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Mammalian cells exposed to ionizing radiation: Structural and biochemical aspects

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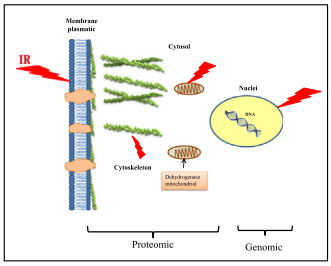
HIGHLIGHTS

- The chronic exposure to IR is a factor that may be hazardous to health.
- The susceptibility of a biological system (HeLa) to ionizing radiation (IR) was study.
- Exposed to 6, 60 mSv/90 s shown alterations nuclear, junctions and actin microfilaments.
- Our investigation, reflects the molecular mechanisms of radiation toxicity.
- And provides the basis for the improvement of clinical radiation in cancer treatment.

GRAPHICAL ABSTRACT



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ABSTRACT

Acute or chronic exposure to ionizing radiation is a factor that may be hazardous to health. It has been reported that exposure to low doses of radiation (less than 50 mSv/year) and subsequently exposure to high doses produces greater effects in people. It has been reported that people who have been exposed to low doses of radiation (less than 50 mSv/year) and subsequently are exposed to high doses, have greater effects. However, at a molecular and biochemical level, it is an unknown alteration. This study, analyzes the susceptibility of a biological system (HeLa ATCC CCL-2 human cervix cancer cell line) to ionizing radiation (6 and 60 mSv/90 s). Our research considers multiple variables such as: total protein profile, mitochondrial metabolic activity (XTT assay), cell viability (Trypan blue exclusion assay), cytoskeleton (actin microfilaments), nuclei (DAPI), and genomic DNA. The results indicate, that cells exposed to ionizing radiation show structural alterations in nuclear phenotype and aneuploidy, further disruption in the tight junctions and consequently on the distribution of actin microfilaments. Similar alterations were observed in cells treated with a genotoxic agent (200 μM H_2O_2/1 h). In conclusion, this multi-criteria assessment enables precise comparisons of the effects of radiation between various line cells. However, it is necessary to determine stress markers for integration of the effects of ionizing radiation.

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1. Introduction

Ionizing radiation (IR) generates both direct and indirect effects to biological molecules. Radiation damage to biological systems is determined by the type of radiation and the total dosage of exposure (Manesh et al., 2015).

In high linear energy transfer (LET) radiation, such as neutrons and alpha particles, most of the cellular damage results from the direct ionization of cellular macromolecules including DNA, RNA, lipids, and proteins (Azorín Nieto and Azorín Vega, 2010).

In contrast, low LET radiation, such as X-rays and gamma rays, indirect damage to biological macromolecules occurs following the generation of reactive oxygen species (ROS). ROS, especially superoxide and hydroxyl radicals from the radiolysis of intracellular H_2O, can have many effects, including the oxidation of biological macromolecules and activation of intracellular signaling pathways, affecting physiologically the cell (Panganiban et al., 2013).

In this work, biological models were carried out to analyze the ionizing radiation effects in HeLa (human cervix cancer cell line) culture cells. These cells exhibit a high proliferative index and can be applied different IR doses. We investigate the signaling mechanisms activated by radiation for the induction of damage in transformed cells. Understanding the molecular mechanisms of radiation damage is critical for the development of radiation countermeasures as well as for the improvement of clinical radiation in cancer treatment.

2. Materials and methods

2.1. Cell culture

The human cervix cancer cell line HeLa ATCC® CCL-2™ was selected for this study. The cells were cultivated at subconfluence in DMEM (Dulbecco's Modified Eagle's Medium, GIBCO, USA) supplemented with fetal bovine serum 10% (GIBCO, USA) and incubated at 37 °C, 5% CO_2.

2.2. Exposure of HeLa cells to ionizing radiation

The HeLa cells were exposed to ionizing radiation from 6 to 60 mSv during 90seg. Exposure was followed by the determination of some parameters such as: morphology, metabolic activity, genomic DNA integrity, actin cytoskeleton and nuclei.

2.3. Metabolic activity determination

The XTT assay is a colorimetric assay that detects the cellular metabolic activities. During the assay, the tetrazolium salt XTT (2,3-bis(2-metoxi-4-nitro-5-sulfofenil)-2-h-tetrazolium-5-carboxanilide) is reduced to a highly colored formazan dye by dehydrogenase enzymes (mitochondria) in metabolically active cells The cells were exposed to IR as previously described. After exposure, 100 μL of XTT detection solution (0.25 mg/mL in 0.1 mM Menadione, Sigma) were added to each well and the plate was returned to the incubator for 90 min. The formazan dye formed in the assay was quantified by measuring the absorbance at 450 nm in a spectrophotometer (Epoch Biotek).

2.4. SDS-PAGE

The separation of proteins from extract was performed in a 10% gel of bisacrylamid/acrylamide, denaturalized conditions (SDS-PAGE), using the technique described by Laemmli (1970).

2.5. Genomic DNA integrity

Genomic DNA extraction was performed according to the AxiPrep™ Multisource Genomic DNA Miniprep kit (AXIGEN Biosciences). DNA samples of cells exposed to IR (6 and 60 mSv) were analyzed on 0.8% agarose gels and stained with Gel Red™ (BIOTUM). The size marker was HyperLadder™ 1 kb (BIOLINE). The bands were visualized in ChemiDoc MP System (UV light at 260–280 nm, BIORAD) and image acquisition with Image Lab™ software (BIORAD)

2.6. Cytoskeleton and nuclei analysis

Control cells and those exposed to IR, were fixed with 4% p-formaldehyde for 20 min, permeabilized with 0.5% Triton X-100 buffer containing 10 mM Tris, 5 mM KCl, 1 mM $MgCl_2$, and later exposed to FITC-phalloidin (Soto Arredondo et al., 2014). The preparations were mounted using Vecta Shield-DAPI (4′,6-diamidino-2-phenylindole), a fluorescent stain that binds strongly to A–T rich regions in DNA (Vector Laboratories, USA) and were observed under a fluorescence microscope (Leica, DMLS) using a 450–490 nm B filter. The image acquisition was done with camera AxioCam ICc1 (Carl Zeiss).

3. Results

The metabolic activity (Fig. 1), indicates that the ionizing radiation at 6 mSv is similar to control (100%), however, the cells exposed to ROS (positive control) was 41%. At 60 mSv the mitochondrial metabolic activity decreased to an 86% compared to control.

Additionally, morphological changes were observed (Fig. 2I). Cells exposed to IR of 60 mSv show evident alterations (Fig. 2B, B′ and C, C′). The protein profile of cells exposed to IR is similar to that of the control cells, however, in proteins of Mr ≥ 60–40 kDa, cells exposed to ROS differ (Fig. 2II lane 2).

In organelles such as cytoskeleton, there are alterations in the arrangement of actin microfilaments and detachments from junctions of cells exposed to ROS and IR (Fig. 3B, C and D).

Regarding the nuclei, the heterochromatin was observed to be homogeneously distributed and some cells in division process. Particularly, genomic DNA shows the integrity in all conditions (Fig. 3II).

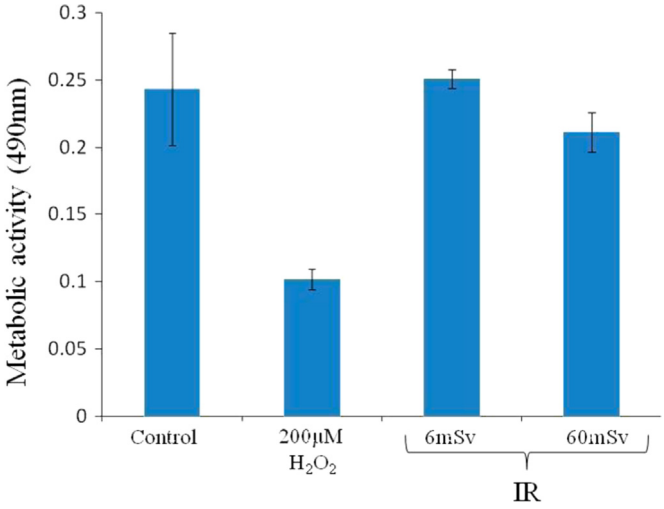

Fig. 1. Metabolic activity of HeLa cells expose to ionizing radiation.