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Nonhomologous End-Joining Repair Plays a More Important Role than Homologous Recombination Repair in Defining Radiosensitivity after Exposure to High-LET Radiation

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DNA double-strand breaks (DSBs) induced by ionizing radiation pose a major threat to cell survival. The cell can respond to the presence of DSBs through two major repair pathways: homologous recombination (HR) and nonhomologous end joining (NHEJ). Higher levels of cell death are induced by high-linear energy transfer (LET) radiation when compared to low-LET radiation, even at the same physical doses, due to less effective and efficient DNA repair. To clarify whether high-LET radiation inhibits all repair pathways or specifically one repair pathway, studies were designed to examine the effects of radiation with different LET values on DNA DSB repair and radiosensitivity. Embryonic fibroblasts bearing repair gene (NHEJ-related *Lig4* and/or HR-related *Rad54*) knockouts (KO) were used and their responses were compared to wild-type cells. The cells were exposed to X rays, spread-out Bragg peak (SOBP) carbon ion beams as well as with carbon, iron, neon and argon ions. Cell survival was measured with colony-forming assays. The sensitization enhancement ratio (SER) values were calculated using the 10% survival dose of wild-type cells and repair-deficient cells. Cellular radiosensitivity was listed in descending order: double-KO cells > *Lig4*-KO cells > *Rad54*-KO cells > wild-type cells. Although *Rad54*-KO cells had an almost constant SER value, *Lig4*-KO cells showed a high-SER value when compared to *Rad54*-KO cells, even with increasing LET values. These results suggest that with carbon-ion therapy, targeting NHEJ repair yields higher radiosensitivity than targeting homologous recombination repair.

INTRODUCTION

The goal of radiotherapy is to eliminate cancer by eradicating tumor cells with various type of ionizing radiation, which induces a variety of DNA lesions. Among these lesions, DNA double-strand breaks (DSBs) are the most toxic since unrepaired or misrepaired DSBs can lead to genomic instability and cell death (*1–3*). There are two major pathways that the cell can repair DSBs in eukaryotes: nonhomologous end-joining (NHEJ) repair (*4*) and homologous recombination (HR) repair (*5*). NHEJ is a ''quick and dirty'' process compared to homologous recombination and is less accurate, with small deletions or insertions often occurring at the repaired break site (*4, 6, 7*). However, although NHEJ can lead to mutations, it enables the cell to survive. An unrepaired DSB is often lethal and leads to chromosome fragment loss at the next mitosis, with the potential loss of hundreds of genes. However, only a small percentage of the genomic DNA contains regions that either code for genes or contain regulatory regions, and therefore the chance of a break occurring in such a region is low, in addition many damaged regions may also be silent and/or nonessential. In view of these considerations, NHEJ appears to be a good repair pathway that can allow the cell to maximize its chances for survival (*7*). Although the ligation of DNA DSBs through the NHEJ process does not require sequence homology. The damaged ends of DNA DSBs cannot simply be ligated together, as DSBs must first be modified before they can be rejoined by a ligation reaction. NHEJ can be divided into five steps: 1. End recognition by the Ku autoantigen protein p70 (Ku70, XRCC6) and p80 (Ku80, XRCC5), and heterodimer binding that serves both to protect ends from degradation by exonucleases; 2. Recruitment of the DNA-dependent protein kinase catalytic subunit (DNA-PKcs), which is a large protein that forms a physical bridge between the two ends; 3. End processing by Artemis, which possesses endonuclease activity to deal with 5′ and 3′ overhangs as well as hairpins, and also processing by polynucleotide kinase (PNK), which trims DNA ''dirty''

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ends; 4. Fill-in synthesis or end bridging by DNA polymerase μ or λ; and 5. Ligation by ligase 4 (Lig4) aided by other proteins, such as XRCC4 and XRCC4-like factor (XLF) (*6*).

Homologous recombination by contrast results in error-free repair of lesions in the late S/G_2 phase of the cell cycle, because homologous recombination can repair DSBs by using homologous undamaged DNA as the repair template. Briefly, the homologous recombination repair process is as follows (*5–7*). The initial step in homologous recombination is the recognition of the lesion and processing of the double-strand DNA ends into 3′ DNA single-strand tails by the Mre11-Rad50-Nbs1 (MRN) complex (*8*). These tails are then coated with replication protein A (RPA) forming a nucleoprotein filament. Then, specific homologous recombination proteins are recruited to the nucleoprotein filaments, such as the Rad51 family of proteins including XRCC3, Rad52, BRCA1 and BRCA2. These single-stranded nucleoprotein filaments then invade undamaged double-stranded DNA on the neighboring sister chromatid, forming a crossover or bubble structure. These bubbles are then expanded with specialized enzymes called helicases, including Bloom syndrome (BLM) and other members of the DNA helicase Q1 (RecQ) family, possibly with the help of Rad54. The objective of this process is to provide an undamaged DNA template with the original base sequence around the break site, so that DNA polymerases can then synthesize across the missing regions, thereby accurately repairing the break. The crossover structure then has to be reversed to reset the chromatin to its original configuration. This is done with specialized nucleases that cut or resolve the junctions, followed finally by connecting or ligating the adjacent ends with ligase 1 (Lig1). The entire process takes several hours to complete. Deletions or mutations in any of these genes can severely impair homologous recombination.

Charged particle therapy, a highly localized dose of energy that is deposited to the tissue, allows for increased radiation dose delivered to the tumor while minimizing radiation exposure to the adjacent normal tissue compared to conventional X-ray therapy. Among the various types of ion species used in charged particle therapy, carbon ions (C-ions) are considered to have optimal properties for producing both physically superior and biologically effective dose localization in the body of densely ionizing high-linear energy transfer (LET) radiation (*9*). The higher LET spectrum of C-ion beams have a number of biological advantages that include a higher relative biological effectiveness (RBE) (*10, 11*), a decreased oxygen enhancement ratio (*12*), the induction of a diminished capacity for DNA repair of cellular radiation injuries (*13*), an efficacy in dealing with radioresistant tumor cells (*TP53*-mutated and *BCL2*-overexpressing cells and cancer stem-like cells) (*14–17*) and a potential suppression of metastases (*18*) when compared with results produced by protons or X rays. These physical and biological characteristics offer theoretical advantages for the treatment of tumors such as adenocarcinomas, adenoid cystic carcinomas, malignant melanomas and sarcomas that are highly resistant to low-LET radiation therapy. The efficacy of C-ion radiation therapy has been demonstrated in the treatment of various cancers (*19, 20*).

While photon beams induce sporadic DNA damage along their tracks, heavy-ion beams such as carbon induce a high density of DSBs along particle tracks. DNA repair of heavy-ion beam-induced DSBs is slower than photon beam-induced damage. In addition, while data suggest the repair of heavy-ion beam-induced DSBs is possible (*13*), it is unclear whether high-LET radiation specifically inhibits one DSB repair pathway or both DSB repair pathways.

To identify the primary target leading to increased radiosensitization after radiation exposure with different LET values, mouse embryonic fibroblast (MEF) cell lines were used, which had NHEJ-related *Lig4* and/or HR-related *Rad54* knockouts (KO) in cells that otherwise had the same genetic background.

MATERIALS AND METHODS

Cell Lines

The cell lines used in these studies were: $p53^{-/-}$ MEF $Lig4^{+/+}$ $Rad54^{-/-}$ (*Rad54*-KO); $Lig4^{-/-}$ $Rad54^{+/+}$ (*Lig4*-KO); $Lig4^{-/-}$ $Rad54^{-/-}$ (double-KO); and $Lig4^{+/+}$ $Rad54^{+/+}$ (wild-type), and were kindly provided by Dr. Frederick W. Alt (Harvard Medical School, Boston, MA) (*21*). Cells were cultured in Dulbecco's modified Eagle medium containing 10% (v/v) fetal bovine serum, 20 m*M* 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid, penicillin (50 units/ml) and streptomycin (50 μg/ml). Cells were cultured at 37°C in a conventional humidified CO_2 incubator.

Irradiation

Exponentially growing cells were irradiated at room temperature with X rays, C-ion beams, iron ion (Fe-ion) beams, neon ion (Ne-ion) beams and argon ion (Ar-ion) beams. X irradiation was performed through a 3.5 mm layer of culture medium and a 1 mm plastic layer in a Nunc™ flask (Thermo Fisher Scientific, Yokohama, Japan) using a 200 kVp X-ray generator (TITAN-225S, Shimadzu, Kyoto, Japan) with a total filtration of 0.5 mm aluminum and 0.5 mm copper. The X-ray dose rate was about 1.3 Gy/min, and was measured using a thimble ionization chamber (PTW-Freiburg, Freiburg, Germany) at the sample position. C-ion beam irradiation [290 MeV/nucleon, 6 cm spread-out Bragg peak (SOBP), 50 keV/μm] was performed through a 3.5 mm layer of culture medium and a 1 mm plastic layer in a Nunc flask at the Gunma University Heavy Ion Medical Center (GHMC, Gunma, Japan) (*22*). C-ion irradiation (13, 50 and 70 keV/μm) was performed through a 1 mm layer in a Nunc flask at GHMC. In addition, C-ion irradiation (18.3 MeV/nucleon beams, 108 keV/μm), Ne-ion beam irradiation (13 MeV/nucleon beams, 437 keV/μm), and Ar-ion beam irradiation (13 MeV/nucleon beams, 1,370 keV/μm) were performed through an 8 μm layer of Kapton® polyimide film (Du Pont-Toray Co. Ltd., Tokyo, Japan) using the AVF cyclotron of the Takasaki Ion Accelerator for Advanced Radiation Application (TIARA) facilities at the Japan Atomic Energy Agency (JAEA, Gunma, Japan) (*23*). Fe-ion beam irradiation (500 MeV/nucleon, 200 keV/μm at the target entrance) was delivered through a 1.3 mm layer in a BD Falcon® flask (BD Biosciences, Tokyo, Japan) using the Heavy Ion Medical Accelerator in Chiba (HIMAC) at the National Institute of Radiological Sciences (NIRS), Chiba, Japan (*9*).

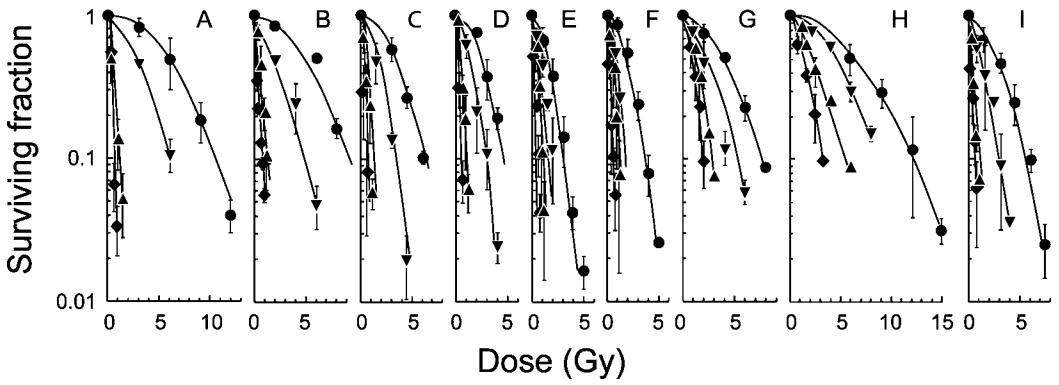

FIG. 1. Cell survival curves after radiation exposure with different LET values in NHEJ and/or homologous recombination KO cells. Panel A: X rays; panel B: 13 keV/μm C-ion beams; panel C: 50 keV/μm C-ion beams; panel D: 70 keV/μm C-ion beams; panel E: 108 keV/μm C-ion beams; panel F: 200 keV/μm Fe-ion beams; panel G: 437 keV/μm Ne-ion beams; panel H: 1,370 keV/μm Ar-ion beams; panel I: 50 keV/μm C-ion beams (6 cm SOBP). Wild-type cells, circle symbol; *Lig4*-KO cells, triangle symbol; *Rad54*-KO cells, inverted triangle symbol; and double-KO cells, diamond symbol. The curves were fitted with least squares to a linear-quadratic equation, and the presented results show the mean and standard errors of three independent experiments.

Colony-Forming Assays

Cell survival was measured using a standard colony-forming assay with three independent experiments. Two or three flasks or Petri dishes were used for each experimental point. Colonies obtained after 1 week were fixed with methanol and stained with a 2% Giemsa solution. Microscopic colonies composed of more than approximately 50 cells were scored as having grown from single surviving cells. Survival curves were analyzed using KaleidaGraph® version 4.1.1 (Synergy Software, Reading, PA) statistical software to fit data weighted by using a linear regression, according to the linear-quadratic formula (*6*):

$$S = \exp(-\alpha D - \beta D^2)$$

D_{10} values were determined by the dose (Gy) required to reduce the surviving fraction to 10% (*10*) and were calculated using the linear-quadratic formula. The relative biological effectiveness values of heavy-ion beams and the sensitization enhancement ratio (SER) values were calculated using D_{10} values according to the following formulas:

$$\mathrm{RBE} = D_{10}\,\text{of X-ray irradiated cells} \,/ D_{10}\,\text{of heavy-ion irradiated cells}$$

$$\mathrm{SER} = D_{10}\,\text{for wild-type cells} \,/ D_{10}\,\text{for cells bearing a DSB repair KO}$$

Statistics

The experimental data were expressed as mean values with standard deviations. The statistical significance was tested with the Student's *t* test. A P value of <0.05 was considered to be statistically significant.

RESULTS

Radiosensitivity after Radiation Exposure with Different LET Values in Cells with a NHEJ and/or Homologous Recombination KO

After exposure with different LET values, radiosensitivity was analyzed using colony formation assays (Fig. 1). Table 1 shows the D_{10} values using different types of radiation in NHEJ and/or homologous recombination KO cells. The

TABLE 1
D_{10} Values for Different Radiation Types in NHEJ and/or Homologous Recombination Knockout Cells

Radiation		LET (keV/μm)	D_{10} Wild-type cells	D_{10} *Lig4*-KO cells	D_{10} *Rad54*-KO cells	D_{10} Double-KO cells
X rays		-	10.36 ± 1.18	1.12 ± 0.15	6.21 ± 0.33	0.55 ± 0.05
Mono-ion beams	C-ion	13	9.28 ± 0.76	1.12 ± 0.25	5.42 ± 0.64	0.67 ± 0.02
		50	6.16 ± 0.83	1.14 ± 0.13	3.24 ± 0.10	0.49 ± 0.11
		70	4.53 ± 0.48	0.83 ± 0.06	2.78 ± 0.05	0.48 ± 0.07
		108	3.39 ± 0.31	0.97 ± 0.12	2.03 ± 0.21	0.60 ± 0.05
	Fe-ion	200	3.79 ± 0.45	1.08 ± 0.24	1.72 ± 0.38	0.57 ± 0.08
	Ne-ion	437	8.03 ± 0.56	2.78 ± 0.04	4.14 ± 0.17	2.00 ± 0.26
	Ar-ion	1,370	13.19 ± 2.35	4.94 ± 0.65	9.06 ± 0.69	3.15 ± 0.40
SOBP beams	C-ion	50	6.17 ± 0.61	0.81 ± 0.01	3.01 ± 0.77	0.68 ± 0.18

D_{10} = dose giving a survival of 10%; C-ion = carbon ion; Fe-ion = iron ion; Ne-ion = neon ion; Ar-ion = argon ion; and SOBP = spread-out Bragg peak.

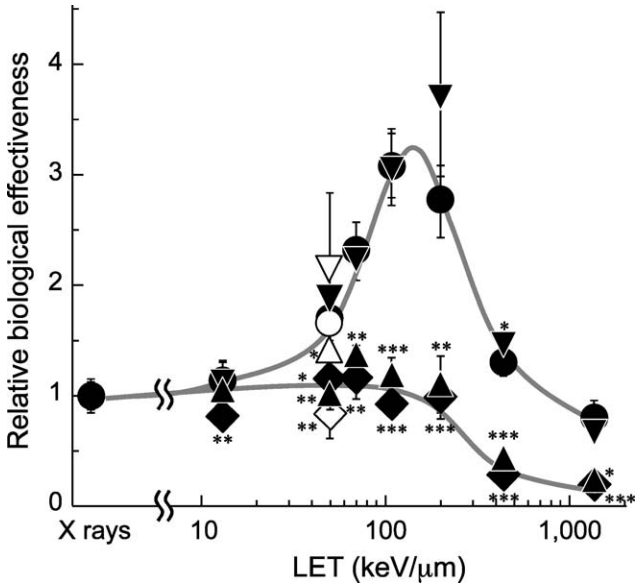

FIG. 2. RBE values with a 10% survival level dose and with different LET values in NHEJ and/or homologous recombination KO cells. Wild-type cells, circle symbol; *Lig4*-KO cells, triangle symbol; *Rad54*-KO cells, inverted triangle symbol; and double-KO cells, diamond symbol. Mono-ion beams, closed symbol; and 6-cm SOBP-beams, open symbols. The presented results are the mean and standard errors of three independent experiments. Data were statistically evaluated with the Student's *t* test with comparisons between wild-type cells and other types of cells (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

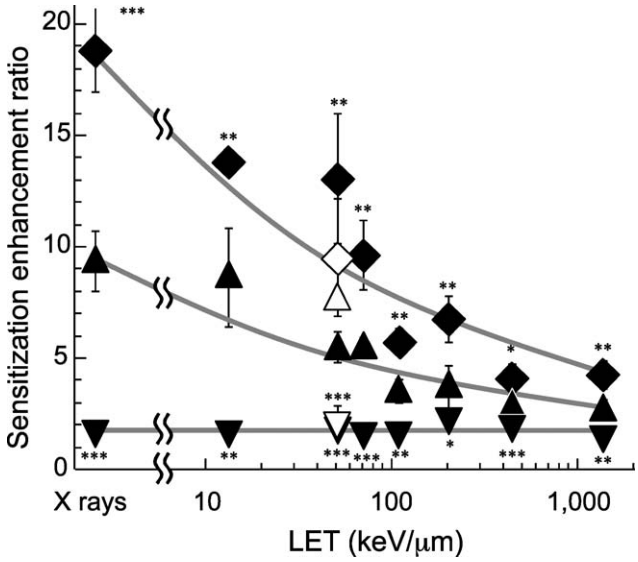

FIG. 3. SER values with radiation possessing different LET values in wild-type cells vs. NHEJ and/or homologous recombination KO cells. Wild-type cells vs. *Lig4*-KO cells, triangle symbol; wild-type cells vs. *Rad54*-KO cells, inverted triangle symbol; and wild-type cells vs. double-KO cells, diamond symbol. Mono-ion beams, closed symbols and 6 cm SOBP-beams, open symbols. The results shown here represent the mean and standard errors of three independent experiments. Data were statistically evaluated with the Student's *t* test between data points indicated by the triangle symbol and by other symbols (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

order of radiosensitivity was double-KO cells > *Lig4*-KO cells > *Rad54*-KO cells > wild-type cells.

For radiation exposures that had LET values of less than 200 keV/μm, double-KO cells and *Lig4*-KO cells showed exactly the same high sensitivity in response to radiation exposure with different LET values (Fig. 1A–F, I). D_{10} values in double-KO cells and *Lig4*-KO cells were less than 0.7 and 1.2 Gy, respectively. The survival curves of double-KO cells and *Lig4*-KO cells were exponential dose responses without a shoulder. Conversely, the survival curve of wild-type cells and *Rad54*-KO cells had shoulders and became steeper with increasing LET. There was no significant difference in D_{10} values between mono-energetic beams and SOBP-beams with 50 keV/μm C-ions in either cell type (Table 1).

With radiation exposures of more than 200 keV/μm, the survival curve for each cell line displayed slightly steeper slopes with increasing LET values (Fig. 1G, H). D_{10} values were higher in Ar-ion irradiated cells than in X-irradiated cells (Table 1).

RBE Values and Radiation with Different LET Values in NHEJ and/or Homologous Recombination KO Cells

RBE values were calculated using the D_{10} values (Fig. 2). Wild-type cells and *Rad54*-KO cells, i.e., NHEJ proficient cells, exhibited maximal RBE values (3.1 and 3.7) at LET values of 108 and 200 keV/μm, respectively. In contrast, *Lig4*-KO cells and double-KO cells exhibited a low, almost constant RBE value ranging from 0.8–1.4 at less than 200 keV/μm. The RBE value for each cell type decreased with increasing LET values over 200 keV/μm (Fig. 2).

SER Values and Radiation with Different LET Values in NHEJ and/or Homologous Recombination KO Cells

To help identify the primary target leading to radiosensitization after radiation exposure with different LET values, the SER values were calculated using the D_{10} values from wild-type cells and from cells with KOs that affected DSB repair (Fig. 3). The SER value for cells with a deficient homologous recombination (wild-type cells/*Rad54*-KO cells) exhibited almost constant values of about 2 (1.5–2.3) regardless of the LET radiation value. Although the SER value for cells with a deficiency in NHEJ (wild-type cells/*Lig4*-KO cells) went down gradually from 9.3 to 2.7 with increasing LET values, a deficiency in NHEJ produced significantly higher SER values than a deficiency in homologous recombination with each LET value. The SER value for cells with deficiencies in both NHEJ and homologous recombination (wild-type cells/double-KO cells) also diminished gradually from 18.8 to 4.0 with increasing LET values.

With a 6 cm SOBP C-ion beam, the SER values with a deficiency in NHEJ and deficiencies in both NHEJ and homologous recombination were 7.7 and 9.5, respectively.

DISCUSSION

The Factor Determining High-RBE Values is NHEJ Repair

In this study, using NHEJ and/or homologous recombination-deficient MEF cells with the same genetic background, cellular radiosensitivity was examined (Fig. 1). Wild-type cells and *Rad54*-KO cells that are NHEJ proficient showed survival curves having a shoulder and a steep slope that peaked at LET values of 108 and 200 keV/μm, respectively. These results suggest that, with or without homologous recombination repair, NHEJ proficient cells have high-RBE values after exposure to C-ion and Fe-ion beams (Fig. 2). These results are consistent with previous reports using homologous recombination-deficient cells such as Chinese hamster *XRCC3*-deficient irs1SF cells (*24*), chicken DT40 *Rad54*-KO cells (*25, 26*) and conditional *Rad51*-KO cells (*25*).

In contrast, double-KO cells and *Lig4*-KO cells that are deficient in NHEJ repair displayed steep survival curves having almost no shoulder and a constant level of radiosensitivity at LET values of less than 200 keV/μm (Fig. 1). Consequently, these cells show no distinct RBE value maximum as a function of LET values (Fig. 2). The LET-RBE response curves that were generated from the D_{10} values were similar to those in previous reports using NHEJ-deficient cells such as MEF *Ku80*-deficient cells (*24*), chicken DT40 *Ku70*-KO cells (*25, 26*), Chinese hamster *Ku80*-deficient xrs5 (*27*) and xrs6 cells (*28*), human *DNA-PK*-mutated M059J fibroblasts (*29*) and *Lig4*-mutated 180BR fibroblasts (*28*). It was reported that the extent of the RBE maximum depends primarily on the cellular DNA DSB repair capacity (*27*).

NHEJ repair is the dominant repair pathway throughout the cell cycle (*30, 31*) and is more essential for cell survival than homologous recombination repair (*7*). High-LET radiation induces complex DNA damage and/or clustered damage in the vicinity of break sites (*32, 33*) that are not easily repaired or are not repaired by NHEJ (*28*). Previously, it was reported that high-LET radiation such as Fe-ion beams results in short DNA fragments (<40 base pairs) being produced along the radiation track (*24, 25*). These short fragments bind Ku proteins involved in NHEJ repair, and this might be a major reason that high-LET radiation suppresses NHEJ repair and results in high-LET radiation induced high-RBE values (*24, 25, 34*).

RBE values decreased with increasing LET values over 200 keV/μm regardless of the DSB repair pathway present in the cell lines (Fig. 2). A possible explanation for this observation might be that the efficiency of generating DSBs decreases under highly lethal conditions (*35*).

Targeting NHEJ Repair Produces High Radiosensitivity to C-Ion Beams

A deficiency in NHEJ led to a high-SER value when compared to a deficiency in homologous recombination, even with increasing LET values (Fig. 3). Another observation was that the SER value for cells with deficiencies in both NHEJ and homologous recombination (Fig. 3, diamond-shaped symbols) were similar to the product of the SER value for cells with a deficiency in NHEJ (Fig. 3, triangle-shaped symbols) and the SER value for cells with a deficient homologous recombination (Fig. 3, inverted triangle-shaped symbols) at each LET value. This suggests that this SER value relationship is independent of LET values, and also suggests that the two repair pathways (homologous recombination and NHEJ) may be independent or almost independent from each other. Further, these results suggest that the main target leading to enhanced radiosensitization was NHEJ repair rather than homologous recombination repair, even with high-LET radiation. After exposures to 6 cm SOBP C-ion beams, the SER value with a deficiency in NHEJ was about eight (Fig. 3). This is a very high value, since the increase in this ratio with most sensitizers is less than two (*36*). Enhancement of this sensitization effect through the use of hyperfractionation was expected with photon radiation therapy. However, there is a tendency toward hypofractionation with C-ion radiation therapy (*37*). Therefore, it is important to be able to identify the candidate agent that will be used to produce a high SER value.

Homologous recombination can only repair DSBs in late S/G_2 phases using the undamaged DNA homologue as a template (*7*). After low-LET radiation, about 20–30% of the DSBs are repaired by homologous recombination in G_2 cells (*31*). High-LET radiation exposure induces short fragments of linear DNA that do not affect homologous recombination-related MRE11 binding efficiency, and which result in the same efficiency of homologous recombination repair in high- or low-LET irradiated cells (*25*). Moreover, it was reported that end resection occurs and promotes homologous recombination repair when rapid repair by NHEJ to rejoin complex DSBs does not occur after exposure to high-LET radiation in G_2 cells (*31*). Recently, it was reported that end-resection also occurs and promotes repair after exposure to high-LET radiation in G_1 cells (*38*). It was proposed that the end-resection activity in G_1 phase may promote microhomology mediated end joining (MMEJ) to repair DSBs that cannot be efficiently repaired by NHEJ (*38*). Because NHEJ repair was suppressed by high-LET radiation, it was reported that the main target of radiosensitization was homologous recombination repair during high-LET irradiation (*24, 25*). However, deficiencies in homologous recombination did not increase with increasing LET values, and there was a low constant SER value of about 2 regardless of the different LET values used (Fig. 3). This point suggests that

the functions in the 2 KO cell lines (NHEJ and homologous recombination) are independent (or almost independent) of each other. The shoulder of the survival curve generated with homologous recombination repair became smaller in the high-dose region after exposure to high-LET radiation, but not after low-LET radiation exposure in NHEJ deficient cells (*26, 28*). It is unknown how much the activation of homologous recombination and MMEJ pathways contributed to cell survival in cells exposed to high-LET radiation.

In addition, it was reported that a backup NHEJ (B-NHEJ), DNA-PK independent NHEJ, acts in *Ku70/80* and/or *Lig4* KO MEF (*39, 40*), and also that DSBs are repaired by DNA-PK dependent NHEJ (D-NHEJ) in human G_1 cells (*7*). It is unknown to what degree the activation of B-NHEJ contributed to cell survival in cells exposed to high-LET radiation.

Advantages of Combining C-Ion Beams and NHEJ Inhibitors

It is important to remember when using radiation therapy that NHEJ is used by all cells and tissues, including dose-limiting late-reacting tissues such as spinal cord and stromal tissues, which give rise to fibrosis and telangiectasia. In attempting to identify drugs that can improve radiation therapy outcomes, targeting NHEJ may likely present greater risks than inhibiting homologous recombination (*7*). However, this disadvantage of tumor nonspecificity may be reduced when NHEJ is targeted by combining it with C-ion beams because of more precise dose distributions. There is also some concern related to immunosuppression because NHEJ is an integral part of the V(D)J immune recombinational pathway (*41*). However, on the positive side the risk of secondary cancers may be reduced through the inhibition of error-prone NHEJ repair.

Cancer stem cells are believed to be a major cause of resistance to radiation, leading to disease recurrence and metastasis to other organs (*42–44*). It has been reported that DNA damage repair in dormant cancer stem cells occurs predominantly through the activation of DNA repair by the NHEJ pathway but not through the homologous recombination pathway (*45*). Therefore, perhaps complete tumor eradication may be expedited by overcoming cancer stem cell radioresistance through the use of NHEJ inhibitors. NHEJ inhibitors such as wortmannin (*46*), Garcinol (*47*) and NU7026 (*48*) have been reported to produce sensitization effects in photon-irradiated cells. In particular, NU7026 was reported to show sensitization effects in heavy-ion irradiated cells (*48*). It is therefore anticipated that the data reported here can contribute to improvements in local effects when using C-ion radiation therapy in combination with a NHEF inhibitor.

In conclusion, the current study suggests that: 1. The determining factor leading to a high-RBE value in the presence of high-LET radiation may be inefficacy or inefficiency in NHEJ repair; and 2. Targeting and suppressing NHEJ repair may result in higher radiosensitivity when used in combination with C-ion beams than suppression of homologous recombination repair.

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ERRATA

Volume **182**, Number 3 (2014); pp. 338–344, in the article ''Nonhomologous End-Joining Repair Plays a More Important Role than Homologous Recombination Repair in Defining Radiosensitivity after Exposure to High-LET Radiation'' by Akihisa Takahashi *et al*.

The running header should read as:

RADIOSENSITIVITY IS DEPENDENT ON NHEJ REPAIR AT HIGH LET

Page 338: Abstract, line 16:

''beams as well as'' should be ''beams, and mono-ion beams''.

Page 340: the first and second formulas should read as:

$$\mathrm{RBE} = D_{10} \text{ of X-ray irradiated cells}/D_{10} \text{ of heavy-ion irradiated cells}$$

$$\mathrm{SER} = D_{10} \text{ of wild-type cells}/D_{10} \text{ for cells bearing a DSB repair KO cells}$$

Page 343: left column, line 4 from the bottom:

''NHEF'' should be ''NHEJ''.