Life Sciences 222 (2019) 228–234

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Both irradiated and bystander effects link with DNA repair capacity and the linear energy transfer

Wenzhi Tu[a,b], Chen Dong[a], Jiamei Fu[a], Yan Pan[a], Alisa Kobayashi[c], Yoshiya Furusawa[d], Teruaki Konishi[d], Chunlin Shao[a,*]

[a] Institute of Radiation Medicine, Fudan University, Shanghai 200032, China
[b] The Comprehensive Cancer Center, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 201620, China
[c] Department of Accelerator and Medical Physics, National Institute of Radiological Sciences, National Institutes for Quantum and Radiological Science and Technology, Inage, Chiba 263-8555, Japan
[d] Department of Basic Medical Sciences for Radiation Damages, National Institute of Radiological Sciences, National Institutes for Quantum and Radiological Science and Technology, Inage, Chiba 263-8555, Japan

ARTICLE INFO

Keywords:
High-LET radiation
DNA repair capacity
Irradiated effects
Bystander effects

ABSTRACT

Aims: In comparison with a low linear energy transfer (LET) radiation, a high-LET radiation induces more complex DNA damage. This study wonders whether radiation-induced bystander effect (RIBE) is dependent of LET.

Materials and methods: Chinese hamster ovary CHO-9 cells and its subline EM-C11 cells (SSB repair deficient) and XR-C1 cells (DSB repair deficient) were irradiated by γ-rays, α-particles, or carbon ions with different LETs of 13, 30 and 70 keV/μm. Cell proliferation, cell death, DNA damage, cell cycle distribution and some protein expressions were measured with the cell counting kit-8 (CCK-8), colony formation, micronuclei (MN), flow cytometry and western blot, respectively.

Key findings: A series of cell responses were induced by these radiations in a LET-dependent manner, including proliferation inhibition, cell death, MN induction, G_2/M phase arrest and the expression of γH2AX protein. These cell injuries were also depended on DNA repair capacity, and XR-C1 cells were the most sensitive to each radiation. Furthermore, when the cells were treated with the conditioned medium (CM) collected from irradiated CHO-9 cells, the MN induction and cell death response in the bystander cells of EM-C11 or XR-C1 increased along with LET of irradiation, and the bystander damage was easier to be induced in EM-C11 and XR-C1 cells than that in CHO-9 cells.

Significance: Both cellular DNA repair capacity and the LET value of radiation could deeply influence damage extents of not only the irradiated cells but also the bystander cells.

1. Introduction

Radiation induced DNA damage could induce important signal transduction processes and lead to cell death, mutation, or genomic instability. Compared to γ-rays irradiation with a low linear energy transfer (LET), α-particles and heavy ions irradiation with high LETs yield the increased clustering and complexity of DNA damage [1–4]. In general, upon induction of DNA damage, DNA repair proteins have important function in maintaining cell survival and genomic integrity by mounting DNA damage response (DDR). Simultaneously, cell cycle arrest is triggered to allow cellular DNA repair and prevents aberrant replication of damaged DNA [5]. Nevertheless, it is also believed that the DNA damage induced by high-LET radiation is more complex and more difficult to repair than that induced by low-LET radiation and thus it leads to more severe biological consequences [6].

Radiation induces DNA damage not only in the directly exposed cells but also in their neighboring nonirradiated cells, termed as radiation-induced bystander effect (RIBE). This phenomenon was first demonstrated by Nagasawa and Little in 1992 [7]. More research progresses about RIBE has been made in recent years. RIBE has been proved existing in both normal cells [8,9] and tumor cells [10–12], and multiple bystanders signaling factors have been identified [12–15]. However, the dependences of RIBE with DNA repair capacity and the LET of radiation are still not disclosed.

In the current study, three cell lines with different DNA repair capacity were exposed to various radiation sources of γ-rays, α-particles

* Corresponding author.
E-mail address: clshao@shmu.edu.cn (C. Shao).

https://doi.org/10.1016/j.lfs.2019.03.013
Received 12 February 2019; Received in revised form 6 March 2019; Accepted 7 March 2019
Available online 08 March 2019

and carbon ions with different LETs. It was determined that not only the DNA repair capacity, but also the LET of radiation has been involved in the DNA damage induced in both irradiated cells and bystander cells.

2. Materials and methods

2.1. Cell culture and conditioned medium transfer

The cell line CHO-9 and its two mutant cell lines EM-C11 and XR-C1 were kindly provided by Professor K. M. Prise (Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, UK). EM-C11 cells are defective in the *XRCC1* gene, which is involved in DNA single-strand break (SSB) repair. XR-C1 cells are defective in the *XRCC7* gene that encodes the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) and thus it is impaired in DNA double-strand break (DSB) repair and V(D)J recombination [16]. All the cells were maintained in F-10 medium (Gibco Invitrogen, Grand Island, NY, USA) with 10% fetal bovine serum (Gibco), 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere with 5% CO_2.

For RIBE study, similar to previous studies [17], the conditioned medium (CM) was harvested from irradiated cells 2 h after radiation with different sources and filtered through a 0.22 μm filter, then it was applied to culture nonirradiated cells of CHO-9, EM-C11 and XR-C1 for 6 h until further analysis.

2.2. Cell irradiation

Exponentially growing cells of CHO-9, EM-C11 and XR-C1 were exposed to 1 Gy of γ-rays, α-particles and carbon ions, respectively. The γ-rays and a-particles were generated from a ^{137}Cs Gammacell-40 irradiator (Nordion International Inc., Kanata, Ontario, Canada) and a ^{241}Am plate source (Atom High Tech Co., Ltd., Beijing, China), respectively. The characteristic of α-particles with a LET of 100 keV/μm has been described before [18]. Carbon ions were accelerated to 290 MeV/u by the HIMAC (Heavy Ion Medical Accelerator in Chiba) facility of NIRS (National Institute of Radiological Sciences, Japan) [19]. Binary filters were used to reduce the beam energy to obtain a variety of LETs in water of 13, 30 and 70 keV/μm for cell irradiation.

2.3. Cell proliferation assay

Cell proliferation was determined with the Cell Counting Kit-8 (CCK-8) (Dojindo, Kumamoto, Japan). After each treatment, 2×10^3 cells in the logarithmic phase were seeded per well into a 96-well micro-plate and cultured for 72 h, and then were analyzed for proliferation using CCK-8 assay. The details have been described in our previous report [20].

2.4. Colony formation assay

Exponentially growing cells were trypsinized and counted. Cells were diluted serially to appropriate concentrations and plated into 60-mm dishes in triplicate and incubated for 7 days at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air. The colonies were stained with crystal violet and these containing at least 50 cells were counted for colony formation. The survival of irradiated cells was normalized to nonirradiated control cells.

2.5. Micronuclei (MN) assay

MN were measured using the cytokinesis-block technique [21]. In brief, cells were treated with 0.83 μg/mL cytochalasin B (Sigma, St Louis, MO, USA) for 20 h and then fixed with methanol/acetic acid (9:1 v/v) for 20 min. Air-dried cells were stained with 0.01% acridine orange (Sigma) and then observed under a fluorescence microscope (Olympus, Tokyo, Japan). MN were scored in at least 1000 binucleated cells and the MN yield, Y_{MN}, was calculated as the ratio of the number of MN to the scored number of binucleated cells.

2.6. Western blot

The cells were treated with lysis buffer, and the total protein was isolated for immunoblotting analysis as described previously [15]. The protein bands labeled with antibodies against γH2AX (Ser 139) (Millipore, Bedford, MA, USA), DNA-PKcs (Santa Cruz Biotechnology, TX, USA), XRCC1 (Santa Cruz Biotechnology), DNA PKcs (phosphor-S2056) (Abcam, Cambridge, UK), actin and tubulin (Beyotime Biotechnology, Jiangsu, China) were visualized using the ChemiDoc XRS system (Bio-Rad Laboratories, Hercules, CA, USA) after incubation with ECL Plus (Millipore).

2.7. Cell cycle analysis

After irradiation, the cells were harvested either immediately or at the indicated time after irradiation. These cells were fixed with 70% ethanol then washed with PBS and stained with propidium iodide (Cell Cycle and Apoptosis Analysis Kit, Beyotime) according to the manufacturer's protocol. The cell cycle distributions were analyzed using a flow cytometer (Gallios, Beckman Coulter, USA) and > 10,000 cells per sample were counted.

2.8. Statistical analysis

The data are expressed as the mean ± SEM of at least three independent experiments and were analyzed by using the unpaired Student's *t*-test. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. LET-related cell proliferation inhibition

To identify the cell characteristics, the expression of DNA repair proteins of XRCC1 and DNA-PKcs were detected in these three cell lines. CHO-9 cells expressed both XRCC1 and DNA-PKcs, whereas EM-C11 cells lacked XRCC1 expression and XR-C1 cells lacked DNA-PKcs expression (Fig. 1A). The expressions of XRCC1 and DNA-PKcs and its phosphorylation activity were increased in CHO-9 cells after 2 h of either low-LET or high-LET irradiation (Fig. 1B). The CCK-8 assay revealed that the cell proliferation rates of CHO-9, EM-C11 and XR-C1 were all inhibited by irradiation. As shown in Fig. 1C, compared to the low-LET γ-rays radiation, the high-LET radiation of α-particles (100 keV/μm) induced a more significant decrease in cell proliferation. Further experiments by irradiating cells with 1 Gy of carbon ions with different LETs of 13, 30 and 70 keV/μm showed that the cell proliferation decreased along with the increase of LET (Fig. 1D). Meanwhile, the colony formation assay illustrates that the clonogenicity of the cells irradiated with γ-rays was significantly higher than that of the cells irradiated with α-particles (Fig. 1E). When the cells were irradiated with 1 Gy of carbon ions with different LETs of 13, 30 and 70 keV/μm, the cell survival reduced gradually along with the increase of LET (Fig. 1F). Moreover, among these cell lines, the XR-C1 cells were the most radiosensitive and the EM-C11 cells took the secondary place to each LET of radiation in cell proliferation inhibition and cell killing effect.

3.2. LET-dependent DNA damage

As a consequence of DNA strand breaks, MN were induced in the irradiated cells. Fig. 2A illustrates that, the amount of MN induced by α-particles was prominently higher than that induced by γ-rays. When these three cell lines were irradiated by carbon ions with different LETs of 13, 30 and 70 keV/μm, the yields of MN significantly increased along

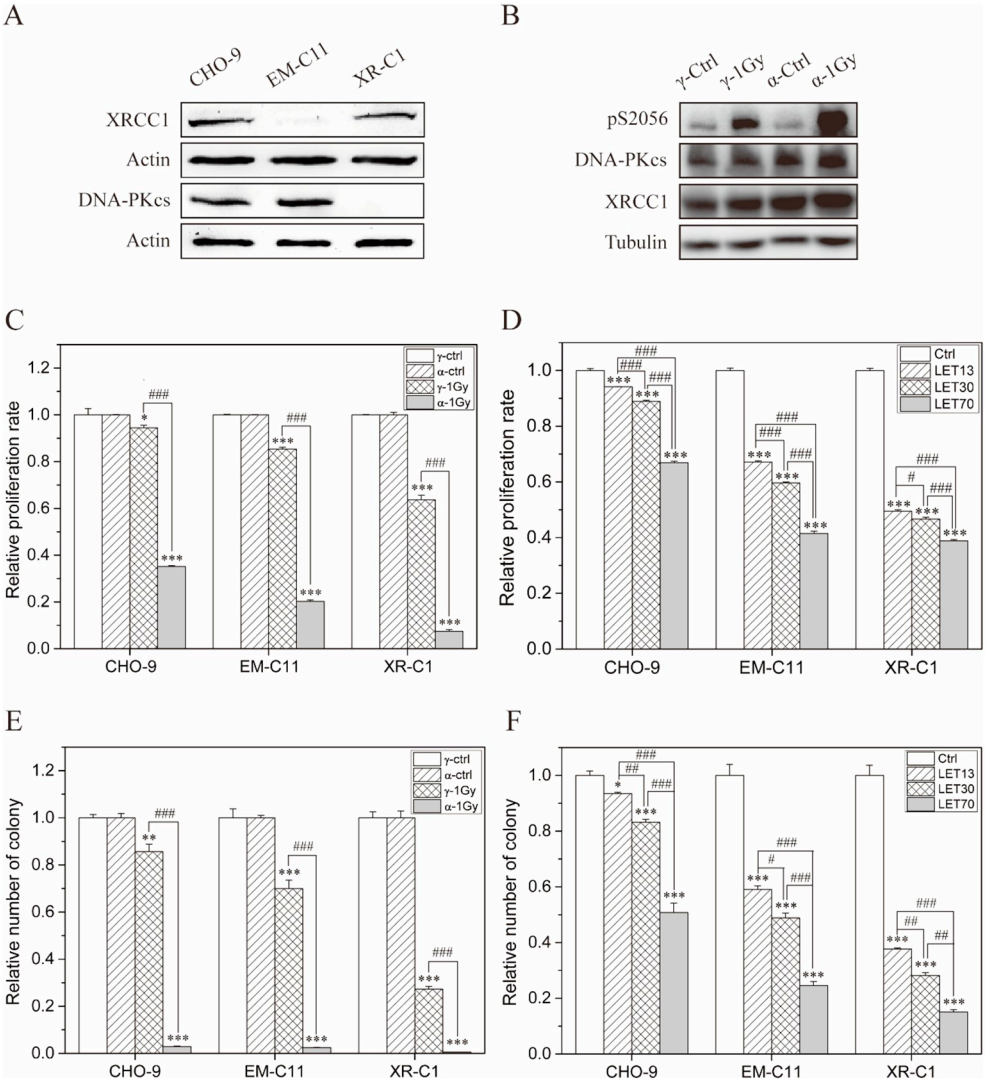

Fig. 1. Proliferation and clonogenic survival of the cells irradiated with different LETs. CHO-9, EM-C11 and XR-C1cells were irradiated with 1 Gy of γ-rays, α-particles or different LETs carbon ions. (A) XRCC1 and DNA-PKcs expressions in these three cell lines. Actin was used as the internal control. (B) The expressions of XRCC1 and DNA-PKcs and its phosphorylation at ser2056 in CHO-9 cells at 2 h after 1 Gy of γ-ray or α-particle irradiation were detected. Tubulin was used as the internal control. (C) Proliferation of the cells irradiated with γ-rays or α-particles. (D) Proliferation of the cells irradiated with carbon ions with different LETs of 13, 30 and 70 keV/μm. (E) Cell survivals after γ-rays and α-particles radiation. (F) Cell survivals after 13, 30 or 70 keV/μm carbon ions radiation. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared to nonirradiated control; $\#P < 0.05$, $\#\#P < 0.01$ and $\#\#\#P < 0.001$ between the indicated groups.

with the LET values (Fig. 2B). Synchronously, as a representative marker of DNA damage, the expression of γH2AX was effectively enhanced at 1 h after different radiation and its level increased with LET from 13 to 100 keV/μm (Fig. 2C and D). Moreover, for different radiation treatment, both inductions of MN and γH2AX in the irradiated XR-C1 cell lines were higher than the other two cell lines of EM-C11 and CHO-9.

3.3. LET-correlative G_2/M phase arrest

A noteworthy G_2/M phase arrest was observed in three cell lines at 8 h after 1 Gy α-particles radiation and it remained until 24 h after radiation (Fig. 3A). In comparison, these cells didn't show obvious G_2/M phase arrest after 1 Gy of γ-rays radiation. For 70 keV/μm carbon ions radiation, an apparent G_2/M phase accumulation was induced at 8 h after irradiation and this cell cycle arrest was almost released at 24 h after radiation (Fig. 3B). But the G_2/M phase arrest was rarely induced by 13 and 30 keV/μm of carbon ion radiation.

3.4. LET-interrelated MN and colony formation in bystander cells

To know the influence of DNA repair capacity and radiation quality on bystander response, we designed two experimental models (Fig. 4A) and treated the bystander cells with the CM harvested from irradiated cells. As shown in Fig. 4B, when CHO-9 cells were treated with the CM from different-LET irradiated CHO-9, EM-C11 and XR-C1 cells, no additional MN was induced in the bystander cells; but when EM-C11 and XR-C1 cells were treated with the CM from irradiated CHO-9 cells, the yields of MN formation in these bystander cells were obviously increased, moreover, this bystander damage induced by α-particles irradiation was memorably higher than that induced by γ-rays irradiation, which is consistent with the results in Fig. 1B where the activity of DNA-PKcs after α-particles irradiation was higher than that of γ-rays irradiation. This result implies that the yield of MN in bystander cells is in a LET-dependent manner. To test this hypothesis, we performed further experiments using the carbon ions with different LETs of 13, 30 and 70 keV/μm. Similar, no bystander response was observed in CHO-9 cells when it was treated with the CM from carbon ion irradiated cells, but MN was induced in the bystander cells of EM-C11 and XR-C1 that treated with CM from irradiated CHO-9 cells (Fig. 4C).

On the other side, when we checked the clonogenicity of bystander cells, it was found that only the survival fractions of bystander EM-C11 or XR-C1 cells were reduced after the treatment of CM from CHO-9 cells irradiated with different-LET particles, and no cell death was observed in the bystander CHO-9 cells (Fig. 4D and E), which are consistent with the situation of bystander MN induction.

It could be concluded that the MN induction and cell death response in the bystander cells of EM-C11 or XR-C1 increased along with LET of irradiation. Somehow, in comparison, the bystander damage was easier to be induced in EM-C11 and XR-C1 cells than that in CHO-9 cells.

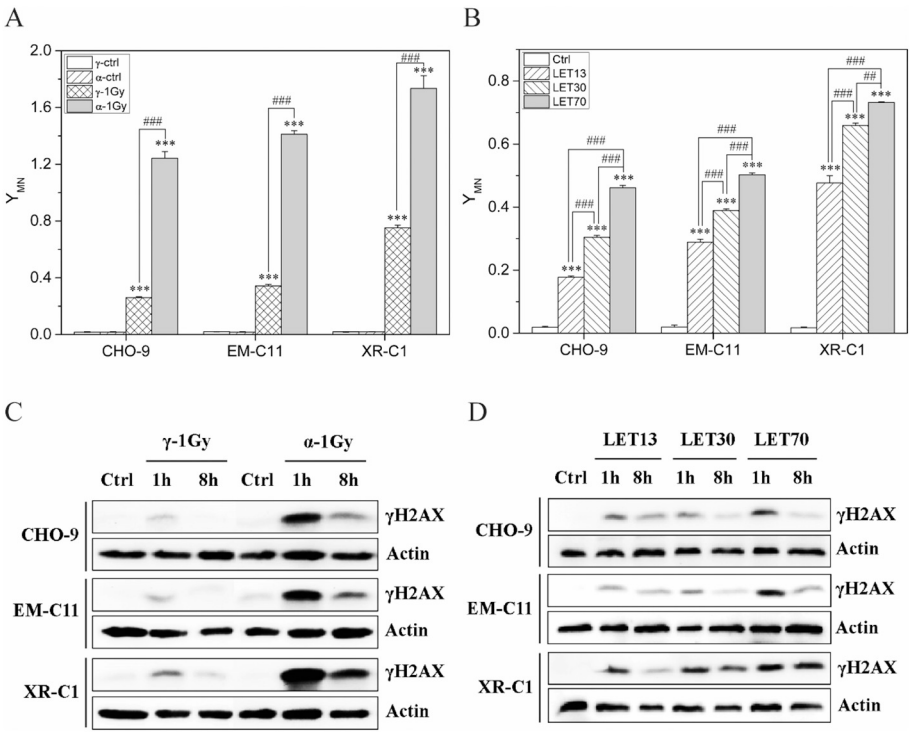

Fig. 2. The yield of MN and the expression of γH2AX protein in the irradiated cells. CHO-9, EM-C11 and XR-C1 cells were irradiated with 1 Gy of γ-rays, α-particles or different LETs carbon ions. (A) The yield of MN in the cells irradiated with γ-rays or α-particles. (B) The yield of MN in the cells irradiated with carbon ions with different LETs of 13, 30 and 70 keV/μm. (C) The expression of γH2AX protein in the cells irradiated with γ-rays or α-particles. Actin was used as the internal control. (D) The expression of γH2AX protein in the cells irradiated with carbon ions of different LETs. Actin was used as the internal control. $***P < 0.001$ compared to non-irradiated control; $##P < 0.01$, $###P < 0.001$ between the indicated groups.

4. Discussion

DNA damage induced by various types of radiation can jeopardize genomic integrity. In comparison with the low-LET radiation, the high-LET radiation has a fierce effect on cell killing by producing complex and clustered DNA damage which is difficult to repair [1]. Within a certain range, DNA damage is fortified and enhanced when LET increases. However, when the LET reaches a higher value, the RBE will not rise and even declines [22]. Our results firmly support the idea that the radiation effects on cell proliferation and survival appear in a LET-dependent manner and are closely related to DNA repair capacity.

It is well known that γH2AX can be used to evaluate radiation-

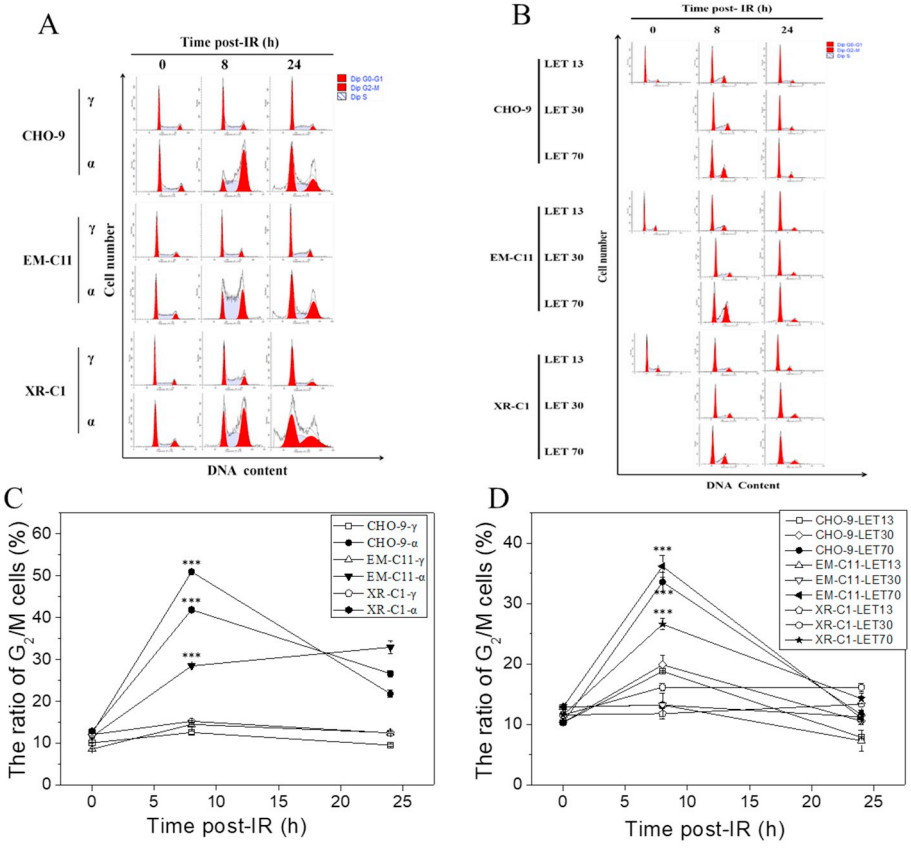

Fig. 3. G_2/M phase arrest in the cells irradiated with different LETs. CHO-9, EM-C11 and XR-C1 cells were irradiated with 1 Gy of γ-rays, α-particles or different LETs carbon ions. (A, C) The histogram of cell cycle assay and quantitative analysis of the cell percentage of G_2/M phase after γ-rays or α-particles radiation. $***P < 0.001$ compared to γ-rays radiation. (B, D) The histogram of cell cycle assay and quantitative analysis of the cell percentage of G_2/M phase after carbon ions radiation with different LETs of 13, 30 and 70 keV/μm. $***P < 0.001$ compared to 13 and 30 keV/μm carbon ions irradiation.

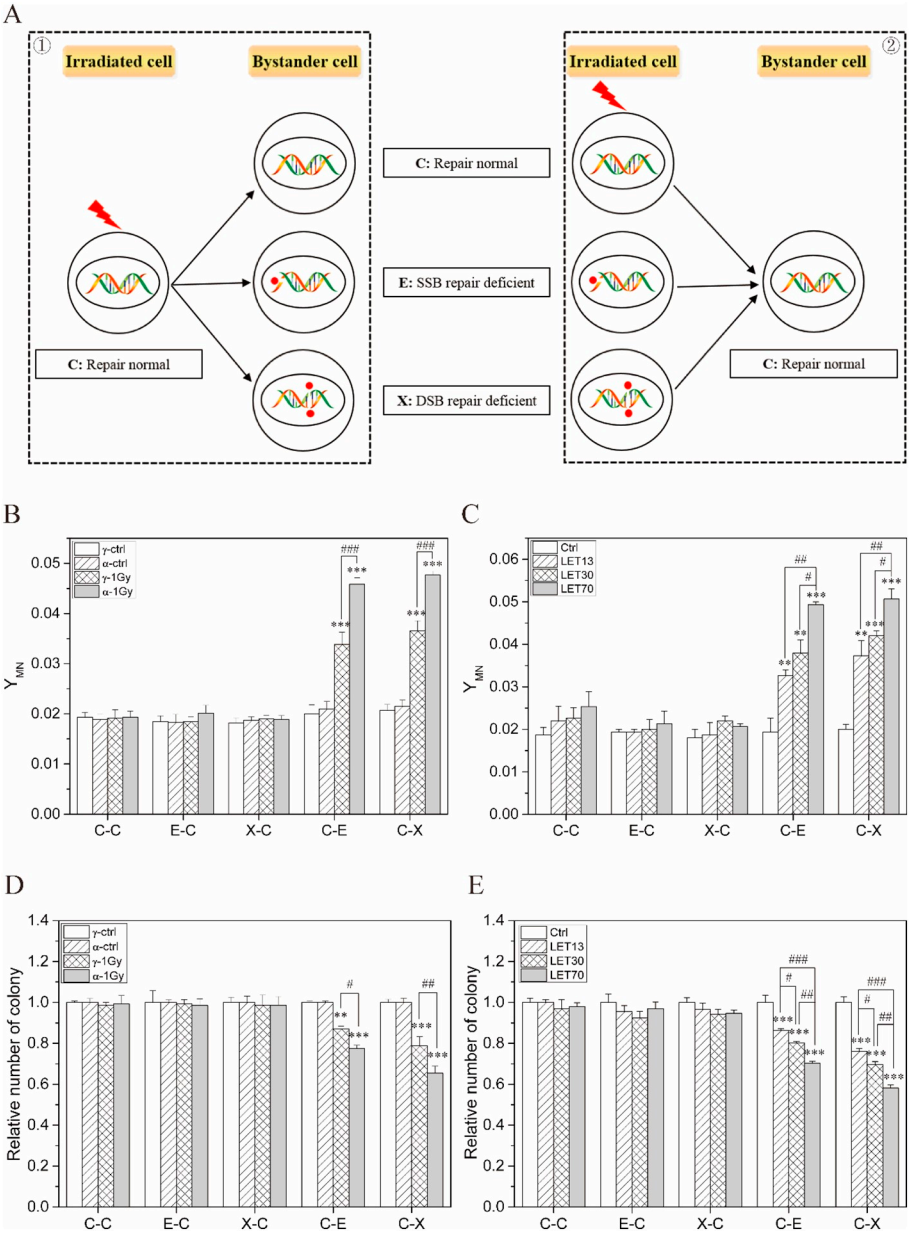

Fig. 4. MN formation and clonogenic survival in the nonirradiated cells that were treated with the CM from irradiated cells. (A) Schematic summary of the experimental models. CHO-9, EM-C11 and XR-C1 cells were irradiated with 1 Gy of γ-rays, α-particles or carbon ions with different LETs of 13, 30 and 70 keV/μm. C, E and X stand for CHO-9, EM-C11 and XR-C1 cells, respectively; C—C, C-E, C-X, *E*-C and X-C stand for the treatment method i.e., the former represents the irradiated cells and the latter represents the corresponding bystander cells (C—C, C-E and C-X in model ①; C—C, E-C and X-C in model ②). (B) The formation of MN in the bystander cells under the situation of γ-rays or α-particles radiation. (C) The formation of MN in the bystander cells under the situation of carbon ions radiation of different LETs. (D) Clonogenic survival of the bystander cells under the situation of γ-rays or α-particles radiation. (E) Clonogenic survival of the bystander cells under the situation of carbon ions radiation. $^{**}P < 0.01$ and $^{***}P < 0.001$ compared to non-irradiated control; $\#P < 0.05$, $\#\#P < 0.01$ and $\#\#\#P < 0.001$ between the indicated groups.

induced DNA damage. Phosphorylation of H2AX at ser139 is one of the earliest events in the cellular response to DNA DSB and it appears rapidly at DSB sites to recruit other DSB repair proteins [23–26]. In the present study, the expression of γH2AX was increased notably at 1 h after irradiation. Strikingly, the high-LET radiation could induce γH2AX accumulation more effective than the low-LET radiation.

Nevertheless, the overexpression of γH2AX was observed in EM-C11 and XR-C1 cells deficient with SSB and DSB repair capacity, respectively. As we know, the γH2AX will be dephosphorylated following to the rapid and robust phosphorylation after radiation and this process is regulated by some protein phosphatases such as PP2A and PP4 [27–29]. XRCC1 has multiple roles in the base excision repair (BER) and DSB repair, and the phosphorylation of XRCC1 at ser371 can be induced by radiation via the activation of DNA-PKcs that further contributes to the phosphorylation of H2AX [30]. In addition, the members of PIKK family including ATM [31] and ATR [32] are also involved in the phosphorylation of H2AX [33]. The loss of XRCC1 and DNA-PKcs make cells difficult to repair the damaged DNA and thus a high level of γH2AX expression is accumulated.

Radiation-induced cell cycle arrest allows time for DNA repair. Our study showed that the ratio of G_2/M phase arrest cells was enhanced at 8 h after irradiations of high-LET α-particles and 70 keV/μm carbon ions. Compared with X-ray, carbon ions irradiation induced stronger G_2/M phase arrest in HeLa cells at 4 Gy [34]. The irradiation of 4.7 ± 0.2 keV/μm protons mainly induced G_1 phase arrest, while 197 keV/μm carbon ions induced G_2/M phase arrest in both CRL5876 and HTB177 cells [35]. Moreover, studies have shown that the G_2/M phase arrest induced by carbon ions irradiation was not related to the inhibition of DNA-PK-mediated NHEJ repair pathway [36]. After 0.5 Gy or 1 Gy carbon ions irradiation, human fibroblasts GM0639 cells showed G_2/M phase arrest in the control group and the chloroquine pretreatment group, while this phenomenon did not occur in the ATM inhibitor (KU55933) pretreatment group, suggesting that ATM may be involved in G_2/M phase arrest induced by carbon ions irradiation [37].

On the other side, it has been well known that the bystander effects can be induced by both high-LET radiation such as α-particles, neutron and heavy ions (carbon, iron, silicon, neon, and argon) and low-LET radiation including X-rays, γ-rays, and β-particles. Radiation quality has been shown to affect the signaling pathways involved in RIBE [38]. Compared to low-LET radiation, high-LET radiation induces more

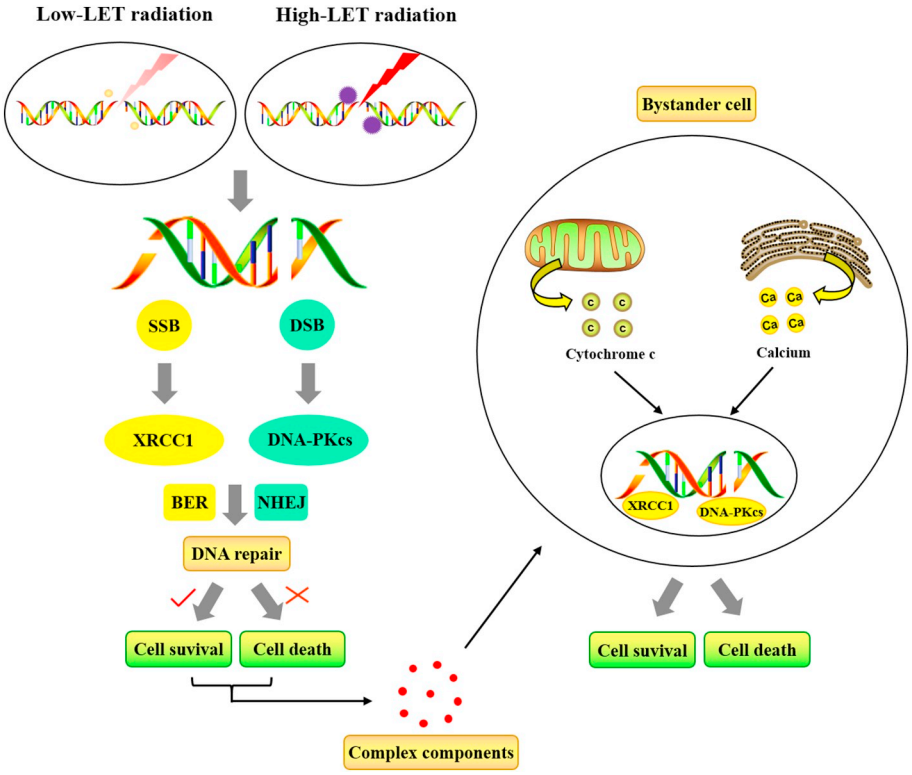

Fig. 5. A schematic representation model that LET and DNA repair capacity are involved in RIBE. In comparison with low-LET radiation, high-LET radiation induced complex DNA lesions. Such DNA damage may be repaired through different signaling pathways including XRCC1-mediated BER and DNA-PKcs-mediated NHEJ repair pathways. Precise repair leads to cell survival whereas incorrect repair leads to cell death or genomic instability. Intriguingly, both living cells, dead cells and dying cells may release complex reactive factors into the surrounding microenvironment that affect the bystander cells. These signal factors may stimulate a series of responses in cellular organelles including mitochondria and endoplasmic reticulum which release cytochrome *c* and calcium ions, respectively, and further triggering DNA damage and repair. The ultimate fate of the bystander cells will probably be survival, death and genomic instability.

complex and irreversible damage. The SSBs and DSBs induced by both low- and high-LET radiation could be repaired via XRCC1-mediated BER and DNA-PKcs-mediated NHEJ pathway. The success or failure of DNA repair determines the ultimate fate of the targeted cells. However, whether the targeted cells are alive, dead or dying, they may release active factors and could further affect and even damage their neighboring cells (Fig. 5). Our results indicate that the high-LET radiation leads to more abundant MN formation in the bystander cells of EM-C11 or XR-C1 than the low-LET radiation, which is consistent with our previous findings that, with 290 MeV/u carbon ions irradiation, the 100 keV/μm radiation was more effective in inducing bystander damage than 13 keV/μm radiation [39].

Importantly, we found that the CM-mediated bystander damage occurred in two DNA repair deficient cell lines of EM-C11 and XR-C1 but not CHO-9 cells. Our previous studies have disclosed that many soluble factors including reactive oxygen species and inflammatory factors of TNF-α and TGF-β can be released from irradiated cells into culture medium and further trigger a series of responses in bystander cells [8–14,39–43]. The current study focuses on a question, what is the role of DNA repair capacity in the bystander response? Several DNA damage and repair factors including ATM [31,44,45], ATR [46] and DNA-PKcs [45] have been suggested to account for the occurrence of RIBE. Those studies demonstrated that the bystander signals could be generated from ATM and DNA-PKcs positive cells but not from the cells with ATM and DNA-PKcs deficiency, indicating that the DNA repair proficiency plays a momentous role in generation of bystander signals. This is in consistent with our finding that the bystander effect was induced by the CM from irradiated CHO-9 but not by the CM from irradiated EM-C11 and XR-C1 cells.

5. Conclusion

In conclusion, the present study clearly demonstrates that the LET-dependent DNA damage responses can be induced in the Chinese hamster ovary cell lines with varying DNA repair capacity, and XRCC1 and DNA-PKcs play vital roles in triggering bystander responses. However, the molecular mechanisms underlying the DNA repair regulated generation of bystander signaling factors are still need further deep investigations.

Acknowledgments

This study was mainly supported by the National Key R&D Program of China (Grant No. 2017YFC0108604), the National Natural Science Foundation of China (Grant Nos. 31770910, 31570850 and 81602663), the Shanghai Municipal Commission of Health and Family Planning Foundation (Grant No. 20164Y0037), and the Research Project with Heavy Ions at NIRS-HIMAC of Japan (Proposal Nos. 15J105 and 17J121).

Conflict of interest

The authors declare that there are no conflicts of interest.

References

[1] M. Hada, A.G. Georgakilas, Formation of clustered DNA damage after high-LET irradiation: a review, J. Radiat. Res. 49 (2008) 203–210.
[2] H. Fakir, R.K. Sachs, B. Stenerlow, W. Hofmann, Clusters of DNA double-strand breaks induced by different doses of nitrogen ions for various LETs: experimental measurements and theoretical analyses, Radiat. Res. 166 (2006) 917–927.
[3] M. Pinto, K.M. Prise, B.D. Michael, Evidence for complexity at the nanometer scale of radiation-induced DNA DSBs as a determinant of rejoining kinetics, Radiat. Res. 164 (2005) 73–85.
[4] K.M. Prise, M. Pinto, H.C. Newman, B.D. Michael, A review of studies of ionizing radiation-induced double-strand break clustering, Radiat. Res. 156 (2001) 572–576.
[5] M.B. Kastan, J. Bartek, Cell-cycle checkpoints and cancer, Nature 432 (2004) 316–323.
[6] B. Rydberg, B. Cooper, P.K. Cooper, W.R. Holley, A. Chatterjee, Dose-dependent misrejoining of radiation-induced DNA double-strand breaks in human fibroblasts: experimental and theoretical study for high- and low-LET radiation, Radiat. Res. 163 (2005) 526–534.
[7] H. Nagasawa, J.B. Little, Induction of sister chromatid exchanges by extremely low doses of alpha-particles, Cancer Res. 52 (1992) 6394–6396.
[8] C. Shao, Y. Furusawa, M. Aoki, K. Ando, Role of gap junctional intercellular communication in radiation-induced bystander effects in human fibroblasts, Radiat. Res. 160 (2003) 318–323.

[9] C. Shao, Y. Furusawa, Y. Kobayashi, T. Funayama, S. Wada, Bystander effect induced by counted high-LET particles in confluent human fibroblasts: a mechanistic study, FASEB J. 17 (2003) 1422–1427.
[10] C. Shao, V. Stewart, M. Folkard, B.D. Michael, K.M. Prise, Nitric oxide-mediated signaling in the bystander response of individually targeted glioma cells, Cancer Res. 63 (2003) 8437–8442.
[11] C. Shao, M. Folkard, B.D. Michael, K.M. Prise, Targeted cytoplasmic irradiation induces bystander responses, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 13495–13500.
[12] M. He, S. Ye, R. Ren, C. Dong, Y. Xie, D. Yuan, C. Shao, Cytochrome-c mediated a bystander response dependent on inducible nitric oxide synthase in irradiated hepatoma cells, Br. J. Cancer 106 (2012) 889–895.
[13] C. Shao, Y. Furusawa, M. Aoki, H. Matsumoto, K. Ando, Nitric oxide-mediated bystander effect induced by heavy-ions in human salivary gland tumour cells, Int. J. Radiat. Biol. 78 (2002) 837–844.
[14] C. Shao, M. Folkard, K.M. Prise, Role of TGF-beta1 and nitric oxide in the bystander response of irradiated glioma cells, Oncogene 27 (2008) 434–440.
[15] M. He, M. Zhao, B. Shen, K.M. Prise, C. Shao, Radiation-induced intercellular signaling mediated by cytochrome-c via a p53-dependent pathway in hepatoma cells, Oncogene 30 (2011) 1947–1955.
[16] Z. Somodi, N.A. Zyuzikov, G. Kashino, K.R. Trott, K.M. Prise, Radiation-induced genomic instability in repair deficient mutants of Chinese hamster cells, Int. J. Radiat. Biol. 81 (2005) 929–936.
[17] W. Tu, C. Dong, T. Konishi, A. Kobayashi, Y. Furusawa, Y. Uchihori, Y. Xie, B. Dang, W. Li, C. Shao, G(2)-M phase-correlative bystander effects are co-mediated by DNA-PKcs and ATM after carbon ion irradiation, Mutat. Res. Genet. Toxicol. Environ. Mutagen. 795 (2016) 1–6.
[18] R. Ren, M. He, C. Dong, Y. Xie, S. Ye, D. Yuan, C. Shao, Dose response of micronuclei induced by combination radiation of alpha-particles and gamma-rays in human lymphoblast cells, Mutat. Res. 741-742 (2013) 51–56.
[19] M. Torikoshi, S. Minohara, N. Kanematsu, M. Komori, M. Kanazawa, K. Noda, N. Miyahara, H. Itoh, M. Endo, T. Kanai, Irradiation system for HIMAC, J. Radiat. Res. 48 (2007) A15–A25 Suppl A.
[20] S. Ye, D. Yuan, Y. Xie, Y. Pan, C. Shao, Role of DNA methylation in long-term low-dose gamma-rays induced adaptive response in human B lymphoblast cells, Int. J. Radiat. Biol. 89 (2013) 898–906.
[21] M. Fenech, Cytokinesis-block micronucleus cytome assay, Nat. Protoc. 2 (2007) 1084–1104.
[22] N. Hamada, T. Imaoka, S. Masunaga, T. Ogata, R. Okayasu, A. Takahashi, T.A. Kato, Y. Kobayashi, T. Ohnishi, K. Ono, Y. Shimada, T. Teshima, Recent advances in the biology of heavy-ion cancer therapy, J. Radiat. Res. 51 (2010) 365–383.
[23] T.T. Paull, E.P. Rogakou, V. Yamazaki, C.U. Kirchgessner, M. Gellert, W.M. Bonner, A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage, Curr. Biol. 10 (2000) 886–895.
[24] J. Fillingham, M.C. Keogh, N.J. Krogan, GammaH2AX and its role in DNA double-strand break repair, Biochem. Cell Biol. 84 (2006) 568–577.
[25] J. Yuan, R. Adamski, J. Chen, Focus on histone variant H2AX: to be or not to be, FEBS Lett. 584 (2010) 3717–3724.
[26] V. Valdiglesias, S. Giunta, M. Fenech, M. Neri, S. Bonassi, gammaH2AX as a marker of DNA double strand breaks and genomic instability in human population studies, Mutat. Res. 753 (2013) 24–40.
[27] D. Chowdhury, X. Xu, X. Zhong, F. Ahmed, J. Zhong, J. Liao, D.M. Dykxhoorn, D.M. Weinstock, G.P. Pfeifer, J. Lieberman, A PP4-phosphatase complex dephosphorylates gamma-H2AX generated during DNA replication, Mol. Cell 31 (2008) 33–46.
[28] S. Nakada, G.I. Chen, A.C. Gingras, D. Durocher, PP4 is a gamma H2AX phosphatase required for recovery from the DNA damage checkpoint, EMBO Rep. 9 (2008) 1019–1026.
[29] G.P. Shouse, Y. Nobumori, M.J. Panowicz, X. Liu, ATM-mediated phosphorylation activates the tumor-suppressive function of B56gamma-PP2A, Oncogene 30 (2011) 3755–3765.
[30] N. Levy, A. Martz, A. Bresson, C. Spenlehauer, G. de Murcia, J. Menissier-de Murcia, XRCC1 is phosphorylated by DNA-dependent protein kinase in response to DNA damage, Nucleic Acids Res. 34 (2006) 32–41.
[31] J.P. Bruce, K. Yip, S.V. Bratman, E. Ito, F.F. Liu, Nasopharyngeal Cancer: molecular landscape, J. Clin. Oncol. 33 (2015) 3346–3355.
[32] E. Tili, J.J. Michaille, H. Alder, S. Volinia, D. Delmas, N. Latruffe, C.M. Croce, Resveratrol modulates the levels of microRNAs targeting genes encoding tumor-suppressors and effectors of TGFbeta signaling pathway in SW480 cells, Biochem. Pharmacol. 80 (2010) 2057–2065.
[33] W.Z. Tu, B. Li, B. Huang, Y. Wang, X.D. Liu, H. Guan, S.M. Zhang, Y. Tang, W.Q. Rang, P.K. Zhou, gammaH2AX foci formation in the absence of DNA damage: mitotic H2AX phosphorylation is mediated by the DNA-PKcs/CHK2 pathway, FEBS Lett. 587 (2013) 3437–3443.
[34] C.X. Di, L.N. Yang, H. Zhang, L.Z. An, X. Zhang, X.F. Ma, C. Sun, X.H. Wang, R. Yang, Z.H. Wu, J. Si, Effects of carbon-ion beam or X-ray irradiation on anti-apoptosis DeltaNp73 expression in HeLa cells, Gene 515 (2013) 208–213.
[35] O.D. Keta, D.V. Todorovic, T.M. Bulat, P.G. Cirrone, F. Romano, G. Cuttone, I.M. Petrovic, A.M. Ristic Fira, Comparison of human lung cancer cell radiosensitivity after irradiations with therapeutic protons and carbon ions, Exp. Biol. Med. (Maywood) 242 (2017) 1015–1024.
[36] S. Sunada, H. Kanai, Y. Lee, T. Yasuda, H. Hirakawa, C. Liu, A. Fujimori, M. Uesaka, R. Okayasu, Nontoxic concentration of DNA-PK inhibitor NU7441 radio-sensitizes lung tumor cells with little effect on double strand break repair, Cancer Sci. 107 (2016) 1250–1255.
[37] L. Xue, D. Yu, Y. Furusawa, R. Okayasu, J. Tong, J. Cao, S. Fan, Regulation of ATM in DNA double strand break repair accounts for the radiosensitivity in human cells exposed to high linear energy transfer ionizing radiation, Mutat. Res. 670 (2009) 15–23.
[38] A. Kobayashi, T. Konishi, Radiation quality effects alteration in COX-2 pathway to trigger radiation-induced bystander response in A549 lung carcinoma cells, J. Radiat. Res. 59 (2018) 754–759.
[39] C. Shao, M. Aoki, Y. Furusawa, Medium-mediated bystander effects on HSG cells co-cultivated with cells irradiated by X-rays or a 290 MeV/u carbon beam, J. Radiat. Res. 42 (2001) 305–316.
[40] Y. Xie, W. Tu, J. Zhang, M. He, S. Ye, C. Dong, C. Shao, SirT1 knockdown potentiates radiation-induced bystander effect through promoting c-Myc activity and thus facilitating ROS accumulation, Mutat. Res. 772 (2015) 23–29.
[41] C. Dong, M. He, W. Tu, T. Konishi, W. Liu, Y. Xie, B. Dang, W. Li, Y. Uchihori, T.K. Hei, C. Shao, The differential role of human macrophage in triggering secondary bystander effects after either gamma-ray or carbon beam irradiation, Cancer Lett. 363 (2015) 92–100.
[42] C. Dong, M. He, R. Ren, Y. Xie, D. Yuan, B. Dang, W. Li, C. Shao, Role of the MAPK pathway in the observed bystander effect in lymphocytes co-cultured with macrophages irradiated with gamma-rays or carbon ions, Life Sci. 127 (2015) 19–25.
[43] J. Li, M. He, B. Shen, D. Yuan, C. Shao, Alpha particle-induced bystander effect is mediated by ROS via a p53-dependent SCO2 pathway in hepatoma cells, Int. J. Radiat. Biol. 89 (2013) 1028–1034.
[44] S. Burdak-Rothkamm, K. Rothkamm, K.M. Prise, ATM acts downstream of ATR in the DNA damage response signaling of bystander cells, Cancer Res. 68 (2008) 7059–7065.
[45] R.T. Hagelstrom, K.F. Askin, A.J. Williams, L. Ramaiah, C. Desaintes, E.H. Goodwin, R.L. Ullrich, S.M. Bailey, DNA-PKcs and ATM influence generation of ionizing radiation-induced bystander signals, Oncogene 27 (2008) 6761–6769.
[46] S. Burdak-Rothkamm, S.C. Short, M. Folkard, K. Rothkamm, K.M. Prise, ATR-dependent radiation-induced gamma H2AX foci in bystander primary human astrocytes and glioma cells, Oncogene 26 (2007) 993–1002.