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Exploring DNA repair deficient CHO cell response to low dose rate radiation

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ABSTRACT

Introduction: DNA double-strand breaks (DSBs) induced by ionizing radiation pose a significant threat to genome integrity, necessitating robust repair mechanisms. This study explores the responses of repair-deficient cells to low dose rate (LDR) radiation. Non-homologous end joining (NHEJ) and homologous recombination (HR) repair pathways play pivotal roles in maintaining genomic stability. The hypothesis posits distinct cellular outcomes under LDR exposure compared to acute radiation, impacting DNA repair mechanisms and cell survival.

Materials and methods: Chinese hamster ovary (CHO) cells, featuring deficiencies in NHEJ, HR, Fanconi Anemia, and PARP pathways, were systematically studied. Clonogenic assays for acute and LDR gamma-ray exposures, cell growth inhibition analyses, and γ-H2AX foci assays were conducted, encompassing varied dose rates to comprehensively assess cellular responses.

Results: NHEJ mutants exhibited an unexpected inverse dose rate effect, challenging conventional expectations. HR mutants displayed unique radiosensitivity patterns, aligning with responses to major DNA-damaging agents. LDR exposure induced cell cycle alterations, growth delays, and giant cell formation, revealing context-dependent sensitivities. γ-H2AX foci assays indicated DSB accumulation during LDR exposure.

Discussion: These findings challenge established paradigms, emphasizing the intricate interplay between repair pathways and dose rates. The study offers comprehensive insights into repair-deficient cell responses, urging a reevaluation of conventional dose-response models and providing potential avenues for targeted therapeutic strategies in diverse radiation scenarios.

1. Introduction

DNA double strand break (DSB) represent the most significant biological damage induced by ionizing radiation. The repair of DNA DSB is crucial for preserving genome integrity within cells. In mammalian cells, upon recognition of damage by sensor proteins, including the MRN complex, ATM and PARP, DNA DSBs are predominantly repaired primarily through two distinct mechanisms [1]: non-homologous end joining (NHEJ) repair, a non-template, potentially error-prone process, and the homologous recombination (HR) repair, a largely error-free process. NHEJ repair is the primary pathway throughout all cell cycle phases, whereas HR repair is predominantly active after DNA synthesis during S and G2 phases. Loss of NHEJ repair results in severe radiosensitivity across cell cycle phases, whereas loss of HR repair leads to mild radiosensitivity, particularly in the exponentially growing population, due to radiosensitivity in only S and G2 phases [2]. Loss of either repair system consequently leads to the accumulation of chromosomal aberrations, causing genome instability that may eventually result in the loss of genes crucial for controlling cell growth and division or the inappropriate activation of genes supporting tumorigenesis. Additionally, other repair systems such as base excision repair (BER), nucleotide excision repair (NER), and Fanconi Anemia repair also contribute to radiation induced DNA damage repair.

The effect of ionizing radiation also depends on its dose rate. For instance, low dose rate (LDR) radiation requires a higher total dose to kill cells compared to acute radiation due to cellular repair and repopulation during the irradiation period. The dose rate effect is most pronounced between 0.01 and 1 Gy per min regarding cell killing, and this effect results from the repair of sublethal damage during irradiation [3–5]. However, the molecular mechanisms in response to LDR radiation, especially those lower than 1 Gy/h, are not well understood compared to acute radiation, and whether exposure to LDR radiation

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enhances cancer risk in humans remains elusive. Previous studies on the effects of LDR irradiation on the life cycle and survival of cells have been limited in terms of the range of dose rates, total doses, and cell lines studied. G1-synchronized NHEJ repair deficient cells showed no dose rate effect between 0.03 Gy/h to acute radiation [6]. In contrast, plateau NHEJ deficient cells exhibited greater radiosensitivity in LDR radiation than acute irradiation (inverse dose rate effect) [7]. Comprehensive studies of LDR irradiation in DSB repair has not been conducted.

We hypothesized that the roles and significance of DNA DSB repair differ in LDR radiation compared to acute radiation exposure. We have employed Chinese hamster ovary origin radiosensitive mutants in NHEJ, HR, Fanconi Anemia, PARP to investigate the mechanisms of LDR radiation induced cell death. To elucidate each pathway's contribution to LDR radiosensitivity, intrinsic radiosensitivity, potentially lethal damage (PLD) repair, sublethal damage (SLD) repair, and cell cycle phase dependent radiosensitivity were also investigated. We identified LDR hypersensitive mutants and the dose rate range causing an inverse dose rate effect in DSB repair mutants, revealing how LDR causes cell death.

2. Materials and Methods

2.1. Cell culture conditions

Chinese hamster ovary (CHO) cells were cultured in alpha-MEM medium supplemented with 10 % fetal bovine serum and antibiotics in a humidified atmosphere with 5 % CO_2. Chinese hamster cell lines, namely, V3, xrs5, XR1, PADR9, irs1SF, and 51D1, deficient for DNA-PKcs, Ku80, XRCC4, PARP1, XRCC3, and Rad51D, respectively were employed as these cells were reported to exhibit sensitivity to acute radiation [8–12].

2.2. Gamma-irradiation

Acute irradiation was conducted using a ^{137}Cs irradiator (nominal 6000 Ci, JL Shepherd) with a dose rate of 2.5 Gy/min. For LDR irradiation, two LDR irradiator facilities were used. The first facility provided dose rates ranging from 2 to 100 mGy/h by placing cells on shelves in a 37 °C warm room above one to twelve ^{137}Cs sources [13]. The second facility delivered dose rates of approximately 500 and 1500 mGy/h by placing the cells on the floor in the 37 °C water bath with or without a lead attenuator, respectively [14]. Dose rates were determined using RadCal 2025 dose-rate meter or Victoreen R meter.

2.3. Clonogenic assay for acute irradiation

Three different irradiation protocols were employed for acute irradiation. Asynchronous cells were irradiated with gamma-ray and immediately subcultured for colony formation. For SLD experiments, asynchronous cells were irradiated with gamma-ray, incubated at 37 °C for specific interval, and then irradiated with the same doses before subculturing for colony formation. For PLD experiments, cells were synchronized into G1 phase by isoleucine depletion media for 1 day [15]. After irradiation, one group of cells were subcultured immediately and another after 6-h incubation. For cell cycle dependent radiosensitivity experiments, cells synchronized by the mitotic shake off method [16] were irradiated at various times and then subcultured for colony formation. 10 μM of BrdU was added for 30 min to determined S phase population by fluorescent immunocytochemistry. Colony was formed 7–10 days after incubation and fixed in 100 % ethanol and stained by 0.1 % crystal violet solution. Colonies containing more than 50 cells were recorded as survivors manually under microscope.

2.4. Three clonogenic assays for LDR irradiation

Total three different types of LDR irradiation and clonogenicity were examined.

The first method was clonogenicity during LDR irradiation. In this method, clonogenicity was examined by single cell colony formation assay. Single cells were plated on T25 flasks with HEPES buffer and placed into incubator for 1 h before irradiation. After cells were attached, cells were irradiated with various doses in LDR irradiator. Cells were maintained in the LDR irradiator for 8 days. After LDR irradiation, survivor colonies were scored and clonogenic curves were obtained. This method determines the limiting dose rates that allow cells to form colonies [17,18].

The second clonogenic assay was carried out as follows. Single cells were plated in T25 flasks and placed into irradiator as with the first method. After LDR irradiation, cells were directly placed into incubator to allow colony formation. By the time of completing LDR irradiation, cells began forming small colonies. This method determines LDR radiosensitivity of initial single cells and assess the repopulation doses [19].

The last clonogenic assay was carried out as follows. Exponentially growing cells were placed into LDR irradiator to reach designated total dose. Subsequently, these cells were trypsinized and replated in low density for colony formation assay. The survival curve for each of the wild-type and mutant cell lines will be established. This method determines a potential LDR irradiation effect to the daughter cells as DNA damage accumulates.

2.5. Cell growth inhibition and cell cycle analysis during LDR irradiation

Cellular growth inhibition was measured by cell number counting. The initial number of plated cells was 10,000. Cells were placed in LDR irradiator. Every day, cell number was recorded. Restriction dose rate was determined by suppression of cellular growth.

Exponentially growing cells were irradiated at LDR for 24 h. Cells were fixed in 70 % ethanol and stained by propidium iodide (PI). Cell cycle was determined by flow cytometer, FACSCalibur BD Biosciences and DNA profile was analyzed by Modfit 3.0 Program.

2.6. Giant cell formation during LDR irradiation

Cellular size was measured by Coulter Counter Z1. Fraction of cells diameter >20 μm was determined by Coulter Counter Z1 after trypsinization.

2.7. γ-H2AX foci assay

Exponentially growing cells were cultured in cell culture flasks. Cells were irradiated at 0.5 Gy/day or 3 Gy/day. After 24 h, cells were fixed in 4 % Paraformaldehyde and permeabilized in 0.5 % TritonX100 solution. γ-H2AX foci were stained with immunocytochemistry. Fluorescence microcopy analysis was conducted at least 50 cells per experiment as previously described [15].

2.8. Statistics

Statistical significance was calculated via GraphPad Prism 8 software. T-test and one way ANOVA were used. P-value less than 0.05 were considered statistically significant.

3. Results

3.1. Characterization of DNA repair deficient cells with acute radiation

Cellular radiosensitivity was confirmed by the clonogenic colony formation assay (Fig. 1A). Among the 8 cell lines tested, CHO wild-type cells were the most radioresistant. FA repair-deficient KO40 and PARP-deficient PADR9 showed almost similar radiosensitivity to the wild-type. Two HR repair-deficient mutants, irs1SF and 51D1, exhibited intermediate radiosensitivity. NHEJ repair-deficient mutants were the

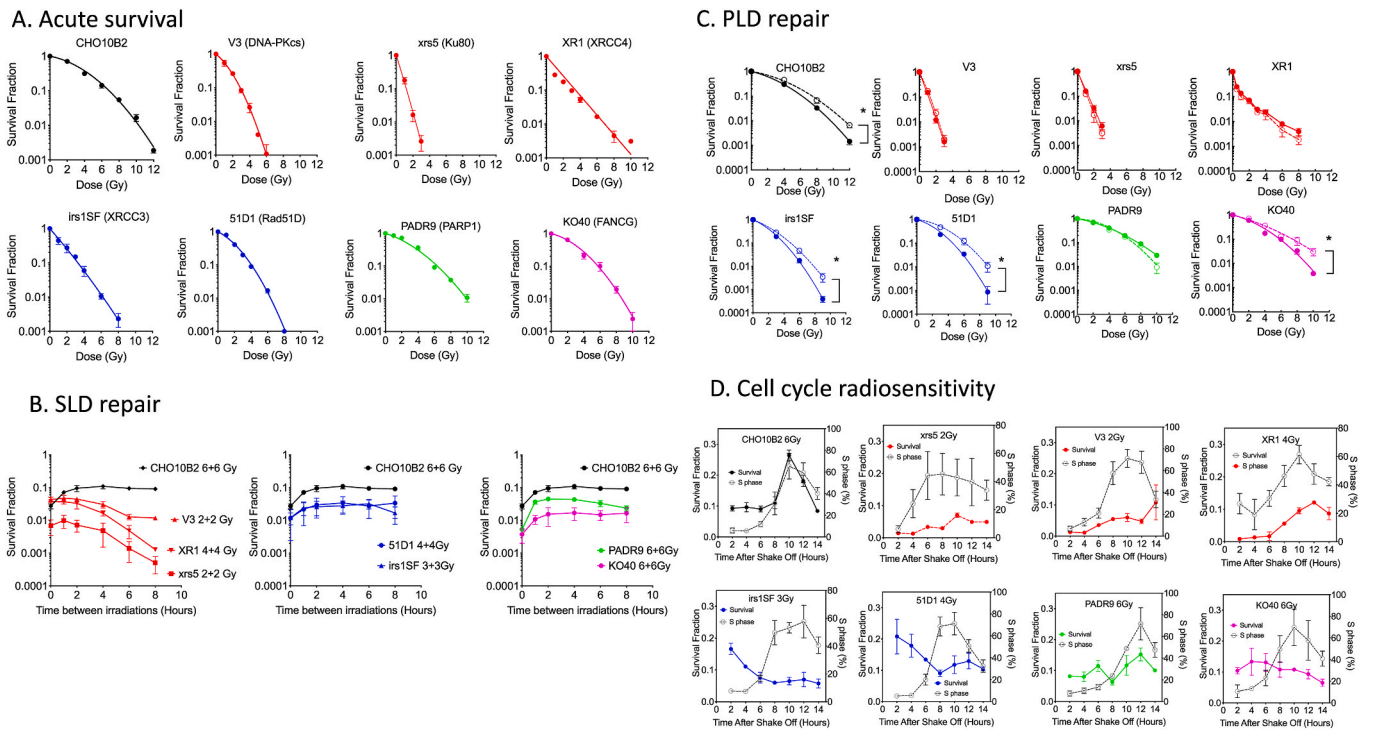

Fig. 1. Cellular radiosensitivity in different conditions.
A. Acute radiation. B. Split dose irradiation condition for SLD repair. C. Delayed subculture condition for PLD repair. D. Cell cycle dependent radiosensitivity. * symbol indicates statistical differences ($P < 0.05$).

most sensitive. The D_{10} values of the mutants are summarized in Table 1.

SLD repair is associated with repair during LDR. The SLD repair capacity was tested by irradiating two split doses with varied repair interval times (Fig. 1B). WT, HR mutants, FA mutants, and PARP mutants showed the lowest cell survival when two irradiations were carried out simultaneously without an interval, and there was an increase in survival fraction within 2 h of an interval. On the other hand, all three NHEJ mutants did not show an increase in survival fraction within 2 h and exhibited a decrease in survival fraction after 4 h. Therefore, except for NHEJ mutants, wild-type, HR mutants, FA mutants, and PARP mutants demonstrated SLD repair capacity (Table 1).

The PLD repair capacity was investigated in G1 synchronized cell culture with or without a 6-h incubation period after irradiation before trypsinization and colony formation (Fig. 1C). PLD repair was observed in CHO wild-type, HR mutants, and FA mutants, as evidenced by a statistically significant increase in cell survival after 6 h of incubation time. However, all three NHEJ mutants and PARP mutants did not show radioresistance after incubation (Table 1).

Cell cycle-dependent radiosensitivity was tested using synchronized populations (Fig. 1D). The S-phase population was assessed through BrdU labeling, with BrdU-positive cells peaking 8–12 h after mitotic shake-off. Consequently, cells in the 2–6 h after mitotic shake-off were predominantly in the G1 phase, while 8–12 h after mitotic shake-off represented the S-phase, and 12–14 h after shake-off was the G2 phase. S-phase radioresistance, observed as an increase in cell survival, was evident in wild-type and NHEJ mutant cells. In contrast, HR mutants exhibited hypersensitivity during both S and G2 phases. KO40 and PADR9 did not show noticeable cell cycle-dependent radiosensitivity compared to other cell lines (Table 1).

Table 1
Characterization of CHO and its DNA repair mutant cell lines.
+++, +, and - indicate severely sensitive, sensitive, and resistant, respectively compared to wild-type cells.

Cell	affected pathway	D10	SLD repair	PLD repair	S/G2 sensitivity	DR10	MMS	Camptothecin	Cisplain	Bleomycin	Etoposide	Mitomycin C
CHO10B2 (WT)	NA	6.9 Gy	yes	yes	resistant	14.1 cGy/h	–	–	–	–	–	–
V3	NHEJ	2.9 Gy	no	no	resistant	3.0 cGy/h	+	–	–	+++	+	–
xrs5	NHEJ	1.2 Gy	no	no	resistant	1.7 cGy/h	+++	+	+	+++	+++	+
XR1	NHEJ	3.5 Gy	no	no	resistant	1.8 cGy/h	+	–	–	+++	+++	–
irs1SF	HR	3.3 Gy	yes	yes	sensitive	3.4 cGy/h	+++	+++	+++	+++	+++	+++
51D1	HR	4.0 Gy	yes	yes	sensitive	4.2 cGy/h	+++	+++	+++	+	+++	+++
KO40	Fanconi repair	5.8 Gy	yes	yes	na	12.5 cGy/h	+++	–	+	–	–	+
PADR9	PARP	6.4 Gy	yes	no	na	5.7 cGy/h	+	–	–	+	+	+

3.2. Clonogenicity of DNA repair deficient cells during LDR irradiation

LDR irradiation was conducted after single cells were plated in flasks. Clonogenicity was confirmed through colony formation, which determined the growth restriction dose rate (Fig. 2A). We defined DR_{10} as the dose rate required to achieve 10 % cell survival. In LDR, NHEJ mutants remained the most radiosensitive group, with DR_{10} values of 1.7 cGy/h for xrs5, 1.8 cGy/h for XR1, and 3.0 cGy/h for V3. xrs5 cells reduced colonies as low as 0.2 cGy/h. HR mutants also exhibited hypersensitivity, with DR_{10} values at approximately 4 cGy/h for irs1SF and 51D1. PARP-deficient cells showed intermediate radiosensitivity, with a DR_{10} value of 5.7 cGy/h. DR_{10} values for wild-type and FA mutants were approximately 13–14 Gy/h.

Data from clonogenicity during LDR irradiation revealed inconsistencies with radiosensitivity obtained from acute irradiation (Fig. 1A). Firstly, PADR9 showed approximately a 2 times higher hypersensitivity in LDR compared to the wild-type. Secondly, the radiosensitivity ratio widened in NHEJ mutants and HR mutants compared to the wild-type. In acute irradiation, D_{10} values for wild-type were approximately 7 Gy, while NHEJ mutants and HR mutants showed between 1.2 Gy and 4 Gy for their D_{10} values, respectively. The ratios were about 2 times in HR mutants and 2–5 times in NHEJ mutants. On the other hand, in LDR, the ratios were approximately 5–8 times for NHEJ mutants and 3–4 times for HR mutants. Including PARP-deficient cells, all mutants except KO40 showed a greater difference in radiosensitivity in LDR irradiation (Table 1).

These LDR radiosensitivities were compared to chronic exposure to DNA damaging agents: Methy Methanesulfonate (MMS) (alkylating agents), Bleomycin (DSB), Camptothecin (Topoisomerase I inhibitor), Etoposide (Topoisomerase II inhibitor), Cisplatin (crosslink), Mitomycin C (crosslink) (Fig. 2B). HR mutants exhibited the strongest hypersensitivities to MMS, Camptothecin, Cisplatin, and Mitomycin C compared to other mutants. NHEJ mutants showed the strongest hypersensitivities to Bleomycin and Etoposide. The spectrum of LDR irradiation sensitivities in mutants was similar to that of Bleomycin and Etoposide (Table 1). Since V3 showed intermediate sensitivity to Etoposide, major DNA damages contributing to LDR radiosensitivities were direct DNA DSBs rather than Topoisomerase II inhibition induced DSBs.

To compare the sensitivities of acute radiation and LDR, cellular radiosensitivities were assessed based on total doses to the cell population. LDR irradiation was halted once the total doses were reached, and cells were further incubated for additional days to allow colony formation (Fig. 2C). This clearly demonstrated the 'repopulation' effect in wild-type cells. At the lowest dose rate of 0.5–1 Gy/day, cells did not exhibit noticeable death, and the majority of CHO wild-type cells could tolerate 12 Gy of irradiation at a dose rate of 3 Gy per day. The apparent dose rate effect with radioresistance was observed at below 3 Gy/day for wild-type and 1 Gy/day for PADR9. NHEJ mutant xrs5 showed radiation induced cytotoxicity with 0.5 Gy/day, but at 3 Gy/day, it exhibited hyper-radiosensitivity compared to other dose rates. This phenomenon is known as the inverse dose rate effect. The inverse dose rate effect was more apparent in XR1 between 3 Gy per day to 1.67 Gy/h. A similar inverse dose rate effect was observed at similar dose rate ranges in two HR mutant cells. The inverse dose rate effect occurred in the 1 Gy/day to 3 Gy/day range in NHEJ and HR mutant cells but not in wild-type and PARP-deficient cells.

Finally, the dose rate effect was investigated using another method of cell plating timing (Fig. 2D). Cells were exposed to LDR to reach designated total doses and then replated for a colony formation assay. This method can overcome the re-population effect. The dose rate effect was observed in wild-type cells at 1.66 Gy/h and below. No noticeable dose rate effect was observed in PADR9 cells. xrs5, 51D1, irs1SF, especially XR1, showed a clear inverse dose rate effect. These cells exhibited hyper-radiosensitivity at lower dose rates.

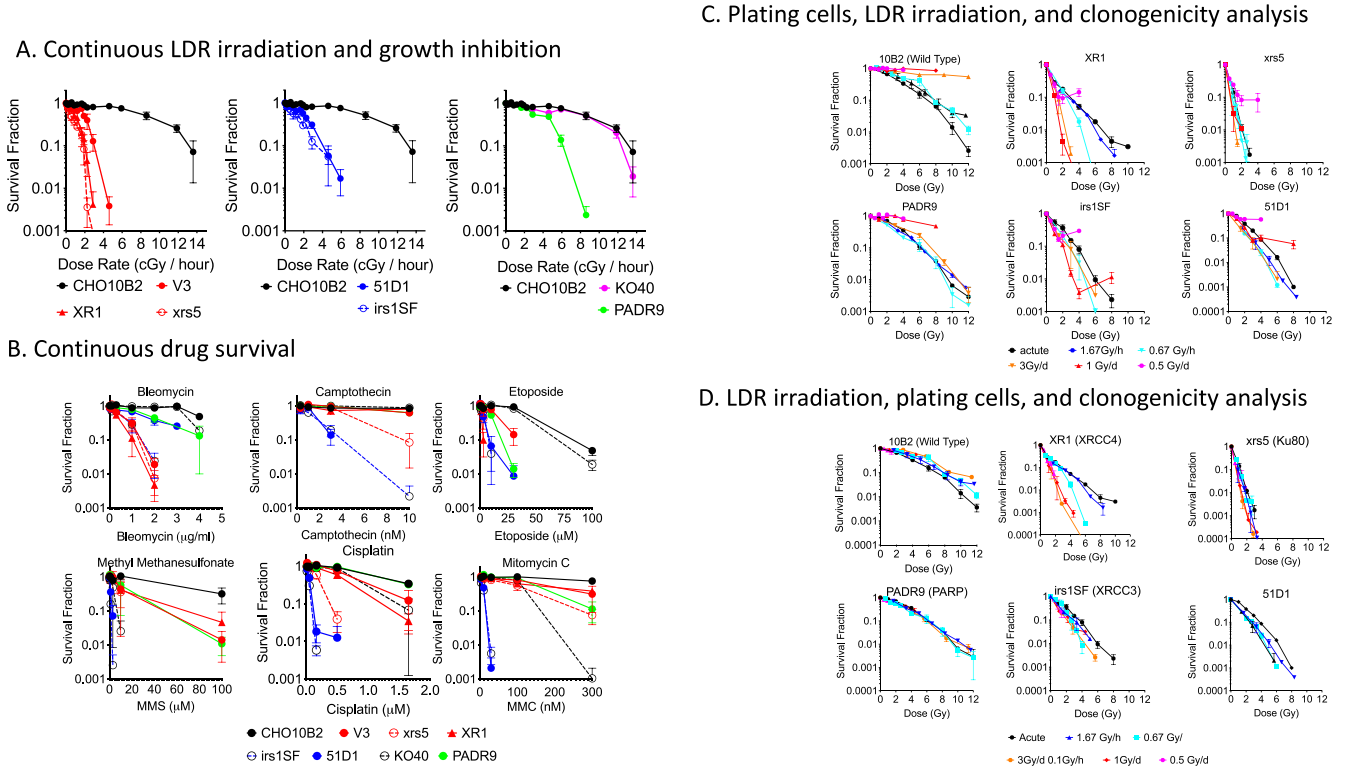

Fig. 2. Continuous treatment radiosensitivity and drug sensitivity.
A. Continuous LDR growth inhibition. B. Continuous DNA damaging drug treatment. C. LDR irradiation colony formation assay: single cells were exposed to LDR irradiation and continued to allow colony formation after LDR irradiation. D. LDR irradiation colony formation assay: LDR irradiation of exponentially growing cells and single cell plating for colony formation.

3.3. Cell growth inhibition, giant cell formation and accumulation of DNA damage in LDR

Our results revealed a clear inverse dose rate effect for cell death in exponentially growing NHEJ and HR-deficient hamster cells (Fig. 2CD). The redistribution of the cell cycle could result in a significant change in the sensitivity of a cell population during irradiation, thus influencing the dose rate effect, as cells vary in radiosensitivity during their life cycle (Fig. 1D). Cell growth was monitored by cell number counting during LDR irradiation, revealing dose rate-dependent growth delay (Fig. 3A). Wild-type cells did not show significant delay until 3 Gy/day. PADR9 showed significant delay from 1Gy/day, while other mutants showed significant delay from 0.5 Gy/day, with poor growth at 3 Gy/day. The dose rate range for the inverse dose rate effect and for the cell growth delay matched in NHEJ and HR mutants, but cell numbers still increased for a few days at this dose rate, indicating that cells were not fully arrested at specific cell cycles during LDR irradiation.

Cell cycle arrest was investigated with a flow cytometer 24 h after LDR started. Within 24 h, cell cycle alteration was observed (Fig. 3B). For WT, the G1 population was reduced, and S and G2/M phases accumulated. Reduction of the G1 phase and an increase in G2/M were significantly observed in NHEJ mutants. This cell cycle alteration should not be complete arrest because cell numbers increased for a few days at this dose rate (Fig. 3A). Cytokinesis failure and giant cell formation were suspected of the observation of G2/M accumulation.

The dose rate-dependent giant cell formation was determined by automatic counting with confirmation under the microscope (Fig. 3C). Cell size was automatically analyzed by Coulter counter, and cells larger than 20 μm diameter were classified as giant cells. Giant cell formation was observed on day 1 for XR1 and xrs5 cells with 1 Gy per day and above which matched with cell cycle arrest (Fig. 3AB). CHO wild-type and PADR9 cells enlarged on day 3 at 3 Gy per day. Irs1SF showed intermediate responses.

Since DNA repair-deficient cells showed hypersensitivity to these LDR conditions, DSBs were associated with this event. During LDR irradiation, DSBs were formed and simultaneously repaired. γ-H2AX foci at the equilibrium state would indicate the presence of DSBs. Two dose rates of LDR irradiation were carried out: 0.5 Gy per day (0.02 Gy/h) which can cause repopulation and 3 Gy per day (0.1 Gy per hour) which can cause inverse dose rate (Fig. 3D). The dose rate of 3 Gy per day induced the accumulation of γ-H2AX foci, especially significant increase in mutant cells. Two tested NHEJ-deficient mutants (xrs5 and XR1) showed a severe two to four-fold increase in foci at both dose rates and significant at the dose rate of 3 Gy/day. HR-deficient cells and PARP-deficient cells also showed increases in γ-H2AX, but the induction was relatively moderate compared to NHEJ mutant cells.

4. Discussion

The comprehensive characterization of DNA repair-deficient cells under acute radiation exposure has provided valuable insights into the intricate interplay between repair mechanisms, dose rates, and cellular responses. Our study, employing clonogenic colony formation assays and subsequent analyses, unveils distinct patterns of radiosensitivity across various cell lines with different DNA repair deficiency (Fig. 1). In agreement with established literature, the clonogenic assays revealed CHO wild-type cells as the most radioresistant among the tested lines (Fig. 1A). Notably, the FA repair-deficient KO40 and PARP-deficient

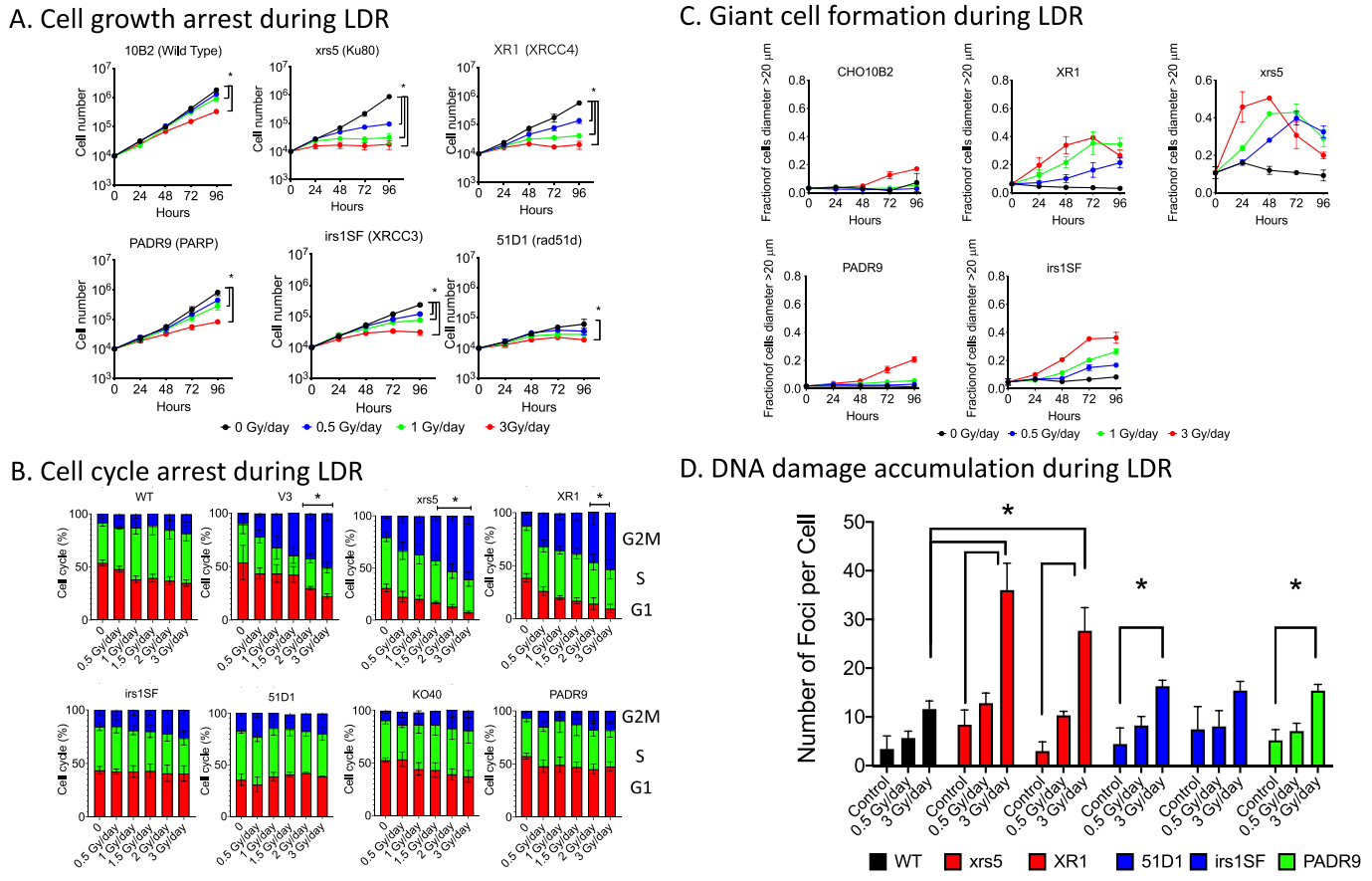

Fig. 3. LDR irradiation and cell growth arrest, giant cell formation, and DNA damage accumulation.
A. Cell growth arrest during LDR irradiation. B. Cell cycle arrest during LDR irradiation, C. Giant cell formation during LDR irradiation. D. DNA damage accumulation during LDR irradiation. * symbol indicates statistical differences ($P < 0.05$).

PADR9 cells demonstrated comparable radiosensitivity in acute irradiation to the wild-type, highlighting the complexity of repair pathways in determining cellular resilience to radiation. KO40 was sensitive to Methyl Methanesulfonate and Mitomycin C (Fig. 2B). PADR9 showed no PLD repair, hypersensitivity to continuous LDR irradiation and no dose rate effect (Fig. 2). Between two DSB repair pathways (NHEJ and HR repair), the intermediate radiosensitivity observed in HR repair-deficient mutants (irs1SF and 51D1) and the heightened sensitivity of NHEJ repair-deficient mutants underscore the critical roles these pathways play in maintaining genomic integrity. HR mutants showed unique S, G2-phases radiosensitivity, sensitivity to major DNA damaging agents, and moderate inverse dose rate effect (Figs. 1D and 2).

NHEJ mutants showed severe sensitivity to radiation and DSB generating drugs (Figs. 1 and 2). Among tested three NHEJ mutants, XR1 showed unique responses. XR1 showed severe radiosensitivity in less than 2 Gy but become mildly radioresistant in higher doses. This was due to cell cycle specific radiosensitivity as XR1 was shown to have strong cell cycle dependent radiosensitivity [20] (Fig. 1D). Although the radioresistance in S phase was expected to cause radioresistance in LDR, XR1 showed hypersensitivity to LDR radiation as the most sensitive xrs5 cells (Fig. 2A). It was repeated in the continuous treatment of DNA damaging agents (Fig. 2B). In the LDR colony formation assay, XR1 showed severe inverse dose rate effects at the LDR irradiation of 1 and 3 Gy per day (Fig. 2CD). The potential explanations about this severe inverse dose rate effects may be related to SLD repair experiments. NHEJ mutants, especially XR1, showed clear reduction of cell survival with split dose irradiation (Fig. 1B). This means the survivors in the initial irradiation at the resistant cell cycle phase shifted into the sensitive cell cycle phase by the time of irradiation. The inverse dose rate effects were observed around the dose rate of 0.1 Gy/h. This dose rate does cause accumulation of cell cycle arrest, but it was more like cell cycle slow down (Fig. 3AB). At this dose rate, XR1 presented giant cell formation and accumulation of γ-H2AX foci (Fig. 3C). Another NHEJ mutant xrs5 did not show the inverse dose rate effect. We assume that this is due to the similar radiosensitivity in xrs5 throughout cell cycle phases (Fig. 1D). Therefore, as previously proposed mechanism of inverse dose rate effect, the inverse dose rate effect can happen when LDR dose was at the proper cell effect such as cytotoxicity and not cell cycle arrest [21]. The inverse dose rate effect was observed around 0.5 Gy/h for HeLa cells and not observed in V79 Chinese hamster cells but it was not more sensitive than acute radiation [21]. We showed that HR and NHEJ mutants have inverse dose rate below 1 Gy per hour and higher sensitivity to LDR radiation than acute radiation exposure. Although it is considered that lower dose rate is less effective [19], in a certain situation like DNA repair deficiency, lower dose rate irradiation may cause more harmful effect than acute radiation exposure.

In conclusion, our study underscores the intricate relationships between repair pathways, dose rates, and cellular responses. The observed inverse dose rate effect challenges conventional paradigms and warrants further exploration. The distinct sensitivities to DNA-damaging agents emphasize the context-dependent nature of cellular responses. This comprehensive characterization contributes valuable insights into the complexities of DNA repair-deficient cell responses under different irradiation conditions, offering potential avenues for targeted therapeutic strategies.

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CRediT authorship contribution statement

Dylan J. Buglewicz: Investigation, Writing – review & editing. **Jeremy S. Haskins:** Investigation, Writing – review & editing. **Alexis H. Haskins:** Investigation, Writing – review & editing. **Cathy Su:** Investigation, Writing – review & editing. **Jeffrey P. Gius:** Investigation, Writing – review & editing. **Takamitsu A. Kato:** Conceptualization, Funding acquisition, Investigation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Takamitsu Kato reports financial support was provided by Dr Akiko Ueno Radiobiology Research Fund. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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