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INVITED REVIEW

How hematopoietic stem cells respond to irradiation: similarities and differences between low and high doses of ionizing radiations

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In this review, we will specifically address the newest insights on the effect of low doses of ionizing radiations on the hematopoietic stem cells, which are prone to long-term deleterious effects. Impact of high doses of irradiation on hematopoietic cells has been widely studied over the years, in line with the risk of accidental or terrorist exposure to irradiation and with a particular attention to the sensitivity of the hematopoietic system. Recently, more studies have focused on lower doses of irradiation on different tissues, due to the increasing exposure caused by medical imaging, radiotherapy or plane travelling for instance. Hence, we will delineate similarities and discrepancies in HSC response to high and low doses of irradiation from these studies.

The scientific understanding of radiation effects is a constantly evolving field. However, it can sometimes be tricky to define doses of radiation, given the different units in which it can be expressed. The "absorbed dose" is expressed in Gray (Gy), while the "equivalent dose" and the "efficient dose" are expressed in Sievert (Sv). Although both correspond to the same units in the International Units System (J/kg), the relationship between these parameters implies several multiplicative factors taking into account the type of radiation, the organ sensitivity or the species [1]. For laboratory studies, the unit commonly used is the Gy, while in epidemiology studying human exposure, it is the Sv, which makes it uneasy to compare them. Moreover, it also depends on the type of radiation, its energy or linear energy transfer (LET), and its relative biological effectiveness (RBE) [2].

Despite this, effects of high doses of irradiation (HDIR) are quite well characterized. Counteracting these effects involves the activation of pathways of the DNA repair response. In humans, the most sensitive tissue is the hematopoietic system: after exposure to irradiation, changes in blood composition might be evidenced with irradiation doses as low as 1 Gy. Death can occur starting at 2 Gy and is certain above 7 Gy in 4 to 6 weeks as a result of hematological failure [3]. At higher doses, other organs (intestines, skin) are also touched. It has long been stated, in particular for HDIR, that irradiation implies either a linear dose-dependent effect or threshold effect, relying mainly on DNA damage. However, debates regarding the effects of low doses of irradiation (LDIR) remain, the definition of a low dose itself being unclear. Doses inferior to 500 mGy, sometimes up to 1 Gy, are often considered low doses, and we refer to that in what follows.

Recently, more concerns over the potential danger of LDIR (below the threshold considered dangerous by the authorities and therefore authorized for medical imaging, for instance) have arisen because of the increased risk of developing leukemia and brain tumors in children who have undergone several computed tomography (CT) scans, with total received doses

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ranging from 0 to 50 mGy in the bone marrow (BM) and 0 to 350 mGy in the brain [4,5]. It became a European priority (RISK-IR European network) to characterize how LDIR affects these organs, considering their sensitivity, in particular for the blood system and hematopoietic stem cells.

Hematopoietic stem cells (HSCs) are responsible for the sustainability of the blood system of an individual lifelong. For such long-term maintenance, they physiologically exhibit intrinsic properties such as self-renewal and multipotency, which allows them to differentiate into all blood cell lineages while keeping a pool of HSCs. However, HSCs are very sensitive to any stress. The smallest perturbation in the system, such as infection (for review, see [6]), blood loss [7], chemical or physical agents [8,9], or genotoxic stress [10], may trigger cell cycle entry and HSC differentiation to sustain blood cell production.

To date, we have been able to isolate populations enriched in HSCs using a combination of cell surface markers. For murine HSCs, the accepted phenotype for a population with long-term reconstitution potential is $Lin^{-}Sca1^{+}cKit^{+}$(LSK) $CD150^{+}CD48^{-}CD34^{-}$ [11,12]. For human HSCs, the purest population is $Lin^{-}CD34^{+}CD38^{low}CD45RA^{-}CD90^{+}$ [13,14], but even in this population, only 10% of the cells are "true" HSCs under the best conditions [15]. Some additional markers such as CD49f [15] or CD133 [16] can also be used to better characterize human HSCs.

HSC properties need to be very carefully regulated and preserved to avoid numerous pathologic conditions such as their exhaustion, causing hematopoietic failure, or their leukemic transformation. Although all mechanisms for preservation of HSCs are not fully understood yet, many pathways have been found to be involved in the maintenance of their quiescence, self-renewal, or differentiation properties under physiological conditions. This regulation can be extrinsic, related to surrounding cells (HSC niches) and environmental conditions in the bone marrow [17]. Several adhesion molecules (such as E-selectin [18], VCAM-1/$\alpha4\beta1$ integrins [19], and JAM-C/JAM-B [20,21]) and secreted growth factors (SCFs [22], CXCL12 [23] ligand of CXCR4, thrombopoietin (TPO) [24], angiopoietin [25], BMP4 [26]) are involved in the retention of HSCs in their niches, therefore contributing to their maintenance. Low levels of oxygen (hypoxia) [27] have also been reported by many groups to be highly essential for the regulation of HSC maintenance, together with hypoxia-inducible factor (HIF) proteins, which regulate, in a cell autonomous way, HSC quiescence and self-renewal properties [28,29].

Other intrinsic factors such as transcription factors (i.e., Gata2, Bmi-1 [30,31], Pbx1 [32], or SCL/TAL1 [33,34]), cell cycle regulators (i.e., p21 [35], p27 [36], or p57 [36,37]), chemokines, cytokines, and growth factor receptors (e.g., cKIT, MPL, CXCR4) also regulate HSC fate. Finally, actors involved in irradiation-induced stress responses also play a role in maintaining HSC stemness under steady-state conditions, as well as after exposure to irradiation or other genotoxic stresses.

In this review, we address the similar and different effects of high and low doses of irradiation (HDIR and LDIR) with respect to the steady-state regulation of HSC properties, focusing on components involved in the DNA-repair response (DRR) and reactive oxygen species (ROS) pathways.

Involvement of DRR and ROS pathways in HSC maintenance

The DRR comprises several pathways depending on the DNA damage generated (for review, see [38]). Under steady-state conditions, these pathways also act to preserve DNA integrity, for instance, during the replication process, and the quiescence and self-renewal properties of HSCs. Several proteins, such as DNA-PKc [39,40], ligase IV [41,42], and ku70/80 [39,42–44] involved in nonhomologous end joining (NHEJ), BRCA2 [45] involved in homologous recombination (HR), XPD [42] and ERCC1 [46–48] involved in nucleotide excision repair (NER), and ataxia telangiectasia mutated (ATM) [49] play a role in maintaining HSC functions. In competitive transplantation experiments, HSC mutants exhibit a loss of functionality. In most knockdown mouse models, the number of HSCs is drastically decreased compared with the number in wild-type (WT) mice. In particular, $atm^{-/-}$ mice exhibit a decreased frequency of HSCs with a defect in their reconstitution capacity. ROS levels are increased in $atm^{-/-}$ compared with WT HSCs. When mice are treated, in drinking water, with *N*-acetylcysteine (NAC), an ROS scavenger, ROS levels of $atm^{-/-}$ HSCs decrease while their reconstitution potential, in particular their long-term reconstitution capacity, is restored [49]. Interestingly, NAC treatment can prevent activation of the p38MAPK signaling pathway [50]. The same effects can be observed after treatment with SB203580, a p38MAPK inhibitor [50]. These defects in $atm^{-/-}$ HSCs can be linked to HSC exhaustion as $atm^{-/-}$ HSCs are less quiescent than WT HSCs, with increased expression of genes involved in cell cycle progression ($p16^{ink4a}$ and $p19^{arf}$). Interestingly, NAC treatment, as well as SB203580 treatment, also induced a decrease in expression of these genes and prevented $atm^{-/-}$ HSC exhaustion. Together, these data indicate that under steady-state conditions, ATM contributes to protecting HSC quiescence and self-renewal potential by controlling ROS levels and p38MAPK activation [49,50].

Under steady-state conditions and independently of ATM, ROS involvement in loss of self-renewal potential is well documented. HSCs have a very specific metabolic activity linked to the hypoxic environment in which they reside. Moreover, HSC intrinsic metabolism is correlated with a low level of ROS [51,52]. Indeed, HSCs rely mostly on anaerobic glycolysis [53] or fatty acid oxidation [54] instead of oxidative phosphorylation. An increase in ROS levels, caused by deletion of several regulators such as AKT [55], mTOR [56], and FoxOs [57,58] proteins, and ATM as mentioned above, induces an exit of quiescence and loss of self-renewal properties. In WT mice, ROS levels increase on serial transplantation [50,59], and NAC treatment of donor mice as well as recipient mice contributes to the reduction of ROS levels and to the protection of HSC reconstitution capacity during serial transplantations [50,60].

Downstream effectors induced by ROS are equally involved in the maintenance of stemness. For instance, inhibiting the degradation of p53 [61] or inducing activation of p38MAPK [62] impairs the functions of HSC, mimicking the effects of elevated ROS. However, important regulators of ROS such as NRF2 [63] can also be involved in an ROS-independent manner in the regulation of HSC properties [64]. Indeed, unlike other tissues, ROS levels are not elevated in *nrf2*$^{-/-}$ mice bone marrow (BM), and NAC is not able to rescue the functional defects caused by NRF2 deficiency. Moreover, HSCs exhibiting the highest self-renewal potential were reported to be mainly quiescent, in the G0 state, and even dormant, dividing only a few times in an entire lifetime [12]. As mentioned above, increased ROS levels induce exit of quiescence of HSCs, and these features are regulated by different members of the Cdkn family, such as p21 [35] and p27 and p57 [36,37], which are themselves targets of effectors previously cited, such as p53 [65].

High doses of ionizing radiations

Radiosensitivity is usually linked to three specificities of a cell: its division speed, the length of its dividing future, and its undifferentiated state [61]. Usually acute radiation lethality arises from anemia or infection caused by hematopoietic failure and pancytopenia. Long-term delayed effects such as leukemia are also predominant after nonlethal irradiation, as witnessed in a significant proportion of the Hiroshima and Nagasaki bombing survivors [66]. They occur when DNA of surviving immature cells is damaged. The probability of developing a malignancy usually increases with the dose.

The mechanisms by which these short- and long-term effects occur are today quite well characterized (Table 1) in hematopoietic stem cells as well as in many other cell types, in several species, and have already been reviewed elsewhere [3]. Mainly, ionizing radiation produces free radicals and ROS and nitrogen oxide species (NOS) [67], which in turn induce mitochondrial and DNA damage, mostly double-strand breaks (DSBs) but also oxidative DNA damage, and eventually lead to apoptosis or senescence if needed. In response to this damage, different pathways, such as the NHEJ, HR, base excision repair (BER), or nucleotide excision repair (NER), and ATM pathways, are activated either to repair the DNA damage or to induce apoptosis, if the damage is significant. Induction of these pathways leads to activation of the p53 and p38MAPK pathways.

Total body irradiation (TBI) of mice with ^{137}Cs at 6.5 Gy induces HSC senescence via the cyclin-dependent kinase inhibitors p16, p21, and p19 specifically [68]. It was also reported to induce a decrease in clonogenicity in selective LSK cells compared with more mature progenitors [69]. HSC frequency and reconstitution potential were also decreased. This is due to the

Table 1. Comparison of effects of high versus low doses of ionizing radiation on hematopoietic stem cells[a]

	HDIR	LDIR
DNA DSB	Yes [70,74]	High LET: yes [82,83] Low LET: no [84,85]
ROS increase	Yes [69,70]	Yes [82–85]
Oxidative DNA damage	Yes [69,70]	Yes (human) [84]
Autophagy	Yes [91]	Yes (mouse) [85]
Apoptosis	Yes [3,73,74]	No [84,85]
Senescence	Yes [68]	Unknown
Cell cycle changes	Yes [73]	No [84,85]
Functional defects	Immediate [69,74]	Mouse : immediate + delayed [82,83,85] human: delayed [84]
Epigenetic modifications	Yes [92]	High LET: yes [81] Low LET: unknown but probable

[a]Contrary to what had been supposed for a long time, LDIR do induce defects, but mostly because of oxidative stress rather than DNA DSBs and apoptosis.

persistent increase in ROS, observed several weeks after irradiation, that leads to oxidative DNA damage and DSBs, as reflected by 8-hydroxy-2-deoxyguanosine (8-OH-dG) and γH2AX staining, which respectively mark guanine oxidation on DNA and phosphorylation of γH2AX following DSBs [70]. These effects were reversible with addition of the antioxidant NAC, indicating the implication of the ROS. Moreover, the authors found that the NOX4 pathway is also involved in these effects using an inhibitor of this protein. Resveratrol, another antioxidant, protects HSCs from HDIR via the Sirt1 pathway; Sirt1 is a histone deacetylase protein involved in responses to stress and aging [71]. Another study on mouse cells revealed that p53 mutations have important consequences on HSC response to irradiation, conferring resistance [72]. Lee and Bernstein [72] hypothesized that it was due to either better DNA damage repair or cell cycle arrest properties. More recently, two teams (Passegué's and Dick's) discussed p53 involvement in HDIR effects on murine and human HSCs, respectively, pointing out some differences in the way they respond. Passegué's group found that after ex vivo irradiation between 2 and 4 Gy, HSC clonogenicity improved compared with that of more mature populations [73]. This seems contradictory to previous articles cited here, but could be explained by the difference in the irradiation protocol (in vivo vs. ex vivo) and lower doses (2 Gy vs. 6 Gy). In addition, HSCs were found to be less vulnerable to apoptosis than multipotent progenitors (MPPs) after irradiation, relying more deeply on p53-mediated growth arrest and NHEJ repair, more commonly used than HR in quiescent cells. However, HSC mobilization and activation in culture, provoking cell cycle entry, attenuating p53 response, and increasing HR, do not change the radiosensitivity or induce increased apoptosis, mimicking only partially MPP behavior. This suggests that quiescence is not the only mechanism driving the specific HSC behavior in response to IR. However, Dick's team found that contrary to mouse cells, human cord blood HSCs ($Lin^-CD34^+CD38^-CD45RA^-CD90^+$) exhibited delayed DSB rejoining, γH2AX foci remaining a longer time, enhanced apoptosis, and diminished clonogenicity compared with more mature populations (MPP, $Lin^-CD34^+CD38^-CD45RA^-CD90^+$ and progenitors, $Lin^-CD34^+CD38^+$) [74]. They confirmed that the p53 pathway is also essential to the irradiation response in human HSCs. Indeed, in both mouse and human cells, inhibition of p53 leads to radioprotective effects, increased clonogenicity, and *in vivo* hematopoietic reconstitution potential. Nevertheless, the mechanisms underlying these protective effects are different, correlating with the way human and mouse HSCs respectively deal with IR: apoptosis is decreased in human HSCs, while cell growth is increased in mouse HSCs. Of note, deletion of downstream effectors of p53 such as PUMA [75] and BCL2 activation [74] suppresses apoptosis and allows mice to survive irradiation and HSC clonogenic potential to be enhanced. However, radioprotection by p53 downregulation comes at a price, as p53-defective HSCs, but not BCL2-overexpressing HSCs, exhibit increased γH2AX foci and defective self-renewal on serial transplantation.

To sum up, HSCs are strongly affected by HDIR and exhibit mostly oxidative stress and DNA damage such as DSBs (Table 1). This damage is then handled by either apoptosis or repair, but it may give rise to errors in the DNA code, mainly through NHEJ, eventually leading to genomic abnormalities in the progeny [73] and even leukemic transformation. Furthermore, discrepancies in the way HSCs deal with DNA damage may differ depending on numerous factors, such as species, HSC ontogenic origin, dose, and type of irradiation. Of note, chemotherapeutic agents such as busulfan, implying chemical instead of physical aggressions, can have similar functional effects but different mechanisms of action [76,77].

Low doses of ionizing radiations

The way HSCs deal with lower doses of irradiation might, however, be different (Table 1). As stated before, doses less than 500 mGy, sometimes up to 1 Gy, are considered low doses.

At high doses, irradiation has either a linear dose-dependent effect or threshold effect, relying mainly on DNA damage. For this type of effect, dose seems to be directly linked to the amount of damage, with the chance of DNA damage becoming extremely low at less than 100 mGy. For the low-dose region, different types of dose–response curves have been proposed, one of them being hormesis [78]. This principle presupposes that a phenomenon giving rise to detrimental effects at high doses (including irradiation) might be beneficial at low doses [79]. Other propositions included "nonlinear quadratic effects," resulting in even lower risks at low doses than linear models [80]. These propositions did not lead to changes in regulatory recommendations for therapy, imaging,or working conditions, considering the lack of statistical robustness and the fact that the potential danger of LDIR had not been proven. However, an increased risk of developing leukemia and brain tumors in children exposed to several CT scans was reported [4,5], suggesting the potential danger of low doses. In this section, we review studies shedding light on the effect of LDIR on hematopoietic tissues and, in particular, HSCs. Mostly, cell autonomous effects are studied.

In mice, one of the most frequently used models for the study of hematopoietic tissues, the effects of

different types of irradiation were evaluated at low doses, uncovering unexpected effects compared with the linear dose–response model. The effect of low doses of high-LET irradiation (heavy ions), as endured by deep space astronauts, was well studied. In 2014, it was reported that ^{56}Fe irradiation has an impact on HSC epigenetic regulation [81]. It increased global DNA methylation and decreased methyltransferase levels specifically in hematopoietic stem and progenitor cells (HSPCs), between 100 and 400 mGy, 4 weeks after exposure. At 22 weeks, results were more visible at higher doses, mainly 400 mGy, but a diminution in *DNMT3b* methyltransferase is still seen at 200 mGy. However, no "usual" effect such as DNA damage or elevation of ROS could be witnessed in any condition with this type of irradiation at those time points. Recently, a team investigated the immediate (2 weeks) and late (3 months) effects of exposure to more or less low doses (50, 100, and 250 mGy and 1 Gy) of ^{16}O irradiation-charged particles, very high energy (600 MeV) [82,83]. For immediate effects, they reported a decrease in HSPC frequency and numbers at all doses, with no great difference between the lower doses and 1 Gy. Interestingly only higher doses (1 Gy) had an impact on mature cells. In vitro functional tests, such as the colony-forming unit in culture (*CFU-C)* and cobblestone area-forming cell (*CAFC*) tests, revealed a decrease in the clonogenic capacity of HSPCs at all doses. Interestingly, for the CAFC test, the effect of the lowest dose, 100 mGy, on HSPC clonogenic potential was detectable only at 5 weeks postirradiation, suggesting an effect on long-term HSPC properties. Several mechanisms were investigated to explain these effects. In particular, a persistent increase in ROS production and, subsequently, an increase in DNA DSBs (γH2AX foci) and cell cycle entry were seen in immature populations. For later time points, the same types of effects were detectable and were exacerbated at lower doses (100 mGy) except for the ROS level in HPCs, which returned to normal levels 3 months postirradiation. In both cases, low doses of irradiation did not induce cell apoptosis. The authors also compared these effects after 3 months of ^{137}Ce irradiation —γ-ray, low LET, and low energy (0.6 MeV); in this case, no difference could be spotted between cells irradiated between 100 mGy and Gy and control cells. This could be compared with the study that found that high-LET (150 keV/μm) irradiation with charged particles has greater RBE than low-LET (2 keV/μm for a 250-keV X-rays) irradiation.

Two other recent studies focused on the effect of even lower doses (20 mGy) of ^{60}Co irradiation—γ-ray, mild energy (4 MeV), low LET (0.2 keV/μm)—on murine and human hematopoietic stem cells [84,85]. This dose was chosen to match the dose received in the bone marrow during a CT scan, on average. In both studies, a hyper-radiosensitivity was evidenced on HSC clonogenic potential at doses around 20 mGy, immediately for murine HSCs and after serial replating for human HSCs, which is less important at higher LDIRs such as 50 and 250 mGy (Figure 1A). This observation of human cells recalls the results reviewed previously on murine HSPCs exposed to ^{16}O irradiation with higher LET [83]. This may also indicate differences in the dose-triggering hyper-radiosensitivity in murine HSCs compared with human HSCs. Lower in vivo reconstitution potentials of irradiated HSPCs were also observed. However, no effect of LDIR on the total number of murine HSCs 4 months after TBI in mice was observed, except in the case of inflammatory conditioning or granulocyte colony-stimulating factor (G-CSF) treatment. This suggests that LDIR affects mostly stressed cells (inflammatory conditions, replicating cells, etc.). In both cases, unlike HDIR (2–2.5 Gy as controls and Table 1), no usual effects of irradiation were seen: neither DSB damage nor DDR pathway activation was observed, as reflected by the absence of γH2AX foci and ATM and p53 phosphorylation; there also were no differences in cell cycle or apoptosis. However, an immediate but transient increase in ROS was observed after irradiation. This oxidative stress was also observed, in human HSCs, as reflected by the presence of 8-OH-dG-stained oxidative DNA lesions and activation of the p38MAPK pathway. In both studies, the NRF2 protein was translocated into the nucleus. Murine cells deficient in NRF2 are more hyper-radiosensitive than WT murine cells, while Keap1 (a repressor of NRF2)-deficient cells exhibit no hyper-radiosensitivity at all, implying that the Keap1-NRF2 pathway is involved in the HSC response to LDIR. Also, in murine HSCs kept in culture, a second wave of ROS was detectable after a week of culture, compared with sham-irradiated cells. Irradiated HSCs transplanted into mice also exhibited an elevated level of ROS several months after irradiation, suggesting a persistent oxidative stress. Metabolic changes were observed in human HSCs: a decrease in metabolic activity, as assessed with TMRE and MTG probes, was observed in the hours following irradiation; in murine HSCs, it was an increase in autophagy (LC3B staining) (Figure 1B). Treatment with the well-known antioxidant NAC improved HSC potential (recovery of clonogenicity and no activation of p38MAPK in human cells, decrease in autophagy in murine cells) (Figure 1A,C). Of note, 20 mGy irradiation using a linear accelerator producing X-rays (1 MeV) had a similar but milder effect on clonogenicity (unpublished data); also, as stated previously, different low doses (50 and 250 mGy) had few or no effects with ^{60}Co irradiation (Figure 1A). This supports the hypothesis that

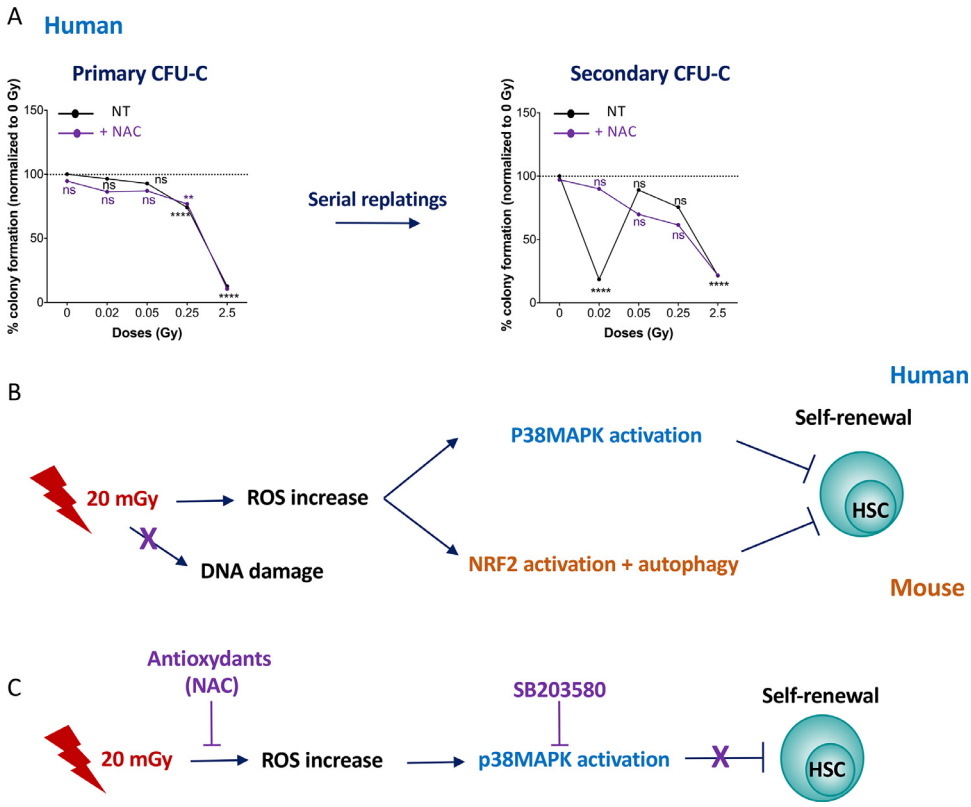

Figure 1. Consequences of exposure to LDIR on properties of HSCs. **(A)** Human HSCs ($CD34^+CD38^{low}CD45RA^-CD90^+$ cells from pools of cord blood samples) were pretreated (*purple*) or not (*black*) with the antioxidant NAC prior to exposure to different low and high doses of IR (source ^{60}Co) from no irradiation to 2.5 Gy, as indicated. Then the HSCs were seeded in serial CFU-C assays. Left: LDIR (20–50 mGy) have no effect on the capacity of HSCs capacities to generate primary CFU-C (n >5). Right: Only 20 mGy- and 2.5 Gy-irradiated HSCs failed to generate secondary CFU-C in serial CFU-C assays (n >3). Two-way analysis of variance test: $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. **(B)** Model explaining the effect on HSCs of exposure to LDIR (20 mGy) in humans (*blue*) and a mouse model (*orange*). **(C)** Model explaining how either antioxidants or p38MAPK inhibitor (SB203580) can protect human HSCs from LDIR.

irradiating particle energy is an important factor in predicting cell response to low doses of irradiation and that there is a sharp nonlinear effect at low doses. Globally, these two groups indicate that oxidative stress, not high enough to induce apoptotic responses, may be the key to understanding how very low doses of irradiation affect HSCs (Figure 1) [84,85].

The type of HSCs might also be an important factor to consider. Different HSCs may respond differently to the same irradiation dose, as indicated by a study comparing cord blood, BM, and peripheral blood HSCs [86].

Discussion

Cellular responses to high and low doses of irradiation can differ, responses to high doses also involving a DNA damage response. However, most studies also agree that the responses to irradiation at both high and low doses have the same features and rely on oxidative stress (Table 1). This could be compared with the fact that a global increase in oxidative stress is also a hallmark of aging in HSCs that leads to similar consequences, notably defects in self-renewal. Indeed, aged HSCs have endured more exits from quiescence than young HSCs, progressively losing self-renewal properties and accumulating unrepaired defects [87].

It is also interesting to look at the impact of LDIR on other types of cells potentially less sensitive than HSCs. For instance, Jones' group recently reported that highly proliferative intestinal stem cells are affected by LDIR (50 mGy, ^{137}Cs irradiation). They found that increased oxidative stress triggers a higher differentiation potential at the expense of stem cell proliferation [88]. p53 mutant stem cells are in this case more able to proliferate. However, antioxidant treatment with NAC was able to reverse this effect and to protect the functionality of intestinal stem cells. In gut and brain, low doses of irradiation also produced an increase in ROS and further activation of NFkB and SOD2 signalization [89]. In mesenchymal stem cells, 80 mGy of X-rays induced persistent γH2AX foci, unlike 1 Gy of irradiation, for which these DSBs are repaired more rapidly, mimicking some of the effects witnessed for HSCs [90].

Obviously, there is room for more investigations of the effects of low doses of irradiation. Indeed, it appears clear that LDIR have substantial effects, especially on stem cells. Moreover, it seems that LET is a more important factor at low doses than at high doses. Hence we suspect that at low doses there might be a specific dose–response curve for each type of irradiation and cell, making it quite difficult to establish a universal model for a specific irradiation dose.

However, the increase in ROS seems to be a major recurrence after LDIR. As discussed, ROS tend to induce defects in self-renewal in quiescent stem cells, such as HSCs, while affecting more deeply the differentiation in proliferating stem cells. As ROS are known to be involved in many pathologic pathways, it would be interesting to dig further in this direction. Nonetheless, ROS are increasingly being studied with respect to their role in physiological signaling and may represent a way to cope with irradiation. After LDIR, the ROS increase may be under the threshold considered as a stress and may not trigger adapted responses. We believe this could be the reason why, in HSCs, low doses result in a differentiation signal at the expense of self-renewal, leading to long-term consequences. Anyway, it may not be useless to consider supplementation in antioxidant molecules to protect HSCs and maybe other types of cells, which might be beneficial for people with more exposure to LDIR, such as those requiring frequent medical imaging, astronauts, and frequent flyers.

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References

1. International Commission on Radiological Protection (ICRP). Recommandations 2007 de la Commission internationale de protection radiologique. ICRP Publication 103. Ottawa, ON: Author; 2007.
2. Radiation Safety for Radiation Workers Manual. Madison: University of Wisconsin;
3. Shao L, Luo Y, Zhou D. Hematopoietic stem cell injury induced by ionizing radiation. Antioxid Redox Signal. 2014;20:1447–1462.
4. Pearce MS. Patterns in paediatric CT use: An international and epidemiological perspective. J Med Imaging Radiat Oncol. 2011;55:107–109.
5. Pearce MS, Salotti JA, Little MP, et al. Radiation exposure from CT scans in childhood and subsequent risk of leukaemia and brain tumours: a retrospective cohort study. Lancet. 2012;380:499–505.
6. Passegué E, Ernst P. IFN-α wakes up sleeping hematopoietic stem cells. Nat Med. 2009;15:612–613.
7. Cheshier SH, Prohaska SS, Weissman IL. The effect of bleeding on hematopoietic stem cell cycling and self-renewal. Stem Cells Dev. 2007;16:707–717.
8. Gardner RV, Lerner C, Astle CM, Harrison DE. Assessing permanent damage to primitive hematopoietic stem cells after chemotherapy using the competitive repopulation assay. Cancer Chemother Pharmacol. 1993;32:450–454.
9. Mauch P, Constine L, Greenberger J, et al. Hematopoietic stem cell compartment: acute and late effects of radiation therapy and chemotherapy. Int J Radiat Oncol Biol Phys. 1995;31:1319–1339.
10. Weiss CN, Ito K. DNA damage: a sensible mediator of the differentiation decision in hematopoietic stem cells and in leukemia. Int J Mol Sci. 2015;16:6183–6201.
11. Kiel MJ, Yilmaz OH, Iwashita T, Yilmaz OH, Terhorst C, Morrison SJ. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. Cell. 2005;121:1109–1121.
12. Wilson A, Laurenti E, Oser G, et al. Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. Cell. 2008;135:1118–1129.
13. Doulatov S, Notta F, Eppert K, Nguyen LT, Ohashi PS, Dick JE. Revised map of the human progenitor hierarchy shows the origin of macrophages and dendritic cells in early lymphoid development. Nat Immunol. 2010;11:585–593.
14. Doulatov S, Notta F, Laurenti E, Dick JE. Hematopoiesis: a human perspective. Cell Stem Cell. 2012;10:120–136.
15. Notta F, Doulatov S, Laurenti E, Poeppl A, Jurisica I, Dick JE. Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. Science. 2011;333:218–221.
16. Radtke S, Gorgens A, Kordelas L, et al. CD133 allows elaborated discrimination and quantification of haematopoietic progenitor subsets in human haematopoietic stem cell transplants. Br J Haematol. 2015;169:868–878.
17. Morrison SJ, Scadden DT. The bone marrow niche for haematopoietic stem cells. Nature. 2014;505:327–334.
18. Winkler IG, Barbier V, Nowlan B, et al. Vascular niche E-selectin regulates hematopoietic stem cell dormancy, self renewal and chemoresistance. Nat Med. 2012;18:1651–1657.
19. Papayannopoulou T, Craddock C, Nakamoto B, Priestley GV, Wolf NS. The VLA4/VCAM-1 adhesion pathway defines contrasting mechanisms of lodgement of transplanted murine hemopoietic progenitors between bone marrow and spleen. Proc Natl Acad Sci USA.. 1995;92:9647–9651.
20. Arcangeli ML, Bardin F, Frontera V, et al. Function of Jam-B/Jam-C interaction in homing and mobilization of human and mouse hematopoietic stem and progenitor cells. Stem Cells. 2014;32:1043–1054.
21. Arcangeli ML, Frontera V, Bardin F, et al. JAM-B regulates maintenance of hematopoietic stem cells in the bone marrow. Blood. 2011;118:4609–4619.
22. Ding L, Saunders TL, Enikolopov G, Morrison SJ. Endothelial and perivascular cells maintain haematopoietic stem cells. Nature. 2012;481:457–462.
23. Sugiyama T, Kohara H, Noda M, Nagasawa T. Maintenance of the hematopoietic stem cell pool by CXCL12–CXCR4 chemokine signaling in bone marrow stromal cell niches. Immunity. 2006;25:977–988.
24. Yoshihara H, Arai F, Hosokawa K, et al. Thrombopoietin/MPL signaling regulates hematopoietic stem cell quiescence and

interaction with the osteoblastic niche. Cell Stem Cell. 2007; 1:685–697.
25. Arai F, Hirao A, Ohmura M, et al. Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. Cell. 2004;118:149–161.
26. Goldman DC, Bailey AS, Pfaffle DL, Al Masri A, Christian JL, Fleming WH. BMP4 regulates the hematopoietic stem cell niche. Blood. 2009;114:4393–4401.
27. Morikawa T, Takubo K. Hypoxia regulates the hematopoietic stem cell niche. Pflugers Arch. 2016;468:13–22.
28. Imanirad P, Dzierzak E. Hypoxia and HIFs in regulating the development of the hematopoietic system. Blood Cells Mol Dis. 2013;51:256–263.
29. Takubo K, Goda N, Yamada W, et al. Regulation of the HIF-1alpha level is essential for hematopoietic stem cells. Cell Stem Cell. 2010;7:391–402.
30. Lessard J, Sauvageau G. Bmi-1 determines the proliferative capacity of normal and leukaemic stem cells. Nature. 2003; 423:255–260.
31. Park IK, Qian D, Kiel M, et al. Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. Nature. 2003;423:302–305.
32. Ficara F, Murphy MJ, Lin M, Cleary ML. Pbx1 regulates self-renewal of long-term hematopoietic stem cells by maintaining their quiescence. Cell Stem Cell. 2008;2:484–496.
33. Correia NC, Arcangeli ML, Pflumio F, Barata JT. Stem Cell Leukemia: how a TALented actor can go awry on the hematopoietic stage. Leukemia. 2016;30:1968–1978.
34. Reynaud D, Ravet E, Titeux M, et al. SCL/TAL1 expression level regulates human hematopoietic stem cell self-renewal and engraftment. Blood. 2005;106:2318–2328.
35. Cheng T, Rodrigues N, Shen H, et al. Hematopoietic stem cell quiescence maintained by p21cip1/waf1. Science. 2000;287: 1804–1808.
36. Zou P, Yoshihara H, Hosokawa K, et al. p57(Kip2) and p27 (Kip1) cooperate to maintain hematopoietic stem cell quiescence through interactions with Hsc70. Cell Stem Cell. 2011;9:247–261.
37. Matsumoto A, Takeishi S, Kanie T, et al. p57 is required for quiescence and maintenance of adult hematopoietic stem cells. Cell Stem Cell. 2011;9:262–271.
38. Biechonski S, Yassin M, Milyavsky M. DNA-damage response in hematopoietic stem cells: an evolutionary trade-off between blood regeneration and leukemia suppression. Carcinogenesis. 2017;38:367–377.
39. Shao Z, Flynn RA, Crowe JL, et al. DNA-PKcs has KU-dependent function in rRNA processing and haematopoiesis. Nature. 2020;579:291–296.
40. Zhang S, Yajima H, Huynh H, et al. Congenital bone marrow failure in DNA-PKcs mutant mice associated with deficiencies in DNA repair. J Cell Biol. 2011;193:295–305.
41. Nijnik A, Woodbine L, Marchetti C, et al. DNA repair is limiting for haematopoietic stem cells during ageing. Nature. 2007;447:686–690.
42. Rossi DJ, Bryder D, Seita J, Nussenzweig A, Hoeijmakers J, Weissman IL. Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. Nature. 2007; 447:725–729.
43. Kenyon J, Gerson SL. The role of DNA damage repair in aging of adult stem cells. Nucleic Acids Res. 2007;35:7557–7565.
44. Qing Y, Wang Z, Bunting KD, Gerson SL. Bcl2 overexpression rescues the hematopoietic stem cell defects in Ku70-deficient mice by restoration of quiescence. Blood. 2014;123:1002–1011.
45. Navarro S, Meza NW, Quintana-Bustamante O, et al. Hematopoietic dysfunction in a mouse model for Fanconi anemia group D1. Mol Ther. 2006;14:525–535.
46. Hasty P, Campisi J, Hoeijmakers J, van Steeg H, Vijg J. Aging and genome maintenance: lessons from the mouse? Science. 2003;299:1355–1359.
47. Niedernhofer LJ, Odijk H, Budzowska M, et al. The structure-specific endonuclease Ercc1-Xpf is required to resolve DNA interstrand cross-link-induced double-strand breaks. Mol Cell Biol. 2004;24:5776–5787.
48. Prasher JM, Lalai AS, Heijmans-Antonissen C, et al. Reduced hematopoietic reserves in DNA interstrand crosslink repair-deficient Ercc1−/− mice. EMBO J. 2005;24:861–871.
49. Ito K, Hirao A, Arai F, et al. Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. Nature. 2004;431:997–1002.
50. Ito K, Hirao A, Arai F, et al. Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. Nat Med. 2006;12:446–451.
51. Bigarella CL, Liang R, Ghaffari S. Stem cells and the impact of ROS signaling. Development. 2014;141:4206–4218.
52. Jang YY, Sharkis SJ. A low level of reactive oxygen species selects for primitive hematopoietic stem cells that may reside in the low-oxygenic niche. Blood. 2007;110:3056–3063.
53. Guo B, Huang X, Lee MR, Lee SA, Broxmeyer HE. Antagonism of PPAR-gamma signaling expands human hematopoietic stem and progenitor cells by enhancing glycolysis. Nat Med. 2018; 24:360–367.
54. Ito K, Carracedo A, Weiss D, et al. A PML-PPAR-delta pathway for fatty acid oxidation regulates hematopoietic stem cell maintenance. Nat Med. 2012;18:1350–1358.
55. Juntilla MM, Patil VD, Calamito M, Joshi RP, Birnbaum MJ, Koretzky GA. AKT1 and AKT2 maintain hematopoietic stem cell function by regulating reactive oxygen species. Blood. 2010;115:4030–4038.
56. Chen C, Liu Y, Liu Y, Zheng P. The axis of mTOR-mitochondria-ROS and stemness of the hematopoietic stem cells. Cell Cycle. 2009;8:1158–1160.
57. Hagenbuchner J, Kuznetsov A, Hermann M, Hausott B, Obexer P, Ausserlechner MJ. FOXO3-induced reactive oxygen species are regulated by BCL2L11 (Bim) and SESN3. J Cell Sci. 2012; 125:1191–1203.
58. Tothova Z, Kollipara R, Huntly BJ, et al. FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. Cell. 2007;128:325–339.
59. Yahata T, Takanashi T, Muguruma Y, et al. Accumulation of oxidative DNA damage restricts the self-renewal capacity of human hematopoietic stem cells. Blood. 2011;118:2941–2950.
60. Hu L, Cheng H, Gao Y, et al. Antioxidant *N*-acetyl-L-cysteine increases engraftment of human hematopoietic stem cells in immune-deficient mice. Blood. 2014;124:e45–e48.
61. Liu Y, Elf SE, Miyata Y, et al. p53 regulates hematopoietic stem cell quiescence. Cell Stem Cell. 2009;4:37–48.
62. Karigane D, Kobayashi H, Morikawa T, et al. p38alpha activates purine metabolism to initiate hematopoietic stem/progenitor cell cycling in response to stress. Cell Stem Cell. 2016;19:192–204.
63. Venugopal R, Jaiswal AK. Nrf1 and Nrf2 positively and c-Fos and Fra1 negatively regulate the human antioxidant response element-mediated expression of NAD(P)H:quinone oxidoreductase1 gene. Proc Natl Acad Sci USA.. 1996;93:14960–14965.
64. Merchant AA, Singh A, Matsui W, Biswal S. The redox-sensitive transcription factor Nrf2 regulates murine hematopoietic stem cell survival independently of ROS levels. Blood. 2011;118:6572–6579.

65. Insinga A, Cicalese A, Faretta M, et al. DNA damage in stem cells activates p21, inhibits p53, and induces symmetric self-renewing divisions. Proc Natl Acad Sci USA. 2013;110:3931–3936.
66. Dainiak N. Hematologic consequences of exposure to ionizing radiation. Exp Hematol. 2002;30:513–528.
67. Azzam EI, Jay-Gerin JP, Pain D. Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury. Cancer Lett. 2012;327:48–60.
68. Wang Y, Schulte BA, LaRue AC, Ogawa M, Zhou D. Total body irradiation selectively induces murine hematopoietic stem cell senescence. Blood. 2006;107:358–366.
69. Wang Y, Liu L, Pazhanisamy SK, Li H, Meng A, Zhou D. Total body irradiation causes residual bone marrow injury by induction of persistent oxidative stress in murine hematopoietic stem cells. Free Radic Biol Med. 2010;48:348–356.
70. Kuo LJ, Yang LX. Gamma-H2AX—a novel biomarker for DNA double-strand breaks. In Vivo. 2008;22:305–309.
71. Zhang H, Zhai Z, Wang Y, et al. Resveratrol ameliorates ionizing irradiation-induced long-term hematopoietic stem cell injury in mice. Free Radic Biol Med. 2013;54:40–50.
72. Lee JM, Bernstein A. p53 mutations increase resistance to ionizing radiation. Proc Natl Acad Sci USA.. 1993;90:5742–5746.
73. Mohrin M, Bourke E, Alexander D, et al. Hematopoietic stem cell quiescence promotes error-prone DNA repair and mutagenesis. Cell Stem Cell. 2010;7:174–185.
74. Milyavsky M, Gan OI, Trottier M, et al. A distinctive DNA damage response in human hematopoietic stem cells reveals an apoptosis-independent role for p53 in self-renewal. Cell Stem Cell. 2010;7:186–197.
75. Shao L, Sun Y, Zhang Z, et al. Deletion of proapoptotic Puma selectively protects hematopoietic stem and progenitor cells against high-dose radiation. Blood. 2010;115:4707–4714.
76. Meng A, Wang Y, Brown SA, Van Zant G, Zhou D. Ionizing radiation and busulfan inhibit murine bone marrow cell hematopoietic function via apoptosis-dependent and -independent mechanisms. Exp Hematol. 2003;31:1348–1356.
77. Meng A, Wang Y, Van Zant G, Zhou D. Ionizing radiation and busulfan induce premature senescence in murine bone marrow hematopoietic cells. Cancer Res. 2003;63:5414–5419.
78. Doss M. Linear no-threshold model vs. radiation hormesis. Dose Response. 2013;11:480–497.
79. Calabrese EJ. Hormesis: improving predictions in the low-dose zone. Exp Suppl. 2012;101:551–564.
80. Gonzalez AJ. Biological effects of low doses of ionizing radiation: A fuller picture. IAEA Bull. 1994;26(6):37–45.
81. Miousse IR, Shao L, Chang J, et al. Exposure to low-dose ^{56}Fe-ion radiation induces long-term epigenetic alterations in mouse bone marrow hematopoietic progenitor and stem cells. Radiat Res. 2014;182:92–101.
82. Chang J, Luo Y, Wang Y, et al. Low doses of oxygen ion irradiation cause acute damage to hematopoietic cells in mice. PLoS One. 2016;11:e0158097.
83. Wang Y, Chang J, Li X, et al. Low doses of oxygen ion irradiation cause long-term damage to bone marrow hematopoietic progenitor and stem cells in mice. PLoS One. 2017;12:e0189466.
84. Henry E, Souissi-Sahraoui I, Deynoux M, et al. Human hematopoietic stem/progenitor cells display ROS-dependent long-term hematopoietic defects after exposure to low dose of ionizing radiations. Haematologica. 2020;105:2044–2055.
85. Rodrigues-Moreira S, Moreno SG, Ghinatti G, et al. Low-dose irradiation promotes persistent oxidative stress and decreases self-renewal in hematopoietic stem cells. Cell Rep. 2017;20:3199–3211.
86. Kato K, Kuwabara M, Kashiwakura I. The influence of gender- and age-related differences in the radiosensitivity of hematopoietic progenitor cells detected in steady-state human peripheral blood. J Radiat Res. 2011;52:293–299.
87. Walter D, Lier A, Geiselhart A, et al. Exit from dormancy provokes DNA-damage-induced attrition in haematopoietic stem cells. Nature. 2015;520:549–552.
88. Fernandez-Antoran D, Piedrafita G, Murai K, et al. Outcompeting p53-mutant cells in the normal esophagus by redox manipulation. Cell Stem Cell. 2019;25:329–341.
89. Veeraraghavan J, Natarajan M, Herman TS, Aravindan N. Low-dose gamma-radiation-induced oxidative stress response in mouse brain and gut: regulation by NFkappaB-MnSOD cross-signaling. Mutat Res. 2011;718:44–55.
90. Pustovalova M, Astrelina capital Te C, Grekhova A, et al. Residual gammaH2AX foci induced by low dose x-ray radiation in bone marrow mesenchymal stem cells do not cause accelerated senescence in the progeny of irradiated cells. Aging (Albany NY).. 2017;9:2397–2410.
91. Lin W, Yuan N, Wang Z, et al. Autophagy confers DNA damage repair pathways to protect the hematopoietic system from nuclear radiation injury. Sci Rep. 2015;5:12362.
92. Giotopoulos G, McCormick C, Cole C, et al. DNA methylation during mouse hemopoietic differentiation and radiation-induced leukemia. Exp Hematol. 2006;34:1462–1470.