

High LET radiobiology at NIRS-current status and future plan

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Abstract

HIMAC synchrotron radiates not only physical beams to cancer patients but also attractiveness to healthy scientists. Our persistent interest to clarify the biologically significant damage caused by high LET radiation needs multilayered approaches including molecular, cellular and tissue levels; all the levels being deeply integrated with physics. The outcome of our research is important for (1) promoting the evidence-based radiotherapy and (2) clarifying the radiation risk in space. We are currently conducting the following experiments; LET-RBE relationship for cell kills, chromatin damages and mutation induction, mitochondrial damages, brain damages at cellular and behavioral level, and fractionated irradiation to skin, gut and tumors. Capacity of cells to repair DNA damages may play an important role in space radiation environment that is characterized as a long-term exposure at low dose rates with mixed LET radiation. Experiments using cells deficient of DNA repair suggested that a process of damage recognition is critical for biological effectiveness of high LET radiation. Specific for space radiation research, we have started a long-term exposure of cultured cells to secondary beams produced at the HIMAC Biology Room. A future plan using HIMAC beams is described.

KEYWORDS: HIMAC, cooperative research, repair gene, long term exposure.

1. Introduction

HIMAC synchrotron was installed and started to accelerate particle beams in 1994 for cancer therapy [1]. Scientific activities including physics and biology also started as a nationwide cooperative research. Machine time available for biology experiments alone is 800 hr per year. Beams are switched from therapy rooms to the biology room at 21:00 when dosimetry for the night run starts. Three to five research groups run irradiation experiments till 7:00 when beams are switched back to therapy room. High LET radiobiology is to clarify mechanisms underlying biological effects of high LET particles and to apply scientific findings for risk/benefit estimation. Radiobiology group in International Space Radiation Laboratory is actively conducting its own research, and also supporting the cooperative research. I here report the outline of current activities and future plans using HIMAC.

2. Current activities

In fiscal year 1999, 66 themes were accepted by a program selection committee and carried out biology experiments in vitro and in vivo. Twenty themes were intra-institutional proposals while 46 themes were applied from domestic and overseas institutions. The following 20 themes were proposed from NIRS (National Institute of Radiological Sciences); i) Evaluation and optimization of therapeutic SOBPs by cultured cells. ii) Experimental studies of therapeutic ratios of heavy particles. iii) Histological and cellular studies of central nervous system after heavy particle irradiation. iv) Response of mouse

intestine to carbon-12 beams. v) Spectrum of the RBE and the OER due to the ion species and radiation quality of heavy-ion beams. vi) Effects of high LET particles on radioresistant cells and the molecular biological factors. vii) Studies on radiation induced brain dysfunction. viii) Chromosome damages in peripheral blood lymphocytes using PCC/FISH analysis: predictability of normal tissue response. ix) Biological and molecular studies of thymic lymphomas induced by carbon ions. x) Evaluation of early and late skin damage after heavy-ion radiation. xi) Effects of heavy ion irradiation on bone metabolism and the related organs. xii) Immunohistochemical study on the brain of fetal and adult rats irradiated with HIMAC beams. xiii) Detection of biological truck structure following exposure to heavy-ions. xiv) Induction of apoptosis in mouse lymphoma cells by HIMAC carbon ion beams and its mechanism. xv) Effect of heavy ion irradiation on nitric oxide production and regulation. xvi) Induction of radical reactions by heavy ion beam and its relation to radiation damage. xvii) Atomic force microscopy of heavy ion radiation-induced chromosome aberrations. xviii) Effects of heavy ions on hematopoietic system and its protection. ix) Radiation effect of carbon beams on rat ocular tissues. xx) Comparison in heavy ion irradiation effects on cell cycle between cell lines. The other 46 themes consist of 6 categories including free radicals, DNA damages, chromosome damages, normal tissue damages, space radiation and others.

Each theme is valid for 3 years after acceptance, but the principle investigator is required to make once a year a progress report which is evaluated by a committee. Some results recently obtained through the above stated activities are introduced in the following sections.

2.1. DNA repair and RBE

Repair of DNA double strand breaks induced by low LET photon plays a significant role in cell lethality. Cells deficient in DNA repair capacity are sensitive to photon radiation [2], but rendered to radioresistant character after being transfected with responsible genes [3, 4]. DNA double strand breaks (dsb) repair is carried out by two mechanisms, i.e., homologous recombination and non-homologous end joining [5]. The former process requires sister chromatids, and effective in the late DNA synthesis and G2 phases while the latter is effective irrespective of cell cycle position. Genes known to repair DNA dsb through homologous recombination are Rad50, Mre11, NBS1, RPA, Rad51, Rad52, Rad54, Rad55, Rad57 while those through non-homologous end joining are DNA-PKcs, Ku70, Ku80, Rad50, Mre11, NBS1, XRCC4 and Lig IV. Biological effectiveness of high LET radiation is believed to stem from unique energy deposition to critical targets within a cell. LET of 100-200 keV/ μ m shows most effective cell lethality per unit dose while DNA dsb does not show the RBE peak at 100-200 keV/ μ m [6]. However, as the RBE peak is not detectable in repair deficient cells [7], dsb repair is significant for determining biological effectiveness of high LET radiation.

2.1.1. DNA-PK - Okumura and Okaichi at Nagasaki University are investigating biological effectiveness of carbon ions on SCID cells that are deficient in a dsb repair gene of DNA-PKcs [3]. Cell survivals determined by colony formation were obtained after irradiation with either 50 keV/ μ m carbon ions or 200 kVp X-rays. SCID cells were more sensitive to X-ray irradiation than hybrid cells that were complemented with DNA-PKcs of human chromosome #8 (Fig. 1). Carbon-ion irradiation reduced survi-

ving fractions more efficiently than X-ray irradiation for not only the hybrid cells but also SCID cells. Doses to reduce surviving fraction down to 50% and 10 % were termed D50 and D10, respectively, and obtained for 4 survival curves. Relative biological effectiveness (RBE) of carbon ions for SCID cells was similar to that for hybrid cells (Table I), indicating that RBE of 50 keV/ μ m carbon ions is independent of DNA-PK. This follows that a repair process carried by DNA-PK does not account for the strong lethality of carbon ions.

2.1.2. HOMOLOGOUS RECOMBINATION AND NONHOMOLOGOUS END JOINING - Furusawa

(NIRS) and Utsumi (Kyoto University) obtained colony survivals of a chicken B cell lymphoma cell line, DT40, after irradiation with 85 keV/ μ m argon-40. DNA repair deficient clones that were prepared by gene targeting at rad54 and ku70 showed increased radiosensitivities than the wild type of DT40 [5]. Survival curves were obtained for the following 8 groups: i) rad54+/Ku70+, 200 kVp X rays; ii) rad54+/Ku70+, argon ions; 3. rad54-/Ku70+, 200 kVp X rays; 4. rad54-/Ku70+, argon ions; 5. rad54+/Ku70, 200 kVp X rays; 6. rad54+/Ku70-, argon ions; 7. rad54-/Ku70-, 200 kVp X rays; 8. rad54-/Ku70-, argon ions. D50 and D10 were calculated for each survival curves and used to obtain RBE of argon ions (Table II).

RBE values for a mutant clone deficient of rad54, i.e., rad-/ku+, were smaller than those for the wild type DT40 cells, i.e., rad+/ku+. A Mutant clone deficient of ku70, i.e., rad+/ku-, showed RBE values prominently smaller than the wild type DT40. As the rad+/ku- clones showed, irrespective of radiation qualities, biphasic survival curves with a radioresistant fraction of 20%, RBE rather increased when the isoeffect level decreased from 50 to 10 % survivals. Mutant clones deficient in rad54 and ku70, i.e. rad-

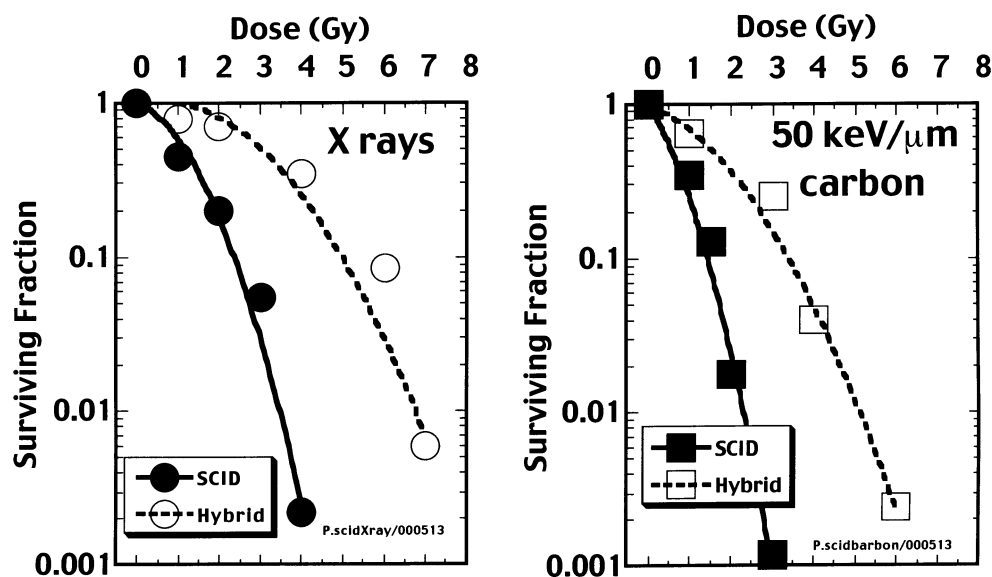


Fig. 1 – Survival curves for mouse SCID cells after 50 keV/ μ m carbon ions and X rays. Closed symbols; parental SCID cells deficient of DNA-PK. Open symbols; SCID cells complemented with human chromosome #8.

Table I – RBE of 50 keV/ μm carbon-12 for mouse SCID cells. D_{50} and D_{10} are isoeffect doses to bring cell survivals to 50% and 10%, respectively. SCIA cells are deficient of DNA-PK, i.e., DNA-PK(-), while DNA-PK (+) SCID cells are prepared by hybridization with human chromosome #8.

Isoeffect	DNA-PK (-)	DNA-PK (+)
D_{50}	2.07	1.95
D_{10}	1.65	1.47

Table II – RBE of 85 keV/ μm argon-40 for Chicken lymphoma cells. D_{50} and D_{10} are isoeffect doses to bring cell survivals to 50% and 10%, respectively.

Iso-effect	rad54+ ku70+	rad54-ku70+	rad54+ku70-	rad54-ku70-
D_{50}	3.44	2.26	0.98	0.98
D_{10}	2.25	2.12	1.45	1.00

/ku-, showed RBE of unity. These results indicate that the DNA repair genes for both homologous recombination and non-homologous end-joining significantly contribute to determine RBE of high LET, and that ku70 gene dominates the contribution. Taken together with the results of SCID cells, it is likely that a process of damage recognition is critical for biological effectiveness of high LET radiation.

2.2. Long term irradiation with HIMAC secondary beams

Suzuki at NIRS continuously irradiated normal fibroblasts with secondary beams of HIMAC synchrotron. NB1RGB human skin fibroblasts in culture bottles were kept in an incubator that was placed at 45 degree off of primary beam (Fig. 2).

Cells were replated once a week for up to one year, and population of doubling number was calculated for each replate. Cells were constantly divided for up to 200 days. No difference in doubling number was detected between the irradiated and non-irradiated cells. Non irradiated cells, however, increased in doubling number after 300 days of culture while irradiated cells showed minimum cell division. This indicates that long term irradiation with the secondary beams prevents normal cells from unscheduled proliferation. Continuous irradiation with Co-60 γ rays at 10 mGy/day rather prolongates the life-span of human embryo cells [8]. Absorbed dose in the incubator was measured with Si-semiconductor and Mg_2SiO_4 -thermoluminescent dosimetry (TLD). Accumulated dose for 223 days was 320 mGy, giving an averaged dose rate of 1.4 mGy/day [9]. Kyan and Uchihori at NIRS identified and measured components consisting of secondary beams. A primary beam used was 290 MeV/u carbon-12. Particles above several hundred keV were identified by measuring events detected with a liquid scintillator and a plastic scintillator that were placed on the out top of incubator for one night. Most of events, i.e. 92-95%, were

attributed to γ rays while protons were 3-5%. Contribution of neutrons was calculated as 1-3% with a possible error factor of 20.

3. Future plan

Radiobiology group at NIRS will further advance basic studies of high LET. The backbone is to clarify the nature of damage caused by high LET radiation. DNA repair genes of mammalian cells responsible for high LET producing RBE larger than unity should be identified along with functional analysis of repair proteins. Capability of microbeams to selectively irradiate individual cells would increase the spatial resolution of initial damages, and clarify the significance of inter- and intracellular communications. Further studies for LET-RBE relationship is necessary to understand effects of mixed LET. As to therapy-oriented research, in vivo and tissue studies should be included to propose therapeutic gain of high LET radiation. Optimization for fractionation schedule with high LET radiotherapy could be provided by experimental approach. Tissue specificity of damage repair and physiological condition such as tumor hypoxia may modify not only fractionation schedule but also design of Spread-Out-Bragg-Peak. Individual radiosensitivity of tumors is least significant at LET of 100-200 keV/ μm , but becomes important for intermediate LET which could increase therapeutic gain in serial organs. Predictive assays using patients' peripheral lymphocytes and tumor biopsies are the initial step for further improvement of predictability. Space radiation is characterized by its specific mode of exposure. A variety of particle species with a wide range of LET spectrum makes space crafts a mixed radiation field with sporadic hits by heavy ions. A long-term flight mission should provide reasonable radiation protection to crews such that risk estimation for somatic and genetic damages is accounted for both design and operation of spacecrafts. Before making

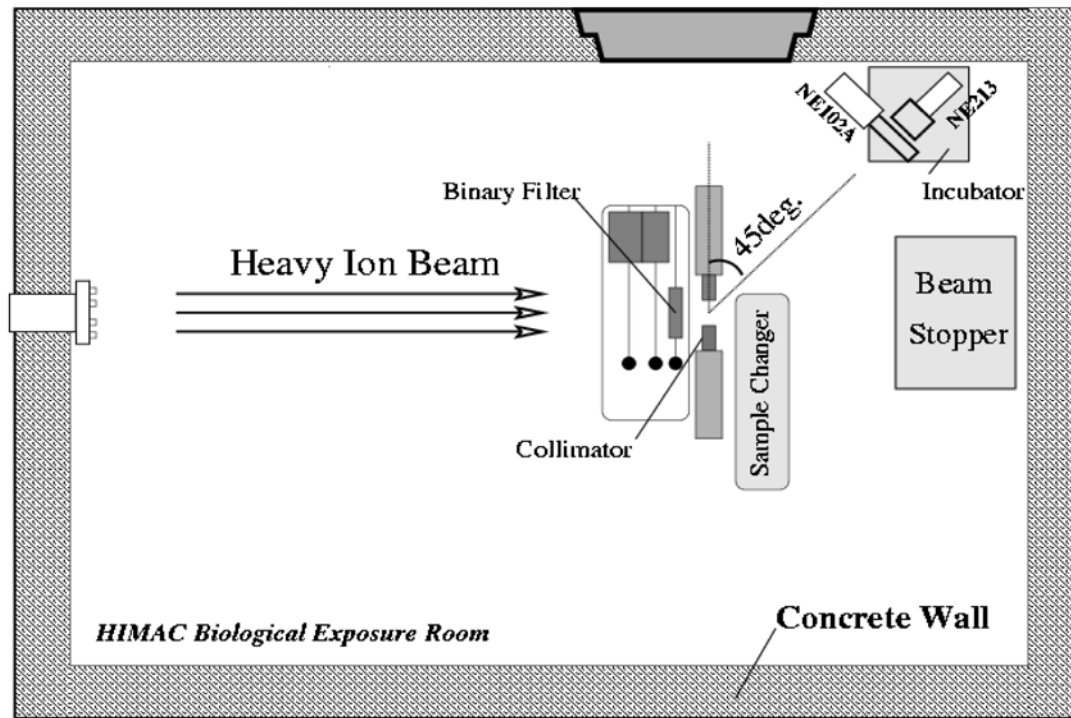


Fig. 2 – Long term exposure of cultured cells in the HIMAC biology room. An incubator is located 45 degree off the primary beam. Human fibroblasts were cultured in the incubator that was intermittently exposed to secondary beams for up to 1 year.

a radiation exposure field with long duration / low dose rate and mixed LET, preparatory experiments using defined primary beams should clarify biological significance of long term exposure. Cyclotron at NIRS is a possible choice for the preparatory experiments. Neural function and carcinogenesis could be most important subjects for risk estimation of space radiation.

4. Conclusion

High LET radiobiology group at NIRS will further extend its activities by conducting prospective research and by integrating separate themes proposed by individual investigators. The activity includes domestic and international cooperation as essential components, and will be valuable for all who contribute to society in 21 century by understanding significance of radiation effects on human beings.

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REFERENCES

- [1] Tsuji H, Morita S, Miyamoto T, Mizoe J, Mukai M, Nakano T, Kato H, Kamada T, Ishikawa A, Matsuoka Y. Preliminary results of phase I/II carbon-ion therapy at the NIRS. *International J. Brachytherapy* 1997; 13; 1-8
- [2] Warters R L, Lyons B W, Chen D J, Sato K. DNA-damage processing in a radiation-sensitive mouse cell line. *Mutation Research* 1993; 293; 91-98
- [3] Komatsu K, Kubota N, Gallo M, Okumura Y and Lieber MR. The scid factor on human chromosome 8 restores V(D)J recombination in addition to double-strand break repair. *Cancer Res.* 1995; 55; 1774-1779
- [4] Chen F, Peterson SR, Story MD, Chen DJ. Disruption of DNA-PK in Ku80 mutant xrs-6 and the implications in DNA double-strand break repair. *Mutation Research* 1996; 362; 9-19
- [5] Takata M, Sasaki MS, Sonoda E, Morrison C, Hashimoto M, Utsumi H, Yamaguchi-Iwai, Y, Shinohara A and Takeda S. Homologous recombination and non-homologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells. *EMBO Journal* 1998; 17; 5497-5508
- [6] Roots R, Holley W, Chatterjee A, Irizarry M and Kraft G. The formation of strand breaks in DNA after high-LET irradiation: a comparison of data from in vitro and cellular systems. *International Journal of Radiation Biology* 1990; 58; 55-69
- [7] Weyrather WK, Ritter S, Scholz M and Kraft G. RBE for carbon track-segment irradiation in cell lines of differing repair capacity. 1999; 11; 1357-1364
- [9] Yasuda H, Suzuki M and Fujitaka N. Utilizing Ion-Beam Secondary Radiation for Experiments on Space Radiation Effects. *J. Health Physics* 1999; 34; 381-386.