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GENOME STABILITY MECHANISMS UNDERLYING RADIATION-INDUCED CHROMOSOMAL ABERRATIONS IN HUMAN CELLS

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ABSTRACT

Background: Ionizing radiation is a major source of DNA damage that can cause chromosomal aberrations and compromise genome stability in human cells. Cellular mechanisms that guard against radiation-induced damage to chromosomal integrity such as DNA damage response signaling and repair pathways are required.

Objective: The objective of this study was to investigate mechanisms of genome stability for radiation-induced chromosomal aberrations in human cells and to evaluate the role of DNA repair pathways in reduction of genomic instability.

Methodology: Human peripheral blood lymphocytes and fibroblast cells were exposed to gamma radiation dose of 0-6 Gy. Cytogenetic analyses including metaphase chromosome assay, micronucleus test and γ-H2AX immunofluorescence were performed to evaluate DNA damage and chromosomal abnormalities. ATM, RAD51 and Ku70 repair proteins were statistically analysed for their expression levels.

Findings: Results showed a dose-dependent increase of chromosomal aberrations. Dicentric chromosomes increased from 1.2% in controls to 31.6% at 6 Gy. γ-H2AX foci as well as DNA repair protein expression increased significantly at moderate doses but decreased at higher exposure levels, indicating that the repair pathways were exhausted.

Conclusions: Mechanisms of genome stability are at work to counteract the radiation-induced chromosomal damage at moderate levels of radiation exposure. Excessive radiation overwhelms the repair mechanisms resulting in persistent genomic instability and chromosomal aberrations.

KEYWORDS: Genome stability, ionizing radiation, chromosomal aberrations, DNA damage response, γ-H2AX, homologous recombination, non-homologous end joining, human cells

1 INTRODUCTION

Genome stability is essential for maintenance of cellular homeostasis, accurate genetic transmission and prevention of disease development. Human cells are constantly exposed to endogenous and exogenous agents able to induce DNA damage, among which ionizing radiation is considered to be one of the most hazardous environmental mutagens [1]. Medical diagnostics, radiotherapy, occupational exposure, environmental contamination, and accidental nuclear events can lead to exposure to radiation. The biological effects of ionizing radiation are primarily attributed to direct ionization of DNA molecules and indirect generation of reactive oxygen species (ROS) that induce severe genomic lesions including single-strand breaks, double-strand breaks (DSBs), base modifications, and chromosomal aberrations [2].

DNA double-strand breaks are the most cytotoxic and mutagenic forms of DNA damage among these lesions, as misrepair can result in chromosomal instability, translocations, deletions, and carcinogenesis [3]. Chromosomal aberrations such as dicentric chromosomes, acentric fragments, ring chromosomes, micronuclei and chromatid breaks induced by radiation are widely used as biomarkers for genomic instability and radiation biodosimetry [4]. Persistent chromosomal abnormalities are a major cause of tumor initiation, aging, reproductive dysfunction and inherited genetic disorders [5].

To counteract genomic instability, human cells possess sophisticated genome maintenance systems collectively known as the DNA damage response (DDR) network. The DDR consists of highly coordinated signaling pathways that are involved in damage detection, cell-cycle arrest, DNA repair, senescence, or apoptosis [6]. Key sensor proteins like

ataxia telangiectasia mutated (ATM), ATM and Rad3-related (ATR) and DNA-dependent protein kinase catalytic subunit (DNA-PKcs) quickly detect radiation-induced DNA lesions and activate downstream repair mechanisms [7]. There are two major pathways that repair DNA double-strand breaks induced by radiation: homologous recombination (HR) and non-homologous end joining (NHEJ). HR is a high-fidelity repair pathway that utilizes a homologous DNA template during the S and G2 phases of the cell cycle, while NHEJ directly ligates DNA ends and functions throughout the cell cycle but with relatively lower accuracy [8]. Defects in these repair systems have been associated with radiosensitivity syndromes, immune deficiencies, neurodegeneration, and increased susceptibility to cancer [9].

γ-H2AX foci formation, chromatin remodelling, oxidative stress modulation and checkpoint activation have been indicated by recent studies to be involved in determining cellular responses to exposure to radiation [10]. Although there has been great advances in radiation biology, the link between genome stability pathways and the development of chromosomal aberrations after different doses of radiation is not fully understood. Therefore, the current study intends to explore the molecular mechanisms of radiation-induced chromosomal aberrations in human cells through the analysis of cytogenetic changes and the activation of DNA repair pathways. Knowledge of these mechanisms may help to improve radiation risk assessment, to optimize cancer therapy and to develop novel radioprotective interventions [11].

2 BACKGROUND WORK

Ionizing radiation is a powerful genotoxic agent and a potent inducer of widespread molecular and chromosomal damage in human cells. Radiation exposure causes direct ionization of DNA molecules and indirect oxidative damage from the generation of reactive oxygen species (ROS), resulting in base modifications, single-strand breaks, and especially DNA double-strand breaks (DSBs), which are considered the most lethal form of DNA damage [12]. Improper repair of DSBs can lead to chromosomal rearrangements, mutagenesis, carcinogenesis and genomic instability [13].

Radiation-induced chromosomal aberrations are commonly used as biomarkers for genome instability and cellular radiosensitivity. Common abnormalities observed are dicentric chromosomes, chromatid breaks, translocations, and micronucleus formation. Dicentric chromosomes are formed from abnormal fusion of chromosome centromeres and contribute to mitotic instability, while chromatid breaks interfere with proper DNA replication [14]. Translocations are associated with activation of oncogenes and progression of cancer, whereas micronuclei formation is a sign of chromosome fragmentation and aberrant mitosis [15].To preserve the integrity of the genome, cells activate highly coordinated DNA damage response pathways involving sensor proteins such as ATM kinase, ATR kinase and DNA-dependent protein kinase catalytic subunit (DNA-PKcs) [6]. These proteins regulate downstream signaling cascades that initiate DNA repair, cell-cycle arrest or apoptosis. Major DNA repair pathways include homologous recombination (HR), a high-fidelity process relying on sister chromatids, and non-homologous end joining (NHEJ), which directly ligates broken DNA ends with moderate accuracy [16]. Oxidative DNA lesions induced by ROS are also repaired by base excision repair (BER) [17].

Recent studies showed that γ-radiation induces dose-dependent chromosomal instability and persistent γ-H2AX foci associated with defective DNA repair [10]. ATM-deficient cells exhibit increased radiosensitivity and defective checkpoint activation [18]. Besides, radiation impacts on chromatin organization and cell-cycle progression, influencing repair efficiency and genome maintenance [19]. However, comprehensive analysis correlating cytogenetic aberrations with genome stability signaling pathways is limited and warrants further investigation [11].

3 MATERIALS & METHODS

3.1 Cell Culture

Human peripheral blood lymphocytes (HPBLs) and human dermal fibroblast cells were used to assess radiation-induced chromosomal aberrations and genome stability responses. Peripheral blood from healthy non-smoking adult donors was obtained with institutional ethical approval and informed consent. Fibroblast cell lines were obtained from authenticated cell repositories and cultured under sterile laboratory conditions as detailed in table 1.

Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin and 2 mM L-glutamine. Cultures were incubated at 37°C in a humidified atmosphere containing 5% CO2. Before irradiation experiments, cell viability was assessed by the trypan blue exclusion assay [20].

Table 1. Cell Culture Conditions

Parameter	**Description**
Cell types	Human peripheral blood lymphocytes and fibroblasts
Culture medium	DMEM + 10% FBS
Antibiotics	Penicillin–streptomycin
Incubation conditions	37°C, 5% CO_2

Cell viability threshold	>95%

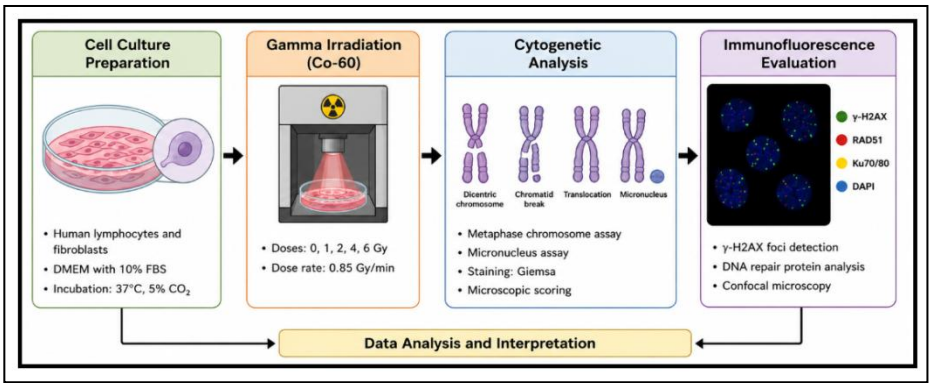

Figure 1. Experimental workflow illustrating cell culture preparation, irradiation, cytogenetic analysis, and immunofluorescence evaluation.

Figure 1 shows the overall experimental workflow used to assess radiation-induced chromosomal instability and genome stability mechanisms in human cells. Firstly, human lymphocytes from peripheral blood and fibroblast cells were cultured in controlled laboratory conditions. Cultured cells were then exposed to gamma radiation at various doses using a Co-60 irradiator. After irradiation, cytogenetic analyses, such as metaphase chromosome assay and micronucleus assay, were carried out to detect chromosomal abnormalities. Finally, immunofluorescence was performed to detect γ-H2AX foci and DNA repair protein expression, allowing the evaluation of DNA damage response and activation of DNA repair pathways.

3.2 Radiation Exposure

Table 2 shows the irradiation of cultured cells with gamma radiation in a Cobalt-60 (Co-60) gamma irradiator under controlled laboratory conditions. Radiation doses of 0, 1, 2, 4 and 6 Gy were delivered at a dose rate of 0.85 Gy/min. Non-irradiated cells were used as controls. After irradiation, cells were incubated for 24 h for the activation of DNA damage response and chromosomal repair processes [10].

Table 2. Radiation Exposure Parameters

Radiation Source	**Dose Range**
Co-60 Gamma Irradiator	0, 1, 2, 4, 6 Gy
Dose rate	0.85 Gy/min
Post-irradiation incubation	24 h

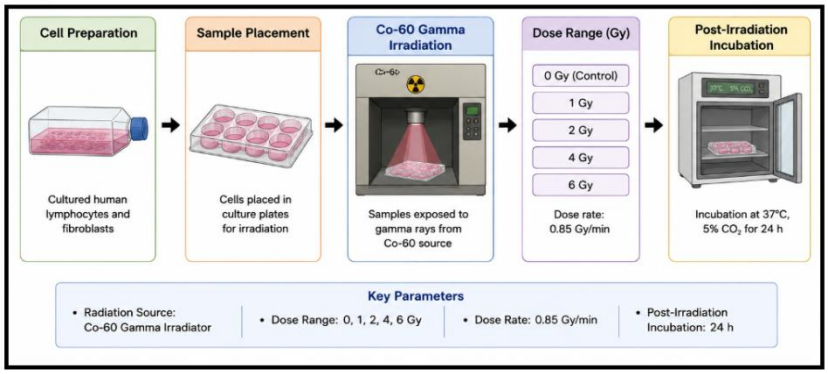

Figure 2. Dose-dependent radiation exposure setup using Co-60 gamma irradiation system

The dose-dependent radiation exposure set-up used in the present study is shown in Fig. 2, using a Co-60 gamma irradiation system. Human lymphocyte and fibroblast cells were first cultured under sterile laboratory conditions and then prepared and transferred into culture plates for irradiation. The samples were then subjected to graded doses of gamma radiation, ranging from 0 to 6 Gy at a constant dose rate of 0.85 Gy/min. Cells were incubated at 37°C with 5% CO 2 for 24 hours following irradiation to allow activation of DNA damage response pathways and cellular repair mechanisms before cytogenetic and molecular analysis.

3.3 Cytogenetic Analysis

Metaphase chromosome assay was done to detect structural chromosomal aberrations. Colchicine (0.1 µg/mL) was added 2 h before harvest to arrest cells in metaphase. The cells were treated with hypotonic KCl solution (0.075 M) and fixed with methanol:acetic acid (3:1). Chromosome spreads were prepared on glass slides and stained in 5% Giemsa. At least 100 metaphases per sample were analyzed microscopically for the presence of dicentrics, chromatid breaks and translocations [15].An additional assay of micronucleus was performed to quantify chromosomal fragments and genomic instability.Micronucleated binucleated cells were scored according to standard cytogenetic criteria.

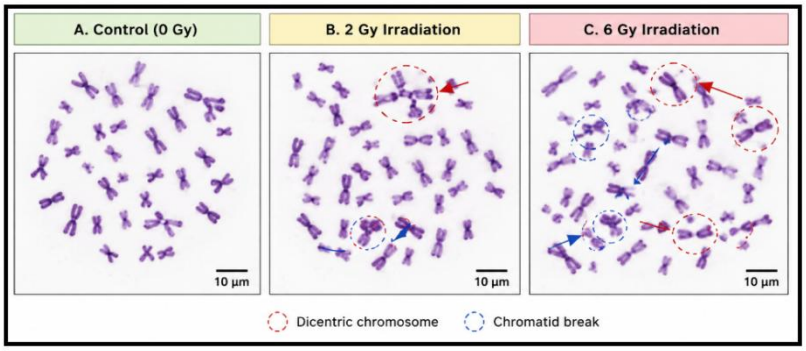

Figure 3. Representative metaphase chromosome spreads showing dicentric chromosomes and chromatid breaks after irradiation

Figure 3 shows representative metaphase chromosome spreads from human lymphocyte cells after exposure to gamma irradiation. The control group (0 Gy) demonstrated normal chromosome organization and no detectable structural abnormalities. On the contrary, irradiated samples exhibited chromosomal damage, which was dose-dependent and involved dicentric chromosomes and chromatid breaks. Moderate exposures to radiation (2 Gy) induced slight structural modifications, whereas higher doses of exposure (6 Gy) resulted in higher frequencies of chromosomal abnormalities and fragmentation. These results suggest that ionizing radiation causes considerable impairment of chromosomal integrity and induces genomic instability by accumulation of unrepaired or misrepaired DNA double-strand breaks.

3.4 Immunofluorescence Assay

Table 3. DNA double-strand breaks assessed by γ-H2AX immunofluorescence staining. The cells were fixed with paraformaldehyde, permeabilized with Triton X-100 and then incubated with primary antibodies against γ-H2AX, RAD51 and Ku70/80 . For confocal microscopy visualization fluorescent secondary antibodies have been used [4].

Table 3. DNA Damage and Repair Markers

Marker	Function
γ-H2AX	DNA damage marker
RAD51	Homologous recombination
Ku70/80	NHEJ pathway

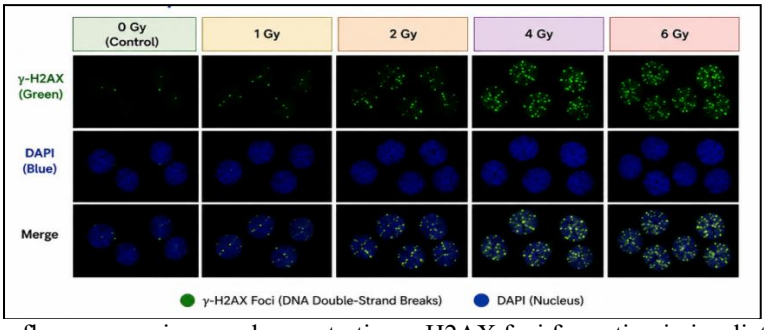

Figure 4. Immunofluorescence images demonstrating γ-H2AX foci formation in irradiated human cells.

Figure 4 shows representative immunofluorescence images of γ-H2AX foci formation in irradiated human cells after increasing doses of gamma radiation. Green fluorescent γ-H2AX foci indicate DNA double-strand breaks, and blue

DAPI staining shows cell nuclei. Control cells showed minimal γ-H2AX signals, whereas irradiated cells exhibited an increase in foci density with increasing doses of radiation. The maximum fluorescence intensity was at 6 Gy indicating the accumulation of extensive DNA damage. These results indicate that gamma irradiation induces dose dependent activation of DNA damage response pathways and genomic instability in human cells.

3.5 Statistical Analysis

Experimental data are reported as mean ± standard deviation (SD). One-way ANOVA followed by Student's t-test was used for statistical comparisons of radiation dose groups. The relationships between radiation dose and frequency of chromosomal aberrations were analysed using the Pearson correlation analysis. Statistical significance was defined as $p < 0.05$.

4. Dataset and Parameters

The experimental data set in Table 1 consisted of cytogenetic and molecular observations obtained from irradiated human peripheral blood lymphocytes and fibroblast cells after exposure to different doses of gamma radiation (0–6 Gy). The parameters analyzed were chromosomal aberration frequency, micronuclei formation, γ-H2AX foci count and DNA repair protein expression levels. The parameters were selected to evaluate genome stability, efficiency of DNA damage response and chromosomal integrity following irradiation [15,10].

Table 4. Dataset Parameters Used in the Study

Parameter	Measurement Purpose
Radiation dose (Gy)	Exposure assessment
Dicentric chromosomes (%)	Chromosomal instability
Chromatid breaks (%)	DNA structural damage
Micronuclei frequency (%)	Genomic instability
γ-H2AX foci count	DNA double-strand breaks
RAD51 expression	Homologous recombination activity
Ku70/80 expression	NHEJ repair pathway

5. RESULTS & DISCUSSION

In the present study, we investigated radiation-induced chromosomal instability and activation of genome stability pathways in human cells irradiated with increasing doses of gamma radiation. The cytogenetic analysis showed significant dose-dependent increases in chromosomal aberrations and the formation of micronuclei. Increased DNA damage by increased γ-H2AX foci formation was also confirmed by immunofluorescence analysis. In addition, DNA repair proteins such as ATM, RAD51, and Ku70 were also differentially expressed following irradiation, indicating the activation of cellular DNA repair mechanisms in response to the genomic stress caused by ionizing radiation.

5.1 Radiation-Induced Chromosomal Aberrations

Gamma irradiation produced a significant induction of chromosomal abnormalities, the extent of which was dose-dependent.

Table 5. Frequency of Chromosomal Aberrations Following Radiation Exposure

Radiation Dose (Gy)	Dicentrics (%)	Chromatid Breaks (%)	Micronuclei (%)
0	1.2	0.8	0.5
1	5.6	3.2	2.8
2	10.4	7.1	5.9
4	18.9	13.5	11.7
6	31.6	22.4	20.3

The results in table 5 showed progressive increases in dicentric chromosomes, chromatid breaks and micronuclei frequency with increasing doses of irradiation. In control cells, minimal chromosomal abnormalities were noticed. In cells exposed to 6 Gy, severe genomic instability was observed, as revealed by 31.6% dicentric chromosomes and 20.3% micronuclei formation. These results suggest that ionizing radiation causes significant chromosomal damage by accumulation of unrepaired DNA double strand breaks and faulty chromosome segregation during mitosis.

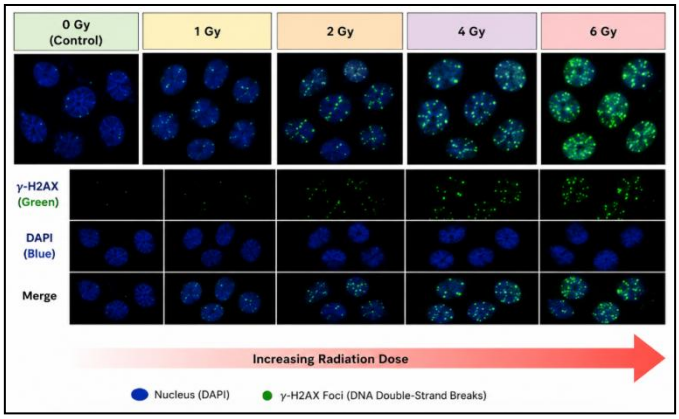

Figure 5. γ-H2AX Foci Formation in Human Cells After Gamma Irradiation

Immunofluorescence analysis of γ-H2AX foci formation increased significantly after radiation (Fig. 5). Control cells exhibited very few γ-H2AX signals while irradiated cells exhibited intense nuclear fluorescence associated with DNA double-strand break accumulation. The highest density of γ-H2AX foci was observed at 6 Gy, indicating extensive genomic damage and activation of DNA damage response signaling pathways. These observations confirm that γ-radiation directly induces DNA lesions and triggers cellular stress responses related to genome instability.

5.2 DNA Repair Pathway Activation

Table 6. Relative Expression of DNA Repair Proteins

Radiation Dose	ATM	RAD51	Ku70
0 Gy	1.0	1.0	1.0
2 Gy	2.8	2.4	2.1
4 Gy	4.2	3.9	3.4
6 Gy	3.1	2.5	2.0

In table 6, DNA repair proteins were significantly upregulated after moderate radiation exposure. The expression of ATM was increased from 1.0 in control cells to 4.2 at 4 Gy indicating strong activation of DNA damage sensing pathways. Similarly, at moderate doses, the expression levels of RAD51 and Ku70 increased indicating activation of homologous recombination and non-homologous end joining repair pathways. But expression levels dropped at 6 Gy, indicating a depletion of repair pathways and a reduced ability of the cells to deal with excessive DNA damage.

5. DISCUSSION

The current study demonstrates that ionizing radiation causes marked chromosomal instability in human cells through dose-dependent accumulation of DNA damage. Higher radiation doses cause severe disruption of chromosomal integrity as reflected by increased frequencies of dicentric chromosomes, chromatid breaks, and micronuclei.

DNA double-strand breaks (DSB) induction was confirmed by activation of ATM/ATR signaling pathways and increased formation of γ-H2AX foci directly after irradiation. Moderate doses of radiation stimulated efficient DNA repair responses through homologous recombination and NHEJ pathways. But too much radiation exposure appeared to swamp cellular repair capacity, resulting in persistent DNA lesions and chromosomal rearrangements.

Reduction of repair protein expression at 6 Gy suggests repair pathway exhaustion and potential apoptosis induction. These findings are consistent with previous reports that defective DNA repair is a major factor in radiation sensitivity and carcinogenesis.

Persistent chromosomal aberrations could serve as biomarkers for radiation biodosimetry and cancer risk assessment. Therefore, understanding the mechanisms involved in genome stability is a prerequisite to improve the outcome of radiotherapy and to develop radioprotective strategies.

7 CONCLUSION AND FUTURE SCOPE

Ionizing gamma radiation caused significant chromosomal instability and DNA damage in human cells in a dose-dependent manner, as shown in the present study. We confirmed that radiation exposure severely affects genomic integrity by increased frequencies of dicentric chromosomes, chromatid breaks, micronuclei formation, and γ-H2AX foci. Activation of DNA damage response proteins, such as ATM, RAD51 and Ku70, indicated that human cells were initiating protective repair mechanisms to maintain genome stability following irradiation. However, too much

radiation exposure reduced the repair efficiency and promoted persistent chromosomal abnormalities, indicating that the repair pathway was exhausted and genomic instability increased.

These results highlight the importance of genome stability pathways in protecting cells from radiation-induced mutagenesis and carcinogenesis. The results also support the potential application of chromosomal aberrations and γ-H2AX biomarkers for radiation biodosimetry and clinical monitoring.

Future studies should explore the molecular regulation of DNA repair pathways with advanced genomics and proteomics approaches. Further studies with larger datasets, stem cells, and cancer cell models could improve understanding of radiation sensitivity and therapeutic resistance. Development of radioprotective agents and personalized radiotherapy strategies may also contribute to minimize radiation-associated genomic damage and improve clinical outcomes. Academic writing frameworks emphasise the need for clear conclusions that connect findings to broader scientific implications and future research directions.

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