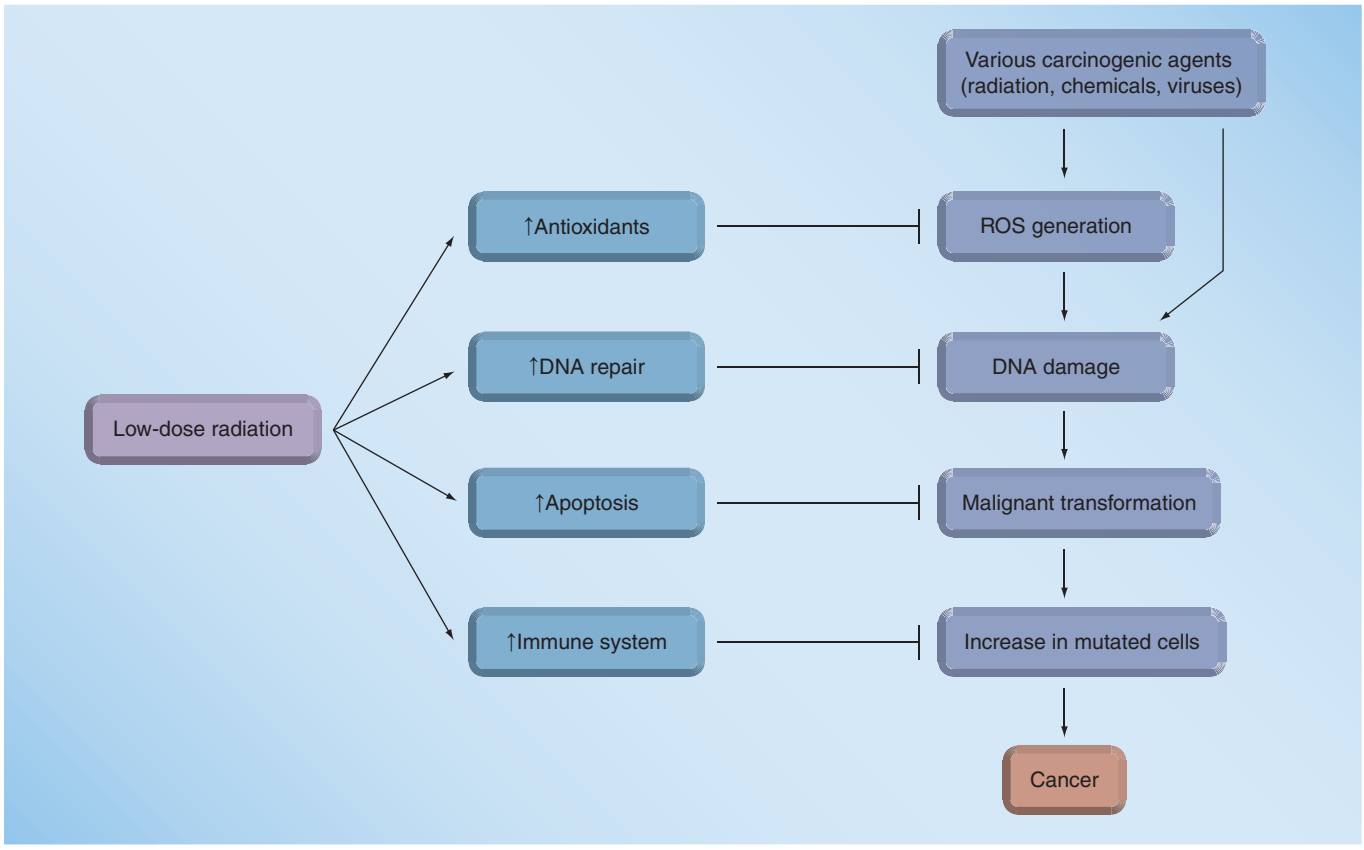


**Figure 1. Factors involved in the anticarcinogenic effects of low-dose radiation.**
ROS: Reactive oxygen species.
Modified from [131].

immune surveillance may ensure elimination of neoplastic cells by stimulating defense mechanisms [28]. Activation of several immune cells such as natural killer (NK) cells, dendritic cells, macrophages and T cells, as well as increase in mast cell activity, has been reported following treatment of tumors with low-dose regimens [4,29]. Decrease in T-regulatory cells, altered cytokine responses such as an increase in IL-2 and IFN-γ secretion, and a decrease in TGF-β levels [5,30,31] and antibody production have also been observed [6].

Experimental studies using low-dose x-rays and γ-rays in different strains of mice have been shown to decrease the growth rate of tumors as well as metastasis, which correlate well with immune enhancement [32–34]. Indeed, fractionated total-body irradiation using low doses of ionizing radiation (LDR) has been used in the treatment of chronic lymphocytic leukemia and advanced stages of indolent low-grade non-Hodgkin's lymphoma (NHL) with good results, which has been shown to be accompanied by enhanced anticancer immunity [35]. Specific activation of the immune system by LDR is one of the contributory mechanisms for enhanced tumor cell killing that strengthens the use of LDR as a standard regimen. This article summarizes the current status on the effects of low doses of ionizing radiation on the immune system that contribute to the efficacy of LDR.

## Tumors & the immune system

Tumors during initial developmental stages have been shown to be antigenic in nature and could potentially be under surveillance by innate immune cells; however, several tumor cells grow fast and become 'in equilibrium' with immune cells, only to escape the immune defense mechanisms by immunoediting and avoiding detection at later stages [28]. The development and establishment of tumor cells in the body suppress the immune system, while activation of both innate (macrophages, NK cells and NKT cells) and adaptive immunity ($CD4^+$ helper T cells [Th1 and Th17] and $CD8^+$ cytotoxic T cells) has been shown to possess anticancer activity. Suppression of the immune system and evasion of self-altered cells are predominant features that, along with other factors, drive the progression and metastasis of cancer [36]. Cancer cells utilize the mechanisms shared by healthy self-cells (self-tolerance) to elude defensive immune responses. Moreover, these altered self-cells utilize several other mechanisms such as sequestration of tissue from surveillance, antigen shedding, lymphocyte killing, secretion of immunosuppressive cytokines, reduced MHC class II expression and costimulatory molecules to evade immune responses [37,38]. Apart from these, cancer cells develop additional mechanisms to escape host immunity such as downregulation of MHC class I expression or peptide presentation, antigenic modulation and

failure of lymphocyte homing, and secretion of cytokines such as inducible IL-2 and TGF-β [39–41]. Although, some nonspecific tumor cell kill is achieved by NK cells, no sufficient immune response is actually mounted against the tumor cells by immune-surveillance mechanisms (Figure 2). These studies suggest that tumors suppress the immune system during carcinogenesis, and therefore upregulation of immune surveillance by LDR offers an effective strategy for anticancer therapy.

## Radiation-induced immune modulation

According to the UNSCEAR 1986 Report, acute doses above 2 Gy, between 2 and 0.2 Gy, and below 0.2 Gy are regarded as high, intermediate and low doses, respectively. It is pertinent to note that much of our understanding on the low-dose effects of radiation has been derived from studies where the total dose used was greater than 0.2 Gy [4,29,42–44], which is higher than the limit of low dose according to the UNSCEAR report; however, the dose rates were extremely low compared with conventional radiotherapy. LDR leads to stimulation of the immune system, while high radiation dose results in its suppression [6]. LDR-induced immune enhancement occurs via the induction of both the antigen-presenting cells (APCs) and T lymphocytes, facilitating intercellular reactions within the immunological synapse [45]. Expression of molecules involved in negative regulation of the immune system, such as CTLA-4, cytokines such as IL-10, IL-4 and c-AMP, as well as protein kinase A, decreases, thereby inducing immune response following treatment with LDR [45]. Upregulation of several other factors such as the 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 signaling molecules (namely calcium, c-GMP and p38MAPK) induce immune activity following LDR treatment [45]. Immune response also varies with target cells, dose range, dose spacing and dose rate. Dose response for stimulation and/or suppression of various lymphocytic components of the immune system show a 'J' or 'inverted J'-shaped curve, while an irregular pattern is observed for macrophages [45]. At lower doses (<0.2 Gy), T-regulatory cells (Tregs) appear to be more sensitive compared with $CD4^+$ and $CD8^+$ cells [30], while at higher doses (>0.2 Gy) Tregs, dendritic cells and NK cells are relatively more resistant than $CD4^+$ and $CD8^+$ cells [46–49]. Therefore, it appears that various cellular components of the immune system show biphasic response to radiation, with some being resistant at higher doses, while others are resistant at lower doses, or *vice versa*. In addition to cellular and humoral immune cells (adaptive immunity), alterations in innate immune cells as well as induced cytokine secretion have also been shown to modulate the therapeutic effects of LDR (Figure 3).

## LDR & innate immunity

Several studies have suggested that induction of innate immune responses primarily involving NK cells, macrophages and dendritic cells mount nonspecific anti-tumor immune surveillance [50,51]. Alterations in innate immune response and suppression of metastasis following treatment with LDR have been reported [2,4,52].

### *NK cells*

Whole-body irradiation has been shown to significantly reduce the formation of pulmonary colonies of syngenic L1 sarcoma cells injected intravenously into BALB/c mice. This inhibition of tumor growth is abrogated by the NK-suppressive anti-asialo GM1 antibody [53], suggesting that NK cells play a role in LDR response. Furthermore, LDR has also been shown to stimulate

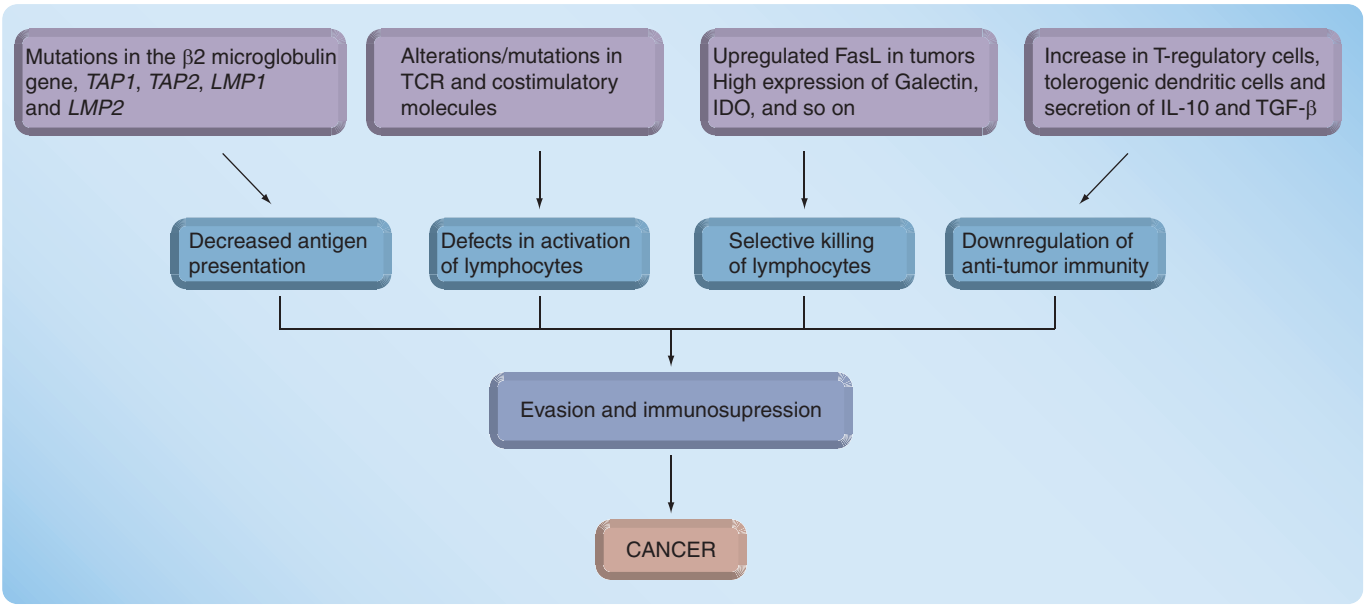


**Figure 2. Immune evasion/suppression during carcinogenesis.**
FasL: Fas-ligand; IDO: Indoleamine 2,3-dioxygenase; LMP: Low-molecular-weight peptide; TAP: Transporter associated with antigen processing; TCR: T-cell receptor.

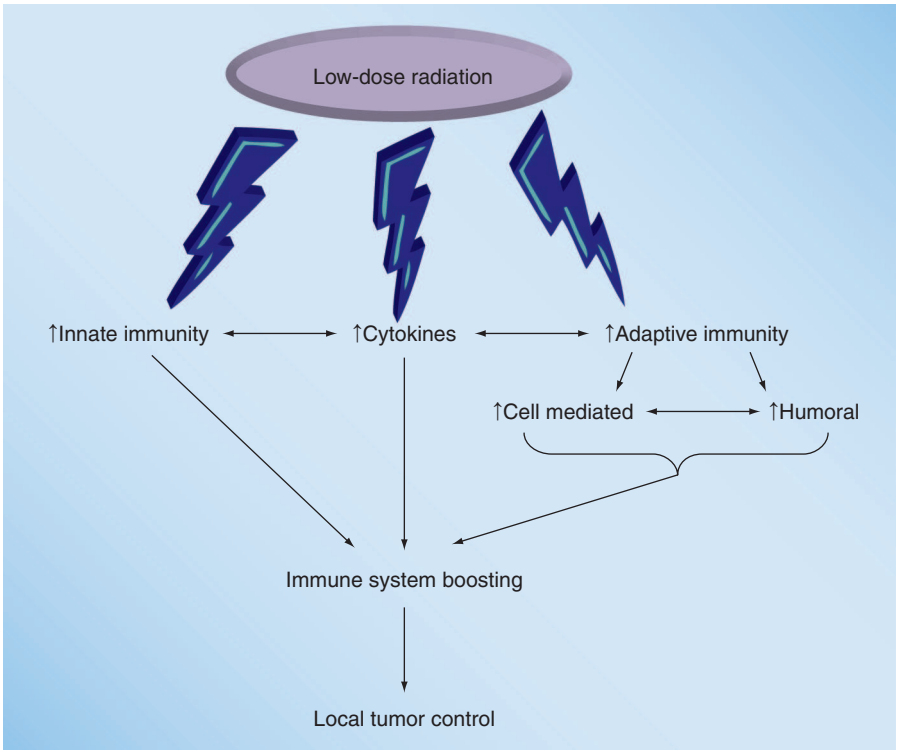


**Figure 3. Immune modulation during low-dose radiation-induced tumor regression.**

NK cell-mediated cytotoxicity [26,54,55]. This cytotoxic activity of NK cells and other cytotoxic cells such as CTLs has been shown to be initiated by Lamp-1 granules that are exocytozed during specific interaction with target cancer cells. In this process, the granule contents, including lethal proteins, namely perforin and granzymes, are delivered to the synapse formed between the CTL and the targeted cancer cells to induce killing [56–58].

### *Macrophages*

Low-dose radiation has been found to enhance concanavalin-A (Con A)-induced proliferation of splenocytes. This activation is not solely caused by the direct activation of splenocytes, as activation of macrophages in the spleen also appears to contribute indirectly [59]. Furthermore, LDR-mediated activation of macrophages is associated with the expression of B7–1 (CD80) and B7–2 (CD86) molecules, as well as secretion of IL-12p70 [60]. Activated macrophages kill susceptible tumor cells by means of a number of cytotoxic factors, of which nitric oxide (NO) plays a prominent role [61–65]. Irradiation of mice with γ-rays stimulates the production of NO by IFN-γ and LPS-treated peritoneal macrophages [66], which enhances the cytotoxic function of macrophages against P815 tumor cells. Upregulated secretion of NO in irradiated C57BL/6 has been found to enhance phagocytic function of peritoneal exudates [59], which can be abrogated by treatment of mice with CGN (lysosome-disrupting and phagocyte-damaging compound), suggesting a role of phagocytosis in LDR-mediated tumor response.

### *Dendritic cells*

Although dendritic cells are considered to be a part of both adaptive and innate immunity, their role in LDR-mediated response is more relevant owing to their antigen-presenting ability. Interestingly, LDR has also been shown to activate dendritic cells and augment T-cell activation. Coculturing of T cells with preirradiated dendritic cells has been found to enhance the proliferation of T cells, which was mainly caused by the secretion of cytokines (IL-2, IL-12 and IFN-γ) from dendritic cells rather than the expression of MHCs or costimulatory molecules on dendritic cells [67]. These observations suggest that low-dose radiation stimulates innate immunity cells, which further activates the cells of adaptive immunity, either by secreting cytokines or by upregulating costimulatory molecules. This probably influences the primary steps of an anti-tumoral immune response that may include dendritic cells attraction to the tumor site, uptake and processing of tumor antigens, migration to the secondary lymphoid organs, presentation to $CD4^+$ and $CD8^+$ T cells and activation of NK cells, and migration of activated tumor-specific T cells to the tumor site, thereby leading to tumor eradication. However, further studies are required to substantiate these suggestions.

## LDR & adaptive immunity

Sporadic data from animal studies have shown that LDR could enhance the immune response through augmentation of the proliferative response of T cells to antigenic, allogenic and mitogenic stimulation with a concomitant increase in cytotoxic effects on the tumor cells [68,69]. This has been linked to alterations in cytokine release, particularly the activation of IFN-γ and IL-2 production [44,52,70], which increases the expression of IL-2 receptors on the T-cell surface, facilitating signal transduction in T lymphocytes, enhancing splenic catecholamine content, lowering the serum corticosterone level and eliminating the radiosensitive subset of suppressor T cells [29].

Low-dose radiation-induced changes in the signaling network constitute a unique pattern distinctly different from those caused by high doses of radiation [45]. Furthermore, LDR stimulates the expression of TCR/CD3 [71], CD2 [72], CD4 [60] and CD28 [60] on lymphocytes and downregulates IL-10 synthesis by T cells as well as decreases CTLA-4 expression [45,73]. LDR-induced higher expression of surface markers both on APCs and T cells leads to a reduction of self-tolerance induced by tumor cells, resulting in an induction of anti-tumor immunity. LDR has also been shown to increase $CD4^+$ T-cell responsiveness reflected by upregulated levels of several chemokines and CD marker genes [74]. Moreover, enhanced $CD8^+$ CTL response following LDR has also been observed [59,75].

A subset of $CD4^+$ T cells, that is, Tregs, are known to protect the body from autoimmunity by controlling hyperimmune responses [76,77]. Different subsets of Tregs have been described, including $CD4^+CD25^+FoxP3^+$ Tregs, which can be naturally occurring (nTregs) or induced by antigen stimulation (iTregs) [78,79]. They control autoreactive T cells and negatively regulate exacerbated immune responses by suppressing $CD4^+$, $CD8^+$, B cells, NK cells, NKT cells, dendritic cells and macrophages [78–82]. Tumors induce Treg expansion by promoting the proliferation of nTregs and/or the conversion of naive $CD4^+CD25^-FoxP3^-$ T cells into $CD4^+CD25^+FoxP3^+$Tregs [83]. Higher number of Tregs during cancer progression is one of the immune-evasion strategies used by cancer cells [84]. Interestingly, a single dose of LDR has been shown to reduce the Treg population, which is directly linked to therapeutic response in the form of reduced tumor burden and prolonged survival [29,30], thereby suggesting a role of Tregs in LDR-mediated response. Further studies are required to establish the role of different subsets of Tregs in the LDR-mediated response. However, repeated LDR exposure has been shown to result in attenuation of arthritis, which appears to be partly caused by radiation-induced upregulation of immunosuppressive Tregs [85,86]. Both these studies suggest that the total dose and dose rate, as well as chronic and acute exposure of LDR, contribute to the sensitivity of Tregs and, therefore, the use of LDR in treating different diseases. Tregs suppress the immune system by a variety of mechanisms, including sequestration of IL-2, as well as secretion of immunosuppressive cytokines [87]. However, a reduction in Tregs following treatment with LDR is a major contributing factor for reduced self-tolerance and enhanced anti-tumor immunity. Disrupting T-cell tolerance can lead to autoimmunity, which is a limitation of tumor immunotherapy. However, in spite of a reduction in tolerance against self-antigens, LDR treatment does not induce but rather reduces autoimmune responses [42,43]. Therefore, LDR appears to not only decrease the chances of autoimmunity but also enhance anti-tumor immunity.

Apart from modulation of T-cell functions, LDR has been shown to enhance the humoral response. Indeed, treatment with LDR has been shown to increase antibody secretion and enhance the ADCC response in tumor-bearing mice, which is well correlated with tumor regression [55]. Activation of cellular immunity by a reductions in apoptosis of T-effector cells, Tregs and T-cell anergy (reduced self-tolerance), as well as induced cytolytic activity of cytotoxic T cells with enhanced ADCC response, collectively contribute to an effective anticancer immune response following LDR exposure.

## LDR-induced cytokine secretion

Natural killer cells, as well as activated macrophages, produce a number of cytokines that mediate the antineoplastic activity of these cells; that is, either directly suppress proliferation of and/or kill tumor targets (e.g., IL-1β, IFN-γ and TNF-α), or stimulate neighboring cells (in a paracrine manner) to secrete cytocidal factors (e.g., IL-1β, IL-12 and IFN-γ). Exposure of mice and rats to a single dose of total-body irradiation with x- or γ-rays at doses ranging from 0.04 to 0.25 Gy results in upregulated secretion and/or expression of mRNAs for IL-1β, IL-12, TNF-α (by macrophages), IFN-γ (by NK cells) and IL-2 (by splenocytes) [6,26,52,60,66,88–93].

Besides stimulating the immune system in normal mice, LDR has been shown to induce anti-tumor immunity in tumor-bearing mice. Tumors induce a predominantly Th2 response, leading to the secretion of anti-inflammatory (immunosuppressive) cytokines [94]. Exposure to low-dose irradiation not only reduces the secretion of immunosuppressive cytokines such as IL-10 and TGFβ [73], but also supports proliferation of cells by the secretion of growth-stimulatory Th1 cytokines such as IFN-γ, IL-2 and TNF-α [95–97]. Likewise, LDR induces a shift in cytokine profile from Th2 to Th1 in tumor-bearing animals, suggesting an enhancement of Th1-mediated anti-tumor immune response [98]. Furthermore, it has also been shown to enhance IL-1β, IL-18, IL-12 and G-CSF secretion [73,95,99].

Phytohemagglutinin and Con A stimulation of lymphocytes from the spleens of LDR-treated polycythemia (similar to leukemia; induced by a strain of the Friend virus complex [FCV-P] virus) mice induced more IL-2 and up to 15-times more IFN-γ than nonirradiated mice infected with FVC-P (composed of a Friend murine leukemia helper virus and a spleen focus-forming virus) [44,100,101]. Altered cytokine expression was found to be associated with an increase in NK-cell activity, implying that both innate and adaptive immune responses were activated [102]. LDR-induced anti-tumor immunity has been shown to enhance the survival (from 40 to >370 days) of animals [102]. Furthermore, treatment of tumor-bearing rats with LDR has been shown to elevate mRNA expression of IFN-γ and IFN-α in splenocytes, while decreasing TGF-β expression. Furthermore, the absence of mRNA expression of IL-4, IL-6 and IL-10 genes (responsible for selectively driving the differentiation of Th2 cells and inhibiting Th1 cells) promotes anti-tumor immunity. The mRNA expression of these genes was not altered when tumor cells were irradiated *in vitro*, suggesting that the mRNA for the genes that encode these cytokines is likely to be derived from normal cells in the host animal [52].

These studies suggest that LDR stimulates the immune system by activating innate immune cells in addition to enhancing T- and B-cell responses by critically balancing the cytokine profile (shift from Th2 to Th1), leading to effective anti-tumor immunity (Table 1).

## Low-dose radiation therapy of cancer

### *Preclinical studies*

Several preclinical studies have shown that low-dose irradiation offers an effective treatment for cancer through stimulation of the immune system [32–34,90,91,95,103–106]. Recent evidences have demonstrated that in animals exposed to single or fractionated low-dose x- or γ-rays, the growth of primary and/or metastatic tumors is inhibited or retarded [2,9,52,107,108]. The anti-tumor properties were detected when whole animals were irradiated prior to inoculation of neoplastic cells, indicating that the immune-surveillance mechanism might be involved [29,52]. Likewise, LDR treatment administered 24 h before implantation

**Table 1. Effects of low-dose radiation on the immune system.**

| Immune system | Modification following LDR regimen | Role of immune system in LDR response | Ref. |
|---|---|---|---|
| Cellular component | | | |
| *Innate immunity* | | | |
| NK | Increase in functionality | Lysis of tumor cells | [53] |
| ADCC | Increase | Lysis of tumor cells | [55] |
| Macrophage | Increase in functionality | Phagocytosis and antigenic presentation | [59] |
| Dendritic cell | Activated | Increase in T-cell proliferation and antigenic presentation | [67] |
| *Adaptive immunity* | | | |
| $CD8^+$ (CTL) | Increase in cytolysis | Lysis of tumor cells | [59] |
| $CD4^+$ | Enhanced responsiveness | Helping other immune cells | [74] |
| Th1 | Increase | Anti-tumor activity | [98] |
| Th2 | No change | Proinflammatory response | [52] |
| T-regulatory | Decrease | Breaking of tumor tolerance during carcinogenesis and induction of anti-tumor immunity | [30] |
| Secretary component | | | |
| *Cytokines* | | | |
| IL-2 | ↑ | T-cell proliferation | [5,31] |
| IL-12 | ↑ | Proinflammatory response | [99] |
| IFN-γ | ↑ | Phagocytosis and antigenic presentation | [97] |
| TGF-β | ↓ | Maturation and proliferation of T and B cells | [30] |
| IL-10 | ↓ | Immunactivation | [30] |
| TNF-α | ↑ | Proinflammatory response | [96] |

ADCC: Antibody-dependent cell-mediated cytotoxicity; CTL: Cytotoxic T lymphocyte; LDR: Low-dose radiation; NK: Natural killer.

of Lewis lung cancer cells has been found to significantly reduce tumor size, leading to an increase in mean survival time and a decrease in the 30-day mortality rate by 40% [33,106]. Similarly, reduction in pulmonary metastasis of B16 melanoma in the form of a reduction in the lung nodules of cancer cells has also been reported [34,90,91].

Low-dose radiation has been shown to enhance the efficacy of chemotherapeutic drugs [103–105], as well as enhance the efficacy of immunotherapy [109]. Moreover, pre-exposure to LDR has been found to reduce the incidence of lymphoma induced by high-dose fractionated total-body irradiation in C57BL/6J mice, which was accompanied by immunologic stimulation [33,110,111]. Taken together, these observations suggest that, owing to its immune-stimulation capacity, LDR can be used as an adjuvant in chemotherapy and immunotherapy of tumors, as well as to reduce the risk of secondary tumor formation during high-dose radiotherapy. Furthermore, it can also be considered as an adjuvant in immunotherapy, such as, for example, to enhance the efficacy of tumor vaccine, and so on.

Interestingly, the induction of thymic lymphoma by a challenging dose of 2 Gy was reduced from 46 to 16% when the animals (mice) were preirradiated with an adapting dose of 1 cGy/day for 5 days, although the adopting dose itself induced the same level (16%) of tumors [112]. These observations suggest that low-dose preirradiation possibly stimulates molecular responses that reduce the manifestations of damage induced by the challenging high dose that would otherwise result in the induction of thymic lymphoma. In a related scenario, reduced incidence of background mammary tumor has been found in older female rats (WAG/Rij) treated with low doses of γ-rays [113,114]. Similar results have been reported for lung and bone cancers in animals [115–117].

Several circumstantial evidences strongly indicate that low-dose preirradiation can modify latency for radiation-induced myeloid leukemia in CBA/H mice after exposure [108]. LDR has also been found to suppress artificial as well as spontaneous lung metastasis in mice, strongly suggesting immune enhancement by LDR [2,3]. Furthermore, growth of implanted tumor cells has also been found to be suppressed during the first 7 days after total-body irradiation, suggesting immune enhancement by LDR [118]. LDR has also been shown to alleviate immune suppression caused by the tumor burden by way of recovery of the NK activity of tumor-bearing mice [4]. Several circumstantial evidences strongly indicate that LDR causes anti-tumor effects through enhancement of the host immune response, unlike high-dose irradiation.

## Clinical studies

Low-dose irradiation has been found to be effective in obtaining higher degrees of latency in patients with different types of cancers, especially CLL and advanced stages of indolent low-grade NHL [29]. Indeed, LDR has been successfully used in the treatment of advanced stages of NHL, where an overall response rate of 70–90% for nodular lymphomas and 50–80% for diffuse type has been reported [119].

An increase in the survival rate of patients with NHL treated with chemotherapy along with LDR has recently been reported. The LDR-treated patients have also shown enhanced anticancer immunity [35]. Both total-body irradiation and half-body irradiation have been successfully used for the treatment of NHL [120–122]. LDR has also been successfully used to treat certain solid tumors such as ovarian cancer and colon cancer besides hematological cancer with no symptomatic side effects [35,123]. Half-body irradiation of an NHL patient appeared to result in control of a nasal tumor that was located outside the radiation field by stimulating the immune system [124].

## Conclusion

Reduction in the incidence of cancer in geographical areas with background radiation suggests that LDR may be used as a cancer-preventive strategy. Furthermore, LDR-induced local tumor control coupled with immune enhancement make LDR a feasible alternative to other conventional therapeutic modalities for the treatment of cancer. Although the induction of genomic instability observed at low doses raises the concern of cancer induction and increased risk for certain pathologies such as cataracts, and cardiovascular, digestive and respiratory diseases [125–128], LDR still appears to be an efficient anticancer treatment with negligible risk as summarized in this article. However, the effects of LDR on other subsets of T cells (Th17) and myeloid-derived suppressor cells have not yet been investigated. Comprehensive understanding of the various mechanisms underlying the modulation of immune response by LDR is likely to provide a fillip to the design of protocols using LDR as a preventive strategy as well as adjuvant to other therapeutic modalities to enhance the therapeutic gain. Since radiosensitivity varies considerably among individuals, development of biomarker(s) of radiosensitivity that are effective at low doses will help in the individualization of treatment protocols using LDR.

## Expert commentary

Geographical, animal and patient data suggest that LDR may be used as a preventive strategy and therapeutic modality, either alone or in combination with other existing therapeutic regimens. The interplay between LDR and the immune system specifically targeting Tregs opens a new area of investigation for redefining the mode of action and treatment protocol for enhancing therapeutic gain. The potential of glycolytic inhibitors such as 2-deoxy-D-glucose in enhancing the efficacy of LDR therapy merits systematic investigation, as recent preclinical studies strongly indicate that 2-deoxy-D-glucose stimulates the immune system in tumor-bearing animals, which was found to be directly linked with therapeutic response during radiotherapy in mice [129,130]. The induction of genomic instability observed at low doses raises the concern of cancer induction and other pathological conditions; however, LDR still appears to be an efficient anticancer treatment with negligible risk, as summarized in this article. Since radiosensitivity varies considerably among individuals, the development of biomarker(s) of radiosensitivity effective at low doses will help in the individualization of treatment protocols using LDR.

## Five-year view

Despite recent advancements in the field of LDR, investigators have continuously strived to find out the intrinsic pathways involved in the response at molecular, cellular and tissue levels. In the past, it has been found that LDR, besides inducing several processes such as antioxidant repair of DNA damage and apoptosis, also activates the immune system [3,6], which plays an important role in the eradication of tumor cells. In the past 5 years, significant advances have been made in the field of LDR-induced immunomodulation, which includes extensive study on the prevention of autoimmunity by LDR in mouse models (which is considered to be one of the most important drawbacks of anti-tumor therapy) [42,43]. Interestingly, antigen-presenting cells were also found to activate T cells as part of their function via several secretory cytokines rather than through MHCs after LDR exposure [67]. Furthermore, $CD4^+$ responsiveness was also found to be increased [74] and activation of $CD8^+$ was also observed [59]. Several studies on macrophage activation were also conducted and the role of glutathione in LDR-induced immune modulation has been established [59,66,96]. Moreover, regression of tumor after LDR has been found to correlate well with the activation of anti-tumor immunity, that is, Th1 response [98]. Finally Tregs, a subset of $CD4^+$ cells that are known to suppress anti-tumor immunity, have been found to be decreased after LDR treatment and correlate well with therapeutic response [30]. All together, these advancements in studies suggest that LDR-mediated immune-modulation is a well-orchestered phenomenon and that all immune components play an important role in the final outcome.

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## Key issues

- Radiotherapy is an important modality in the treatment of cancer, either employed alone in inoperable tumors or following surgery in many neoplasms. Despite significant advances in medical physics, resistance of tumors to therapy in addition to systemic and normal tissue toxicity has limited the success of anticancer treatment.
- Several investigations during the last decade have shown that low-dose radiation (LDR) is an even more effective in cancer therapy than the conventional daily doses of 1–2 Gy. While, high doses of radiation are known to suppress the immune system, low doses have been shown to enhance immune response, thereby reducing systemic toxicity besides enhancing anti-tumor response.
- Epidemiological data have shown that inhabitants of high background radiation in India (Kerala), Brazil, China, the USA, the Misasa Radon spa area of Japan and many other parts of the world have lower cancer mortality than those living in the areas of normal background radiation. A significantly lower rate of cancer mortality among the population residing in the Guangdong (China) area has been found to correlate well with immune enhancement, mainly in the form of enhanced sensitivity of peripheral blood lymphocytes to mitogenic stimulation. Similar results have been reported in occupational radiation workers, patients exposed to LDR used for diagnostic purposes and in experimental studies with animals.
- Activation of several immune cells such as NK cells, dendritic cells, macrophages and T cells, as well as an increase in mast cell activity, has been reported following the treatment of tumors with low-dose regimens. A decrease in T-regulatory cells, altered cytokine response such as increases in IL-2 and IFN-γ secretion, and decreases in TGF-β levels and antibody production have also been observed.
- Experimental studies with low-dose x-rays and γ-rays in different strains of mice have been shown to decrease the growth rate of tumors as well as metastasis, which correlate well with immune enhancement. Indeed, fractionated total-body irradiation using low doses of ionizing radiation (LDR) has been used in the treatment of chronic lymphocytic leukemia and advanced stages of indolent low-grade NHL with good results, which has been shown to be accompanied by enhanced anticancer immunity.

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•• of considerable interest

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