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Radiation Injury and hydrogen peroxide

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Abbreviations:

MnSOD	manganese superoxide dismutase
CuZnSOD	cupper zinc superoxide dismutase
e_{aq}^-	aqueous electron
$H_2O^{excitation}$	water in excitation state
siRNA	small interfering RNA

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Abstract

Radiation injuries are believed to be caused by reactive oxygen species and DNA is the critical target. Antioxidant enzyme, MnSOD has been shown to alleviate radiation injuries. The accumulation of hydrogen peroxide raises the question whether both inhibit superoxide and hydrogen peroxide will maximize the radiprotection. Here I address an experiment to test that hypothesis. By modulating the catalase and MnSOD activity, the role of hydrogen peroxide and superoxide under radiation will become clear.

Introduction

Radiation is present all around us in a variety of sources; cosmic rays, naturally occurring isotopes, diagnostic and therapeutic medical procedures. Radiation applications in medicine are aimed to diagnosis and treat disease. However, some radiation exposure of normal tissue, especially in radiotherapy is unavoidable. Radiation injury in normal tissue is a major limitation in radiotherapy and a critical issue of quality of life in cancer survivors after radiotherapy.

Ionizing radiation

Radiation energy higher than 33 eV is capable to eject one or more orbital electrons from an atom or molecule and is called ionizing radiation. The importance of ionizing radiation is the amounts of energy deposit in biologic tissue are capable to produce radicals, break a strong chemical bond, and subsequently alter properties of biomolecules. Ionizing radiation produces more detrimental radiation injuries then non-ionizing radiation and this paper focuses on the former one.

Direct and indirect action of radiation

The interaction between the incident ionizing radiation and the critical targets in the cell can be categorized into direct and indirect action. Direct action depicts the radiation directly ionizes or excites the molecules of the critical target and leads to form or break a chemical bond. Alternatively, radiation may interact with other molecules (water is the most abundant molecule in a cell), produce radicals and reactive species that diffuse and damage the molecule of the critical targets. In this indirect action, radicals are produced and mediate the radiation damage. It is estimated that about two thirds of the biologic damage by x-rays is caused by indirect action[1].

Radiolysis of water

Water is the most abundant molecules in a cell. In the indirect action of radiation, water interacts with ionizing radiation will result in the production of certain quantities (table 1) of oxidizing species such as the hydroxyl radical, OH, and hydrogen peroxide, H_2O_2*.

Radiolysis of $H_2O \rightarrow H^{\bullet}, HO^{\bullet}, e_{aq}^-, H_2, H_2O_2, H_3O^+$ (1)

Initial excitation and ionization (10^{-12} s)

$H_2O \rightarrow H_2O^{\bullet +} + e^-$ (2)

$e^- + nH_2O \rightarrow e_{aq}^-$ (3)

$H_2O \rightarrow H_2O^{excitation}$ (4)

Appearance of primary radicals (10^{-9} s)

$H_2O^{\bullet +} \rightarrow H^+ + HO^{\bullet}$ (5)

$H_2O^{\bullet +} + H_2O \rightarrow H_3O^+ + HO^{\bullet}$ (6)

$H_2O^{excitation} \rightarrow H^+ + HO^{\bullet}$ (7)

Chemistry begins (10^{-6} s)

$H^{\bullet} + HO^{\bullet} \rightarrow H_2O$ (8)

$e_{aq}^- + HO^{\bullet} \rightarrow OH^-$ (9)

$e_{aq}^- + e_{aq}^- + 2H_2O \rightarrow H_2 + 2\ OH^-$ (10)

$H^{\bullet} + H^{\bullet} \rightarrow H_2$ (11)

$HO^{\bullet} + HO^{\bullet} \rightarrow H_2O_2$ (12)

Primary products	G-value	G-value (uM/Gy)
$HO^{\bullet}$	2.8	0.28
e_{aq}^{-}	2.7	0.27
$H^{\bullet}$	0.57	0.057
H_2O_2	0.71	0.071
H_2	0.47	0.047

Table 1, G-values on radiolysis of water. Chemical yield in water; the concentration at the end of a short pulse of ionizing radiation*.

Under the presence of molecular oxygen, superoxide is produced very quickly[2].

$$e_{aq}^{-} + O_2 \rightarrow O_2^{\bullet -} \qquad k = 1.9 \times 10^{10}\ M^{-1}\ s^{-1} \qquad (13)$$

$$H^{\bullet} + O_2 \rightarrow HO_2^{\bullet} \qquad k = 2.1 \times 10^{10}\ M^{-1}\ s^{-1} \qquad (14)$$

*Buettner GR. (2005) Radiation Chemistry. class note in 77:222 Free radical and Radiation Biology. chapter 7; pp 2-3.

The superoxide will dismutate into oxygen and hydrogen peroxide.

$$O_2^{\bullet-} + HO_2^{\bullet} + H^+ \rightarrow O_2 + H_2O_2 \qquad k = 9.7 \times 10^7\ M^{-1}\ s^{-1} \qquad (15)$$

$$HO_2^{\bullet} + HO_2^{\bullet} \rightarrow O_2 + H_2O_2 \qquad k = 8.3 \times 10^5\ M^{-1}\ s^{-1} \qquad (16)$$

$$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \rightarrow O_2 + H_2O_2 \qquad k < 0.3\ M^{-1}\ s^{-1} \qquad (17)[3]$$

Superoxide dismutase (SOD) catalyzes the reaction (17) and $k_{SOD} = 2 - 4 \times 10^9\ M^{-1}\ s^{-1}$ *

DNA is the critical target

DNA damage induced by ionizing radiation leads to cell death is the paradigm of classical radiobiology[1]. A dose of radiation that induces 67% cell death and 37% cell viable in mammalian cells usually lies between 1 and 2 Gy. The DNA lesions within each cell immediate after such radiation dose are estimated approximately: > 1000 base damage, about 1000 single-strand breaks, and about 40 double-strand break. Cell killing relates better to double strand break[4].

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*Buettner GR. (2005) Reactive Oxygen Species. class note in 77:222 Free radical and Radiation Biology. chapter 4; pp 7.

Radiation injuries

Radiation injuries are harmful effects of biologic tissues exposed to radiation. In normal tissue, radiation kills the clonogenic cells that maintain a sufficient number of mature cells to maintain the organ function[5]. The organ function after radiation depends on the numbers of clonogenic cells survived. In radiation biology, clonogenic assay is a good method to measure the radiation injury in vivo.

Radiation injuries in different tissues exhibit different damage. Hematopoietic tissue produces blood elements and radiation will cause leucopenia, thrombopenia and finally anemia. In the digestive track, radiation will lead to mucosal ulceration, atrophy, diarrhea, electrolytes imbalence and infection. In lung, kidney, liver, central nerve system radiation will lead to specific organ failure.

Radioprotection by antioxidant enzymes

Reactive oxygen species (ROS) produced in radiation and the antioxidants reduce radiation damage in animal models have been investigated for 50 years[6]. Epperly showed MnSOD transgene expression exhibited radioresistance and only mitochondrial localization produced radioresistance[7, 8]. Cu/ZnSOD overexpressing cells showed similar levels of overall antioxidant activity, however, there was no significant irradiation protection (figure 1). Delanian showed that CuZnSOD can treat the radiation-induced fibrosis by intramuscular injection of liposomal CuZnSOD after radiotherapy[9].

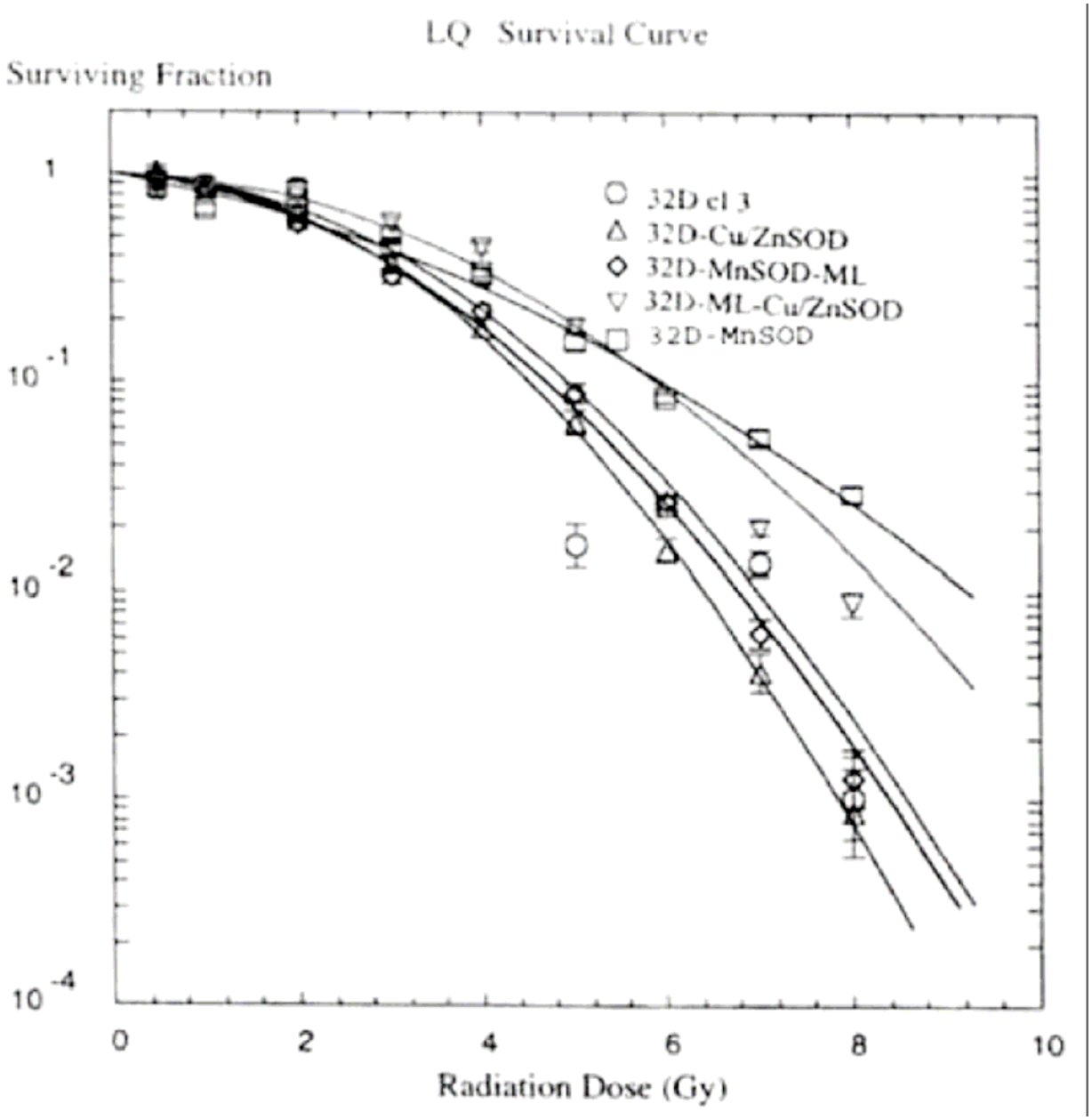

Figure 1, clonogenic irradiation survival curves for 32D cl 3 and subclones transfected and overexpressing the nonradioprotective Cu/ZnSOD human transgene, radioprotective MnSOD human transgene, MnSOD transgene with deleted mitochondrial localization signal (MnSOD-ML), showing no radioprotection and the construct of Cu/ZnSOD with spliced mitochondrial localization signal of MnSOD and MLCu/ ZnSOD, showing irradiation protection. 32D cl 3 cells expressing MnSOD or the Cu/ZnSOD-ML plasmid have an increased radioresistance in comparison to 32D cl 3 cells. Cells from 32D cl 3 and a clone expressing MnSOD (1F2), MnSOD-ML, Cu/ZnSOD, or ML-Cu/ZnSOD were irradiated to doses ranging from 0 to 8 Gy and plated in methylcellulose. Seven days later, colonies of >50 cells were counted and data was analyzed using linear quadratic and single-hit, multi-target models. 32D-MLCuZnSOD and 1F2

were more radioresistant than the other cell lines as reflected in the increased shoulder on the survival curve. There was no significant difference in the D_0 for any of the cell lines.

Hypothesis

Hydrogen peroxide is the key ROS that mediates Radiation injury.

MnSOD and CuZnSOD have been tested to increase radioresistance or diminish radiation injuries. Both enzymes catalyze the dismutation of superoxide.

Hydrogen peroxide and superoxide are distinct ROS

Hydrogen peroxide and superoxide both are ROS produced under radiation. However, they show distinct properties. Superoxide is believed to be produced (leaked) from mitochondrial electron transport chain and can dismutate into hydrogen peroxide and molecular oxygen. It is kept at a low steady state in mitochondria with half-life 1 microsecond[10]. Hydrogen peroxide is more stable with half-life 1 millisecond and is permeable to mitochondrial membranes. Hydrogen peroxide is rather a mild oxidant that modifies TP53, Jun, Fos, NFkB[11-13].

Epperly showed MnSOD transgene expression exhibited radioresistance [7, 8]. The overexpression of SOD will catalyze and dismutate superoxide into hydrogen peroxide and molecular oxygen. In this situation, hydrogen peroxide may accumulate and play an important role in redox state and redox environment.

Catalase modulates the redox status under radiation

Hachiya and Akashi provided the evidence that oxidizing species produced during radiation can be modulated under different level of catalase activity [14]. In figure 2A, they observed the distinct antioxidant enzymes mRNA levels in the two cell lines. They also showed

that the mRNA level of MnSOD, CuZnSOD, GSH-Px are the same. The increased catalase mRNA level in HP100-1 is further confirmed by the enzyme activity (figure 2B).

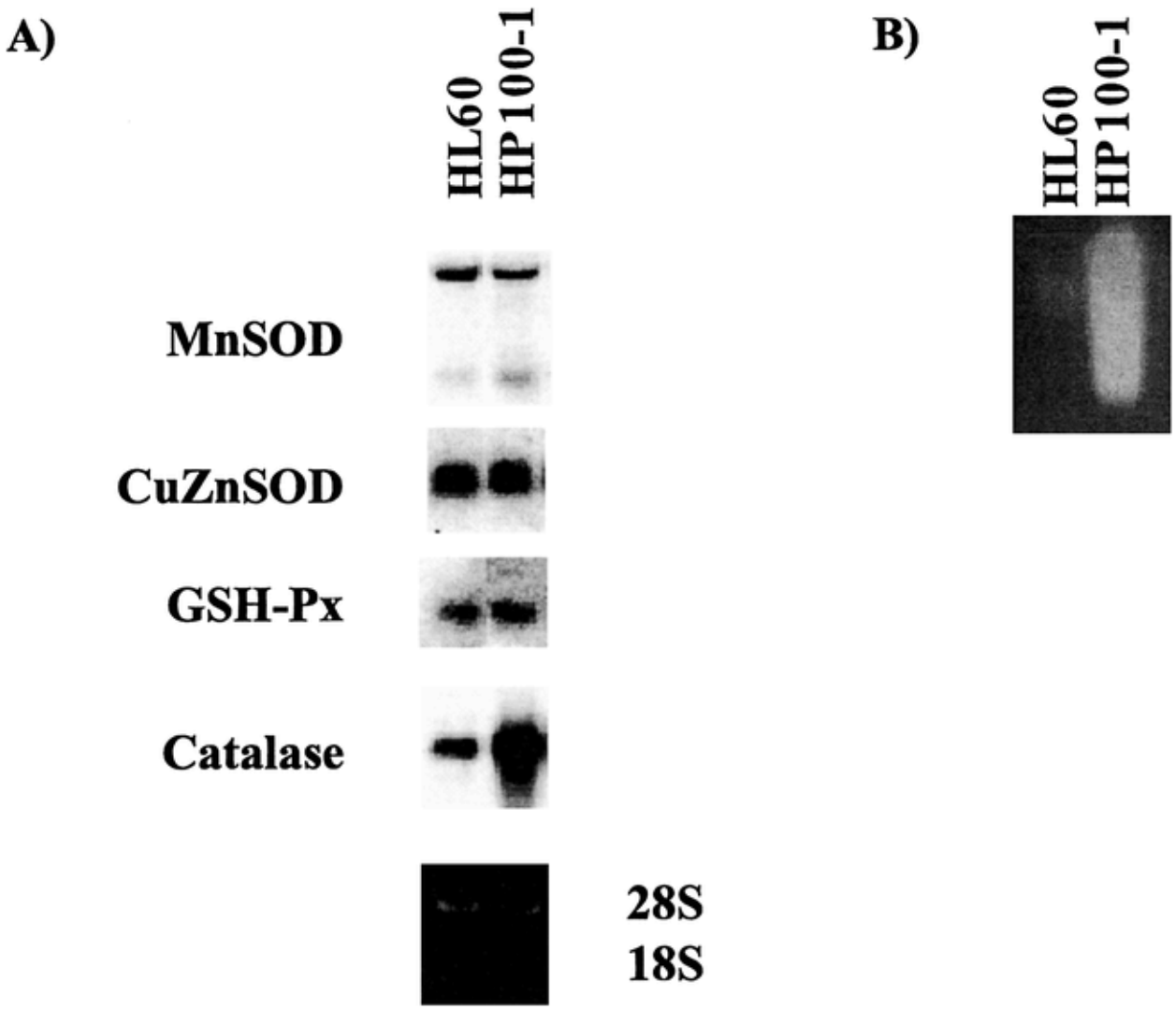

Figure. 2. Levels of antioxidant enzyme mRNAs and catalase activity in HL60 and HP100-1 cells. Panel A: Cells were harvested and total RNA was prepared. The basal levels of MnSOD, CuZnSOD, GSH-Px and catalase mRNAs were determined by Northern blot analysis. The bottom panel shows the ethidium bromide-stained formaldehyde gel before Northern blotting; levels of 28S and 18S ribosomal RNA were comparable in each lane (15 μg/lane). Panel B: Cells were harvested and sonicated in ice-cold potassium phosphate buffer. Samples (30 μg/lane) were electrophoresed in a non-denaturing polyacrylamide gel followed by staining with diaminobenzidine

They further measured the levels of oxidizing species under radiation in these 2 cell lines. Oxidative sensitive dye, 2′,7′-dichlorofluorescein diacetate (DCFH-DA, Molecular Probes, Eugene, OR) was used to estimate the amount of oxidizing species in this experiment. Figure 3 showed that in HP100-1, radiation did not increase fluorescence significantly. In HL60, the more radiation dose prescribed, the more oxidizing species can be detected in this oxidation dye.

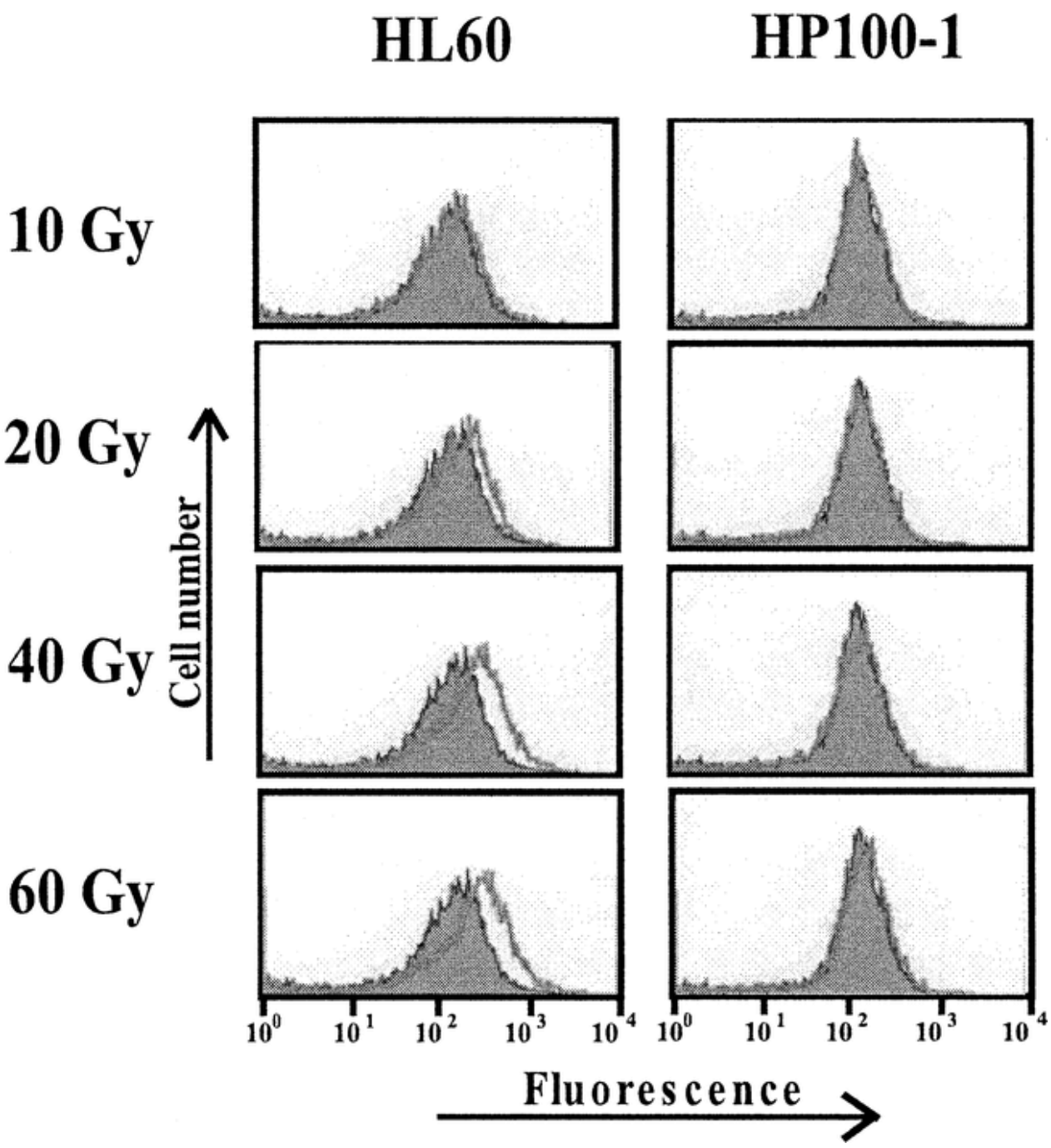

Figure. 3. Production of H_2O_2 after irradiation in HL60 and HP100-1 cells. Cells were incubated with 10 μ*M* DCFH-DA, a peroxide-sensitive fluorescent probe, for 15 min and then irradiated at indicated doses. After 15 min, the levels of intracellular H_2O_2 were analyzed by flow cytometry (FACSCalibur). Gray histograms: untreated cells; white histograms: irradiated cells [14]

Experiment design

In order to address the hypothesis, I will pick 2 or more human cancer cell lines.

- modulate the catalase activity by
  - transient adenoviral transfection,
  - plasmid liposomes,
  - microinjection the active or inactive catalase protein.
  - siRNA
  - Catalase inhibitor: 3-amino-1, 2, 4-triazole
- Measure the catalase activity by native gel.
- Exposed cells to different radiation dose.
- Perform colony formation assay to estimate the radiation toxicity.

I am supposed to observe decrease radiation toxicity as catalase activity goes up. Furthermore, combined expression of catalase and MnSOD are expected to provide more radioprotection than MnSOD alone. But this combination will not total protect cells from radiation because only two-third of the biologic damage by x-ray are estimated through indirect action (ROS production). However, the individual effect of superoxide and hydrogen peroxide will be available.

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