3.3 Evidence for beneficial low level radiation effects
and radiation hormesis

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Abstract

Low doses in the mGy range cause a dual effect on cellular DNA. One effect concerns a relatively low probability of DNA damage per energy deposition event and it increases proportional with dose, with possible bystander effects operating. This damage at background radiation exposure is orders of magnitudes lower than that from endogenous sources, such as ROS. The other effect at comparable doses brings an easily observable adaptive protection against DNA damage from any, mainly endogenous sources, depending on cell type, species, and metabolism. Protective responses express adaptive responses to metabolic perturbations and also mimic oxygen stress responses. Adaptive protection operates in terms of DNA damage prevention and repair, and of immune stimulation. It develops with a delay of hours, may last for days to months, and increasingly disappears at doses beyond about 100 to 200 mGy. Radiation-induced apoptosis and terminal cell differentiation occurs also at higher doses and adds to protection by reducing genomic instability and the number of mutated cells in tissues. At low doses, damage reduction by adaptive protection against damage from endogenous sources predictably outweighs radiogenic damage induction. The analysis of the consequences of the particular low-dose scenario shows that the linear-no-threshold (LNT) hypothesis for cancer risk is scientifically unfounded and appears to be invalid in favor of a threshold or hormesis. This is consistent with data both from animal studies and human epidemiological observations on low-dose induced cancer. The LNT hypothesis should be abandoned and be replaced by a hypothesis that is scientifically justified. The appropriate model should include terms for both linear and non-linear response probabilities. Maintaining the LNT-hypothesis as basis for radiation protection causes unreasonable fear and expenses.

Ionizing radiation and endogenous toxins at low doses

All agree that cellular responses to low values of absorbed doses of ionizing radiation are not readily predictable by extrapolation of responses observed at high doses. One reason for this unpredictability is in the physics of energy distribution in low-dose exposed tissues. In case of penetrating radiation, particle tracks arise stochastically throughout the exposed tissue with the relatively low density at low doses [1]. These tracks generate on the one hand unevenly distributed ionizations and excitations of constituent molecules along the track path, as well as bursts of reactive oxygen species (ROS) [2]. In case of exposure to internal emitters, the distribution of particle tracks is determined by the distribution of the emitter in tissue [3].
The lower the radiation fluence or number of particle emitters in a given tissue mass, the less crowded are the particles in the exposed mass and with them the more heterogeneous is the distribution of ionized molecules and of ROS bursts.

The other reason is the presence of compounds that may aggravate or reduce radiation effects; there is especially the abundant and constant metabolic generation of ROS and of other endogenous toxins, on top of which low-dose radiation acts [2, 4]. The quotient between the rates of endogenous and radiogenic ROS production at background radiation exposure strongly favors the former. In fact, the average production rate of endogenous DNA double strand breaks (DSB) per cell per day in the body is about $10^3$ times higher than that of radiogenic DSB from background irradiation assumed overwhelmingly to be low-LET type. However, at low-LET irradiation the probability of radiation induced DSB per primary DNA alteration of any type is about $10^5$ times higher than that caused endogenously [5]. This data set attests not only that endogenous DNA damage far outweighs radiation induced DNA damage at background level exposure, but also that irradiation is far more effective in causing DSB than are endogenous ROS.

**Ratio of DNA damage and cancer probabilities**

Radiation induced DNA damage increases with absorbed dose [6]. Such cellular effects come through direct energy deposition events from traversing particle tracks by which DNA damage rises proportional with dose. Dependent of the amount of energy deposited per cell, bystander effects in non-irradiated neighboring cells may add to this damage in tissue at low doses [7, 8]. By measuring damage in multicellular systems, values of damage per exposed cell or defined micromass are calculated averages. This implies that any bystander phenomenon that may have occurred is coregistered and expressed in the oberved values from which these calculations were made [9]. A dose of 1 mGy of low-LET radiation, such as 100 kVp x-rays, causes on average the following effects per potentially oncogenic stem cell with an average mass of 1 nanogram: 1 particle track; about 150 ROS; 2 DNA alterations of any kind; $10^2$ DSB; $10^4$ chromosomai aberrations; and the probability of an oncogenic transformation of the hit cell with lethal outcome is about $10^{-13}$ to $10^{-14}$ [10, 11, 12]. In other words, the ratio of the probabilities for radiation induced lethal cancer and the corresponding DSB is about $10^{11}$ to $10^{12}$. This means that the statement of even one DSB to pose a risk of causing a lethal cancer to develop from the affected cell is unreal and, in fact, scientifically unfounded.
Adaptive Responses, Protection

A sudden suprabasal yet non-lethal rise of toxin concentration in a biological target tends to elicit stress responses and to stimulate adaptation usually in terms of protective mechanisms in the sense of hormesis [13]. Increasing evidence in the literature over the past 25 years indicates that adaptive protection responses occur in mammalian cells in vivo and in vitro after single as well as protracted exposures to X- or γ-radiation at low doses. Not only the occurrence of adaptive protection but also the nature of some biochemical mechanisms involved have been reported [4, 11, 12, 14, 15, 16, 17]. There appear to be two principal types of adaptive protection, one is to prevent and repair DNA damage and in doing so to keep cells alive and functioning properly. The other is to remove damaged cells from tissue by inducing apoptosis, terminal differentiation, and immune responses and thus to reduce genomic instability in the tissue system and eliminate mutated cells.

Contrary to the immediate begin of repair after DNA damage has occurred, adaptive protection develops as adaptive response relatively slowly within a few hours, may last for several weeks to months, and resemble physiological stress responses that protect against accumulation of DNA damage in tissue. This damage may be from any source such as from metabolically generated or environmental toxins or renewed irradiation [18]. Such protective responses occur in various ways. They appear to depend on mammalian species, individual genomes, cell types, cell cycle, and cell metabolism. Adaptive protection categories after single low-dose, low-LET irradiation, are as follows:

Damage prevention
Stimulation of the radical detoxification system that appears to reach a maximum at about 4 hours after irradiation and lasts for several hours to even weeks, depending on tissue and cell type. In mouse bone marrow in vivo, there was a delayed and temporary reduction of the incorporation of DNA precursors and of thymidine kinase activity to some 70 % of control with a concomitant rise of free glutathione; the effect slowly declined over a period of about 6 hours. [10, 19, 20, 21]. In other low-dose irradiated rodent tissues, increased levels of superoxide dismutase (SOD) occurred in parallel with decreased lipid peroxidation lasting for weeks [22, 23] and an elevated level of glutathione up by a factor of close to five in spleen cells was involved in an increase in natural killer cell activity [24]. ROS detoxification was also linked to gene activation. Thus, mRNAs for glutathione synthesis-related proteins in the
mouse liver became elevated after low-dose gamma irradiation [25]. The increase in intracellular glutathione caused by low-dose in RAW 264.7 cells had its maximum between 3 and 6 hours after exposure; this effect was mediated by transcriptional regulation of the gamma-glutamylcysteine synthetase gene, predominantly through the AP-1 binding site in its promoter [26].

*Damage repair*

Protection against high-dose induced chromosomal aberrations in human lymphocytes increased to a maximum about 4 hours after a conditioning low-dose low-LET irradiation; the protection also operated against other DNA damaging agents [27, 28]. This protection covered up to about 30% of the damage seen in non-conditioned controls and varied between individuals and cells types; it was absent in some individuals and is probably determined genetically [29, 30]. Where it operates, it appears to last up to about 3 days, as reported for various human cells *in vivo* as well as in culture [15]. This adaptive response probably involves a several-fold enhancement of the DNA repair rate [31, 32] with the slow component of DSB repair being much faster at 0.5 Gy x-rays than that seen at 2 Gy [33]. Another adaptive response of this type appeared regarding micronuclei formation in human fibroblasts [34]. In these cells, conditioning doses from 1 to 500 mGy were equally effective; this also indicated that at the lowest dose, when approximately 40% of the cells did not experience an energy deposition event, a bystander effect was involved in causing the adaptive protection [35]. A similar set of data in fibroblasts showed constancy of the adaptive protection over a dose from 1 to 100 mGy gamma-rays using the micronucleus assay [36]. The degree of inhibition of DNA synthesis and cell growth in rat glial cells in culture by a high dose of x-rays was reduced by about one fourth to one third at several hours following a conditioning low-dose exposure, when the cells were obtained from young rats. The adaptive response decreased with age of the donor rats. This adaptive response involved protein-kinase C (PCK), DNA-dependent protein-kinase (DNA-PK), and phosphatidylinositol 3-kinase (PI3K), as well as the activity of the ataxia-telangiectasia gene (ATM) [37].

*Damage removal by apoptosis*

Damaged cells may be induced into apoptosis by intra- and intercellular cellular signaling. Apoptosis also may occur within hours after high-dose irradiation. Low-dose induced apoptosis of pre-damaged cells with replacement by healthy cells may be a major route of *in vivo* removal of oncogenically transformed cells [38, 39, 40, 41, 42, 43, 44]. Low-dose induced apoptosis is assumed to operate also through intercellular
signaling from normal cells, which may also be activated by transformed cells in culture [45, 46]. Non-growing human fibroblasts in culture with DSBs from low-dose low-LET irradiation readily lost this damage to the level of DSBs in non-irradiated control cells after induction of proliferation; this damage removal was mainly due to apoptosis [47]. Low-dose induced enhancement of DNA repair may be responsible for the observation in rat thymocytes, where the incidence of radiation-induced apoptosis first declined at low doses and only rose with higher doses [48]. The induction of apoptosis apparently requires a certain level of DNA damage.

**Stimulation of immune response**

Removal of damaged cells occurred in vivo by way of a low-dose induced immune competence [49, 50]. This was, in another study, associated with a reduction in the incidence of cancer metastases to less than one third of control concomitantly with an increased number of circulating cytotoxic lymphocytes [51]. Such response had its maximum in vivo at about 0.2 Gy [52]. Low-dose induced immune competence may last for several weeks [53].

**Protection and cell cycle**

Damaged cells also may exit the system by premature differentiation and maturation to senescence [54]. This was observed to occur also via bystander effect in microbeam experiments directed to single cells in complex tissue [55]. The various mechanisms of protection may be directly or indirectly linked to transient changes in the activity of the G1 cell cycle checkpoint [56]. Another mechanism in this category of damage removal is known to occur in a number of tissue culture cell types by way of hypersensitivity to low-dose radiation that disappears at higher doses [57, 58]. This hypersensitivity in some cells was linked to the cell cycle [59, 60] and it disappeared in a number of culture cells within about 4 hours, but not immediately, after a single low-dose, low-LET irradiation [61]. Radiation-induced predisposition to genetic instability in culture cells also declined following low-dose irradiation [62]. These data indicate prevention of damage removal by way of low-dose induced DNA repair.

**Reduction of carcinogenesis**

The coordinated action of these protective responses, in one form or another may be responsible for the observation of a reduction of spontaneously occurring cancers. In fact, single low doses of low-LET radiation in tissue culture cells initiated with a delay of 1 day,
but not immediately, a significant reduction of spontaneous clonogenic transformation to about one third of control [63, 64, 65]. There is indication that this low-dose suppression of oncogenic transformation is not in response to cellular glutathione [66]. It can involve bystander phenomena likely through extra-cellular signaling exchange [67]. In mice heterozygous for the Trp-53 gene, a single low dose of low-LET radiation given at the age of about 2 months significantly delayed the appearance of "spontaneous" lymphoma and spinal osteosarcoma later in life [68]. A review on tumor development following low-dose, low-LET irradiation in rodents showed the existence of a threshold dose [69]. This is supported by a recently published study of induction of lymphomas, solid tumors, and ovarian tumors in BC3F1 female mice that at the age of 1 month or 3 months received single whole body doses up to 32 cGy of low-LET radiation; the threshold dose was 4 cGy [70]. Several human epidemiological studies also indicate either a threshold or a reduced cancer incidence below control following a single low-dose irradiation [5, 15, 16, 40].

**Low-dose induced changes in gene expression**

The above listed categories of adaptive protection involve changes in gene expression [4, 25, 26, 37, 71]. An example for DNA repair gene activation refers to the telangiectasia gene [37]. Human fibroblasts in culture showed DNA repair in the course of adaptive protection against micronucleus formation following acute high-dose irradiation; the repair was more effective in the gene poor chromosome than in the gene rich chromosome of the cells [72]. Another data set showed that exposure of human skin fibroblasts in culture to a single dose of 20 mGy γ-radiation caused more than 100 genes to change their expression within 2 hours. This gene group included stress response genes and was different from the group of genes in parallel cultures that concomitantly responded to 500 mGy [73]. A similar pattern of expression amongst a total of 1574 genes developed in the γ-irradiated mouse brain more at 30 min. than at 4 hours, with 30 % of the genes exclusively affected by 0.1 Gy [74].

**A common pattern**

Despite the disparity of the examined systems and responses, there appears to be a common pattern in the data. In fact, adaptive protection following low doses of low-LET radiation appears to be the consequence of changed cellular signaling and to be ubiquitous. Adaptive protection is a physiological expression of cellular capabilities to maintain integrity of tissue structure and function in the face of various exposures to potentially toxic agents including ROS, be they from endogenous sources or from ionizing radiation [5, 75, 76]. One
might speculate that DNA damage accumulation from any source eventually conditions a cell to become susceptible to apoptosis induced by low doses including that from background radiation exposure [2]. In this sense, background radiation exposure comes into focus as a possible trigger for maintaining tissue homeostasis.

Regarding their dependence on absorbed dose, the above listed categories of adaptive protection are schematically summarized in Figure 1. Except for apoptosis and terminal cell differentiation, all the above protective responses to single exposures tend to be expressed maximally after less than 0.1 and not after more than 0.5 Gy X- or γ-radiation [10, 77, 78] and to increasingly fail with higher doses depending on type of adaptive protection in a given cell system, as summarized previously [5, 10, 11, 12]; in most mammalian cells so far examined, the expression of adaptive protection had a maximum above 5 mGy and below about 200 mGy.

Fig. 1:
Single low-dose induced adaptive responses have a protecting function through various mechanisms. Note that mechanisms of DNA damage prevention and repair and the immune stimulation decrease after a maximum at doses between 0.1 – 0.2 Gy, in contrast to apoptosis incidence that increases with dose. Absorbed dose is in Gy and also in terms of microdosimetry, in that the mean energy deposition per particle traversal per defined micomass (specific energy $\bar{z}_i$) (ICRU 1983) is multiplied by the number of such events ($N_H$) in the number of exposed micomasses ($N_E$).
Regarding the duration of their effectiveness, Figure 2 gives a schematic summary of available published data. The time scales of duration of adaptive protection of various kinds are crucially important for the assessment of dose rate effects [79]. Depending on radiation type and dose rate, energy deposition events per defined micromass such as a cell happen at certain average time intervals. The time interval between repetitive energy deposition events in a defined biological target at a given dose rate may determine to what degree damage or adaptive protection prevails.

![Graph of Low-Dose (low-LET) Induced Adaptive Protection](image)

Fig. 2:
Single low-dose induced adaptive responses have different times of duration depending on protective mechanisms, that begin with a delay of several hours and may last for up to months regarding immune response. Note that repair in response to radiation damage begins immediately after damage has occurred.

Since DNA damage and cancer in mammals arise mainly from non-radiogenic sources, it is justified to relate the low-dose induced various adaptive protection mechanism mainly to non-radiogenic, i.e., "spontaneous" DNA damage and cancer in addition to their potential effect against radiogenic damage and cancer, as presented in more detail in a model elsewhere [5, 11, 12, 17]. A summarizing graphical display applying the model of risk evaluation after single low-dose irradiation is shown in Figure 3. It illustrates in principle that low-doses induce adaptive protection against DNA damage and its accumulation in tissue, mainly from endogenous, i.e., "spontaneous" sources and thus counterbalances effects from radiation.
exposure. The net risk of cancer, then, becomes lower than predicted by the LNT-hypothesis, or even negative with more benefit than damage to the low-dose exposed system.

**Fig. 3:**
The dual effect of single low-dose irradiation is schematically analyzed according to a simplified model (see also text). This encompasses as a function of dose D, i.e. of \( \frac{N_H}{N_E} \) for a given radiation quality, the following probabilities: a) of DNA damage induction per energy deposition event \( z_i \) (see Fig. 1), \( p_{\text{ind}} \), with a potential contribution from bystander effect, in red; this function appears linear with dose beyond the contribution from bystander effect; b) of the net protection provided by dose dependent mechanisms, \( p_{\text{prot}}(\frac{N_H}{N_E}) \), plus of apoptosis \( p_{\text{apo}} \), both against spontaneous cancer with the probability \( p_{\text{spo}} \) per affected cell, in green.

The net cancer risk derives from the difference between cancer induction and prevention at the various dose levels; the solid curve of net cancer risk is without protection from apoptosis, and the dotted curve of net cancer risk is with protection from apoptosis.

**Summary**

1) Ionizing radiation causes DNA damage in mammalian cells proportional with dose with additional possible bystander effects. 2) At background radiation exposure levels, DNA damage comes overwhelmingly from non-radiation sources. 3) The probability of radiation induced adaptive protection measurably outweighs that of damage from doses well below 200 mGy low-LET radiation. 4) The delayed and temporary adaptive protection at low doses involves damage prevention, damage repair, and immune responses. They appear to operate
primarily against DNA damage from non-radiation sources. Moreover, apoptosis and terminal cell differentiation also occur at higher doses and tend to remove susceptible damaged cells as does the low-dose induced stimulation of the immune system. Cell removal reduces genomic instability and mutated cells from tissue. 5) At higher absorbed doses in tissue, cell and DNA damage appear increasingly to overrule, negate, or annihilate the more subtle signaling effects seen after low doses that lead to adaptive protection, whereas apoptosis and terminal cell differentiation continue to function. 6) The linear-dose-risk function appears invalid and should be replaced by a function that includes both linear and non-linear terms. Basic research data and human epidemiological data conform to threshold or hormesis in the low-dose range.

References


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