VIII-II-3. Original Research

OR1 Influence of Manipulating Hypoxia in Solid Tumors on Radiation Dose-Rate Effect *in Vivo*, with Reference to That in Quiescent Tumor Cell Population

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BACKGROUNDS AND PURPOSES: Intensity modulated radiotherapy (IMRT) and stereotactic irradiation have become common as a new radiotherapy technique for treatment of malignancies. Both modalities generally use multiple arc or fixed-portal radiation beams, and radiation beams are exposed intermittently. These techniques often require 30 min or a longer time in one treatment session for precise positioning of patients. Prolongation of irradiation time may reduce a radiation effect, and evokes a major concern for the dose rate effect. Thus, it is needed to clarify the effect of the reduction of dose rate on the radiosensitivity of tumors *in vivo*.

When using low linear energy transfer (LET) radiation, lowering the dose rate is thought to reduce late effects in normal tissue much more than it decreases tumor control. Thus, the "therapeutic ratio" increases as the dose rate decreases, because the therapeutic ratio is equal to the ratio of tumor control to normal tissue complications. Further, the difference between early and late effects for low dose-rate radiotherapy, as well as improving the therapeutic ratio, allows complete treatment in a short period of time, minimizing the effects of tumor repopulation. In other words, decreasing the dose rate increases the therapeutic ratio, limited only by tumor cell repopulation. This is the primary rationale for low dose-rate radiotherapy using low LET radiation. High LET radiation is more effective than low LET X- or gamma-radiation at inducing biologic damage. High LET radiation results in a greater relative biological effectiveness (RBE) value for cell killing, a reduced oxygen effect, and a reduced dependence on the cell cycle and the irradiation dose rate.

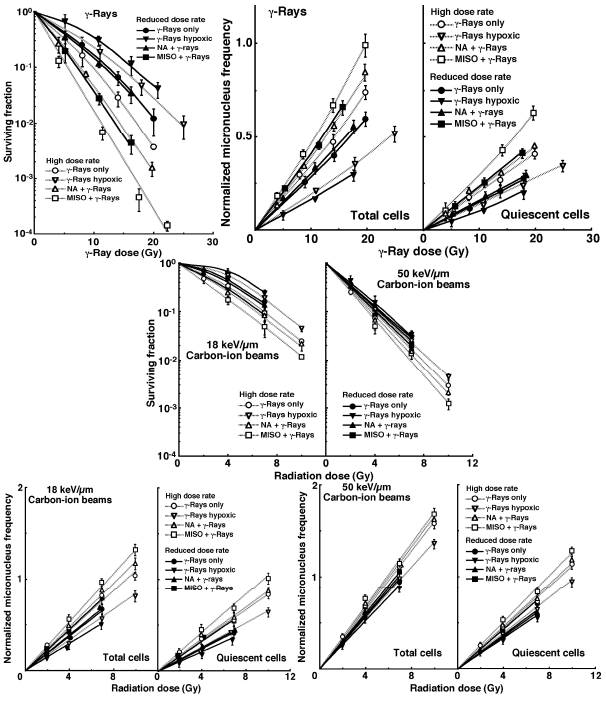
Manipulating hypoxia in solid tumors during irradiation apparently influences tumor radiosensitivity under high dose-rate (HDR) irradiation using low LET radiation. However, its significance in solid tumors irradiated at a reduced dose rate (RDR) is less clear *in vivo*, whether low or high LET radiation is employed.

Thus, in this study, we tried to elucidate the effect of manipulating hypoxia in irradiated solid tumors at a RDR with low-LET gamma-rays or high-LET 290 MeV/u accelerated carbon-ion beams *in vivo*, compared with HDR irradiation. Further, the responses of the total (= proliferating (P) + quiescent (Q)) and Q cell populations in irradiated solid tumors were separately detected with the method for selectively detecting the response of Q cells within solid tumors. This is the first attempt to clarify the direct relationship between the irradiation dose rate effect and the oxygen effect *in vivo*, referring to the response of the Q cell population in irradiated solid tumors. This is the first attempt to clarify the direct relationship between the irradiation dose rate effect and the oxygen effect *in vivo*, referring to the response of the Q cell population in irradiated solid tumors.

MATERIALS AND METHODS: Tumor-bearing mice were continuously given 5-bromo-2'-deoxyuridine (BrdU) to label all P cells. They received gamma-rays or accelerated carbon-ion beams at HDR or RDR with or without tumor clamping to induce hypoxia. Some mice without clamping received nicotinamide, an acute hypoxia-releasing agent or misonidazole, a hypoxic cell radio-sensitizer before irradiation. The responses of Q and total (= P + Q) cells were assessed by the micronucleus frequency using immunofluorescence staining for BrdU.

RESULTS: The clearer decrease in radiosensitivity in Q than total cells after RDR gamma-ray irradiation was suppressed with carbon-ion beams, especially with a higher linear energy transfer value. Repressing the decrease in the radiosensitivity under RDR irradiation through keeping tumors hypoxic during irradiation and enhancing the decrease in the radiosensitivity by nicotinamide were clearer with gamma-rays and in total cells than with carbon-ion beams and in Q cells, respectively. Inhibiting the decrease in the radiosensitivity by misonidazole was clearer with gamma-rays and in Q cells than with carbon-ion beams and in total cells, respectively.

CONCLUSION: Manipulating hypoxia during RDR as well as HDR irradiation influences tumor radio-sensitivity, especially with gamma-rays [1].



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休止期腫瘍細胞への効果も加味した固形腫瘍内酸素化状況の操作の照射線量率効果独自研究に対する影響の解析

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独自研究

OR2 Usefulness of Hexamethylenetetramine as an Adjuvant to Radiotherapy and Cisplatin Treatment for Solid Tumors: its inDependency of *p53* Status

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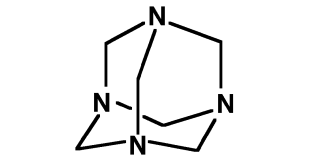
BACKGROUNDS AND PURPOSES: Formaldehyde preserves or fixes tissue or cells by irreversibly cross-linking primary amine groups in proteins with other nearby nitrogen atoms in protein or DNA through a -CH2- linkage. An acid-dependent formaldehyde donor, hexamethylenetetramine (HMTA), has been used as an antiseptic for urinary tract infections and it has been supposed to be characterized as a non-carcinogen in animals. In the hypoxic condition in a solid tumor, pyruvate generated by glycolysis induces a low pH environment that produces formaldehyde through dissociation of HMTA. Except for surgical resection, chemotherapy using cis-diamminedichloroplatinum (cisplatin) and radiotherapy are the most frequently employed anticancer therapeutic modalities in real clinics. Based on these clinical backgrounds, using a p53 wild-type SCC VII tumor, the combined effect of HMTA with gamma-ray irradiation or cisplatin treatment was examined, resulting in producing almost similar radio- and cisplatin sensitivity enhancing effects to TPZ [1]. Accordingly, in this study, using two different solid tumors identical in genetic background except for p53 status and our method for selectively detecting the response of the quiescent (Q) cell populations within solid tumors [2], we evaluated the usefulness of combined use of HMTA in solid tumor treatment based on its dependency on p53 status. In addition, the usefulness of continuous administration of HMTA was also evaluated.

MATERIALS AND METHODS: Human head and neck squamous cell carcinoma cells transfected with mutant *TP53* (SAS/mp53), or with neo vector as a control (SAS/neo), were inoculated subcutaneously into both the hind legs of Balb/cA nude mice. The tumor-bearing mice received 5-bromo-2'-deoxyuridine (BrdU) continuously to label all proliferating (P) cells in the tumors. Then, they received HMTA, intraperitoneally or continuously, combined with or without gamma-ray irradiation or cisplatin treatment. Immediately after treatment following HMTA, the response of Q cells was assessed in terms of the micronucleus frequency using immunofluorescence staining for BrdU. The response of the total (= P + Q) tumor cells was determined from the BrdU non-treated tumors.

RESULTS: A higher toxicity of HMTA to Q cells than total cells, especially in SAS/neo, was made less clear by continuous administration. There was no apparent difference in the radio- and cisplatin-sensitivity enhancing effects by HMTA combination between SAS/neo and SAS/mp53 tumors, with a slightly greater effect in SAS/mp53. In both SAS/neo and SAS/mp53 tumors, continuous HMTA administration produced higher radio- and cisplatin-sensitivity enhancing effects than intraperitoneal single administration.

CONCLUSION: The use of HMTA as an adjuvant to radiation or cisplatin might be promising in curing solid tumors with large fraction of hypoxic cells and also with frequent loss-of-function in p53. [3].



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固形腫瘍に対する放射線・シスプラチン治療の併用処置としての　　独自研究
ヘキサメチレンテトラミンの有用性：p53 status への非依存性

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OR3 Assessment of Environmental and Social Impacts Originating from the Use of Nuclear Energy

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INTRODUCTION: Japan Atomic Energy Commission recently released a new strategy contributing to reduction of greenhouse gas emission in Japan by constructing new nuclear power plants as well as increasing availability. According to a public opinion poll, however, 66 % of respondents were worrying about safety aspect of nuclear energy. The decision whether or not our society relies on nuclear energy should be made by the public taking into account correct information on its merits and demerits. For the purpose to provide scientific information for the public decision, our group has been investigating environmental and social impacts originating from the use of nuclear energy. Main themes are: a) analysis of radiological consequences of hypothetical accidents at nuclear facilities as well as actual accidents such as Chernobyl-4 (1986), JCO criticality accident (1999), TMI-2 (1979) *etc.*, b) monitoring of radioactivity in the environment around nuclear facilities and radioactivity measurements in food and other materials, c) retrospective radiation dosimetry for Hiroshima-Nagasaki bombings and nuclear tests in Semipalatinsk *etc.*, d) analysis of social issues related with nuclear energy and e) hosting of open-seminars on various aspects of nuclear safety issues.

RECENT TOPICS

The following three open-seminars were held in 2009-10:

-107th: Aug 7, 2009: Rehabilitation of Narodychi district contaminated by the Chernobyl accident. "Outline of NANOHANA project" by K. Tomura and "The consequences of Chernobyl accident in the agriculture of Zhytomyr region and counter-measures against them" by M. Didukh.

-108th: Oct 26, 2009: Global warming and carbon dioxide emission. "Discussion on IPCC-4 report as well as the opinion that carbon oxide emission is causing global warming" by H. Koide. "To what extent the global warming can be explained by carbon dioxide emission?" by H. Watanabe.

-109th: Apr 26, 2010: 65 years since Hiroshima-Nagasaki. "What happened 65 years ago" by H. Hasai. "As a hibakusya and a scientist" by S. Sawada. Abstracts of these seminars as well as previous ones are on: http://www.rri.kyoto-u.ac.jp/NSRG/.

Countermeasure for global warming is recently used as the main slogan to enlarge the use of nuclear energy because nuclear power plants do not emit carbon dioxide during operation. Koide, analyzing the IPCC-4 report, pointed out that the long-term meteorological observation did not support the IPCC opinion that the causal relationship between global warming and carbon dioxide emission was confirmed [(1)]. According to his opinion, global warming is merely one of many factors that are destructing the environment. Promotion of nuclear energy will inevitably accelerate consumption of energy, which will make the environmental situation worse.

International collaboration project, "Historical review of nuclear disasters during the process of nuclear development program by the former USSR" was launched in 2008 for three years under a support of KAKENHI. The project leader, Imanaka intends to investigate consequences of a series of radiological incidents that occurred in the former USSR: Techa river contamination in 1948-1951, the explosion of radioactive waste storage at the Mayak complex in 1957, various accidents at USSR nuclear submarines as well as the Chernobyl accident in 1986 [(2)]. Articles prepared through the project are being put one by one on: http://www.rri.kyoto-u.ac.jp/NSRG/en/kkn2008.html

The Rokkasho Reprocessing Plant began its active test using real spent nuclear fuels in March 2006. Because of a series of serious troubles that occurred at the glass-solidification facility for high level liquid waste, currently no perspective is given for its completion. Koide analyzed various problems related with the plutonium nuclear fuel cycle policy in Japan [(3)].

An Excel Basic program, FPCOMP.xls was developed to calculate fission product composition at any time after fission reaction. It was applied to estimate radiation exposure from local fallout deposition in villages around the Semipalatinsk Nuclear Test Site, Kazakhstan [(4)] as well as from "black rain" areas in Hiroshima and Nagasaki.

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原子力利用にともなう環境影響ならびに社会的影響に関する研究　　独自研究

（京大・原子炉）今中哲二、小出裕章