Low-dose X-rays leave scars on human hematopoietic stem and progenitor cells: the role of reactive oxygen species

Masayuki Yamashita[1] and Toshio Suda[2,3]

[1]Division of Stem Cell and Molecular Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan; [2]International Research Center for Medical Sciences, Kumamoto University, Kumamoto, Japan and [3]Cancer Science Institute, National University of Singapore, Singapore

E-mail: TOSHIO SUDA - sudato@keio.jp

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After Röntgen's discovery in 1895, an X-ray became a game changer in medicine.[1] It was discovered as an invisible ray of light that passes through many objects, including human bodies, and visualizes the internal organs and structures as silhouettes. As now seen in medical radiography, such as chest X-rays and computed tomography (CT) scans, X-rays have enabled investigation of deep tissues in humans that had been otherwise impossible without surgical intervention, contributing to the early detection and treatment of many diseases. However, as is often the case with new medicine, X-rays were shown to have a biohazard effect.[2] They are identified as a type of ionizing radiation (IR): a stream of high energy photons that are strong enough to ionize atoms and disrupt molecular bonds in biomolecules, including DNA. As DNA encodes an essential blueprint of a cell, the DNA-damaging property of X-rays can be toxic. This effect, although used for killing cancer cells in radiotherapy, has raised concerns about the effect of X-rays on normal tissues and whether the benefits exceed the risks.

Modern medicine relies heavily on radiography to assess human health. The annual doses of X-rays people receive are increasing. A recent study estimated that around 2% or 4,000,000 of the non-elderly adults in the US receive 20 milligray (mGy) or more per year due to medical requirements.[3] Historically, risks associated with low-dose IR are considered to be almost negligible as it does not cause any acute toxicity, nor does it increase the risk of carcinogenesis, based on empirical linear fits of existing human data determined at high doses, such as those of Japanese atomic bomb survivors.[4] Indeed, low-dose IR rarely induces DNA double strand breaks (DSB), which often cause mutations and are considered to be the most relevant lesion for the deleterious effects of IR.[5] However, even though low-dose X-rays rarely cause DSB, they are reportedly less easy to repair than those induced by high-dose X-rays.[6] Importantly, recent evidence suggests that cumulative doses of 50 mGy X-ray (doses equivalent to 5-10 brain CT scans when given in childhood) have long-term detrimental effects on human health, including a more than 3-fold increase in the risks of acute lymphoblastic leukemia and myelodysplastic syndrome.[7] Furthermore, mouse studies demonstrate that low-dose X-rays affect function of long-lived tissue-specific stem cells, including hematopoietic stem cells (HSC).[8,9] Thus, understanding the persistent effect of low-dose X-rays on human tissue-specific stem cells is of particular importance in precisely evaluating the risks posed by radiography on public health.

In this issue of *Haematologica*, Henry *et al.* compared the effects of low and high doses of X-rays on hematopoietic stem and progenitor cells (HSPC) obtained from human umbilical cord blood (CB) (Figure 1).[10] HSPC sustain themselves *via* self-renewing ability, and give rise to all of the blood lineage cells, such as innate and acquired immune cells, erythrocytes and platelets, through multi-lineage differentiation. They found that a single dose of 20 mGy X-rays is sufficient to impair the self-renewing capacity of CB HSPC. Intriguingly, this effect is independent of canonical DNA damage response (DDR), as a 20 mGy dose fails to induce DSB markers γ–H2AX and 53BP1 foci, or DDR hallmarks phospho-ATM and -p53, all of which are induced by a 2.5 Gy dose. Instead, the authors demonstrate that it is mediated by reactive oxygen species (ROS), a highly reactive oxygen byproduct mainly generated *via* the cell respiratory process of oxidative phosphorylation (OXPHOS) in mitochondria, and p38/MAPK14, a key enzyme that, upon elevation of ROS, sends a signal to HSPC to inhibit their self-renewing potential.[11] Thus, the results of Henry *et al.* indicate that low-dose X-rays impair human CB HSPC function through ROS and p38/MAPK14, but not *via* canonical DDR *via* ATM or p53.

The high sensitivity of HSC to elevated levels of ROS is well established, first in ATM deficiency and later in the contexts of other stress conditions.[11-13] Similarly, p38/MAPK14 activation in response to ROS elevation is identified as a common downstream pathway responsible for impairment of self-renewal in HSC.[11,12] In contrast, what is often unclear is the upstream mediator that causes ROS elevation. In the context of low-dose IR, mouse studies have uncovered the hypersensitivity of HSC and esophageal stem cells to low-dose IR that is mediated by ROS elevation, although the molecular link between low-dose IR and elevated ROS has not yet been investigated.[8,9] It is estimated that approximately 90% of ROS can be generated during OXPHOS in mitochondria,[14] mainly through functions of complexes I and III.[15] Interestingly, the results shown by Henry *et al.* indicate that ROS elevation in human CB HSPC upon exposure to 20 mGy X-rays is closely associated with loss of mitochondrial membrane potential, which reflects a decrease in proton gradient across the cristae and often correlates with mitochondrial dysfunction.[10] Apart from nucleus, mitochondria are the only organelle in mammalian cells that contain DNA, which can also be damaged by low-dose IR.[16] Mitochondrial DNA (mtDNA) encodes proteins that consist of complexes I and ATP synthase, both of which are essential for proper electron transport and OXPHOS. Of note, these components are located in the so-called "common deletion" region of mtDNA that is commonly deleted upon exposure to low-dose IR. mtDNA is not protected by histones, and is thus potentially more susceptible to IR-induced damage compared to nuclear DNA. Moreover, mtDNA is located in matrix inside inner membranes where ROS is generated, and is thus more greatly affected by IR-induced oxidative stress

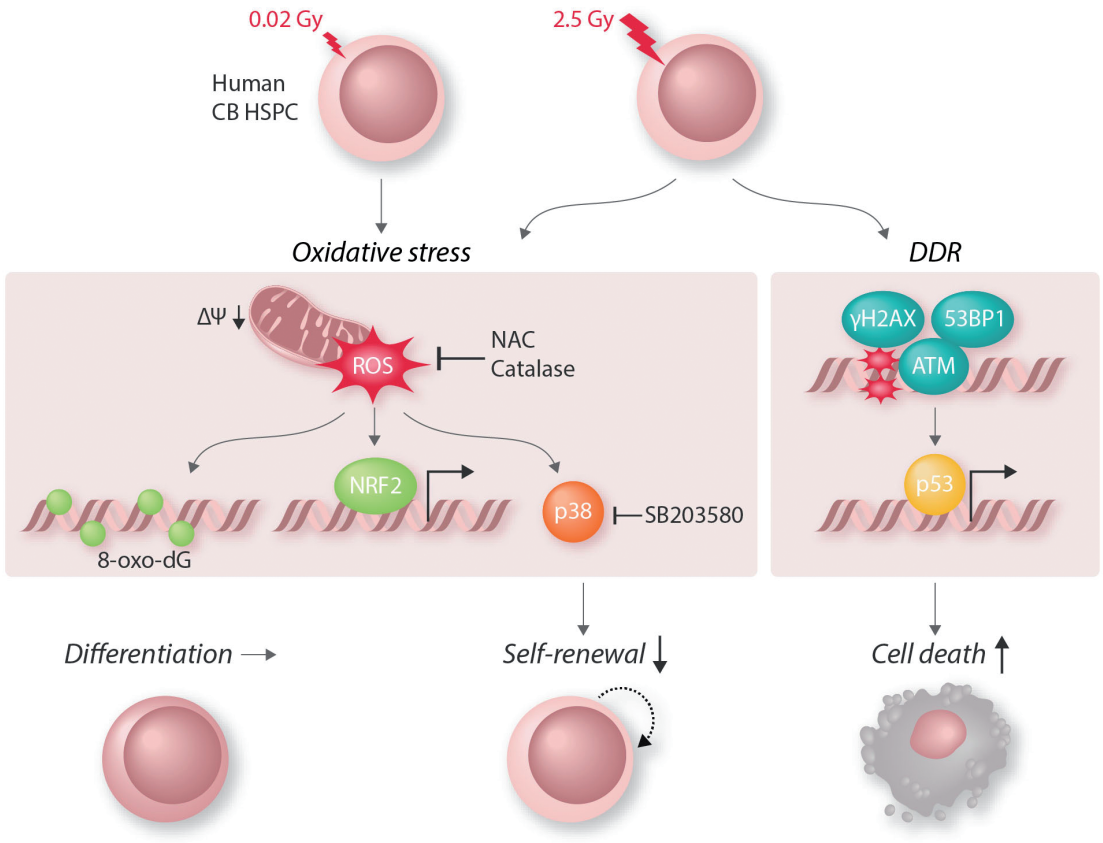

Figure 1. Response of human cord blood (CB) hematopoietic stem and progenitor cells (HSPC) to low- and high-dose X-ray irradiation demonstrated by Henri *et al.*[10] A low dose of 0.02 Gy (20 mGy) X-rays induces reactive oxygen species (ROS) elevation coupled with decrease in mitochondrial membrane potential ($\Delta\Psi$), which leads to increase in oxidative stress represented by formation of 8-oxo-deoxyguanosine (8-oxo-dG) in DNA, nuclear expression of NRF2, and activation of p38/MAPK14. The p38/MAPK14 activation mediates a decline in self-renewing capacity of HSPC without affecting their differentiating potential. The low-dose X-rays do not induce γ-H2AX and 53BP1 foci that represents nuclear DNA double strand breaks (DSB), or canonical DNA damage response *via* phosphorylation of ATM and p53. In contrast, a high dose of 2.5 Gy X-ray irradiation causes both ROS elevation and nuclear DSB. As a result, ROS inhibition either by N-acetylcysteine (NAC) or catalase, or p38 inhibition by SB203580, can reverse the detrimental effect by low dose, but not high dose, of X-rays on self-renewal capacity of HSPC.

than nuclear DNA. Damage in mtDNA is not so simple as that in nuclear DNA, as a cell can contain more than 1,000 copies of mtDNA. Furthermore, numbers of mitochondria are dynamically changed by fusion and fission, which play critical roles in maintaining functional mitochondria *via* inter-mitochondrial complementation and quality control.[17] In addition, damaged mitochondria can be removed by autophagy, which contributes to maintenance of self-renewal capacity of HSC.[18,19] Henry *et al.* show that mitochondrial mass in HSPC does not seem to change after irradiation of 20 mGy X-rays.[10] Although this observation should be validated by other methods,[20] it supports the idea that changes in mitochondrial mass are unlikely to be the cause of ROS elevation. Rather, it is tempting to speculate that damage in mtDNA induced by low-dose IR causes persistent changes in mitochondrial function that lead to initial elevation of ROS and long-term impairment of HSC function. This would be consistent with the results reported by Rodrigues-Moreira *et al.*, which demonstrate that low-dose X-rays cause biphasic elevations of ROS and the second wave of ROS elevation causes persistent reduction in self-renewing capacity of mouse bone marrow HSC.[9] Mitochondrial dysfunction, but not constant elevation of ROS, is implicated in age-associated decline in HSC function.[18] Since involvement of mtDNA remains unknown, investigating whether aged HSC have mtDNA damage would be of particular interest. Collectively, identifying molecular 'scars' left by low-dose X-rays on HSC would help provide a precise evaluation of the long-term detrimental effects by medical radiographic examination and also find common mechanisms that underlie hematopoietic aging and disease.

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Busy signal: platelet-derived growth factor activation in myelofibrosis

Anna E. Marneth[1] and Ann Mullally[1,2,3]

[1]Division of Hematology, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston; [2]Dana-Farber Cancer Institute, Harvard Medical School, Boston and [3]Broad Institute, Cambridge, MA, USA.

E-mail: ANN MULLALLY - amullally@partners.org

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The pathogenesis of myelofibrosis, a bone marrow (BM) disorder characterized by megakaryocytic hyperplasia and the deposition of extracellular matrix components such as reticulin, remains incompletely understood.

Using a mouse model of myelofibrosis (i.e. Gata-1^{low} mice), Kramer *et al.*[1] sought to identify key signaling molecules that play a role in early myelofibrosis development. GATA-1 is a transcription factor that is key to megakaryocyte development, and its downregulation results in expansion and abnormal maturation of megakaryocytes.[2] Importantly, low GATA-1 expression has been demonstrated in patients with myelofibrosis,[3] and GATA-1 mutations are found in megakaryocytic leukemias.[2]

New key findings

Unlike several widely used myelofibrosis mouse models that rely on BM transplantation to engender fibrosis, primary Gata-1^{low} mice gradually develop myelofibrosis spontaneously.[4] Due to its slow progression, this model allows for analysis at prefibrotic (5 months), early fibrotic (10 months), and overtly fibrotic (15 months) stages. A strength of the study by Kramer *et al.* is the application of an unbiased approach (i.e. RNA sequencing) to interrogate the changes that occur in receptor tyrosine kinase pathways during the development of myelofibrosis. Using bulk RNA sequencing on unfractionated BM (including stromal cells), the authors identified the platelet-derived growth factor (PDGF) pathway as significantly up-regulated in early fibrotic Gata-1^{low} mice compared to wild-type mice. Additionally, the authors analyzed protein expression of PDGF receptors and ligands on BM sections at the three aforementioned time points; this allowed them to study the PDGF pathway in a spatio-temporal manner.

In addition to demonstrating increased transcript expression of PDGF receptor a (Pdgfra) and Pdgfrb, as well as the ligand Pdgfb, in fibrotic Gata-1^{low} mice, the authors employed a novel technique called *in situ* proximity ligation assay to determine protein localization. They found that the receptor PDGFRβ and ligand PDGF-B are in close proximity in the setting of overtly fibrotic BM, suggesting binding of the ligand to the receptor and increased PDGF-B signaling. Furthermore, their data suggest that the most important cell types involved in PDGF signaling are megakaryocytes, which express PDGFRα and secrete the ligand PDGF-B, and spindle-shaped stromal cells which express PDGFRβ (Figure 1).

Despite these findings, Kramer *et al.* did not detect increased PDGFRβ tyrosine phosphorylation, a marker of receptor activation. They suggest that the phosphatase TC-PTP (PTPN2) may play a role in dephosphorylation of PDGFRβ and show that TC-PTP is in close proximity to PDGFRβ in fibrotic Gata-1^{low} mice. There are two main potential explanations for these findings. Either: (i) PDGF receptor activation is transient and rapidly down-regulated; or (ii) PDGF receptor activation is rapidly reset by phosphatases such as TC-PTP after ligand binding. Rapid downregulation would call into question the importance of the PDGF pathway in myelofibrosis, while a rapid reset may increase signaling in the presence of ligand and potentially contribute to the development of myelofibrosis. Further investigation of PDGF signaling in human myelofibrosis will be required to fully resolve this question.