Project Research

Project 13

PR13 Project Research on a Study on Biological Character and use of the Particle Induced by the Boron Neutron Capture Reaction

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Objectives and Participating Research Subjects

In this project, we are intending to develop the new application using the characteristics of the particles from the neutron capture reaction.

PRS-1 Analysis of mutation in the mammalian cells induced by BNCR (boron neutron capture reaction)
(Y. Kinashi *et al.*)

PRS-2 Analysis of double strand breaks in the mammalian cells induced by BNCR
(S.Takahashi *et al.*)

PRS-3 Development of the PARP repressor reinforced in its function by BNCR
(Y.Uto *et al.*)

PRS-4 Development of the model animal shouing the blood vessel damage by BNCR
(R. Wate *et al.*)

Main Results and Contents

PRS-1 studied gamma-H2AX focus data and investigated the DNA-dsb (double strand breaks) damage and association of the mutation induction following the neutron radiation beam used for BNCT in KUR. The mutagenic effects of BNCT on the normal tissue cells that do not take up the boron compounds may cause the genetic instability and second cancer decade years after BNCT.
The mutagenicity measured by the frequency of mutations induced by neutron irradiation with or without boron compound. The HPRT locus was examined in Chinese hamster ovary (CHO) cells irradiated with neutrons of KUR.

The frequency of mutations after neutron irradiation with 10ppm BPA was about 5 times and more increased than 1Gy gamma-ray irradiation. the gamma-H2AX foci size at after 1Gy of neutron irradiation with 10ppm BPA. After 2 hours of neutron irradiation, about 20% of cells had big foci,

The result suggestted that The mutation induction might be increased due to the inferiority of the ability of DNA damage repair following BNCR.

PRS-2 was not able to carry out its experiments because KUR (Kyoto University Research Reactor) has been stopping during this year.

PRS-3 was not able to carry out its experiments because KUR has been stopping during this year.

PRS-4 was not able to carry out its experiments because KUR has been stopping during this year.

PR13-1 Association of the DNA-double Strand Breaks Damage and the Mutation Induction Following the Neutron Radiation Beam used for BNCT

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INTRODUCTION: We have previously reported the increased mutagenicity of thermal neutrons and the dose rate effect of the neutron radiation beam [1]. Clinically, the mutagenic effects of BNCT on the normal tissue cells that do not take up the boron compounds may cause the genetic instability and second cancer decade years after BNCT. In this study, we analyzed gamma-H2AXfocus data and investigated the DNA-dsb (double strand breaks) damage and association of the mutation induction following the neutron radiation beam used for BNCT in Kyoto University Research Reactor (KUR).

MATERIALS & METHODS: The mutagenicity measured by the frequency of mutations induced by neutron irradiation with or without boron compound. The hypoxanthine-guanin-phosphoribosyl-transferase (HPRT) locus was examined in Chinese hamster ovary (CHO) cells irradiated with neutrons of KUR. A stock solution of 10B-para-boronophenylanine (BPA) was used for this experiment. Cell suspensions were incubated with BPA at 10 ppm concentration 1 hour before neutron irradiation. To determine mutation frequencies, each treated culture was incubated with non-selective medium for 7-9 days to allow phenotype expression. Then, $2x10^5$ cells were added to each dish containing 6-thioguanine and incubated for 10-14 days, after which time the mutant colonies were counted. The mutation frequency is expressed as the number of resistant colonies divided by the total number of viable cells as determined by cloning efficiency at the time of selection. DNA double-strand breaks (DSBs) in CHO cells following neutron irradiation were detected using the gamma H2AX foci assay. Immunofluorescence staining was performed as described previously (2). Briefly, irradiated cells were incubated 30 min.or 120 min., and washed with cold PBS. After fixation with 3.6% formalin Immunofluorescence images were acquired using a fluorescence microscope (KEYENCE, BZ-9000), and then analyzed using the BZ-9000 optional software and Image-J (National Institutes of Health).

RESULTS and DISCUSSION: Table 1 shows the mutation frequency in the HPRT locus in CHO cells after 1Gy neutron irradiation. The frequency of mutations after neutron irradiation with 10ppm BPA was about 5 times and more increased than 1Gy gamma-ray irradiation.

Table 1 HPRT mutation frequencies of CHO cells exposed to 1Gy of gamma-rays, neutron and BNCR

	Gamma ray	Neutron	BNCR (10ppm **B**PA)
HPRT mutation frequency / 105 cells	1.2±1.5	3.2±2.5	6.8±3.5

Figure 1 shows the gamma-H2AX foci size at after 1Gy of neutron irradiation with 10ppm BPA. After 2 hours of neutron irradiation, about 20% of cells had big foci (>5.0 μm2), On the other hand, the cell with the big focus was less than 1% at 2 hours after gamma ray irradiation.

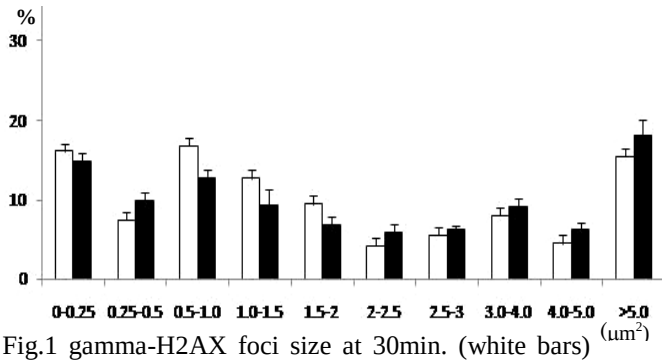

Fig.1 gamma-H2AX foci size at 30min. (white bars) and 120min. (black bars) after 1Gy of neutron irradiation with 10ppm BPA.

In this study, we found that the large foci which are a serious DNA damage increased by BNCR. The mutation induction might be increased due to the inferiority of the ability of DNA damage repair following BNCR.

REFERENCES:

[1] K. Kinashi *et al.*, Appl. Radiat. Isot. , **88** (2014) 153-156.
[2] K. Kinashi *et al.*, Radiation Oncology, **6:106** (2011)