Low-dose X-ray exposure does not harm human stem cells

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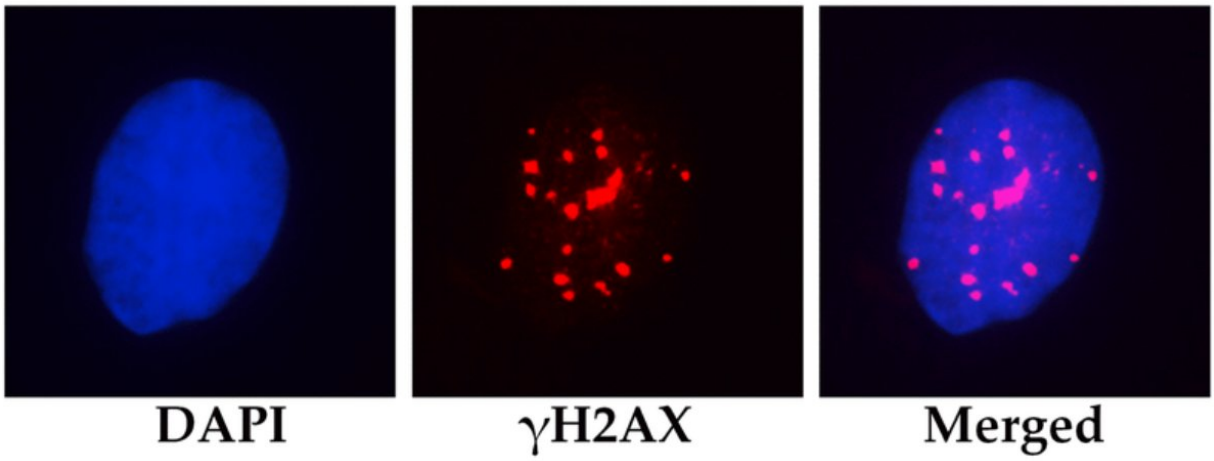

Fig. 1. Mesenchymal stem cell nucleus on a microphotograph. Cell nucleus highlighted by a fluorescent stain called DAPI (blue); γH2AX foci, that is, the accumulations of the protein marking DNA damage (red dots); and the first two images merged together. Credit: MIPT

Biophysicists have shown that following low-dose exposure to X-rays (at 80 milligrays), stem cells remain healthy, proliferate, and do not accumulate DNA damage to be passed on to their progeny. The paper was published in the journal *Aging*.

"The 80-milligray dose is equivalent to the radiation exposure during a medical diagnostic imaging procedure such as a CT scan, which is

routinely used in conjunction with stem cell therapy," explains Sergey Leonov, director of the Phystech School of Biological and Medical Physics at MIPT, who also heads the institute's Laboratory for the Development of Innovative Drugs. "Our research helps predict the side effects and health risks for patients, who are increasingly often undergoing both stem cell therapy and diagnostic X-ray procedures at the same time."

The field of regenerative medicine, which is now making rapid advances, holds the promise of using stem cells to replace or restore damaged human tissues and organs. Stem cells have a high potential for division and self-renewal, and are capable of differentiating into various cell types. They are present in most organs and tissues in an adult organism and can identify damage sites, migrate toward them, replace damaged cells, and promote healing. However, stem cells are believed to be harmed by frequent medical diagnostic procedures involving the use of ionizing radiation, such as CT scans and mammography. According to this view, X-rays cause damage that is accumulated in stem cells and passed on to their progeny. This supposedly leads to cell death, accelerated cellular aging, and malignant transformations.

The international research team, including Andreyan Osipov from Burnasyan Federal Medical Biophysical Center and MIPT's Sergey Leonov and Anastasia Tsvetkova, ran a series of experiments aimed at obtaining the much needed data on the delayed effects of low-dose radiation exposure. They showed that low-dose X-ray exposure does not induce genome instability, premature aging, or the accumulation of DNA damage in the progeny of irradiated cells.

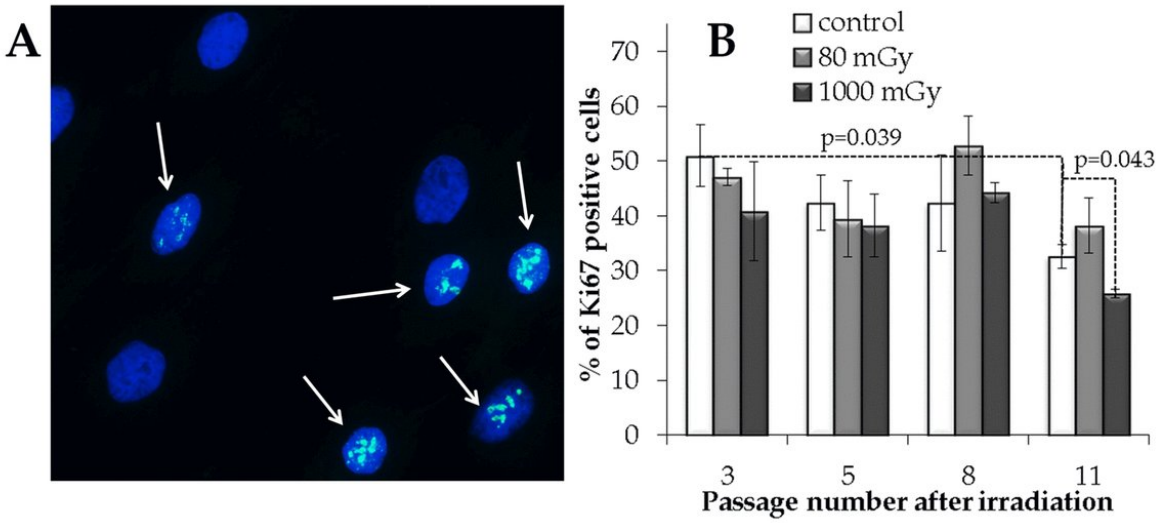

Fig. 2. The arrows in A indicate cells fluorescently stained for proliferation that have suffered DNA double-strand breaks. The bar chart (B) illustrates the change in the number of proliferating cells from the third to the 11th passage for control cells and the cells exposed to low- and intermediate-dose irradiation — at 80 mGy and 1,000 mGy, respectively. Credit: MIPT

Cellular response to X-ray irradiation

During a regular X-ray exam, a dose of about 0.001-10 milligrays—depending on the procedure—is delivered to the patient. Receiving 100 mGy is considered low-dose exposure, while 1,000 mGy is regarded as an intermediate dose. The effects of larger-dose exposure have been studied extensively. It is known to cause a dose-dependent increase in the incidence of DNA lesions, including the so-called double-strand breaks in which both strands of the double helix are severed. These breaks can lead to cell death, oncogene activation, and anti-oncogene inactivation.

In contrast, the effects of low-dose radiation exposure during routine X-

ray exams are still poorly understood. The regulatory agencies responsible for radiation protection currently use the so-called linear no-threshold model to estimate radiation risks. Under this model, ionizing radiation is harmful to living cells, no matter how low the dose. However, this crude assumption does not reflect the actual state of affairs: We are regularly exposed to natural background radiation, and its absence even has adverse effects on the ability of cells to repair DNA damage.

Criteria for assessing the effects of low-dose exposure

Among the various types of DNA lesions caused by ionizing radiation, double-strand breaks get most of the attention of researchers, because their long-term effects on cells are the most pronounced. Their repair takes long, and uncorrected double-strand breaks lead to serious cytogenetic abnormalities, tumor suppressor gene inactivation, oncogene activation, and cell death.

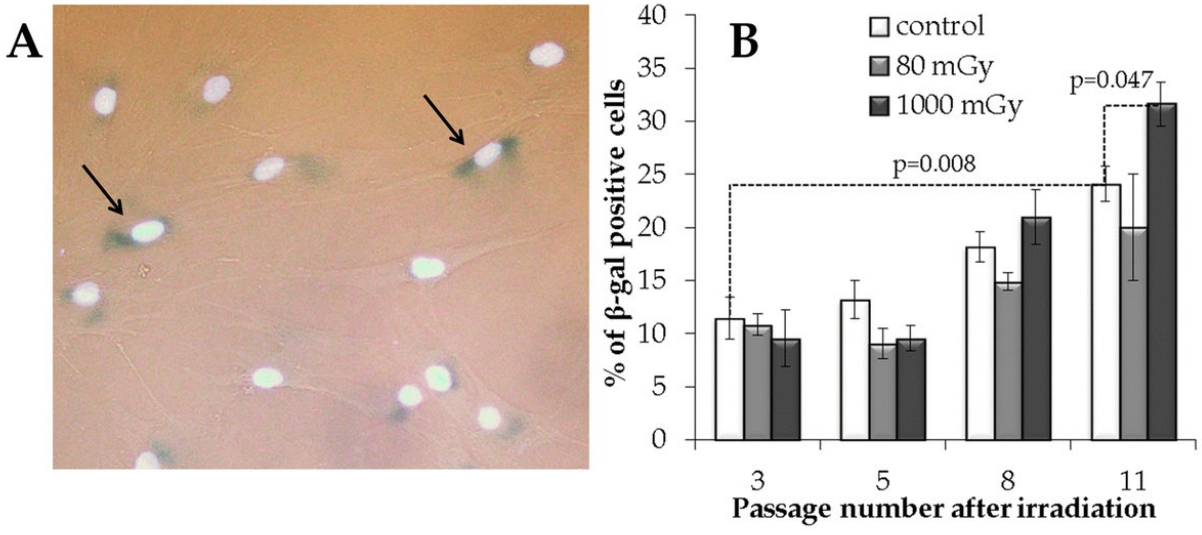

Fig. 3. The arrows in A indicate cells stained for senescence, with the cytoplasm

marked in blue and cell nuclei in white. The bar chart (B) illustrates the change in the number of senescent cells from the third to the 11th passage for control cells and the cells exposed to low- and intermediate-dose irradiation. Credit: MIPT

For a long time, no method was available for evaluating the generation of DNA double-strand breaks due to low-dose radiation exposure. The classical methods could only describe the effects of large-dose exposure. Now, advances in immunocytochemistry, have given biophysicists the tools needed to quantify double-strand breaks caused by low-dose X-ray exposure and look into how they are distributed in the nucleus and repaired by the cell.

As proteins involved in the correction of DNA damage accumulate at the site of a double-strand break, they can be observed with a microscope thanks to immunofluorescence staining—a technique that involves treating antibodies with fluorescent dyes. This enables scientists to visualize DNA lesions as bright dots called foci. One of the proteins widely used as a DNA damage marker is the histone variant called γH2AX.

Effects on progeny cells

The cell has two main mechanisms for repairing double-strand breaks. The first one, called homologous recombination, is a slow but virtually error-free way of restoring the lost information in a damaged DNA sequence. The other one, nonhomologous end joining, may lead to a loss of genetic information, resulting in errors and mutations. And yet it is this faster but inaccurate mechanism that is used to repair eight out of 10 breaks occurring in an irradiated cell.

The researchers found that 24 hours after low-dose X-ray treatment, more γH2AX foci are observed in stem cells, compared to those exposed to an intermediate dose of radiation. However, this was only true for cells undergoing division and not for quiescent cells (see fig. 2). DNA double-strand breaks are known to occur naturally during cell division. Such breaks are usually mended correctly via homologous recombination. When the progeny of the cells were examined 11 passages, or "cellular generations," after the exposure to low-dose radiation, the researchers found them to be no different from the progeny of the control cells, which were not treated with X-rays. Furthermore, the progeny of the cells that received low-dose irradiation did not exhibit genome instability, proliferation abnormalities, or accelerated senescence (see figs. 2 and 3).

Andreyan Osipov, professor of the Russian Academy of Sciences and the head of the Experimental Radiobiology and Radiation Medicine Department at Federal Medical Biophysical Center, comments on the team's findings: "Our research suggests that the presence of γH2AX foci in cultured human stem cells 24 hours after low-dose X-ray irradiation is associated with cell division processes and does not lead to delayed effects related to aging. This is an important conclusion to draw, because γH2AX foci are now actively used in biodosimetry. Misunderstanding the biological significance of residual foci might lead to a severe overestimation of the risks associated with low-dose exposure."

More information: Margarita Pustovalova et al, Residual γH2AX 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* (2017). DOI: 10.18632/aging.101327

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