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Chapter

Space Radiation-Induced Hematopoietic Stem Cell Injury

Huihong Zeng, Mengzhen Yue and Lijian Shao

Abstract

Space radiation is an unavoidable health risk during space activities. Hematopoietic cells are sensitive to radiation including proton and oxygen radiation and so on. Understanding the mechanisms responsible for detrimental effects of space radiation is important to achieve countermeasures protecting hematopoietic stem cells (HSCs), which generates different hematopoietic populations. However, the biological effects of various sources of space radiation on HSCs are not understood well. Induction of cellular apoptosis, reactive oxygen species (ROS), and DNA damage upon space radiation is believed to be critical mediators for HSC damage. In this chapter, we will mainly discuss the biological effectiveness of proton and oxygen radiation on the numbers and function of HSCs. Space radiation-induced apoptosis, ROS, and DNA damage were examined as well, which will provide foundation to develop novel strategies protecting HSCs from space radiation.

Keywords: space irradiation, proton irradiation, oxygen irradiation, apoptosis, reactive oxygen species, hematopoietic stem cells, bone marrow

1. Introduction

Human spaceflight and exploration began in the 1960s. Manned spaceflight activities have continually expanded in frequency and scope since that time, and plans are now forming for long-duration flights to deep-space destinations. However, numerous risk factors have potential to negatively affect the astronauts' health during deep-space missions, especially microgravity and space radiation. Exposure of astronauts to space radiation is relatively unpredictable yet inevitable. Space radiation comes from two major sources: solar particle events (SPE) emanating from the sun and galactic cosmic rays (GCR) originating from sources outside the solar system.

SPE mainly includes protons and can lead to moderate- to high-dose rate exposures to ionizing radiation during long-term space mission [1, 2]. Astronauts may receive cumulative doses from 1 to 3 gray (Gy) during an SPE [3, 4]. Especially, proton radiation contributes to more than 80% of SPE [1, 2, 5].

GCR contains high atomic number and energy (HZE) particles, such as ^{56}Fe, ^{28}Si, ^{16}O, ^{12}C, and so forth. HZE particles are characterized by dense tracks of ionization, a property quantified as high-linear energy transfer (LET). The properties of HZE particles are consistent with their stronger toxicities and higher energy to normal tissues than photon and proton radiation [6, 7]. Previous studies have documented that the value of RBE in relation to γ-ray radiation was 1.25 for ^{56}Fe, 1.4 for ^{28}Si, and 0.99 for ^{12}C using a mouse model [6, 8]. Among HZE particles, ^{56}Fe has

high-linear energy transfer that might heavily contribute to GCR in space [9]. There are many different components including ions, hydrogen, helium, and so on in spacecraft [10]. Based on measurements in the Mars Science Laboratory from 2011 to 2012, the irradiation dose of GCR in the spaceflight was approximately 481 ± 80 μGy per day [11]. For a 600- to 900-day Mars mission, the total radiation doses from GCR reside between 0.33 and 0.49 Gy. Therefore, the total radiation dose from SPE and GCR will reach to 1.0 Gy or above. Although doses and dose rates of space radiation are low, it will still result in space dose accumulation in the body and high risk to astronauts' health during a long-term space mission [9, 12].

Radiation-induced tissue damage in the body has long been understood since Wilhelm Röntgen discovered X-ray in 1895 [13]. Hematopoietic and gastrointestinal systems have been shown to be the two most sensitive compartments of the body to radiation. It has been well-documented that radiation (including space radiation) also induces dysfunction of the brain, manifesting as behavioral and cognitive disabilities [14, 15]. The detrimental effects of X-ray radiation in the body were firstly reported by Warren and Whipple [16] and Shouse et al. [13]. They reported that exposing dogs to high doses of X-rays resulted in death from severe hematopoietic suppression and damage. The detrimental effects of radiation on human health were heavily realized after the use of the first atomic bombs in 1945. Many people in Hiroshima and Nagasaki who survived the initial bomb blast later died from radiation exposure in the event. Long-term toxic impacts of the atomic bomb on humans were observed as well, such as the high risk of hematopoietic malignancies. Further studies proved that hematopoietic failure was one of the primary reasons in radiation-induced death when animals experienced a moderate to high dose of total-body irradiation. This is supported by a study in the 1940s showing that shielding the spleen or one entire hind leg with lead or transplantation of splenocytes protected mice from the lethal effect of irradiation [17]. The importance of hematopoietic cells under radiation was also supported by studies showing that intravenous infusions of bone marrow (BM) cell suspensions protected mice from the effects of radiation [95]. Initially, investigators suggested that a humoral factor from the spleen and BM cell suspensions might benefit the radioprotective effects [18], while later studies proved that it was attributed to the transplanted hematopoietic cells [19–21]. When Till and McCulloch discovered hematopoietic stem cells (HSCs) in the 1960s, those cells protecting animals from IR-induced lethal hematopoietic damage were HSCs [22, 23]. Remarkable progress has been subsequently made in understanding of the mechanisms by which radiation causes hematopoietic damage.

However, the effects of space radiation on the hematopoietic system have yet to be fully understood, leading to a lack of effective countermeasure strategies thus far. In the present chapter, we mainly focus our discussion on the biological effectiveness of space radiation, such as proton and oxygen, whereby space radiation induces HSC injury, and the implication of HSC injury to IR-induced BM suppression in mouse. In addition, genomic instability, malignancies, and intestinal, brain, behavioral, and cognitive effects induced by space radiation will not be discussed here, which were extensively discussed by other investigators [24, 25].

2. Composition of the mouse hematopoietic system

Hematopoietic stem cells often replenish the whole blood system throughout the life span of the body and maintain hematopoietic homeostasis. In Till and McCulloch's landmark work [22, 23, 26], they utilized colony-forming unit-spleen (CFU-S) assay to identify functional HSCs even while these HSCs were a mixed

population with different proliferating and self-renewal abilities. This pioneering research not only provided strong evidence to show in vivo HSC existence but also stimulated many investigators to define and isolate HSCs and characterize their properties in mice and humans.

In the decades since Till and McCulloch's study [22, 23, 26], multiple cell surface markers have been used to separate HSCs from other populations. For example, lineage cell surface markers, such as Ter119, CD4, CD8, B220, Gr-1, Mac-1, and NK1.1, are not detected neither in mouse multipotent progenitors (MPPs) nor in hematopoietic progenitor cells (HPCs). Both HSCs and MPPs express c-Kit and Scal-1, which are therefore named lineage negative (Lin-), c-Kit$^+$, and Sca-1$^+$ as LSK cells (**Figure 1**). HPCs express c-Kit, but not Sca-1, and are termed Lin$^-$c-Kit$^+$Sca-1$^-$. In 2005, Kiel et al. further utilized CD150 and CD48 surface markers to distinguish HSCs and MPPs [27]. HSCs express CD150, but not CD48, and termed CD150$^+$CD48$^-$LSK cells. MPPs are CD150$^{+/-}$CD48$^+$LSK cells. Investigators also used different strategies to identify HSCs. Combination of CD34 or Thy^{lo}Flk-2$^-$ with LSK surface markers was used to isolate HSCs, named as CD34$^-$LSK and Thy1^{lo}Flk-2$^-$LSK cells, respectively [26]. Trumpp's group combined CD34, CD135, CD150, CD48, and LSK markers to further differentiate HSC into long-term HSCs (CD34$^-$CD135$^-$CD150$^+$CD48$^-$LSK cells) and short-term HSCs (CD34$^+$ CD135$^-$CD150$^+$CD48$^-$LSK cells). In addition, HSCs have a feature with highly expression of ATP-binding protein like ABCG2 and can efflux DNA-binding dye Hoechst 33342. The Hoechst-effluxing side population (SP) cells were therefore used to indicate HSC population in the case of some circumstances [28], such as 5FU and radiotherapeutic stress conditions.

It is well-documented that the hematopoietic system is organized in a hierarchical manner (**Figure 1**). The rare long-term HSCs are located at the top of the hierarchy and have the capacity to self-renew, proliferate, and differentiate into various lineages of mature blood cells though immature MPPs and HPCs [29]. The most important feature of HSCs is quiescent to maintain their self-renewal ability

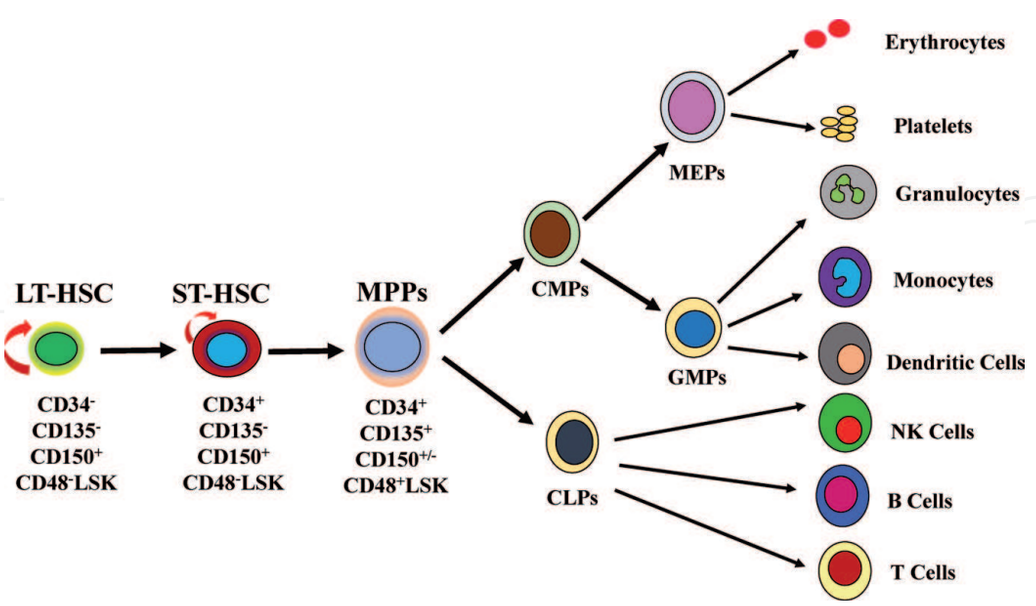

Figure 1.
Composition of mouse hematopoietic system. Long-term hematopoietic stem cell (LT-HSCs, CD34$^-$CD135$^-$CD150$^+$CD48$^-$LSK cells) is located at the top of the hierarchy and has the ability to self-renew, proliferate, and differentiate into short-term HSCs (ST-HSC, CD34$^+$CD135$^-$CD150$^+$CD48$^-$LSK cells) and multipotent progenitors (MPPs, CD34$^+$CD135$^+$CD150$^{+/-}$CD48$^+$LSK cells), which can further differentiate into different progenitors, such as common lymphoid progenitors (CLPs), common myeloid progenitors (CMPs) and their progeny megakaryocyte/erythroid progenitors (MEPs), and granulocyte/monocyte progenitors (GMPs). Progenitors can proliferate different lineages of mature blood cells.

and potentially provide lifelong hematopoiesis. The dominant HSCs have ability to protect the whole blood system against different stress conditions [30]. Under sublethal irradiation, HSCs have been long-term damaged, which can be easily ignored in clinic because of normal cell counts from the bone marrow and peripheral blood. Damaged HSCs induced by photon-irradiation have impaired self-renewing ability, leading to bone marrow failure and death [31]. However, whether low doses of space radiation trigger long-term HSC damage remains unknown. Comparing to HSCs, MPPs and HPCs have limit or lack self-renewal ability even though they are proliferating populations. The property of MPPs and HPCs with proliferation provides a beneficial role in case of normal hematopoiesis and stress hematopoiesis. For example, in case of blood loss or infection, MPP and HPC quickly proliferate to meet the requirement of mature cell production, trying to maintain normal hematopoiesis. Under radiotherapy and chemotherapy, MPPs and HPCs can be easily depleted with acute myelosuppression because of their proliferating feature. This will lead to HSC activation, proliferation, and differentiation to reestablish MPP and HPC populations and rebuild hematopoiesis, which might result in HSC exhaustion.

3. Proton radiation and hematopoietic stem cells

During spaceflight missions outside low Earth orbit, there remains the possibility of astronauts receiving damaging doses of space radiation. Because the dose-depth distribution of SPE spectrum protons is relatively low [32], during an SPE the skin and organs near the surface of the skin will receive higher doses than deeper organs such as the bone marrow. In addition, compared to traditional radiation therapy with X-rays (photons) or electrons, proton therapy has potential benefit for clinical cancer treatment because of its favorable distribution of the radiation dose, leading to selectively increased radiation dose to the cancerous tissues while lowering the dose to normal tissues [33, 34]. However, the hematopoietic system is highly sensitive to ionizing radiation, and exposure to even a relatively low dose of SPE may still be able to result in substantial damage to the system [35, 36]. Therefore, understanding the biological effects of proton radiation is immediately needed.

One of the characteristics of the radiation-induced hematopoietic syndrome is a decline in blood cell counts, resulting from radiation-induced cell killing in circulating blood cells and suppression of hematopoietic stem and progenitor cells in the bone marrow [37–39]. Studies have reported that whole-body exposure to protons causes acute effects on the hematopoietic system in animal models [40–45]. The decreased WBCs, lymphocytes, and neutrophils were detected starting at 4 hours after 0.25–3 Gy proton radiation, with the lowest numbers observed on day 4 [43, 44]. The reduction in WBCs and lymphocytes was still evident in mice after exposure to 2.0 Gy of protons (230 MeV) [41]. This might be due to the high sensitivity of lymphocytes to proton radiation, which is consistent with the data from γ-radiation [46]. Two Gy of proton radiation induced the acute decrement of peripheral blood cells, which was shown completely recovered in the long term. The dynamic changes of peripheral blood counts might result in ignoring the negative effects of proton radiation on hematopoietic system. However, the abnormalities of splenic WBCs and lymphocytes were still detected at more than 100 days after low dose of proton radiation [47]. Taken together, proton radiation has not only acute injury but also long-term harmful effects on the hematopoietic system.

It has been well established that exposure to a significant dose of total-body γ-irradiation (TBI) induces not only the acute radiation hematopoietic syndrome but also long-term bone marrow injury [48, 49]. The acute radiation hematopoietic syndrome induced by γ-irradiation is primarily attributed to the induction

of apoptosis of HPCs, while γ-irradiation-induced long-term BM suppression is mainly ascribed to the persistent damage to HSCs. While the effects of γ-irradiation on the hematopoietic system have been extensively documented, much less is known about the effects of proton irradiation [35, 36].

Previously, mice were exposed to two different doses (0.5 and 1.0 Gy) of proton radiation and examined the acute and long-term effects on BM HSPCs at 2 and 22 weeks after proton radiation, respectively (**Table 1**). Results showed that exposure of mice to 1.0 Gy of proton radiation resulted in a significant decrease in the number of WBCs and PLTs from peripheral blood 2 weeks after the exposure [50]. It was demonstrated that 1.0 Gy of oxygen ion radiation (^{16}O TBI) significantly decreased the cell counts of peripheral blood leukocytes when measured 2 weeks after exposure in male C57BL/6 mice [51]. Interestingly, the decrease of peripheral blood cell counts was not observed 2 weeks after 0.5 Gy proton TBI or 0.1 and 0.25 Gy ^{16}O TBI [51]. The threshold dose of protons (50 or 70 MeV) to induce a decline in WBC counts in female ICR outbred mice was previously estimated to be between 0.25 and 0.5 Gy [43]. The threshold dose identified from this previous study is lower than the result from our study. It may have resulted in part from the use of different strains of mice, the time when the mice were studied after radiation or the difference in energies of protons between the two studies. For example, the linear energy transfer values for 50, 70, and 150 MeV protons would be 1.26, 0.96, and 0.55 keV/μm [52]. Because of the dose-depth distribution of SPE protons, only relatively large or higher-energy spectrum SPEs may lead to BM exposure to these doses of protons in astronauts. However, the doses of SPEs that can cause significant HSPC damage have been observed, raising the possibility that astronauts might experience reductions in circulating blood cell counts and BM HSPC damage if they encounter such an SPE. The dose-depth distribution in mice exposed to protons is different from that in humans [1]. It was found that a dose of 0.5 Gy protons (150 MeV) significantly reduced hematopoietic stem/progenitor cell function. The effects of protons at this energy and at doses below 0.5 Gy are unknown. Since the dose to the blood-forming tissues of human subjects will likely be low, future studies need to examine the hematopoietic effects of proton doses below 0.5 Gy.

Proton TBI can acutely induce the decrease of all lineages of peripheral blood cells, since all lineages of blood cells are generated from hematopoietic stem cells through their differentiation into various lineages of progenitors. We have shown that the exposure to both 0.5 and 1.0 Gy proton TBI damaged not only HPCs but also LSK cells, leading to the defect in their numbers and function, which were supported by the decreased abilities to form in vitro colonies including BFU-E,

	Number of cells		Function of HSCs		
	HPC	LSK cells	BFU-E	CFU-GM	CFU-GEMM
2 weeks	↓	↓	↓	↓	↓
22 weeks	no change	↓	↓	↓	↓

Note: C57BL/6 mice were exposed to 1.0 Gy of proton total body irradiation. The indicated parameters were measured at 2 and 22 weeks after the exposure.
↓Indicate that the parameter is decreased when compared to non-irradiated mice.
No change means that the parameter is similar to that from non-irradiated mice.

Table 1.
Acute and long-term effects of proton irradiation on hematopoietic cells.

CFU-GM, and CFU-GEMM [50]. The in vivo functional defect of hematopoietic stem and progenitor cells after proton exposure will be further investigated through bone marrow transplantation in future studies. We have also reported that a 1.0 Gy dose of ^{16}O TBI significantly decreased peripheral blood counts and BM HSPCs 2 weeks after the exposure [51]. Therefore, the mechanisms of space radiation-induced acute damage to the hematopoietic system should be investigated further.

In our previous studies, we firstly show that exposure to proton radiation causes long-term hematopoietic injury at 22 weeks after the exposure [53]. Our data provide the first direct evidence that exposure of mice to 1.0 Gy dose of proton radiation results in not only a sustained reduction in the frequency of BM HSCs but also in the long-term inhibition of HSCs clonogenic function to form BFU-E, CFU-GM, and CFU-GEMM colonies in vitro (**Table 2**). In contrast, the number and frequency of HPCs returned to normal levels at 22 weeks postradiation. Another question that needs to be addressed is whether proton radiation-induced HSC damage leads to hypoplastic syndrome after hematopoietic stress. Myeloid leukemia could be induced by low and/or moderate doses of γ-irradiation and ^{56}Fe heavy ion radiation in mice [54, 55]. It has been shown that proton radiation induced minor myeloid leukemia but have high possibility to induce hepatocellular adenoma and malignant lymphoma in CBA mice [56], which is in contrast with the effects of γ-irradiation, showing that 3 Gy of γ-irradiation caused around 25% of CBA mice developing acute myeloid leukemia. These differences are due to the differential biological effectiveness between proton and γ-irradiation. To closely mimic space environment, investigators used minipig animal models to expose to electron solar particle event (eSPE) [57]. Comparing to eSPE, proton solar particle events (pSPE) have stronger negative effect on the numbers of peripheral WBCs, lymphocytes, neutrophils, and monocytes with a factor of 2.79. These data suggest that different hematopoietic populations have differential radiosensitivity to proton irradiation.

Protons have a higher linear energy transfer and are denser ionizing radiation than photon. Therefore, protons deposit high energy at the end of their range termed "Bragg peak" and cause heavily damage to the target tissues, cells, and molecules. Compared to γ-irradiation, proton radiation causes larger γ-H2AX foci [58], leads to hypermethylated DNA [59], has different transcriptome profiles [60], and modulates different signaling pathways [61]. More detailed investigation into what unique biological effects proton radiation has is called for to instruct proton studies.

Because an SPE contains protons of multiple energies below 150 MeV, some groups have developed cell culture or animal models of exposure to broad energy spectra of protons to better simulate an SPE. Previously, some differences were

	HPC			LSK cells		
	Apoptosis	ROS	DNA damage	Apoptosis	ROS	DNA damage
2 weeks	↑	no change	no change	↑	↑	no change
22 weeks	no change	no change	no change	no change	↑	↑

Note: C57BL/6 mice were exposed to 1.0 Gy of proton total body irradiation. The indicated parameters were measured at 2 and 22 weeks after the exposure.
↓ or ↑ Indicate that the parameter is decreased or increased when compared to non-irradiated mice, respectively. No change means that the parameter is similar to that from non-irradiated mice.

Table 2.
Induction of cellular apoptosis, ROS, and DNA damage after proton irradiation.

found in peripheral blood cell counts between mice exposed to one-energy protons (230 MeV, 2 Gy) and mice exposed to SPE-like protons at the same dose level [41]. Exposure of minipigs to SPE-like protons at a skin dose as low as 0.5 Gy (estimated dose to the BM: 0.42 Gy) caused decrement in peripheral blood cell counts up to at least 2 weeks after exposure [45]. Hence, further studies with SPE-like proton exposures at low doses are warranted. Since we used only two radiation doses (0.5 and 1 Gy), we were not able to prepare dose-response curves for the effects observed. In one of our separate studies, mice were irradiated with fully modulated beams containing particles from 0 energy to 150 MeV and a uniform dose versus depth profile. Doses of protons were 0.1, 0.25, and 0.5 Gy. Bone marrow cells were collected at 2 weeks after irradiation and examined as described in the current manuscript. We found a dose-dependent decrease in LSK cells, together with an increase in ROS levels and apoptosis in these cells (data not shown). In summary, our study has demonstrated that acute exposures to medium doses of proton TBI induced damage to HPCs and LSK cells in a mouse model.

Radiation-induced cell damage might be mediated by induction of apoptosis, DNA damage, and oxidative stress [48, 62]. We therefore assessed those parameters in HPCs and LSK cells 2 weeks after proton exposure [50]. Our data indicate that HPCs and LSK cells may respond differently to proton radiation. Exposure to 1.0 Gy protons resulted in an increase in cellular apoptosis in HPCs. Irradiated LSK cells, on the other hand, showed both increased apoptosis and oxidative stress. Neither of the two cell types showed enhanced DNA damage or cell cycling 2 weeks after proton exposure. Importantly, LSK cells in mice bone marrow from acute and long-term proton exposure cause the significant induction of oxidative stress [50, 53]. In the previous studies, it has been reported that the long-term increase in ROS production in LSK cells was observed after ^{16}O radiation and γ-rays [48, 63, 64]. For example, increasing levels of ROS production were detected at 2 months after 6.0 Gy of total-body γ-irradiation, which might be related to irradiation-induced DNA damage, leukemia, and senescence in irradiated hematopoietic stem and progenitor cells. Both proton and gamma radiation may induce residual negative effects on the bone marrow, which might be mediated by overproduction of chronic reactive oxidative stress in HSCs [63, 65, 66]. Taken together, present data indicate that irradiation-induced oxidative stress in HSCs might be a critical factor in the hematopoietic cell response to space radiation.

Reactive oxygen species (ROS) plays an important role in determining the fate of normal stem cells. Low levels of ROS are required for stem cells to maintain their quiescence and self-renewal capacities. Increases in ROS production cause stem cell proliferation, differentiation, apoptosis, and cell death, leading to their exhaustion. Regulating ROS production in stem cells is important to maintain tissue homeostasis and repair damaged area during the life span of an organism. It has been reported that the levels of ROS were closely related to the proper functional hematopoietic stem cells. There are multiple different ways for ROS production in cells, such as mitochondria oxidative phosphorylation, glycolysis, NADPH oxidases (NOXs) enzyme, peroxisomal and cytochrome P450 metabolism, and so on. Mitochondria oxidative phosphorylation is not a major source to generate ROS in hematopoietic stem cells under homeostasis. This is because (1) HSCs locate bone marrow hypoxic niche with low levels of oxygen; (2) HSCs have small amount of mitochondrial and immature mitochondrial; (3) HSCs have high level of pimonidazole, which is a hypoxia marker; and (4) HSCs have capacity to response to hypoxia by increasing hypoxia-inducible factor 1α (HIF-1α) expression [67]. Subsequently, increasing levels of HIF-1α benefit HSCs to use anaerobic glycolysis, instead of mitochondrial oxidative phosphorylation, to produce energy along with reducing ROS production. Previous studies have shown that increasing expression of NOX enzyme might

contribute to γ-irradiation-induced ROS production in HSCs [66], which was supported by increasing NOX4 expression, rather than other isoforms of NOXs in γ-ray irradiated HSCs [66]. Diphenyliodonium (a selective NOX inhibitor) treatment can partially restore the functional impairment in γ-ray irradiated HSCs by decreasing irradiation-induced ROS production in HSCs [66]. For space radiation circumstance, the higher level of NOX4 expression was observed in proton-irradiated HSCs than that in unirradiated HSCs. These data indicated that NOX enzyme, especially NOX4, might be involved in the induction of ROS production in proton-irradiated HSCs. The importance of ROS production in space radiation-induced HSC injury should further be assessed by using NOX4 inhibitor or other antioxidants, such as N-acetylcysteine and gamma-tocotrienol in future studies. There are some other potential unanswered questions including (1) whether these chronically oxidative-stressed HSCs induced by proton radiation experience senescence, (2) whether these space-irradiated HSCs have chromosomal instability, and (3) whether the chromosomal aberrant HSCs after space radiation result in the leukemia development, which was evidenced in mice after γ-irradiation exposure [38, 65, 68].

One of HSC properties is its self-renewal ability, which is sustained via its slow cycling and quiescence. By using BrdU-chasing assay and H2B-GFP mice model, it has been shown that dominant HSCs divide only once every 145 days, which ensures self-renewal capacity along with providing whole life blood homeostasis and avoiding HSC exhaustion [69]. It was reported that loss of FOXO3a resulted in increasing ROS production and accelerating HSC cycling, which is along with the defect of HSC self-renewal capacity and the exhaustion of HSCs. N-acetylcysteine (NAC), an antioxidant, can protect FOXO3a mutant HSCs from oxidative stress and restore HSC dormancy. The same phenotype was also seen in the case of loss of Bmi-1 and TSC1 in mice [70–72], which is due to increasing ROS production and cycling in HSCs. Upon proton radiation, data have shown that there were far fewer HSCs in G0 phase and higher numbers of HSCs in G_1 phase than nonirradiated controls. Proton radiation-induced HSC cycling is consistent with upregulation of positive cell cycle regulators cyclin D1 and cyclin D3. Although HSC proliferation might compensate for the decreased number of HSCs after proton radiation, it will be at risk of loss self-renewal of HSCs.

Additionally, it has demonstrated that the persistent increase of DNA damage in proton-irradiated HSCs, but not in HPCs, was associated with proton radiation-induced ROS production in HSCs. Unrepaired DNA damage in proton-irradiated HSCs might negatively affect HSC self-renewal, proliferation, and differentiation, leading to long-term functional damage in HSCs. Taken together, these findings provide strong evidence showing that proton TBI induces not only acute hematopoietic injury but also long-term BM suppression and HSC damage. These detrimental effects of proton radiation on hematopoietic cells are closely related to the induction of oxidative stress in irradiated HSCs. The proton exposure-induced acute and long-term hematopoietic damage might be ameliorated through using antioxidants, which should be investigated in the future.

4. Oxygen radiation and hematopoietic stem cells

As we discussed above, GCR contains various HZE particles including ^{56}Fe, ^{28}Si, ^{16}O, and ^{12}C, which have more detrimental effects on normal tissues than do photon and proton radiations during spaceflight. Oxygen (^{16}O) radiation has relatively high-charge and high-linear energy transfer (LET), leading to a high relative biological effectiveness. In this section, we will mainly discuss the biological negative effects of ^{16}O on hematopoietic stem cells in long-duration space missions.

Hematopoietic cells in the body are the most radiosensitive cells to radiation [73, 74]. Exposure to γ-irradiation causes both acute and long-term damage in hematopoietic stem and progenitor cells (HSPCs), which is due primarily to radiation-induced cellular apoptosis and senescence in HSPCs [37–39, 65]. Using porcine and mice model, it has documented that proton radiation induced both acute and long-term hematopoietic damage. We have described the acute and residual effects of proton radiation on hematopoietic stem cells showing that numbers and function of bone marrow HSCs in mice were detrimentally affected. The negative effects of proton radiation mainly contribute to increasing the production of oxidative stress and DNA damage in irradiated HSCs [53]. ^{56}Fe radiation causes significant alterations in the expression of repetitive elements and DNA methylation, and 0.1-0.4 Gy of ^{56}Fe radiation resulted in significant epigenetic changes in hematopoietic stem and progenitor cells in a mouse model [75]. Using cultured human hematopoietic stem and progenitor cells, it was found that ^{12}C radiation induced chromosome aberrations and cellular apoptosis [76]. 0.3–0.9 Gy of ^{28}Si radiation triggers a significant increase of cellular apoptosis in irradiated mice HSCs at 4 weeks after the exposure, which results in the deficiency of numbers and clonogenic function of irradiated HSCs [77]. These findings indicate that GCR, including different forms of ionizing radiation, induces acute and residual injury in hematopoietic stem cells. However, it remains elusive whether ^{16}O radiation induces acute and long-term hematopoietic effects and what main factors are involved in the negative effects on HSCs under ^{16}O exposure.

In one of our experiments, C57BL/6 J mice were exposed to 0.1, 0.25, and 1.0 Gy ^{16}O (600 MeV/n) total-body irradiation (TBI) and analyzed the effects of ^{16}O radiation on peripheral blood and BM 2 weeks after the exposure [51] (**Table 3**). Since hematopoietic cells are known to be radiosensitive, it is not surprising that a significant decrease was observed in peripheral WBC and platelet counts in mice exposed to 1.0 Gy of ^{16}O. In comparison to ^{16}O radiation, peripheral blood cell counts, including numbers of WBCs and platelets, were almost recovered to normal levels at 2 weeks after 1.0 Gy of γ-ray radiation in BALB/c mice [78]. This might due to (1) different animal species used and (2) different biological effectiveness of ^{16}O and γ-ray radiation along with high LET properties of ^{16}O. ^{16}O TBI causes cellular apoptosis in hematopoietic progenitors but not hematopoietic stem cells at 2 weeks postexposure. To monitor how fast HPCs recover from ^{16}O TBI, apoptotic assay was performed at 3 months after 0.1, 0.25, and 1.0 Gy of ^{16}O TBI, showing that the apoptotic levels in HPCs and HSCs after the exposure are similar to those in nonirradiated mice. These data suggest that HPCs have a slower recovery than HSCs after ^{16}O TBI.

	Number of cells		Function of HSCs		
	HPC	LSK cells	BFU-E	CFU-GM	CFU-GEMM
2 weeks	↓	↓	↓	↓	↓
3 months	↓	↓	↓	↓	↓

Note: C57BL/6 mice were exposed to 1.0 Gy of oxygen total body irradiation. The indicated parameters were measured at 2 weeks and 3 months after the exposure. ↓ Indicate that the parameter is decreased when compared to non-irradiated mice.

Table 3.
Acute and long-term effects of oxygen irradiation on hematopoietic cells.

Previous studies have demonstrated that the functional defect of mouse HSCs was observed under either radiation or chemotherapeutic drug treatment. For example, the reconstitution capacity of mouse HSCs has detrimental effects under 1.0 Gy of total-body γ-ray radiation along with apparent myeloid bias differentiation [78, 79]. One dose of 5-fluorouracil treatment significantly decreased the numbers and engraftment ability of mouse HSCs at day 10 after the exposure [80]. To investigate the effects of low doses of ^{16}O TBI on HSC function, in vitro colony-forming assays using bone marrow cells were performed at 2 weeks after 0.1–1.0 Gy doses of ^{16}O exposure. It shows that low doses of ^{16}O TBI not only decreases numbers of HSCs but also abates the in vitro colony-forming abilities, such as decreased numbers of BFU-E, CFU-GM, and CFU-GEMM from irradiated bone marrow cells. These data suggest that the function of HSC after ^{16}O TBI was negative affected despite low doses of ^{16}O used. Using in vitro cell culture model, previous studies have shown that ^{16}O radiation has more dramatic effects on chromosomal aberrations, micronuclei formation, cell survival, and apoptosis than photon radiation [81, 82]. It has been documented that 6.5 Gy total-body γ-irradiation decreased the numbers of bone marrow HSCs up to 50% at 2 weeks postexposure, which was observed at 2 weeks after 1.0 Gy of total-body ^{16}O radiation [38]. These results indicate that ^{16}O radiation has a higher RBE than photon radiation.

To explore long-term effect of ^{16}O TBI on hematopoietic cells, 0.05, 0.1, 0.25, and 1.0 Gy ^{16}O (600 MeV/n) radiations were used to irradiate C57BL/6 J mice. Irradiated mice were analyzed for the long-term effects of ^{16}O radiation on peripheral blood cells and bone marrow cells 3 months postexposure [83]. Although there are the same numbers of peripheral blood cells at 3 months after 0.05 to 1.0 Gy of ^{16}O TBI as nonirradiated controls, numbers of HPC and HSCs from irradiated mice were significantly lower than those from nonirradiated controls. The changes of peripheral blood cell counts after oxygen radiation are similar to the effects of other types of ionizing radiation, such as 0.5 and 1.0 Gy of γ-TBI [84, 85]. Peripheral blood cell counts were back to normal levels 2 months after sublethal doses of γ-ray exposure [86]. Recovery of peripheral blood cell counts may neglect the effects of irradiation on bone marrow HSCs [84], which will result in overlooking the long-term bone marrow suppression after radiation.

We have demonstrated that 0.1 to 1.0 Gy of ^{16}O TBI, but not 0.05 Gy dose, resulted in a dramatic impairment in both numbers and function of bone marrow HSCs in mice at 3 months after exposure. Comparing to ^{16}O TBI, exposure of mice to 0.5 and 1.0 Gy of γ-TBI did not negatively affect the numbers and function of HSC in mice at 3 months after exposure. The phenotype from ^{16}O- and γ-TBI might be related to their RBE along with higher RBE levels of ^{16}O TBI than γ-TBI [81]. The long-term detrimental effects of ^{16}O TBI on bone marrow hematopoietic stem cells have also been seen in 6.5 Gy sublethal doses of γ-rays and 1.0 Gy low dose of proton radiation, showing a reduction in HSC reserves and a defect in HSC function [48, 85, 87, 88]. When comparing the effects of different radiation sources on HPCs, we have shown that acute exposure to low doses of ^{16}O TBI triggered a significant reduction in numbers of HPCs at 2 weeks after exposure. However, numbers of HPCs in irradiated mice recovered back to normal levels 2 weeks after either γ-ray or proton exposure [51]. These data indicate that ^{16}O-irradiated HPCs have a slower recovery than proton- and photon-irradiated HPCs.

We have previously demonstrated that exposure of mice to 1.0 Gy of ^{16}O TBI leads to an increased rate of apoptosis at 2 weeks postexposure in irradiated HPCs but not HSCs. This is consistent with HPC colony-forming ability assay, showing lower numbers of BFU-E, CFU-GM, and CFU-GEMM when compared to those in nonirradiated controls [51]. When we further examined HPC colony-forming abilities at 3 months after same dose of ^{16}O TBI, it showed that numbers of various

colonies were still much lower than those in nonirradiated controls [83]. Notably, the decreased colony-forming abilities after ^{16}O TBI were in a dose-independent manner, which suggests "hit and damage." These data suggest that oxygen irradiation has features with a high-linear energy transfer and strong relative biological effectiveness.

A cobblestone area-forming cell (CAFC) assay is a surrogate in vitro hematopoietic stem cell functional assay. We measured HSC in vitro CAFC-forming ability at 3 months after 0.1, 0.25, and 1.0 Gy of ^{16}O radiation, showing that irradiated mice HSCs had a significant reduction of CAFC numbers independent of radiation doses (**Table 4**). These unusual dose-response curves have also been seen in the studies of ^{28}Si radiation, such as effects of ^{28}Si radiation on synaptic plasticity and contextual fear memory [89, 90]. However, when mice were exposed to 0.05 Gy of ^{16}O TBI, numbers of CAFC were comparable to nonirradiated controls, indicating that HSC function was not affected after exposure to 0.05 Gy ^{16}O TBI [83]. These long-term negative effects of ^{16}O TBI on HSCs are also observed in other different types of ionizing radiation [53, 63, 91]. Mice were exposed to 1.0 Gy of proton total-body irradiation, leading to a significant decrease in the CAFC-forming ability in HSCs at 22 weeks postexposure [53]. 6.5 Gy of γ-ray total-body irradiation caused a reduction in HSC colony-forming ability 2 months postexposure [38]. As we discussed previously, peripheral blood cell counts were back to normal levels at 3 months after low-dose ^{16}O TBI, while the numbers and function of bone marrow HSCs were significantly decreased after exposure. Proliferation of progenitors (such as myeloid, lymphoid, and erythroid progenitors) might contribute to the recovery of peripheral blood cells after radiation. These results suggest that low doses of ionizing radiation can induce long-term HSC suppression, while ^{16}O TBI has stronger abilities to induce the long-term HSC suppression than other types of ionizing radiation.

Cellular apoptosis and ROS production are crucial mediators in irradiation-induced cell damage. We exposed mice to 1.0 Gy of ^{16}O, and it triggered an aberrant increase in ROS production in HPCs and HSCs 2 weeks after exposure [51]. Meanwhile, increasing levels of apoptosis were significant in irradiated HPCs, but not LSK cells and HSCs, when compared to nonirradiated controls. Whether the different acute responses of HPCs and HSCs to ^{16}O TBI are related to ROS production has yet to be determined. Induction of ROS production persisted up to 3 months after ^{16}O TBI [83], which is congruent with the decreased expression of the antioxidant genes GPX2 and SOD3 in 1.0 Gy ^{16}O-irradiated HSCs when compared to nonirradiated HSCs. Previous studies utilized proton and γ-ray radiation to prove that HSC functional impairment might be attributable to the accumulation of residual ROS [53, 63, 92, 93]. This is evidenced by a decrease in in vivo

	HPC			LSK cells		
	Apoptosis	ROS	DNA damage	Apoptosis	ROS	DNA damage
2 weeks	↑	↑	no change	no change	↑	↑
3 months	no change	no change	no change	no change	↑	no change

Note: C57BL/6 mice were exposed to 1.0 Gy of oxygen total body irradiation. The indicated parameters were measured at 2 weeks and 3 months after the exposure.
↑Indicate that the parameter is increased when compared to non-irradiated mice. No change means that the parameter is similar to that from non-irradiated mice.

Table 4.
Induction of cellular apoptosis, ROS, and DNA damage after oxygen irradiation.

engraftment capacity and in vitro colony-forming ability using bone marrow cells after radiation. The importance of ROS overproduction on functional HSCs was not only supported under radiation stress condition but also supported by other genetic animal models. Deletion of Foxo3, ATM, TSC1, and Bmi-1 in mice leads to the impairment of numbers and function of HSCs along with increasing ROS production. Application of antioxidants, such as N-acetyl cysteine (NAC), on these mutant mice significantly ameliorated the HSC functional deficiency [71, 94–96]. We have provided data showing ^{16}O-irradiated HSCs had higher levels of ROS production than nonirradiated animals in both acute and long-term studies. It is well accepted that mitochondrial oxidative phosphorylation and NADPH oxidases are two main sources to produce ROS in mammalian cells. Because HSCs reside in hypoxic environmental niche in the bone marrow and have higher expression of HIF1α in response to hypoxia, HSCs produce ROS mainly through glycolysis and NOX enzyme. We have previously shown that proton and γ-ray radiation induced significantly upregulation of NOX4 in irradiated HSCs [53, 66]. The NOX4 inhibitor diphenyliodonium can partially protect functional HSCs from γ-irradiation-induced long-term damage. Therefore, antioxidants, such as NOX4 inhibitors and NAC, should be further tested whether inhibiting ROS production can decrease ^{16}O TBI-induced ROS production to accelerate the functional recovery of HPCs and HSCs after ^{16}O irradiation exposure.

Under radiotherapy and chemotherapy stress conditions, dominant HSCs might be activated from quiescent status to provide the need for stressed hematopoietic system. However, frequent HSC activation might cause its loss of self-renewal ability, differentiation, and death with bone marrow failure syndrome [69, 97]. We have shown that proton and γ-irradiation can efficiently activate quiescent HSCs [53, 65], leading to the redistribution of different cell cycle phases and stem cell functional defects. Data from genetic mice models, such as depletion of FOXO3a and Lkb1, showed that HSCs had fast cycling with loss of HSC self-renewal ability and HSC exhaustion [94, 98–100]. There are fewer numbers of HSCs in G_0 and higher numbers in G_1/G_2SM at 2 weeks after ^{16}O TBI [51]. Additionally, we observed that around 15% of irradiated HSCs had more than two γH2AX foci per cell 2 weeks after ^{16}O exposure [51], which is positively correlated with the increased ROS production in ^{16}O TBI HSCs. Taken together, all of ROS production, DNA damage, and HSC cycling after ^{16}O TBI might contribute to HSC defect induced by oxygen radiation, which will be tested in our future studies.

Note:

1. Due to the large difference in size between mice and human subjects, the dose-depth distribution in mice exposed to protons is different from that in humans [1]. The dose to the blood-forming tissues in humans will be lower, whereas the dose to the blood-forming tissues in mice is likely very similar to the dose at the surface. This difference in dose-depth profile should be taken into considerations when translating risk of hematopoietic damage in response to space radiation in a mouse model to the human situation.

2. During long-term space flight (such as to Mars), astronauts will encounter either a chronic, fractionated dose of space radiation or they will accumulate 1–3 Gy of radiation exposure. Presently, it is impossible to achieve chronic or fractionated doses of space radiation due to the limited access to the facilities capable of producing synthetic space radiation. In most research to date, investigators have used either acute radiation exposure or higher doses of space radiation in animal models. Cautions should therefore be taken when applying those findings from animal studies to human.

3. The protons and oxygen nuclei in the studies described here were all delivered within a few minutes, and most charged particle exposures during space flight occur at a very low-dose rate and/or are fractionated. Though we acknowledge that the high-dose rates we used are a limitation of our studies, low-dose rate exposures were not possible because of practical constraints. Future studies with low-dose rate or fractionated exposures should provide further insight into dose-rate dependence of hematopoietic stem/progenitor cell response to high-energy charged particle radiation.

5. Conclusion

In summary, proton and oxygen space radiation have detrimental effects on the hematopoietic system even with at low doses, which will have potential implications for health outcomes during long-duration space missions. Increasing ROS production might be a major mediator on space radiation-induced HSC damage. Knowledge gained from this chapter could aid in planning countermeasure strategies to protect against hematopoietic effects of radiation exposure during space travel. To minimize the health negative effects of deep-space travel, decreasing oxidative stress might be a good approach to mitigate the adverse effects of proton and HZE particle exposure on the hematopoietic system.

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Conflict of interest

The authors declare no conflicts of interest.

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References

[1] Cengel KA, Diffenderfer ES, Avery S, Kennedy AR, McDonough J. Using electron beam radiation to simulate the dose distribution for whole body solar particle event proton exposure. Radiation and Environmental Biophysics. 2010;**49**(4):715-721

[2] Townsend LW. Implications of the space radiation environment for human exploration in deep space. Radiation Protection Dosimetry. 2005;**115**(1-4):44-50

[3] Simonsen LC, Cucinotta FA, Atwell W, Nealy JE. Temporal analysis of the October 1989 proton flare using computerized anatomical models. Radiation Research. 1993;**133**(1):1-11

[4] Parsons JL, Townsend LW. Interplanetary crew dose rates for the August 1972 solar particle event. Radiation Research. 2000;**153**(6):729-733

[5] Moore FD. Radiation burdens for humans on prolonged exomagnetospheric voyages. FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology. 1992;**6**(6):2338-2343

[6] Datta K, Suman S, Trani D, Doiron K, Rotolo JA, Kallakury BV, et al. Accelerated hematopoietic toxicity by high energy (56)Fe radiation. International Journal of Radiation Biology. 2012;**88**(3):213-222

[7] Boerma M, Sridharan V, Mao XW, Nelson GA, Cheema AK, Koturbash I, et al. Effects of ionizing radiation on the heart. Mutation Research. 2016;**770**(Pt B):319-327

[8] Suman S, Datta K, Trani D, Laiakis EC, Strawn SJ, Fornace AJ Jr. Relative biological effectiveness of 12C and 28Si radiation in C57BL/6J mice. Radiation and Environmental Biophysics. 2012;**51**(3):303-309

[9] Cucinotta FA, Wu H, Shavers MR, George K. Radiation dosimetry and biophysical models of space radiation effects. Gravitational and Space Biology Bulletin. 2003;**16**(2):11-18

[10] Walker SA, Townsend LW, Norbury JW. Heavy ion contributions to organ dose equivalent for the 1977 galactic cosmic ray spectrum. Advances in Space Research. 2013;**51**(9):1792-1799

[11] Zeitlin C, Hassler DM, Cucinotta FA, Ehresmann B, Wimmer-Schweingruber RF, Brinza DE, et al. Measurements of energetic particle radiation in transit to Mars on the Mars Science Laboratory. Science. 2013;**340**(6136):1080-1084

[12] Hamilton SA, Pecaut MJ, Gridley DS, Travis ND, Bandstra ER, Willey JS, et al. A murine model for bone loss from therapeutic and space-relevant sources of radiation. Journal of Applied Physiology. 2006;**101**(3):789-793

[13] Shouse SS, Warren SL, Whipple GH. II. Aplasia of marrow and fatal intoxication in dogs produced by roentgen radiation of all bones. The Journal of Experimental Medicine. 1931;**53**(3):421-435

[14] Raber J, Yamazaki J, Torres ERS, Kirchoff N, Stagaman K, Sharpton T, et al. Combined effects of three high-energy charged particle beams important for space flight on brain, behavioral and cognitive endpoints in B6D2F1 female and male mice. Frontiers in Physiology. 2019;**10**:179

[15] Iancu OD, Boutros SW, Olsen RHJ, Davis MJ, Stewart B, Eiwaz M, et al. Space radiation alters

genotype-phenotype correlations in fear learning and memory tests. Frontiers in Genetics. 2018;**9**:404

[16] Warren SL, Whipple GH. Roentgen ray intoxication: I. Unit dose over thorax negative-over abdomen lethal. Epithelium of small intestine sensitive to X-rays. The Journal of Experimental Medicine. 1922;**35**(2):187-202

[17] Jacobson LO. Evidence for a humoral factor (or factors) concerned in recovery from radiation injury: A review. Cancer Research. 1952;**12**(5):315-325

[18] Jacobson LO. Hematopoietic responses to radiation injury. Annual Review of Medicine. 1956;**7**:345-352

[19] Ford CE, Hamerton JL, Barnes DW, Loutit JF. Cytological identification of radiation-chimaeras. Nature. 1956;**177**(4506):452-454

[20] Nowell PC, Cole LJ, Habermeyer JG, Roan PL. Growth and continued function of rat marrow cells in x-radiated mice. Cancer Research. 1956;**16**(3):258-261

[21] Trentin JJ. Mortality and skin transplantability in x-irradiated mice receiving isologous, homologous or heterologous bone marrow. Proceedings of the Society for Experimental Biology and Medicine. 1956;**92**(4):688-693

[22] Becker AJ, Mc CE, Till JE. Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. Nature. 1963;**197**:452-454

[23] Till JE, Mc CE. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiation Research. 1961;**14**:213-222

[24] Lorimore SA, Coates PJ, Wright EG. Radiation-induced genomic instability and bystander effects: Inter-related nontargeted effects of exposure to ionizing radiation. Oncogene. 2003;**22**(45):7058-7069

[25] Morgan WF. Non-targeted and delayed effects of exposure to ionizing radiation: II. Radiation-induced genomic instability and bystander effects in vivo, clastogenic factors and transgenerational effects. Radiation Research. 2003;**159**(5):581-596

[26] McCulloch EA, Till JE. The radiation sensitivity of normal mouse bone marrow cells, determined by quantitative marrow transplantation into irradiated mice. Radiation Research. 1960;**13**:115-125

[27] 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**(7):1109-1121

[28] Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC. Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. The Journal of Experimental Medicine. 1996;**183**(4):1797-1806

[29] Weissman IL, Anderson DJ, Gage F. Stem and progenitor cells: Origins, phenotypes, lineage commitments, and transdifferentiations. Annual Review of Cell and Developmental Biology. 2001;**17**:387-403

[30] Wilson A, Laurenti E, Trumpp A. Balancing dormant and self-renewing hematopoietic stem cells. Current Opinion in Genetics & Development. 2009;**19**(5):461-468

[31] Wang Y, Schulte BA, Zhou D. Hematopoietic stem cell senescence and long-term bone marrow injury. Cell Cycle. 2006;**5**(1):35-38

[32] Nelson GA. Space radiation and human exposures, a primer. Radiation Research. 2016;**185**(4):349-358. Epub 2016/03/29

[33] Raju MR. Proton radiobiology, radiosurgery and radiotherapy. International Journal of Radiation Biology. 1995;**67**(3):237-259

[34] Loeffler JS, Smith AR, Suit HD. The potential role of proton beams in radiation oncology. Seminars in Oncology. 1997;**24**(6):686-695

[35] Chambers KA, Harrington NP, Ross WM, Filion LG. Relative alterations in blood mononuclear cell populations reflect radiation injury in mice. Cytometry. 1998;**31**(1):45-52

[36] Harrington NP, Chambers KA, Ross WM, Filion LG. Radiation damage and immune suppression in splenic mononuclear cell populations. Clinical and Experimental Immunology. 1997;**107**(2):417-424

[37] Shao L, Sun Y, Zhang Z, Feng W, Gao Y, Cai Z, et al. Deletion of proapoptotic Puma selectively protects hematopoietic stem and progenitor cells against high-dose radiation. Blood. 2010;**115**(23):4707-4714

[38] Wang Y, Schulte BA, LaRue AC, Ogawa M, Zhou D. Total body irradiation selectively induces murine hematopoietic stem cell senescence. Blood. 2006;**107**(1):358-366

[39] Yu H, Shen H, Yuan Y, XuFeng R, Hu X, Garrison SP, et al. Deletion of Puma protects hematopoietic stem cells and confers long-term survival in response to high-dose gamma-irradiation. Blood. 2010;**115**(17):3472-3480

[40] Kajioka EH, Andres ML, Li J, Mao XW, Moyers MF, Nelson GA, et al. Acute effects of whole-body proton irradiation on the immune system of the mouse. Radiation Research. 2000; **153**(5 Pt 1):587-594

[41] Gridley DS, Rizvi A, Luo-Owen X, Makinde AY, Coutrakon GB, Koss P, et al. Variable hematopoietic responses to acute photons, protons and simulated solar particle event protons. In Vivo. 2008;**22**(2):159-169

[42] Ware JH, Sanzari J, Avery S, Sayers C, Krigsfeld G, Nuth M, et al. Effects of proton radiation dose, dose rate and dose fractionation on hematopoietic cells in mice. Radiation Research. 2010;**174**(3):325-330

[43] Maks CJ, Wan XS, Ware JH, Romero-Weaver AL, Sanzari JK, Wilson JM, et al. Analysis of white blood cell counts in mice after gamma- or proton-radiation exposure. Radiation Research. 2011;**176**(2):170-176

[44] Luo-Owen X, Pecaut MJ, Rizvi A, Gridley DS. Low-dose total-body gamma irradiation modulates immune response to acute proton radiation. Radiation Research. 2012;**177**(3):251-264

[45] Sanzari JK, Cengel KA, Steven Wan X, Rusek A, Kennedy AR. Acute hematological effects in mice exposed to the expected doses, dose-rates, and energies of solar particle event-like proton radiation. Life Sciences and Space Research. 2014;**2**:86-91

[46] Wagemaker G. Heterogeneity of radiation sensitivity of hemopoietic stem cell subsets. Stem Cells. 1995;**13**(Suppl 1):257-260

[47] Gridley DS, Pecaut MJ. Whole-body irradiation and long-term modification of bone marrow-derived cell populations by low- and high-LET radiation. In Vivo. 2006;**20**(6B):781-789

[48] Shao L, Luo Y, Zhou D. Hematopoietic stem cell injury induced by ionizing radiation. Antioxidants & Redox Signaling. 2014;**20**(9):1447-1462

[49] Shao L, Wang Y, Chang J, Luo Y, Meng A, Zhou D. Hematopoietic stem cell senescence and cancer therapy-induced long-term bone marrow injury. Translational Cancer Research. 2013;**2**(5):397-411

[50] Chang J, Wang Y, Pathak R, Sridharan V, Jones T, Mao XW, et al. Whole body proton irradiation causes acute damage to bone marrow hematopoietic progenitor and stem cells in mice. International Journal of Radiation Biology. 2017;**93**(12):1312-1320

[51] Chang J, Luo Y, Wang Y, Pathak R, Sridharan V, Jones T, et al. Low doses of oxygen ion irradiation cause acute damage to hematopoietic cells in mice. PLoS One. 2016;**11**(7):e0158097

[52] Cucinotta FA, Plante I, Ponomarev AL, Kim MH. Nuclear interactions in heavy ion transport and event-based risk models. Radiation Protection Dosimetry. 2011;**143**(2-4):384-390

[53] Chang J, Feng W, Wang Y, Luo Y, Allen AR, Koturbash I, et al. Whole-body proton irradiation causes long-term damage to hematopoietic stem cells in mice. Radiation Research. 2015;**183**(2):240-248

[54] Major IR, Mole RH. Myeloid leukaemia in x-ray irradiated CBA mice. Nature. 1978;**272**(5652):455-456

[55] Shimizu Y, Kato H, Schull WJ. Risk of cancer among atomic bomb survivors. Journal of Radiation Research. 1991;**32**(Suppl 2):54-63

[56] Kennedy AR, Davis JG, Carlton W, Ware JH. Effects of dietary antioxidant supplementation on the development of malignant lymphoma and other neoplastic lesions in mice exposed to proton or iron-ion radiation. Radiation Research. 2008;**169**(6):615-625

[57] Sanzari JK, Wan SX, Diffenderfer ES, Cengel KA, Kennedy AR. Relative biological effectiveness of simulated solar particle event proton radiation to induce acute hematological change in the porcine model. Journal of Radiation Research. 2014;**55**(2):228-244

[58] Desai N, Davis E, O'Neill P, Durante M, Cucinotta FA, Wu H. Immunofluorescence detection of clustered gamma-H2AX foci induced by HZE-particle radiation. Radiation Research. 2005;**164**(4 Pt 2):518-522

[59] Goetz W, Morgan MN, Baulch JE. The effect of radiation quality on genomic DNA methylation profiles in irradiated human cell lines. Radiation Research. 2011;**175**(5):575-587

[60] Tian J, Zhao W, Tian S, Slater JM, Deng Z, Gridley DS. Expression of genes involved in mouse lung cell differentiation/regulation after acute exposure to photons and protons with or without low-dose preirradiation. Radiation Research. 2011;**176**(5):553-564

[61] Di Pietro C, Piro S, Tabbi G, Ragusa M, Di Pietro V, Zimmitti V, et al. Cellular and molecular effects of protons: Apoptosis induction and potential implications for cancer therapy. Apoptosis. 2006;**11**(1):57-66

[62] Dorr H, Meineke V. Acute radiation syndrome caused by accidental radiation exposure - therapeutic principles. BMC Medicine. 2011;**9**:126

[63] Li H, Wang Y, Pazhanisamy SK, Shao L, Batinic-Haberle I, Meng A, et al. Mn(III) meso-tetrakis-(N-ethylpyridinium-2-yl) porphyrin mitigates total body irradiation-induced long-term bone marrow suppression. Free Radical Biology & Medicine. 2011;**51**(1):30-37

[64] Zhang H, Zhai Z, Wang Y, Zhang J, Wu H, Li C, et al. Resveratrol

ameliorates ionizing irradiation-induced long-term hematopoietic stem cell injury in mice. Free Radical Biology & Medicine. 2013;**54**:40-50

[65] Shao L, Feng W, Li H, Gardner D, Luo Y, Wang Y, et al. Total body irradiation causes long-term mouse BM injury via induction of HSC premature senescence in an Ink4a- and Arf-independent manner. Blood. 2014;**123**(20):3105-3115

[66] 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 Radical Biology & Medicine. 2010;**48**(2):348-356

[67] Takubo K, Goda N, Yamada W, Iriuchishima H, Ikeda E, Kubota Y, et al. Regulation of the HIF-1alpha level is essential for hematopoietic stem cells. Cell Stem Cell. 2010;**7**(3):391-402

[68] Pazhanisamy SK, Li H, Wang Y, Batinic-Haberle I, Zhou D. NADPH oxidase inhibition attenuates total body irradiation-induced haematopoietic genomic instability. Mutagenesis. 2011;**26**(3):431-435

[69] Wilson A, Laurenti E, Oser G, van der Wath RC, Blanco-Bose W, Jaworski M, et al. Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. Cell. 2008;**135**(6):1118-1129

[70] Tothova Z, Kollipara R, Huntly BJ, Lee BH, Castrillon DH, Cullen DE, et al. FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. Cell. 2007;**128**(2):325-339

[71] Chen C, Liu Y, Liu R, Ikenoue T, Guan KL, Liu Y, et al. TSC-mTOR maintains quiescence and function of hematopoietic stem cells by repressing mitochondrial biogenesis and reactive oxygen species. The Journal of Experimental Medicine. 2008;**205**(10):2397-2408

[72] Schuringa JJ, Vellenga E. Role of the polycomb group gene BMI1 in normal and leukemic hematopoietic stem and progenitor cells. Current Opinion in Hematology. 2010;**17**(4):294-299

[73] Waselenko JK, MacVittie TJ, Blakely WF, Pesik N, Wiley AL, Dickerson WE, et al. Medical management of the acute radiation syndrome: Recommendations of the Strategic National Stockpile Radiation Working Group. Annals of Internal Medicine. 2004;**140**(12):1037-1051

[74] Moroni M, Coolbaugh TV, Lombardini E, Mitchell JM, Moccia KD, Shelton LJ, et al. Hematopoietic radiation syndrome in the Gottingen minipig. Radiation Research. 2011;**176**(1):89-101

[75] Miousse IR, Shao L, Chang J, Feng W, Wang Y, Allen AR, et al. Exposure to low-dose (56)Fe-ion radiation induces long-term epigenetic alterations in mouse bone marrow hematopoietic progenitor and stem cells. Radiation Research. 2014;**182**(1):92-101

[76] Becker D, Elsasser T, Tonn T, Seifried E, Durante M, Ritter S, et al. Response of human hematopoietic stem and progenitor cells to energetic carbon ions. International Journal of Radiation Biology. 2009;**85**(11):1051-1059

[77] Chang J, Feng W, Wang Y, Allen AR, Turner J, Stewart B, et al. (28)Si total body irradiation injures bone marrow hematopoietic stem cells via induction of cellular apoptosis. Life Sciences and Space Research. 2017;**13**:39-44

[78] Stewart FM, Zhong S, Wuu J, Hsieh C, Nilsson SK, Quesenberry PJ. Lymphohematopoietic engraftment in minimally myeloablated hosts. Blood. 1998;**91**(10):3681-3687

[79] Stewart FM, Zhong S, Lambert JF, Colvin GA, Abedi M, Dooner MS, et al. Host marrow stem cell potential and engraftability at varying times after low-dose whole-body irradiation. Blood. 2001;**98**(4):1246-1251

[80] Haug JS, He XC, Grindley JC, Wunderlich JP, Gaudenz K, Ross JT, et al. N-cadherin expression level distinguishes reserved versus primed states of hematopoietic stem cells. Cell Stem Cell. 2008;**2**(4):367-379

[81] Habermehl D, Ilicic K, Dehne S, Rieken S, Orschiedt L, Brons S, et al. The relative biological effectiveness for carbon and oxygen ion beams using the raster-scanning technique in hepatocellular carcinoma cell lines. PLoS One. 2014;**9**(12):e113591

[82] Pathak R, Dey SK, Sarma A, Khuda-Bukhsh AR. Cell killing, nuclear damage and apoptosis in Chinese hamster V79 cells after irradiation with heavy-ion beams of (16)O, (12)C and (7)Li. Mutation Research. 2007;**632**(1-2):58-68

[83] Wang Y, Chang J, Li X, Pathak R, Sridharan V, Jones T, 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**(12):e0189466

[84] Chang J, Wang Y, Shao L, Laberge RM, Demaria M, Campisi J, et al. Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. Nature Medicine. 2016;**22**(1):78-83

[85] Xu G, Wu H, Zhang J, Li D, Wang Y, Zhang H, et al. Metformin ameliorates ionizing irradiation-induced long-term hematopoietic stem cell injury in mice. Free Radical Biology & Medicine. 2015;**87**:15-25

[86] Testa NG, Hendry JH, Molineux G. Long-term bone marrow damage in experimental systems and in patients after radiation or chemotherapy. Anticancer Research. 1985;**5**(1):101-110

[87] Farres J, Martin-Caballero J, Martinez C, Lozano JJ, Llacuna L, Ampurdanes C, et al. Parp-2 is required to maintain hematopoiesis following sublethal gamma-irradiation in mice. Blood. 2013;**122**(1):44-54

[88] Chua HL, Plett PA, Sampson CH, Joshi M, Tabbey R, Katz BP, et al. Long-term hematopoietic stem cell damage in a murine model of the hematopoietic syndrome of the acute radiation syndrome. Health Physics. 2012;**103**(4):356-366

[89] Raber J, Marzulla T, Stewart B, Kronenberg A, Turker MS. 28Silicon irradiation impairs contextual fear memory in B6D2F1 mice. Radiation Research. 2015;**183**(6):708-712

[90] Raber J, Rudobeck E, Campbell-Beachler M, Allen AR, Allen B, Rosi S, et al. (28)Silicon radiation-induced enhancement of synaptic plasticity in the hippocampus of naive and cognitively tested mice. Radiation Research. 2014;**181**(4):362-368

[91] Epperly MW, Wang H, Jones JA, Dixon T, Montesinos CA, Greenberger JS. Antioxidant-chemoprevention diet ameliorates late effects of total-body irradiation and supplements radioprotection by MnSOD-plasmid liposome administration. Radiation Research. 2011;**175**(6):759-765

[92] Chai X, Li D, Cao X, Zhang Y, Mu J, Lu W, et al. ROS-mediated iron overload injures the hematopoiesis of bone marrow by damaging hematopoietic stem/progenitor cells in mice. Scientific Reports. 2015;**5**:10181

[93] Cho J, Yusuf R, Kook S, Attar E, Lee D, Park B, et al. Purinergic P2Y(1)(4) receptor modulates stress-induced

hematopoietic stem/progenitor cell senescence. The Journal of Clinical Investigation. 2014;**124**(7):3159-3171

[94] Miyamoto K, Araki KY, Naka K, Arai F, Takubo K, Yamazaki S, et al. Foxo3a is essential for maintenance of the hematopoietic stem cell pool. Cell Stem Cell. 2007;**1**(1):101-112

[95] Nitta E, Yamashita M, Hosokawa K, Xian M, Takubo K, Arai F, et al. Telomerase reverse transcriptase protects ATM-deficient hematopoietic stem cells from ROS-induced apoptosis through a telomere-independent mechanism. Blood. 2011;**117**(16):4169-4180

[96] Nakamura S, Oshima M, Yuan J, Saraya A, Miyagi S, Konuma T, et al. Bmi1 confers resistance to oxidative stress on hematopoietic stem cells. PLoS One. 2012;**7**(5):e36209

[97] Pietras EM, Warr MR, Passegue E. Cell cycle regulation in hematopoietic stem cells. The Journal of Cell Biology. 2011;**195**(5):709-720

[98] Nakada D, Saunders TL, Morrison SJ. Lkb1 regulates cell cycle and energy metabolism in haematopoietic stem cells. Nature. 2010;**468**(7324):653-658

[99] Lorenz E, Uphoff D, Reid TR, Shelton E. Modification of irradiation injury in mice and guinea pigs by bone marrow injections. Journal of the National Cancer Institute. 1951;**12**:197-201

[100] Main JM, Prehn RT. Successful skin homografts after the administration of high dosage X radiation and homologous bone marrow. Journal of the National Cancer Institute. 1955;**15**:1023-1029